O PAPEL DA VITAMINA D COMO UM NOVO MARCADOR NA INFERTILIDADE DAS MULHERES

THE ROLE OF VITAMIN D AS A NEW MARKER ON THE WOMEN INFERTILITY

دور فيتامين D كعلامة دالة جديدة لعقم النساء

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RESUMO

O colecalciferol (vitamina D) é essencial para o bom funcionamento do corpo humano. Esta vitamina pertence a uma vitamina lipossolúvel, responsável por estimular a absorção intestinal de cálcio e fósforo. Recentemente, foi observado que a vitamina D pode estar relacionada a casos de infertilidade feminina. Este estudo teve como objetivo examinar o efeito da vitamina D na infertilidade feminina analisando parâmetros hormonais e bioquímicos. Estrogênio, progesterona, FSH, LH, colesterol total, triglicerídeo, HDL, LDL, VLDL, vitamina D e IMC foram testados em 60 mulheres com infertilidade e 40 mulheres férteis como grupo controle. Os resultados mostraram uma elevação altamente significativa na concentração de LH, triglicerídeo, LDL e IMC em mulheres inférteis em comparação com as mulheres saudáveis com um significativo $P=0,025$, $P=0,01$, $P=0,05$ e $P=0,001$, respectivamente. Além disso, uma baixa elevação significativa na concentração de estrogênio, progesterona, FSH, HDL e vitamina D em mulheres inférteis quando comparadas às mulheres saudáveis em um nível significativo de $P=0,01$, $P=0,039$, $P=0,05$, $P=0,05$ e $P=0,001$, respectivamente, também foi observada. Essa vitamina teve uma forte relação positiva com a progesterona, FSH, colesterol total, HDL e LDL. Também foi comprovada uma correlação inversa significativa para LH, estrogênio, TG, VLDL e IMC. Pode-se concluir que existe uma diminuição significativa do nível de vitamina D em mulheres inférteis em comparação com o grupo de mulheres saudáveis, principalmente com IMC elevado. Também pode ser deduzido que a vitamina D pode ser um novo marcador de aumento de infertilidade e risco de aborto.

Palavras-chave: *infertilidade, vitamina D, aborto, hormônios femininos.*

ABSTRACT

Cholecalciferol (Vitamin D) is essential to the proper functioning of the human body. This vitamin belongs to fat-soluble vitamins, responsible for stimulating the intestinal absorption of calcium and phosphate. It has been recently observed that vitamin D may be related to cases of female infertility. This study aimed to examine the effect of vitamin D on female infertility by analyzing hormonal and biochemical parameters. Estrogen, progesterone, FSH, LH, total Cholesterol, Triglyceride, HDL, LDL, VLDL, vitamin D, and BMI were tested in 60 women with infertility and 40 fertile women as a control group. The results showed a highly significant elevation in LH concentration, Triglyceride, LDL, and BMI in infertile women, compared to the healthy women at a significant $P=0.025$, $P=0.01$, $P=0.05$, and $P=0.001$, respectively. Also, a significant low elevation in the concentration of estrogen, progesterone, FSH, HDL, and vitamin D in infertile women when compared to the healthy women at a significant level of $P=0.01$, $P=0.039$, $P=0.05$, $P=0.05$, and $P=0.001$, respectively was observed. This vitamin had a strong positive relationship with progesterone, FSH, total cholesterol, HDL, and LDL. It was also proved a significant inverse correlation to LH, estrogen, TG, VLDL, and BMI. It could be concluded that there is a significant decrease in the level of vitamin D in infertile women compared with the group of healthy women, especially with high BMI. It could also be deduced that vitamin D can be a new marker of increasing infertility and miscarriage risk.

Keywords: *Infertility, Vitamin D, Miscarriage, Female hormones.*

يعتبر الكولكالسيفيرول (فيتامين D) ضرورياً لتنظيم سير عمل جسم الإنسان. ينتمي هذا الفيتامين إلى مجموعة الفيتامينات القابلة للذوبان في الدهون و هو المسؤول عن تحفيز امتصاص الأمعاء للكالسيوم و الفوسفات. لقد لوحظ مؤخراً أن فيتامين D قد يكون مرتبطاً بحالات العقم عند النساء. هدفت هذه الدراسة إلى قياس تأثير فيتامين D على العقم لدى النساء من خلال قياس المتغيرات الهرمونية و الكيموحيوية. حيث تم قياس هورمون الاستروجين و هورمون البروجسترون و FSH و LH و الكوليستيرول الكلي و ثلاثي أسيل كليسيرول و HDL و LDL و VLDL و فيتامين D و مؤشر كتلة الجسم في 60 امرأة مصابة بالعقم و 40 امرأة سليمة كمجموعة سيطرة. أظهرت النتائج ارتفاعاً معنوياً في تركيز LH و ثلاثي أسيل كليسيرول و LDL و مؤشر كتلة الجسم لدى النساء المصابات بالعقم، مقارنة بالنساء السليمات عند مستوى احتمالية $P=0.025$ ، $P=0.01$ ، $P=0.05$ ، $P=0.001$ على التوالي. أيضاً، ظهر إنخفاض معنوي في تركيز هورمون الاستروجين و هورمون البروجسترون و FSH و HDL و فيتامين D لدى النساء المصابات بالعقم مقارنة بالنساء السليمات عند مستوى احتمالية $P=0.01$ ، $P=0.039$ ، $P=0.05$ ، $P=0.05$ ، $P=0.001$ على التوالي. كان لهذا الفيتامين علاقة ارتباط إيجابية قوية بهورمون البروجسترون و FSH و الكوليستيرول الكلي و HDL و LDL. كما تم إثبات وجود علاقة ارتباط عكسية معنوية مع كل من LH و هورمون الاستروجين و TG و VLDL و BMI. يمكن الإستنتاج أن هناك انخفاضاً ملحوظاً في مستوى فيتامين D لدى النساء المصابات بالعقم مقارنة مع النساء السليمات، خاصة مع ارتفاع مؤشر كتلة الجسم. يمكن أيضاً إستنتاج أن فيتامين D يمكن أن يكون علامة دالة جديدة لزيادة مخاطر العقم والإجهاض.

الكلمات الدالة: العقم، فيتامين D، الإجهاض، الهورمونات الإنثوية

1. INTRODUCTION:

Infertility is a common health problem all over the world. It is defined as the inability to conceive after 12 months or more of marriage, even after treatment or in the case of using fertility aids outside the body (Miyashita *et al.*, 2016; Jungwirth *et al.*, 2013; Gurunat *et al.*, 2011). Vitamin D is essential to the proper functioning of the human body. This vitamin is a fat-soluble steroid group responsible for stimulating the intestinal synthesis of calcium and phosphate (Pludowski *et al.*, 2018; Holick, 2007).

The pregnant woman requires a healthy bone and muscles to retain this one of the most important vitamins. Therefore, the appropriateness of a vitamin D requirement of a pregnant woman healthy and complete their pregnancy (Webb, 2006). Iron, folic acid, or zinc deficiency, as well as vitamin B12, affects fertility in women. It has been recently observed that vitamin D may be related to female infertility cases, and studies are still very few to clarify its role. Most of the daily requirements for vitamin D3 are formed from the biosynthesis of vitamin D3 under the skin. Many environmental factors affect skin production with vitamin D3, such as lack of exposure to sunlight caused by season, clouds, or air pollution. The condition of the skin and its pigmentation are also significant factors (Ranjana, 2017).

The production of vitamin D3 by the skin is often insufficient to ensure that the recommended daily amount is met. Especially in industrialized countries where the World Health Organization has defined vitamin D deficiencies as having a serum level of less than (20 ng/dl) (Ranjana, 2017; Gaskins *et al.*, 2017), over the past couple of years, a growing interest in researching the relationship between the deficiency and sterility of

vitamin D. And studies are still very few to clarify its role. Further studies are required to urgently use quantitative methods to explicitly measure vitamin D to check its functionality in the treatment of infertility in women. And investigate the possible therapeutic benefits of vitamin D addition (Cie'slińska *et al.*, 2018; Ranjana, 2017).

The risk of sterility is also proportional to age. As a result of the work defect in the uterus and poor quality of the eggs, it was noted that the infertility rate exceeds (20.3%) in pairs over 40 years in addition to the vast increase in the female fertility rate after 37 years of age (Feichtinger *et al.*, 2019). As with age, eggs with an abnormal genetic structure are created by defects in the splitting and lack of adhesion between structural chromosomes. That prevents natural fertilization and chromosome in aborted women. Obesity also plays an essential role in reproductive disorders, especially in women. It is associated with ovulation, menstrual disorders, infertility, and multiple abortions. Pregnancy changes differ depending on the origin and duration of infertility, the woman's age, pregnancy history, and availability of different therapies for unexplained infertility (Feichtinger *et al.*, 2019; Rodrigu *et al.*, 2018). As for Iraq, there are no accurate statistics on infertility.

Due to the lack of documented records, infertility significantly impacts the psychological and social point of view. Due to changes in lifestyle and environmental stress, the rate of infertility has increased considerably. This disease has become the third most dangerous disease after contracting diseases, Cardiovascular, and cancer diseases (Cong *et al.*, 2016; Jangir *et al.*, 2014).

This research aimed to examine the effect of vitamin D on female infertility by analyzing hormonal and biochemical parameters for early

diagnosis. It can improve treatment response and prevent female infertility by maintaining vitamin D levels at a normal level.

2. MATERIALS AND METHODS:

2.1. Sample collection

2.1.1 Group of Infertile Patients

Sixty infertile women have been registered in this research. Specialists in Hospitals diagnosed it, and a laboratory test was carried out in the laboratories of the hospitals and external laboratories for the period from 13/4/2019 to 17/5/2020. Their ages ranged from 18-45 years, and the BMI is between (17-37.5 kg/m²). Clinical data as age, sex, weight, height, the number of years of marriage, and the number of abortions were obtained for each case in a questionnaire prepared for this purpose. Excluded conditions included diabetes, high blood pressure, and thyroid disease.

2.1.2 Control Group (Reference Group)

This study was attended by forty young fertile women (control group), ages ranging from 17-45 years, and the BMI is between (24-26.1 kg/m²).

2.1.3 Blood Collection

Two groups were tested for blood over 12-hour fasting (values for 5ml), early follicular cycles (cycle days two or three) for estrogen (E2), progesterone, follicle-stimulating hormones (FSH) and lutein's hormone (LH) and vitamin D. Luteal prolactin (cycle day 21). Into the test tube (gel and clot activator tube for serum separation) by drawing (5ml), and centrifuged for serum separation within an hour of blood collection, and the serum was stored in a deep freezer at a temperature of 70°C for subsequent analysis. Samples were analyzed in batches of 100 to be omitted between analytical variations. Samples were permitted to reach room temperature before the study.

2.1.3.1. Materials

This study was performed using a special kit for each variable from the German company Cobas to estimate the following hormonal and chemical variables: Estrogen hormone E2, progesterone, follicle-stimulating hormone (FSH), ovulation hormone, or called luteinizing hormone

(LH), vitamin D, and lipid profile were also measured, which include total cholesterol, triacylglycerol TG, and high-density lipoprotein (HDL) cholesterol.

2.1.3.2. Procedures

The commercial kits (Roche kits) were then measured by Cobas E411. The lipid profile also included Total Cholesterol, Triglyceride (TG), High-Density-Lipoprotein (HDL) analysis, and was measured using commercial kits (Roche Kits) by Cobas C311.

2.1.3.3. Hormones and vitamin D measurement methods

The reagents used are ready and available in the device. After separating the serum in a centrifuge for five minutes at 4000 rpm, it put the separated serum into a type test tube. Hitachi tube then, we put the tube on the required number inside the E411 Cobas device, then the clinical information was entered. The required tests, and the number in which the serum was placed, then press the STAR button, and then the results appear automatically.

2.1.3.4. Lipid profile measurement methods

The reagents used are ready and available in the device. After separating the serum in a centrifuge for five minutes at 4000 rpm, we put the separated serum into a Hitachi tube. After that, the test tube was put on the required number inside the C311 Cobas device, then entered the clinical information, the required tests, and the number in which the serum was placed; then pressed the START button, and then the results appeared automatically.

2.1.3.5. Indirect Procedures

Low-Density-Lipoprotein (LDL) and Very-Low-Density-Lipoprotein (VLDL) were determined indirectly using the Friedewald equations.:

$$\text{LDL Con. (mg/dl)} = \text{Cholesterol Con.} - \text{HDL Con.} - \text{TG/5} \quad (\text{Eq.1})$$

$$\text{VLDL Conc. (mg/dl)} = \text{Triglyceride/5} \quad (\text{Eq. 2})$$

2.1.3.6. BMI Procedures

BMI was calculated using the following

equation:

$$\text{BMI (Kg/m}^2\text{)} = \text{Weight (Kg)} / \text{Height (m}^2\text{)}.$$

(Eq. 3)

2.1.3.7. Statistical analysis

SPSS software has been used to analyze the data. The T-test and Duncan-tests were already used to compare parameters between the total control number and patients based on occupancy at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively, and the test of Pearson correlation coefficients. The Duncan-tests is used to indicate the differences when comparing more than two groups of the same chemical parameter (a, b, ab means the difference, and if all are the same, it indicates no significant statistical difference), which is identical to the p-value (Kirkpatrick and Feeney, 2012).

2.1.4 Ethical approval

The research has been carried out and agreed upon by the author's Institutional Review Board following all applicable national legislation, institutional policy, and the values of the Helsinki Declaration.

2.1.5 Informed Consent

All participants rights were protected, and oral informed consent was obtained according to the Helsinki Declaration.

3. RESULTS AND DISCUSSION:

3.1. The level of hormonal and biochemical parameters of infertile women (primary and secondary) compared with the control group

The findings in Table 1 indicate that hormone and biochemical variables were significantly lower estrogen hormone (E2), causing menstrual disruptions and ovulation in women who had infertility than in healthy women (Dag and Dilbaz, 2015). The explanation for this may be due to the presence of a pituitary gland defect (Taraborrelli *et al.*, 2015), which affects the ovaries. Therefore, it may cause any loss of ovarian function or harm to the decrease in the estrogen hormone and adversely affect the female ovulation process. Thus, decreasing the probability of pregnancy and the probability of infertility. Cortisol and sex hormone-bound globulin is increased by the estrogen hormone (Li *et al.*, 2017; Gilbert, 2019).

Also, progesterone hormone concentration revealed a significant decrease, which could lead to an increase in the risk factor of infertility and an increased risk of endometrial cancer. Was found in women who had infertility compared with healthy females, possibly due to lack of ovulation (Dosouto *et al.*, 2019; Momenimovahed *et al.*, 2019). Similarly, women who have low progesterone hormone levels and who are successful in conceiving are more likely to have a miscarriage. Irregular uterine bleeding, or to have an ectopic pregnancy and fetal death (Taraborrelli *et al.*, 2015). With a highly significant level of the ovulation hormone (LH) occurring in women with infertility relative to healthy women, a significant decrease in the concentration of the follicle-stimulating hormone (FSH), because long-term loss of ovulation due to hyperandrogenemia, which could contribute to premature action on granulocytes in the mid-Follicular process through early cessation of follicle growth and development, leading to the absence of ovulation and sterility (Gilbert *et al.*, 2019; Kesmodel, 2012).

Also, the TG, LDL, and BMI concentrations were considerably increased and the high-density lipoprotein (HDL) concentration in infertile women was decreased significantly compared to healthy women. Maybe because of the connection between the abnormal metabolism of lipids and the infertility of women as the high body mass index is linked to the high triglyceride level and the high-level of free fatty acids in the follicular fluid is harmful and not suitable for egg maturity in this fat-rich environment. Dyslipidemia is also seen in obese women by a high concentration of triglycerides, free fatty acid concentration, higher concentration of lipoprotein (LDL) density, and low concentration of lipoprotein (HDL) (Fontana and Torre, 2016).

Vitamin D levels in infertile women was decreased significantly compared to healthy women. The reason may be that disorder in vitamin D is associated with early ovarian failure. It is characterized by menopausal disorder, metabolic deficiency, and increased genital serum levels in women younger than 40 years of age or miscarriage (Voulgaris *et al.*, 2017; Wasim, 2015; Stanford, 2013). In addition to vitamin D deficiency, it is normally responsible for stimulating sexual appetite in women who suffer from primary ovarian insufficiency (Wasiewicz *et al.*, 2018).

3.2. A comparison of the level of hormonal, biochemical parameters, and Vit. D in infertile women with different BMI

Table 2 shows decreased LH and FSH levels in infertile women, depending on BMI, as the higher BMI. Obesity is linked to higher leptin blood levels (Broughton and Moley, 2017; Al-Turki *et al.*, 2017). The leptin hormone inhibits the process of steroidogenesis in granulosa cells in obese women. This deficiency may affect the level of sexual hormones (LH and FSH). This leads to an imbalance in the central nervous system (CNS), leading to the recruitment of inactive ovarian follicles. A small number of them enter the maturity stage. And the resulting egg is of low quality in addition to the effect on follicle growth (Al-Taie and Al-Jawadi, 2019; Baig *et al.*, 2019; Hill and Elias, 2018; Ranjana, 2017).

Besides, the follicle-stimulating hormone decreases as BMI increases. This is because, as well as being a risk factor for multiple metabolic disorders. Obesity affects the fertility of women and the rise in obesity has hormonal changes that may disrupt the sub glands function. The hypothalamus is directly affecting ovarian function and impair ovarian follicle growth, the production, and fertilization of qualitative and quantitative Oocysts (Kamyabi *et al.*, 2015). Significant increases in cholesterol and TG concentrations with increased body mass index in sterile women with BMI (39.9-35 Kg/m²) as shown in Table (2). The reason may be that dietary fat may affect the follicular fluid or the ovarian cell lipid composition. Higher body mass index is associated with a high triglyceride level and free fatty acid in the follicular fluid. That reflects changes in the blood and correlates with the body mass index, meaning that the increased fatty acid associated with the egg quality (Poor Cumulus Oocyte Morphology) (Benjamin *et al.*, 2017; Valckx *et al.*, 2012). No significant difference in the concentrations of estrogen, progesterone, HDL, LDL, and VLDL statistically. However, there has been a substantial decrease in vitamin D levels in women with infertility with an increase in BMI.

The low vitamin D levels are because obese women have a lower level of vitamin D relative to normal-weight patients since the level of vitamin D in follicular fluid is strongly associated with BMI and may play a major role in females infertility. This is primarily due to the presence of VDR (Vitamin D (1,25-dihydroxy vitamin D3) receptor) and ((CYP27B) Cytochrome P450, family 27 subfamily B member1) in various tissues of the female reproductive system (Blomberg *et al.*, 2018), leading to impaired follicle development and ovulation impairment (Oostingh *et al.*, 2019; Kokanalij *et al.*, 2019).

3.3. Number of miscarriage with Vitamin D

The results in Figure (1) showed a significant decrease in the level of Vit. D in infertile women with repeated miscarriages compared to the control group at ($P = 0.001$), highlighting that Vit. D can be an essential regulator of an immune reaction for the body during pregnancy, as it's supposed that Vit. D stimulates the immune changes necessary to prevent pregnancy loss and strengthen the mother's immune system, and it does not happen. It might harm an unborn baby and end the pregnancy by abortion, as vitamin D receptors are found in the ovaries, uterus, placenta, hypothalamus, and pituitary gland (Li *et al.*, 2017; Zhang *et al.*, 2015; Barragan *et al.*, 2015).

As normal vitamin D levels, reduce the incidence of miscarriage, especially in pregnancy during the first trimester, women who have more likely low levels of Vit. D would have antibodies to phospholipids and antinuclear antibodies and increased natural killer cells (NK) than of women with a normal vitamin D level, which confirms the immune role of the vitamin in preserving the fetus. And that the presence of vitamin D receptors and enzymes responsible for vitamin D hydroxylation work to determine localized vitamin D synthesis in the human placenta; Thus will shed light on the possible mechanism between the vitamin state D and persistence of pregnancy (Grieger and Norman, 2020; Samimi *et al.*, 2017).

3.4. The correlation of vitamin D for infertile women with hormonal and biochemical parameters

Correlation between infertility and vitamin D is shown in Table 3, which confirms that vitamin D, is a sign that increases risk factors of infertility. The correlation between infertility and vitamin D positively correlated with estrogen, progesterone, FSH, total cholesterol, HDL, and LDL, especially in FSH. On the other hand, the correlation between infertility and vitamin D has a significant negative correlation with LH, TG, VLDL, and BMI. Especially in BMI, where the higher the body mass index, the decreased the level of vitamin D in women with infertility. The reason may be that obese people are less able to convert vitamin D into its active form. Vitamin D is synthesized or absorbed in the skin upon exposure to sunlight. Or absorbed by the body from its food sources or nutritional supplements, it is distributed in the fatty tissues. However, its blood levels are low because it is distributed in the fats accumulated in the body and did not reach the blood.

4. CONCLUSIONS:

The study proved a significant decrease in vitamin D levels in infertile women compared with the healthy women's group. Also, when comparing the level of hormonal variables for infertile women, of both types (primary and secondary), with healthy women, there was a significant decrease in the level of estrogen hormones, progesterone hormones, and follicle-stimulating hormones, as well as a significant decrease in the level of high-density lipoprotein (HDL). At the same time, it showed a significant increase in triglyceride levels, LDL, and BMI. Studies have also shown that the level of vitamin D in infertile women with a high BMI; is much lower than the level of vitamin D in infertile women with a normal body mass index. As it has been proven that there is an inverse relationship between BMI and vitamin D. Also, there is a strong inverse correlation between miscarriage times in infertile women and vitamin D levels compared to healthy women.

This study found vitamin D is a new risk factor to increase women infertility and miscarriage. It is associated with hormonal derangements and dyslipidemia, responsible for infertility and impaired ovarian follicular development, qualitative and quantitative development of the Oocyte. Hence it should be primarily targeted in managing these women before starting any therapy to correct their hormonal imbalance; by measuring the level of vitamin D and treating vitamin deficiency through nutritional and drug supplements under medical supervision.

5. CONFLICT OF INTERESTS:

The authors declare that there is no conflict of interest regarding the publication of this article.

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Table 1. The level of hormonal and biochemical parameters of infertile women (primary and secondary) compared with the control group.

Hormonal and biochemical parameters	Control Group Mean $\pm$ SD	Infertility group Mean $\pm$ SD		P-value
		Primary	Secondary	
Estrogen (E2) (pg/mL)	84.43 $\pm$ 10.03b	53.30 $\pm$ 9.23a	44.68 $\pm$ 7.68a	0.01**
Progesterone (ng/mL)	11.604 $\pm$ 3.52b	8.54 $\pm$ 8.33a	7.86 $\pm$ 10.51a	0.039*
FSH (mIU/mL)	8.434 $\pm$ 2.65b	6.70 $\pm$ 2.66a	7.25 $\pm$ 2.96a	0.05*
LH (mIU/mL)	5.48 $\pm$ 1.72b	7.19 $\pm$ 3.04a	7.00 $\pm$ 3.83a	0.025*
Total cholesterol (mg/dL)	158.85 $\pm$ 24.93a	160.08 $\pm$ 36.68a	157.46 $\pm$ 23.39a	N
Triglyceride (TG) (mg/dL)	90.28 $\pm$ 31.38a	95.50 $\pm$ 35.84b	100.22 $\pm$ 45.74ab	0.01**
HDL (mg/dL)	50.70 $\pm$ 6.74a	48.40 $\pm$ 7.72ab	46.39 $\pm$ 8.20b	0.05*
LDL (mg/dL)	83.01 $\pm$ 21.79b	90.58 $\pm$ 32.71a	88.73 $\pm$ 20.37a	0.05*
VLDL (mg/dL)	19.61 $\pm$ 8.63a	18.94 $\pm$ 7.57a	22.44 $\pm$ 9.06a	N
Vit. D (ng/dL)	39.94 $\pm$ 6.46a	11.94 $\pm$ 5.82b	11.83 $\pm$ 5.78b	0.001***
BMI (Kg/m ²)	25.28 $\pm$ 0.39b	26.46 $\pm$ 3.23b	30.25 $\pm$ 4.54a	0.001***

*Significant differences at $P \leq 0.05$; **Significant differences at $P \leq 0.01$; ***Significant differences at $P \leq 0.001$, N=No significant differences; a, b, ab denote Duncan-test

Table 2. A comparison of the level of hormonal, biochemical parameters, and Vit. D in infertile women with different BMI

Hormonal and biochemical parameters	BMI (Kg/m ²) (Number of Women) Mean± SD				P-value
	20-24.9 (13)	25-29.9 (27)	30-34.9 (14)	35-40 (6)	
Estrogen (E2) (pg/mL)	52.4±13.4a	51.6±11.70a	46.70±18.62a	51.55±10.21a	N
Progesterone (ng/mL)	8.64±5.00a	9.27±9.65a	9.42±12.62a	9.64±8.52a	N
LH (mIU/mL)	8.65±0.72ab	6.42±1.18a	6.90±0.17a	7.14±1.65b	0.05*
FSH (mIU/mL)	5.82±1.17ab	4.70±2.67a	4.25±3.43a	6.64±3.56b	0.05 *
Total cholesterol (mg/dL)	139.52±35.65b	166.83±31.78a	158.70±16.61ab	163.5±25.19ab	0.01**
Triglyceride (TG) (mg/dL)	94.11±13.87b	100.13±21.56a	102.29±29.14a	110.50±29.72ab	0.05*
HDL (mg/dL)	43.08 ±6.06a	49.73±7.84a	48.28±9.34a	43.67±3.20a	N
LDL (mg/dL)	77.47±31.92a	95.42±28.05a	90.13±18.15a	97.73±22.56a	N
VLDL (mg/dL)	18.76±6.79a	21.66±8.55a	20.52±9.95a	22.10±7.94a	N
Vit. D (ng/dL)	11.26±3.23b	10.30±0.89a	9.75±7.52a	9.33±0.61ab	0.05*

*Significant differences at P≤0.05; **Significant differences at P≤0.01, N=No significant differences; a, b, ab denote Duncan-test.

Table 3. The correlation of vitamin D for infertile women with hormonal and biochemical parameters.

Hormonal and biochemical parameters	r-value	p-value
LH (mIU/mL)	-0.0766	0.05*
FSH (mIU/mL)	0.36975	0.003**
Progesterone (ng/mL)	0.00832	0.05*
Estrogen (E2) (pg/mL)	0.03875	0.05*
Total cholesterol (mg/dL)	0.09434	0.05*
Triglyceride (TG) (mg/dL)	-0.13496	0.03*
HDL (mg/dL)	0.19880	0.01**
LDL (mg/dL)	0.09583	0.05*
VLDL (mg/dL)	-0.14995	0.02*
BMI (Kg/m ²)	-0.04446	0.01**

*Significant differences at P≤0.05; **Significant differences at P≤0.01

Figure 1. Number of miscarriage with Vitamin D.