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ESTIMATION OF ESTRADIOL LEVEL AND SOME BIOCHEMICAL INDICATORS IN NATURALLY PREGNANT WOMEN AND PREGNANT WOMEN AFTER TAKING STEROIDS

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Abstract

This study measures the level of Estradiol and a number of biochemical variables, including (cholesterol, triglycerides, high-density lipoproteins, low-density lipoproteins,and very low-density lipoproteins). The study also measures the total antioxidant index, as well as the level of malondialdehyde in naturally pregnant women, as well as pregnant women. after taking steroids.

A total of (100) samples of naturally pregnant women and pregnant women after taking steroids were studied. These represent different age groups ranging between (15-45) years of age. Blood samples were collected from women admitted to Azadi Teaching Hospital as well as outpatient medical women's clinics for the period from 12/25/2021 to 5/5/2022. The samples were divided into (40) samples for naturally pregnant women as a control group, (30) samples for pregnant women using steroids, and (30) samples for non-pregnant women after taking steroids.

*There was a high probability level (P≤0.01) with high statistical differences in the level of (Estradiol, triglycerides, very low-density proteins) in naturally pregnant women by comparison with pregnant women after taking steroids.

* There was an increase at the probability level (P> 0.05) and no statistical differences appeared in the level of (malondialdehyde, total cholesterol, and low-density lipoproteins) in naturally pregnant women by comparison with pregnant women after taking steroids.

* A decrease in the probability level (P ≤ 0.01) with high statistical differences in the level of total antioxidant index in naturally pregnant women by comparison with pregnant women after taking steroids.

* There was a decrease at the probability level (P > 0.05) and no statistical differences in the level of high-density lipoproteins appeared in naturally pregnant women by comparison with pregnant women after taking steroids.

The effect of women's age on the biochemical variables above-mentioned was studied, and the following was found:

* A high statistical variations at the level of the (p≤0.01) in the levels of Estradiol for the age group 36-45 years in pregnant women are naturally pregnancy compared to the age groups (15-25) (26-35) years. In non-pregnant women after taking steroids and pregnant women after taking steroids compared with the age group (26-35) years. The two age groups (15-25) (36-45) years excelled significantly in non-pregnant women after taking steroids and pregnant women after taking steroids compared to the age group (26-35) years.

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* It was found to be no statistical differences at the probability level (P> 0.05) in the MDA for the three age groups respectively for pregnant women with steroids and non-pregnant women after taking steroids compared with naturally pregnant women, where the highest percentage was in the age group (36-45) years for both naturally pregnant women and pregnant women with steroids and non-pregnant women after taking steroids compared with the age group (15-25) and (26-35) years.
* There is a decrease with differences in statistical terms at the probability level (P≤0.01) in the TAC at the age group (36-45) years for naturally pregnant women more than the two age groups (15-25) and (26-35) years, as well as for the two groups (pregnant women after taking steroids and non-pregnant women after taking steroids).
* There were no significant differences in statistical terms at the probability level (p>0.05) in the TC of the three age groups in blood serum of pregnant and non-pregnant women and pregnant women with steroids.
* An increase with significant differences in statistical terms in the level of TG, VLDL at the probability level (p ≤ 0.01) for the three age groups in the blood serums of naturally pregnant women and steroids compared with the age groups of the non-pregnant group.
* There was a decrease and no significant differences in statistical terms appeared at the probability level (p > 0.05) in the level of (HDL) in the serums of naturally pregnant women and pregnant women after taking steroids and non-pregnant women after taking steroids for the three age groups, where the highest percentage decrease was recorded in the age group (G3 = 36-45).
* There was an increase with no significant differences in statistical terms at the probability level (p> 0.05) in the level of (LDL) in the serums of naturally pregnant women and pregnant women after taking steroids compared with non-pregnant women after taking steroids for the three age groups, where the highest percentage of increase was recorded in the age group (G3=36-45).

1. Introduction

Infertility in women is a state of disorders that occur to women, for many reasons that may be known or unknown. There is no comprehensive definition of infertility in women because it depends on the social and physical characteristics that vary according to the environment and cultural situation. The term "infertility" refers to the inability of a group of women to carry the fetus throughout the entire pregnancy, whereas the term "clinical infertility" refers to a defect affecting the reproductive system that results in failure to become pregnant after a continuous period of at least a year and without the use of any contraindications. (Mustafa et al., 2019).

Women can become pregnant and have pregnancies repeatedly, but this process is incomplete and excludes infertility caused by surgery, such as the removal of the ovaries or other procedures (Yatsenko & Rajkovic, 2019). If the woman does not give birth to a full-grown child who is born alive and in excellent health, neither one nor both spouses can be called fertile (Campbell,2019).

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1.1 Factors leading for infertility in Women

The causes and factors of female infertility are classified as acquired or hereditary by the ASRM American Society for Reproductive Medicine. Ovulation disorders are one of the most common causes of a woman's inability to have a pregnancy, and it is represented in 30% of infertility cases in women. However, approximately 70% of these cases can be successfully treated by medications. One of the causes of ovulation failure is ovulation disorders, which mean irregular ovulation or no ovulation at all. It may be caused by female hormonal disorders in the hypothalamus or the pituitary gland in the brain or disorders of the ovaries themselves (Upadhyay et al., 2020). Infertility can be influenced significantly by the hypothalamus and the disturbance in the production of its hormones GnRH releasing the inducers of gonadotrophins (Farzana & Afshar, 2020). Infertility can be primary infertility and this condition does not occur when the woman is pregnant at all. It can also be secondary infertility where the woman has a previous pregnancy. In certain circumstances, after giving birth to her first child without issues, the pregnant then experiences a miscarriage during the first few months of her subsequent pregnancy (Sun et al., 2020).

1.1 Hormone Estradiol;

Estrogen is a female hormone secreted by the ovaries as the primary sex hormone. It stops being secreted in females when reaching menopause. natural estrogens are steroid hormones, while synthetic estrogens are non-steroidal (Shamma et al., 1992). It is produced in females by the ovaries and in smaller quantities than the adrenal cortex and placenta during pregnancy. Some estrogen is also produced in smaller quantities by other tissues such as the liver, adrenal glands, and breasts. These secondary sources of estrogen are of particular importance in postmenopausal women and fat cells also produce estrogen (Wang & Moenter, 2020).

Follicle-stimulating hormone (FSH) stimulates the production of estrogen from the ovaries by the granulocytes of the ovarian follicles. This hormone has several functions, including controlling and directing sexual development, including physical changes associated with puberty in the female (secondary sexual characteristics). It also affects the ovulation process in the menstrual cycle, breastfeeding after pregnancy (Trevisan et al., 2018). There are three forms of estrogen, namely Estraiol (E3), Estradiol (E2) and Estrone (Estrone) (E1). The main type of estrogen production is estradiol, which is most important in non-pregnant females in the age stage that begins with menstruation until menopause. It has a major role in developing and determining the characteristics of women and also facilitating the process of fertilization and preparing the uterus for pregnancy. It also contributes in forming and producing proteins and also in raising the concentration of blood calcium (Trevisan et al., 2018 and Wang & Moenter, 2020). Ovulation is activated by Lutinizing hormon and Estradiol and the sensory receptors important for the creation and development of the follicle are stimulated by E2 estradiol (Trevisan et al., 2018). In infertile females, the percentage of fat increases. This increase makes them more at risk of stroke and heart disease, the reason for this is a disorder in fat levels, including an increase in the level of TGs and a decrease in the level of HDL-c. Calonge et al., (2018) note that different Lipoproteins when transferred between tissues have a very important role in fertility and that the only

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Lipoprotein present in the follicular fluid around the developing egg in the ovary is HDL-c (High density lipoprotein -Cholesterol). According to Busso et al., cholesterol is important to prolong the primary substance for the creation of steroids and aids in the maturation, ovulation, and luteinization phases of the follicle (Jain et al., 2020).

Because they reduce the effects of oxidative stress and function as an antioxidant before other molecules that can interact with free radicals do, antioxidants aid in the removal of nitrogen and oxygen. This provides protection against the oxidative process (Lu et al., 2018). Thus, the high levels of oxidative stress that cause poor fertility are caused by an imbalance between reactive oxygen species (ROS) and antioxidants (infertility) (Verma et al., 2018).

1.2 Objective of the study

The main objectives of the research were defined as follows:

1. Studying the effect of estradiol E2 levels.
2. Studying the effect of TC cholesterol, TG, HDL, LDL and VLDL.
3. Studying the effect of levels of malondialdehyde (MDA) and total antioxidant (TAC)

2. Materials and Methods

2.1 The sample of the study

Blood samples were collected from women visiting the outpatient clinic (gynecology) and Azadi Teaching Hospital for the period from 12/25/2021 until 15/5/2022. The required information was retrieved, and the models were divided into a control group, which included (40) blood samples from pregnant married women in a natural way without taking steroids. The group of patients included (30) blood samples from married women with primary and secondary infertility from married women who are not pregnant after taking steroids and (30) blood samples from pregnant women with steroids and their ages ranged between 15) - 45). This is after confirming that they are infertile by the specialist doctor. (5 ml) of intravenous blood was drawn for patients and healthy patients by medical syringes that are used only once. The blood was put in glass tubes (Gel tube) clean and sterile cover tight and free of anticoagulants, left at room temperature for (10) minutes until coagulation.

To obtain the blood serum, the tubes are placed in a centrifuge for a period of time of (10 min) and at a speed of (4000 cycles / minute), then the Serum is withdrawn by a micropipette to be placed in clean, sterile plastic tubes with a cap.

2.2 Measurement of serum Estradiol concentration

Several methods were adopted by the (mindray) device in estimating the level of estradiol hormone concentration by a toolkit prepared by the company (mindray) and according to the leaflet attached to the toolkit.

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2.3 Measurement of serum Total Cholesterol concentration

The enzymatic method adopted color (Allain et al.,1974) using ready-made analysis kit from the French company (Biolabo). The enzyme cholesterol esterase in the analysis kit activates the decomposition of cholesterol ester, which is present in the blood serum to cholesterol and fatty acids. Then, cholesterol is oxidized by the enzyme cholesterol oxidase and the presence of oxygen to produce hydrogen peroxide. This interacts with phenol and 4- amino antipyrene through the enzyme peroxidase to be pink quinone amine, which is absorbed at the wavelength (500nm) and the intensity of the color is proportional to the concentration of total cholesterol in the blood serum.

2.4 Measurement of serum Triglyceride concentration

The concentration of triglycerides in the blood serum was estimated using the diagnostic kit prepared by the company (BioLABO) (Fabiny & Ertingshausen, 1971 and Labbe ,1996)

2.5 Measurement of serum Low density lipoproteins-cholesterol (LDL-C)

LDL-C value was measured according to (Benjamin et al.,2019), according to the following equation:
LDL-C(mg/dl) = Total cholesterol -(HDL + VLDL)

2.6 Measurement of serum High density lipoprotein-cholesterol (HDL-C)

The percentage of HDL in the blood serum was calculated by adopting the ready-made work kit manufactured by the French company Biolaba (Friedewald et al., 1972) and depending on the enzymatic method. The principle of work depends on the enzymatic method, in which chylomicrons and lipoproteins of LDL and VLDL are precipitated by adding Phosphotugstic acid. Ultimately, HDL-c remains in the blood serum after the centrifugation process.

2.7 Measurement of very low Density Lipoprotein –Cholesterol(VLDL-C) Concentration in serum:

VLDL was derived from Friedwalds equation:
VLDL = triglyceride/5 (Sochor et al., 2012).

2.8 Measurement of malondialdehyde in serum (MDA)

Malondidehyde is a measurement of lipid peroxide through the reaction of thiobarbituric- TBA with MDA (ibid). TBA reacts with MDA under conditions of temperature and low pH, where the reaction occurs in an acidic medium to form the pink color of the complex [TBA] 2-malondialdehyde. The absorbance is measured at the wavelength 532nm. This is consistent with the percentage of lipid peroxide in the sample.

2.9 Measuring the level of total antioxidant capacity (TAC) in the blood serum

This was based on the FRAP method, which is an easy way to calculate the capacity of antioxidant power that reduces the complex [Fe(III)-TPTZ] ferric tripyridyltriazine to TPTZ] ferrous tripyridyltriazin –[Fe

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(II) with intense blue color absorbed at wavelength (593nm). FRAP values are calculated by comparing the absorption change in the reaction mix test with those containing Fe (II) ions in known concentrations (Benzie & Strain 1996).

3. Results and Discussion

Values of estradiol hormone concentrations Table (1): E2 level in naturally pregnant women and pregnant women after taking steroids

The group	**E2 (mean ± SD)**
naturally pregnant	3057±182.5
Pregnant women with steroids	130±22.1
P-value = (0.0005)**	

Table (1) shows that there is an increase with high statistical differences at the level of probability (P≤ 0.01) for naturally pregnant women and the result was (3057±182.5) picogram / ml compared to pregnant women after taking steroids whose result was (130± 22.1) picogram / ml in its effect on the concentration of the hormone estradiol. The reason for this is its important role in the hormone estradiol during the different stages of pregnancy. This is shown in its negative effect on the production and formation of GnRH hormones and their secretion from Hypothalamus and FSH to prevent the formation of new eggs during pregnancy (Klein et al., 1996). Moreover, In addition, the hormone estradiol is very important in the preparation of the uterine environment at the beginning of pregnancy, with the help of progesterone (McLachlan & Arnold, 1996), as well as its role in the high levels of proteins transporting hormones T4 and T3, especially in the early stages of pregnancy. Thus, this causes an increase in the levels of these hormones during this stage of pregnancy, because of their sensory and major importance in the success of pregnancy and the development of fetuses (Robbins & Nelson, 1958). On the other hand, its decrease is attributed to the beginning of an increase in the level of the hormone prolactin, which has a negative effect on the production of estradiol, as well as its role in cooperation with other hormones in the construction and development of milk gland tissue, for example, cortisone and growth hormones (Shahar et al.,2002).

Table (2) The level of E2 in naturally pregnant women and pregnant women after taking steroids and non-pregnant women after taking steroids according to age groups

Age group	**E2 Non-pregnant after taking anabolic steroids (Mean ± SD)**	**E2 natural pregnancy (mean ± SD)**	**E2 Pregnant women after taking anabolic steroids (Mean ± SD)**
15-25 years old	170.8b±18.40	2990.0a±50.31	142.2b±10.21
26-35 years old	47.5b±10.10	2968.0a±32.19	125.7b±18.31
36-45 years old	79.5b±15.12	3372.0a±50.12	130.5b±10.11
P-value = 0.0006)**			
**(High Statistical Difference)			

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It is noted from Table (2), there is a rise with high statistical differences at the level of probability (P≤ 0.01) for the age group (36-45) in naturally pregnant women, whose value amounted to (3372.0 ± 50.12) pg / ml, compared with the two age groups (15-25) (26-35), whose results were (2990.0a ± 50.31) (2968.0a ± 32.19) pg / ml, respectively. The two age groups (15-25) (36-45) years outperformed their results (170.8b±18.40) (79.5b±15.12) pg / ml significantly in non-pregnant women after taking steroids compared to the age group (26-35) years, which amounted to (47.5b±10.10) pg / ml, either in pregnant women after taking steroids, the two age groups (15-25) (36-45) years outperformed and their results were (142.2± 10.21) (130.5± 10.11) pg / ml respectively compared to the age group (26-35) years, which amounted to (125.7± 18.31) pg / ml Estradiol concentrations It is believed that this is due to the fact that estradiol is of great importance in maintaining pregnancy as well as its continuation because the process of implantation of fetus in the endometrium is based on the balance between both estradiol and progesterone and that this explains the reason for the increase in E2 in the age group (36-45) years, as most of the abortions recommended for pregnancy to occur are in the imbalance between the hormones estradiol and progesterone in the advanced stages of pregnant life (Guyton & Hall, 2006). The level of cholesterol decreases in females with high estradiol, which is the main source of synthesis and formation of sex hormones during pregnancy. In addition to having an impact on sexual and reproductive processes, estradiol also promotes the formation of bone tissue (Collins et al , 1995). It is also thought that a number of factors, including hormonal factors, hereditary factors, and physiological ones like stress, all contribute to the decline of the hormone estradiol (Nelson, 2003).

3.1 Concentrations of malondialdehyde

Table (3): The level of MDA in naturally pregnant women and pregnant women after taking steroids

the group	**MDA (nmol/ml) (mean ± SD)**
natural pregnancy	6.48±1.98
Pregnant women with steroids	5.34 ± 1.83
P-value = (0.386)^{ns}	

It can be seen from the results in Table (3) The level of (MDA) is elevated and there were no statistical differences at the probability level (p > 0.05) in the blood serums of pregnant women with natural pregnancies, compared with the group of pregnant women with steroids.

These results were in agreement with Tiwari et al., (2016). The reason for the high MDA is that pregnant women are exposed to oxidative stress, which is the process of peroxidation of fats and thus producing high levels of MDA. The cause of oxidative stress in pregnant women is the placenta, which forms the link between the fetus and the mother's bloodstream. It performs various functions, including nutrition, respiration, and excretion of fetal waste. The high metabolic rates of the placenta and the high numbers of mitochondria in it, in addition to the molecular pressure of oxygen in pregnant women, produce high levels of active oxygen types ROS, causing the formation of lipid peroxide and increasing its proportions in tissues, leading to an increase in the level of MDA as the final product in the lipid peroxide process

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(ibid). Placental progesterone plays a role in the formation of ROS and this interacts with unsaturated fatty acid present in membranes or is interacted with lipoproteins and thus begins peroxidation. Hypertension and preeclampsia can result from oxidative deterioration, which is brought on by lipid peroxide and some forms of active oxygen (Saikumar et al., 2013).

Table (4): The level of MDA in naturally pregnant women and pregnant women after taking steroids and non-pregnant women after taking steroids according to age groups

Age group	MDA not pregnant after taking anabolic steroids	MDA natural pregnancy (mean ± SD)	Pregnant MDA after taking anabolic steroids
G1	6.03a±1.14	5.37a ± 0.43	7.93a±1.48
G2	5.59a±1.19	4.74a ± 1.32	6.27a±1.08
G3	.18a±0.39 8	7.88a ± 1.47	9.25a±1.06
P-value = (0.121)^{ns}			

The results shown in Table (4) showed that the level of MDA rises and no high differences in statistical terms appeared at the level of probability ($p > 0.05$) for the three age groups respectively for pregnant women with steroids and non-pregnant women after taking steroids compared with pregnant women with natural pregnancy. The highest percentage reached in the age group (36-45) for both pregnant women with natural pregnancy and pregnant with steroids and non-pregnant women after taking steroids compared with the age group (15-25) and (26-35). This is consistent with the results of the study reached by (Castro et al., 2012) and (Hussein et al., 1996) who reported an increase in the percentage of malonedidehyde with age for pregnant women. The increase in the proportion of MDA with age is due to the high production of free radicals leading to an increase in the process of lipid peroxidation. When this happens, the ratios of antioxidants and active oxygen classes lose equilibrium, which causes oxidative stress (Abou-Seif & Youssef, 2001).

3.2 Total antioxidant concentration values

Table (5): the level of TAC in naturally pregnant women and pregnant women after taking steroids

the group	**TAC (Mean ± SD)**
natural pregnancy	0.296 ± 0.063
Pregnant women with steroids	0.367 ± 0.081
P-value = (0.019)**	

The results of the study showed, as shown in Table (5), the level of TAC decreases and shows high statistical differences at the level of probability ($P \leq 0.01$) in the blood serums of pregnant women

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during a natural pregnancy compared with pregnant women after taking steroids. The reason for this may be due to the fact that in many clinical cases the decrease in antioxidants is related to the inability of the antioxidant systems to compensate for the excess by oxidative stress. This leads to the deterioration and disorder of proteins, including cell membranes and enzymes, the role of which is to reduce the activity and levels of antioxidant enzymes for oxidation. This supports several studies stating that the metabolic syndrome and chronic stress affect the decreased susceptibility of antioxidant enzymes (Rujito et al., 2015).

It is possible that the reason for this decrease is the high percentage of free radicals and their collection within the body, leading to a rise in oxidative stress as a type of physiological condition during pregnancy, as free radicals increase. The result, therefore, is an increase in the proportions of MDA, which breaks down and deflates the use of oxidative compounds, thus reducing their levels. These results are consistent with (Patil et al., 2009) and (Yassin et al., 2015).

Table (6): TAC level in naturally pregnant women, pregnant women after taking steroids, and non-pregnant women after taking steroids, according to age groups

Age group	TAC of non-pregnant women after taking anabolic steroids (Mean ± SD)	TAC natural pregnancy (mean ± SD)	TAC of pregnant women after taking anabolic steroids (Mean ± SD)
G1	0.3885ab ± 0.03516	0.3743ab ± 0.03261	0.4457a ± 0.04521
G2	0.3708ab ± 0.02616	0.2835c ± 0.02521	0.3572b ± 0.03315
G3	0.3668ab ± 0.02721	0.2318bc ± 0.02631	0.3265b±0.03415
P-value = (0.002)**			

Table (6) shows the total antioxidants for both naturally pregnant women and pregnant women after taking steroids and non-pregnant women after taking steroids. It was found that there was a decrease with high statistical differences at the level of probability (P≤0.01) in the age group (36-45) for naturally pregnant women more than the two age groups (15-25) and (26-35), as well as for the two groups (pregnant women after taking steroids and non-pregnant women after taking steroids).

It has been pointed out by (Carbone et al., 2003) that the antioxidant activities decrease in older women when compared with younger women because of the high percentage of free radicals with age. Also, Khan et al., (2010) indicated that the decrease in antioxidant activities is attributed to the accumulation of free radicals and the inhibition of antioxidant systems that can lead to the accumulation of H2O2 (Ghara et al., 2014). The reason for this decrease can be ascribed to the fact that as a woman ages, the percentage of free radical production increases and results in the loss of the balance between the effectiveness of antioxidants and free radicals, and thus the percentage of antioxidants in the blood will decrease (Hussein et al., 1996).

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3.2 Fat concentration values

Table (7): the lipid levels in the blood serums of naturally pregnant women and pregnant women after taking steroids

variants	Pregnant women after taking steroids(mean ± SD)	natural pregnancy (mean ± SD)	(P-value)
TC	157.8 ± 19.4	156.9 ± 19.1	(0.902)^{ns}
TG	112.7±19.9	140.9 ± 20.1	0.002))**
HDL-C	39.87±3.21	38.45 ±3.13	0.128))^{ns}
LDL	89.4 ± 10.11	81.7 ± 11.12	(0.316)^{ns}
vldl	29.54 ± 2.16	30.7 ± 2.21	(0.006)**

The results shown in Table (7) displays the concentrations of fats and lipoproteins for naturally pregnant women and pregnant women after taking steroids. The results showed that the level of (total cholesterol, low-density lipoproteins) has increased and no statistical differences have appeared at the probability level ($p>0.05$). The results of (triglycerides, very low density lipoproteins) increased and show high statistical differences at the probability level ($p\leq0.01$). It was found that there was a decrease and no statistical differences appeared at the probability level ($p> 0.05$) in the level of high-density lipoproteins in the serum of naturally pregnant women and pregnant women after taking steroids. These results were consistent with the findings reported by (Ryckman et al., 2015), which indicated an increase in the level of TC, T.G, VLDL and a clear decrease in the level of HDL in pregnant women. This is due to the increase in total cholesterol resulting from the imbalance in the process of fat metabolism and also the rise in the activity of the enzyme Cholesterol acyl- transferase, which stimulates the absorption of cholesterol from the intestine.

During pregnancy, the placenta creates large amounts of steroid hormones, in which case the Cholesterol is increasing to produce these hormones (Herrera, 2002). TG increases in pregnant women due to a decrease in the activity of the enzyme lipoprotein lipase. This decrease leads to a high level of TG in the serum, leading to a high level of VLDL. This is because it contains a large amount of triglycerides, leading to a decrease in the percentage of (HDL). Increasing the level of estrogen in the serum of pregnant women leads to the activation of the hepatic structure of TG, which is one of the important reasons that work on fetal development, as ketosis and glycerol are avoided (Jayanta et al., 2006).

The study indicated that the decrease in the proportion ofHDL is due to a decrease in the activity of the enzyme hepatic lipase

This is because it rich in triglycerides. Thus, it is one of the main substances that hepatic lipase works on, and as a result, it accelerates the process of removing high-density lipoproteins (HDL) from the circulatory system, leading. This in turn leads to a decrease in its percentage in the serum. This study showed that it is in agreement with the study of (Jayanta et al., 2006).

The results of this study indicated that they agree with (Hussein & Al-Samarrai, 2012) who found a high level of VLDL in the serum of pregnant women, due to the decrease in the activity of the enzyme

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lipoprotein lipase. This leads to an increase in the level of TG and at the same time to an increase in the level of VLDL.

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