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Investigating the Prevalence of Vitamin D Deficiency and Its Effects on Female Fertility in Albyda City, Libya

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التحقيق في مدى انتشار نقص فيتامين د وآثاره على الخصوبة لدى النساء في مدينة البيضاء، ليبيا

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Abstract:

Research suggests a strong connection between a woman's vitamin D level and her reproductive health, including infertility and hormonal imbalance. This research aimed to assess how common vitamin D deficiency is among women and to offer a detailed analysis of the relationship between vitamin D status and infertility in females. Furthermore, it was aimed to examine the correlation between serum vitamin D, reproductive hormones (LH, FSH and prolactin) and BMI in 380 women of reproductive age (18-45). The study included 260 women in an infertility group and 120 healthy women as a control group. Serum 25(OH)D levels, body mass index (BMI), and hormonal parameters were evaluated. Most participants (60%) were found to have vitamin D deficiency, defined as levels below 20 ng/mL. This deficiency occurred significantly more often in infertile women ($p = 0.001$). In addition, a statistically significant relationship was observed between low vitamin D levels and obesity ($p < 0.05$). Spearman's analysis indicated a weak negative correlation between vitamin D status and the study groups ($r_s = -0.177$, $p = 0.001$). However, no significant associations were detected between vitamin D and other measured variables across the entire sample. A possible link between vitamin D and FSH was identified only among obese participants ($p = 0.013$). Overall, the results highlight that vitamin D deficiency is widespread in women of reproductive age and may be associated with infertility and hormonal disturbances, particularly in those who are obese.

Keywords: Vitamin D, Infertility, Body mass index, Reproductive Hormones, FSH, LH, Prolactin, Nonparametric statistics.

الملخص

تشير الدراسات الحديثة إلى وجود ارتباط وثيق بين مستوى فيتامين د لدى المرأة وصحتها الإنجابية، بما في ذلك العقم واضطراب التوازن الهرموني. وقد صُممت هذه الدراسة بهدف التحقيق في مدى انتشار نقص فيتامين د بين النساء، وتقديم فهم شامل للعلاقة بين مستويات فيتامين د والعقم لدى النساء. كما هدفت إلى

فحص الارتباط بين فيتامين د، والهرمونات التناسلية (FSH ،LH)، والبرولاكتين (ومؤشر كتلة الجسم لدى 380 امرأة في سن الإنجاب (18–45 سنة). شملت العينة 260 امرأة ضمن مجموعة العقم و120 امرأة سليمة ضمن المجموعة الضابطة. تم قياس تركيز 25(OH)D في المصل، ومؤشر كتلة الجسم، بالإضافة إلى الملف الهرموني. أظهرت النتائج أن غالبية النساء (60%) يعانين من نقص فيتامين د (<20 نانوغرام/مل)، وكان هذا النقص أكثر شيوعًا وبدرجة معنوية لدى مجموعة النساء العقيمات (p = 0.001) كما أظهرت التحاليل وجود ارتباط معنوي بين نقص فيتامين د والسمنة. (p < 0.05) وكشف اختبار ارتباط سبيرمان عن علاقة عكسية بين فيتامين د ومجموعات الدراسة (rs = -0.177, p = 0.001)، بينما لم يظهر ارتباط ذو دلالة إحصائية بين فيتامين د والمتغيرات الأخرى في العينة الكلية. ومع ذلك، برز ارتباط محتمل بين فيتامين د وهرمون FSH في النساء البدينات فقط (p = 0.013) تؤكد هذه النتائج أن نقص فيتامين د شائع بين النساء في سن الإنجاب، ويرتبط بالعقم واختلال التوازن الهرموني، خاصة لدى النساء البدينات.
الكلمات المفتاحية: فيتامين د، العقم، مؤشر كتلة الجسم، الهرمونات التناسلية، FSH، LH، البرولاكتين، الإحصاءات غير البارامترية.

INTRODUCTION

Vitamin D is crucial for many parts of reproduction in both sexes, according to mounting evidence from animal and human studies over the past decade (Anagnostis, P., et al., 2013). This includes pregnancy, lactation, conception and sperm function. Although the exact molecular roles of vitamin D in relation to fertility in men and women are still unknown, it is clear that sub-fertile groups have a higher rate of vitamin D insufficiency than the general adult population (Luk, J., et al., 2012). This provides more evidence that vitamin D insufficiency may have a role in infertility in humans.

The vitamin D receptor is found in numerous bodily tissues, This has contributed to growing evidence that inadequate vitamin D levels are associated with a higher risk of various conditions, including autoimmune disorders, certain cancers, cardiovascular diseases, diabetes, and mental health issues.Recent years have seen a surge in interest in vitamin D among scientists, food industry professionals, policymakers, and the general public, Recent findings on vitamin D's broader influence on health—extending beyond its established role in bone and mineral metabolism—have expanded understanding of its impact on multiple physiological outcomes (Hilger et al., 2014).

Vitamin D has attracted attention for a number of reasons, including its effects on metabolism and health, and the fact that the prevalence of vitamin D insufficiency varies greatly among countries, with rates ranging from 2% to 90% (Hilger, J., et al., 2014). Vitamin D deficiency—commonly defined as serum 25(OH)D levels below 20 ng/mL—is a widespread global issue affecting individuals of all ages and is particularly prevalent among patients attending fertility clinics (Thomson et al., 2012).A low level of 25-hydroxyvitamin D (25(OH)D) has also been linked to obesity, metabolic abnormalities, and endocrine problems in polycystic ovary syndrome (PCOS) women. Taking vitamin D supplements may help these women with their menstrual cycle irregularities and metabolic issues (Thomson, R. L., et al., 2012). So far, most of the obesity-related disorders in the Middle East, such as diabetes mellitus, hypothyroidism, and the whole metabolic syndrome, have been covered in the most recent vitamin D research that have expanded beyond its traditional role of calcium homeostasis. The reported association between vitamin D deficiency and infertility is one reason to conduct more research on vitamin D, but there is still an overwhelming number of unanswered questions about its role in health and disease, despite the abundance of recent literature on this topic (Paffoni, A., et al., 2014).

The presence of Vitamin D receptors (VDRs) in human and animal granulosa cells and cumulus ophorus cells provides evidence supporting the important role of vitamin D in the appropriate regulation of the female reproductive cycle (Mohan, A. *et al*., 2023). Vitamin D inadequacy or insufficiency, together with obesity and infertility, represents a significant health concern for women globally. It is estimated that close to one billion people globally have inadequate vitamin D levels. Evidence from both observational and clinical studies suggests that individuals with infertility and low vitamin D status often experience reduced live birth rates. Consequently, vitamin D supplementation is especially advised for patients diagnosed with polycystic ovary syndrome (PCOS) (Zhao, Fu, and Chen, 2023).

Vitamin D deficiency and infertility are global health concern. Both are linked to metabolic disorder; However, limited data exist on the vitamin D status of women with infertility issues in Al-Beyda – Libya. This study aims to investigate the prevalence of vitamin D deficiency among women of reproductive ages. AdditionallyIt aims to examine the association between vitamin D deficiency, body mass index (BMI), and reproductive hormones, specifically follicle-stimulating hormone (FSH), luteinizing hormone (LH), and prolactin Abdulsadiq, S. M., Akrim, Z. S. M., & Almaedani, A. 2023).

MATERIALS AND METHODS

Study Design and Participants

A sample of approximately 380 individuals was recruited through random sampling from the target demographic of married females aged 18 to 45. They were divided into two groups: the infertile participants group and the control group.

A total of 260 women with infertility were randomly selected from Albyda city for inclusion in the study.namely from the infertility center located there. A total of 120 healthy married women were randomly selected from the community as a control group. All pregnant or breastfeeding women were excluded from the control group.

By corporation with the fertility center at ALBYDA city and after their approved, 260 files were recruited from the archives of the center. They are collected from the patients who are registered in AL-Bayda infertility center from the period of the year 2019 until the year 2025. Their ages ranged from 19 – 45 years and the BMI is between (15-49 Kg/m²). Basic information was collected from their files including their age and body mass index BMI. And for the control group, a total of 120 healthy women were recruited at this group, information about the study was given to them also an informed consent form was sent for them. Data collected through a combination of clinical assessments and laboratory analysis. Their ages ranged from 18-45 years and the BMI is between (16-35.6 Kg/m²).

Sample Collection

Afterward, a 5 mL venous blood sample was obtained under resting conditions. from participants via venepuncture of the antecubital vein into a vacutainer tube. And their biochemical indicators were analysed.The measurements included serum 25-hydroxyvitamin D [25(OH)D], luteinizing hormone (LH), and prolactin (PRL), follicle-stimulating hormone (FSH), along with participants' age and body mass index (BMI).The samples taken from the control group participants were analysed utilising the CLIA technique. Utilising three distinct CLIA-certified firms, the Cobas e411, Elecys 2010 and the Maccura i1000, which analyse serum samples to detect levels of 25(OH)D, FSH, LH, and prolactin. All the test done at Al Bayda Specialized Laboratory at Al-Beyda city.

Serum 25(OH)D levels below 20 ng/mL were classified as vitamin D deficiency, while concentrations between 20 and 30 ng/mL were considered insufficient. Levels ranging from 30 to 100 ng/mL were regarded as adequate.

Statistical Analysis

Data were analysed using SPSS version 27. All parameters were evaluated for normality using the Shapiro-Wilk test, indicating that most were not normally distributed ($p < 0.05$); hence, we employed nonparametric statistical tests. Continuous variables were summarized using medians and interquartile ranges, while categorical data were presented as frequencies and percentages. Differences between two categorical groups were assessed using the chi-square test. For continuous data, the Mann–Whitney test was applied when comparing two groups, and the Kruskal–Wallis test was used for comparisons across more than two groups. Spearman's rank correlation coefficient was utilized to evaluate the relationships between serum 25(OH)D levels and selected hormonal parameters, as well as BMI. Statistical significance was defined as a p-value less than 0.05.

RESULTS

To assess the normality of the distribution of data, the Shapiro-Wilk test was conducted. As a result, the data found to be not normally distributed, p value <0.05 (Table 1). Therefore, suitable statistical analysis was applied to evaluate this nonparametric data, including the Mann-Whitney U test, Kruskal-Wallis H test, Chi-square test, and Spearman's correlation test.

Table 1: Normality test expressed by and Shapiro-Wilk**.**

Tests of Normality			
	Shapiro-Wilk		
	Statistic	df	Sig.
vitamin D level (ng/mL)	.908	380	**.000**
Age in years	.976	380	**.000**
Body mass index	.967	380	**.000**
Luteinizing hormone (IU/L)	.510	380	**.000**
Follicle stimulating hormone (IU/L)	.514	380	**.000**
Prolactin	.869	380	**.000**

Demographic Sample Profile of the Studied Groups

Table 2 illustrate the basic characteristics of women in the two groups. Including the level of vitamin D, their ages, body mass index, and reproductive hormones (FSH, LH, and prolactin).

Table 2: Demographic Characteristics of the Studied Groups

Study groups						
Descriptive statistics	Control (n = 120)			Infertility (n = 260)		
	Median	Max	Min	Median	Ma x	Min
Vitamin D level	19.75	70.1	5.6	16.47	70.9	2.9
Age in years	30	45	17	32	45	19
BMI	24.65	35.6	16	29	59	15
FSH	7.5	89.4	1.4	7.26	50	0.2
LH	7	86.3	1.2	6.73	55.9	0.1
Prolactin	17.85	67.3	1.2	18.4	100	1

Table 3 and Figure 1 illustrate that the majority of participants (228 individuals, 60%) exhibited vitamin D deficiency. Moreover, 23% of participants (88 individuals) had an insufficient level of vitamin D, while only 17% of participants had sufficient level of vitamin D.

Table 3: Frequency and Percentages of Vitamin D Levels in the Overall Sample (N = 380)

Vitamin D categories	**Control Group**	**Infertility Group**
Deficient (< 20 ng/ml)	228	60%
Insufficient (20-29.9 ng/ml)	88	23%
Sufficient (30-100 ng/ml)	64	17%
Total	380	

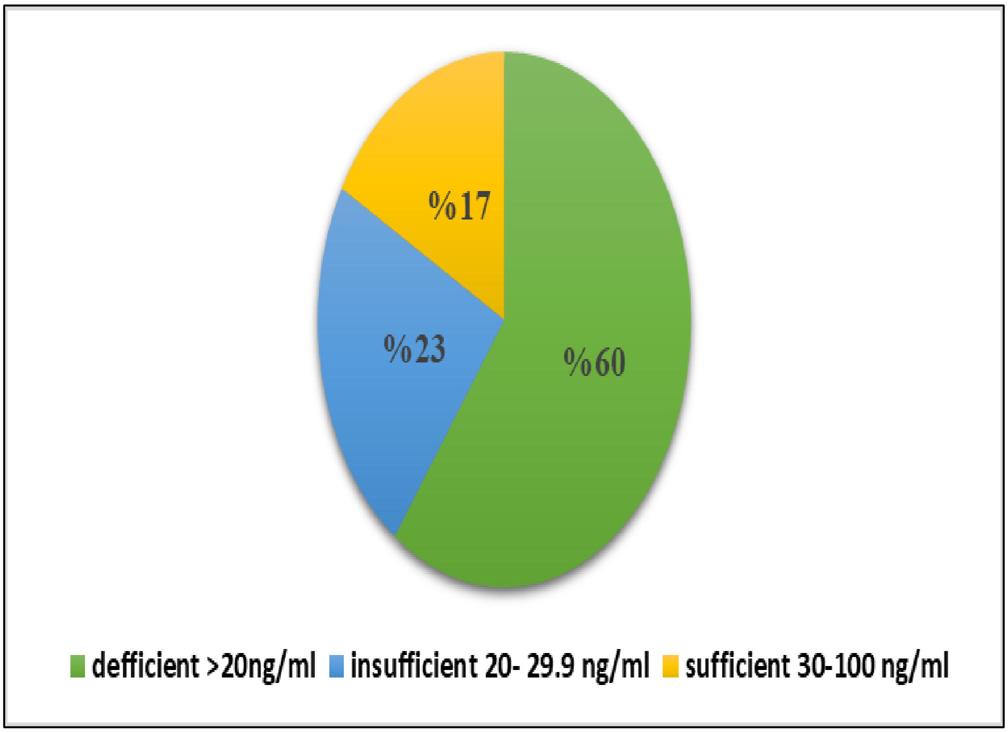

Figure 1: Percentages of Vitamin D Levels in the Overall Sample (N = 380)

Table 4: Mann-Whitney U test for the variables between control and infertility group

Variable	Control (mean)	Infertility (mean)	U Value	P value
vitamin D ng/ml	219.06	177.32	12173	**0.001***
BMI Kg/m²	138.03	214.72	9304	**0.00****
FSH IU/L	195.88	188.02	14954	0.517
LH IU/L	195.16	188.35	15041	0.574
Prolactin ng/ml	181.89	194.47	14567	0.299

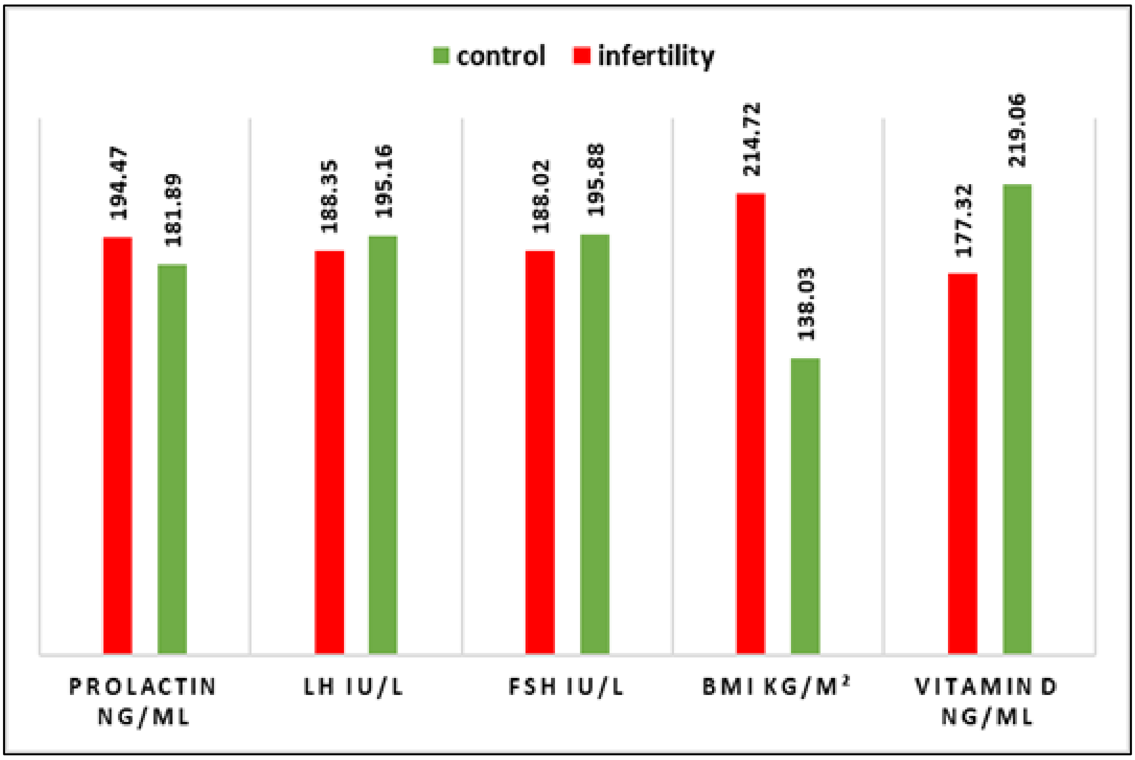

Figure 2: The Difference in the Means in Control and Infertility groups

Association between Vitamin D Categories and Study group

The association between vitamin D levels (deficiency, insufficiency and sufficiency) and the study groups (control and infertility) was conducted by chi-square independence test. With a p value of 0.001 and $\chi^2(2, N = 380) = 13.559$, the connection was determined to be statistically significant, showing a substantial difference in vitamin D levels across research groups. The range of vitamin D levels across the research groups is shown in Figure 3. When comparing the female infertility group to the control group, the frequency of vitamin D insufficiency was much greater. Vitamin D deficiency affected around 65% of infertile women and 49.20% of control women. Conversely the proportion of female in sufficient category was higher in control group with 26.70% and only 12.30% of infertility women had a sufficient level of vitamin D. The insufficient level for control and infertility group was 24.20% and 22.70% respectively**.**

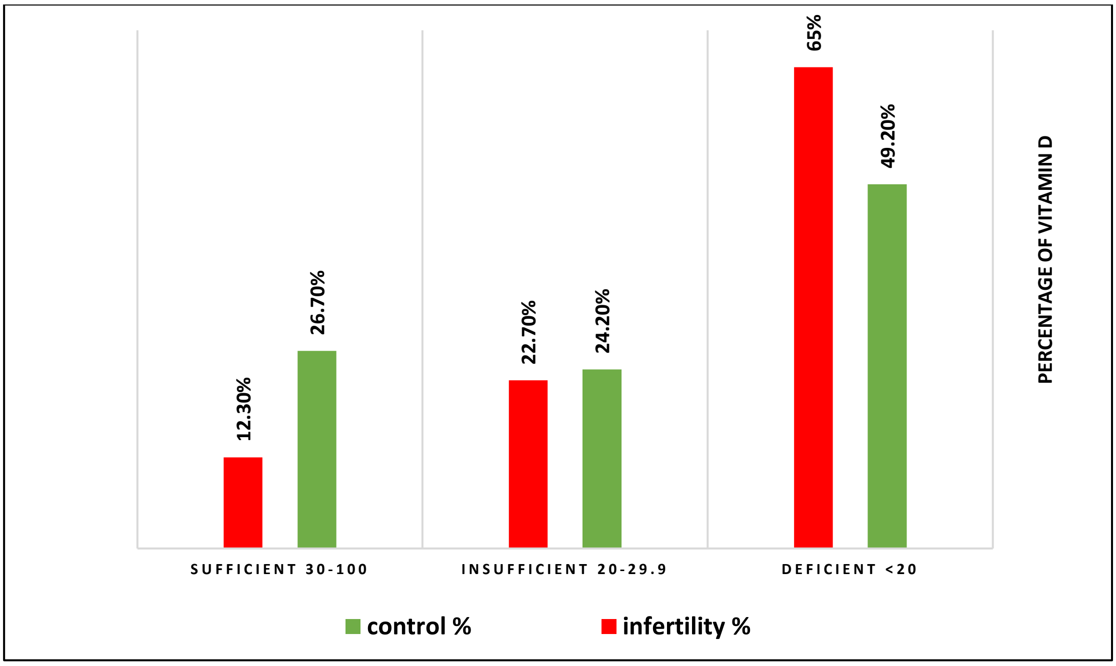

Figure 3: percentages of vitamin D in control and infertility group

Kruskal-Walli's test: Differenced based on Vitamin D categories

The Table 5 and Figure 4 show how different clinical and hormonal factors vary among the vitamin D level groups based on the Kruskal-Walli's test. The results indicated a statistically significant difference in the distribution of participants across study groups and vitamin D level categories, with a p-value of **0.001**. Likewise, body mass index (BMI) exhibits significant differences across vitamin D categories (**p = 0.046**). On the other hand, no statistically significant differences were detected in age (p = 0.345), FSH (p = 0.535), LH (P = 0.864), and Prolactin (p =0.242).

Table 5: Differences based on Vitamin D categories by Kruskal-Walli's test

Variable	Vit D Deficient	Vit D Insufficient	Vit D sufficient	P
Study group	201.3	187.8	155.5	**0.001**
Age	185.8	205.4	186.5	0.345
BMI	196.3	197.8	159.5	**0.046**
FSH	194.9	179.5	189.7	0.535
LH	192.4	185	191.2	0.864
Prolactin	191.3	176.5	206.7	0.242

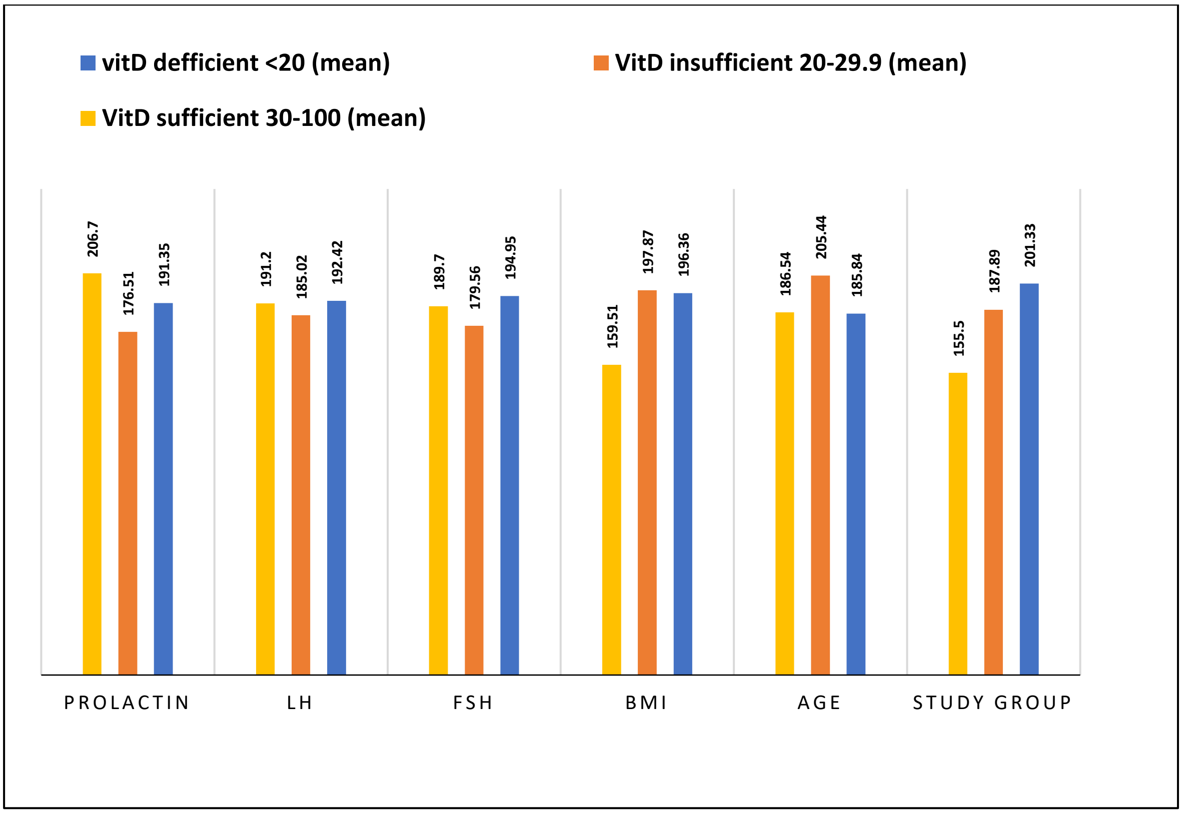

Figure 4: Difference based on vitamin D categories

Association Between Vitamin D categories and BMI categories within Infertility group

As shown in the following Figure 5, the majority of infertility participants across BMI categories had vitamin D deficiency, With the highest deficiency observed among obese participants (66.7%), followed by the normal weight participants (66.7%), overweight (61.7%) and the underweight (63.6%).

Regardless of the variation in the distribution of vitamin D level across the different categories of BMI, the Chi-square test result indicate to non-statistically significant association between BMI categories and vitamin D levels with p value of **0.541**.

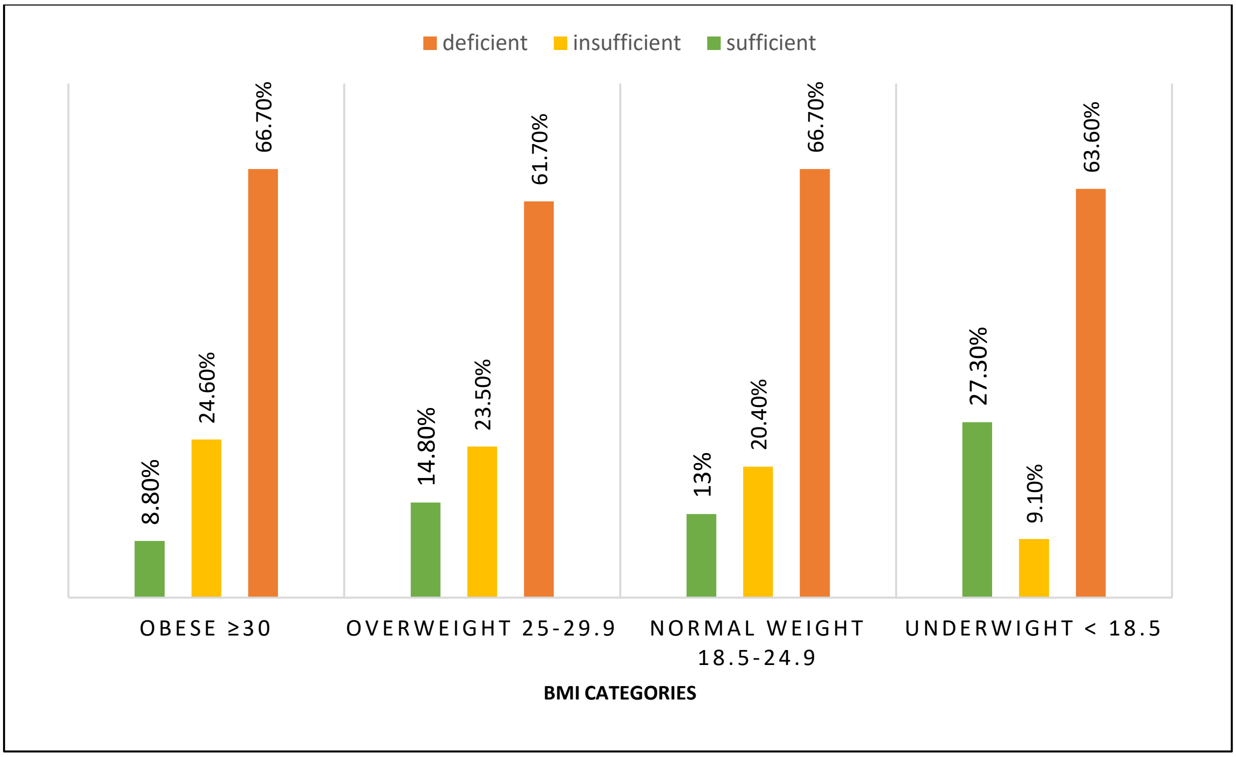

Figure 5: percentages of distribution of Vitamin D across BMI in infertility group

Differences on hormonal profile and Vitamin D based on BMI categories among Infertile participants

To explore whether vitamin D level and reproductive hormone levels (FSH, LH, Prolactin) varied significantly across the BMI categories among the infertile participants, the Kruskal-Walli's test was conducted**.**

Table 6: Differences on vitamin D levels and hormonal profiles based on BMI categories among infertility participants.

Variables	Underweight < 18.5 (mean)	normal weight 18.5 - 24.9 (mean)	overweight 25-29.9 (mean)	obese ≥30 (mean)	H (df)	p-value
Vitamin D	122.68	124.98	139.27	127.64	1.677 (3)	0.642
FSH	158.45	133.17	135.56	122.95	3.104 (3)	0.376
LH	152.23	153.94	136.48	113.05	12.815 (3)	**0.005**
Prolactin	182.18	131.58	138.33	119.44	8.551 (3)	**0.036**

As shown in Table 6 and Figure 6, the mean rank for vitamin D levels varies little throughout BMI groups. Nevertheless, the outcome (p = 0.642) was not statistically significant. Similarly, there were no statistically significant variations in FSH levels across BMI groups. On the other hand, LH levels among infertile individuals exhibit statistically significant variations between BMI categories, with a test statistic of H (3) = 12.815 and a p value of 0.005, indicating a statistically significant correlation. Prolactin levels also varied significantly across BMI groups (H (3) = 8.551, p = 0.036), with the underweight group having the highest mean rank (182.18) and the obese group having the lowest (119.44).

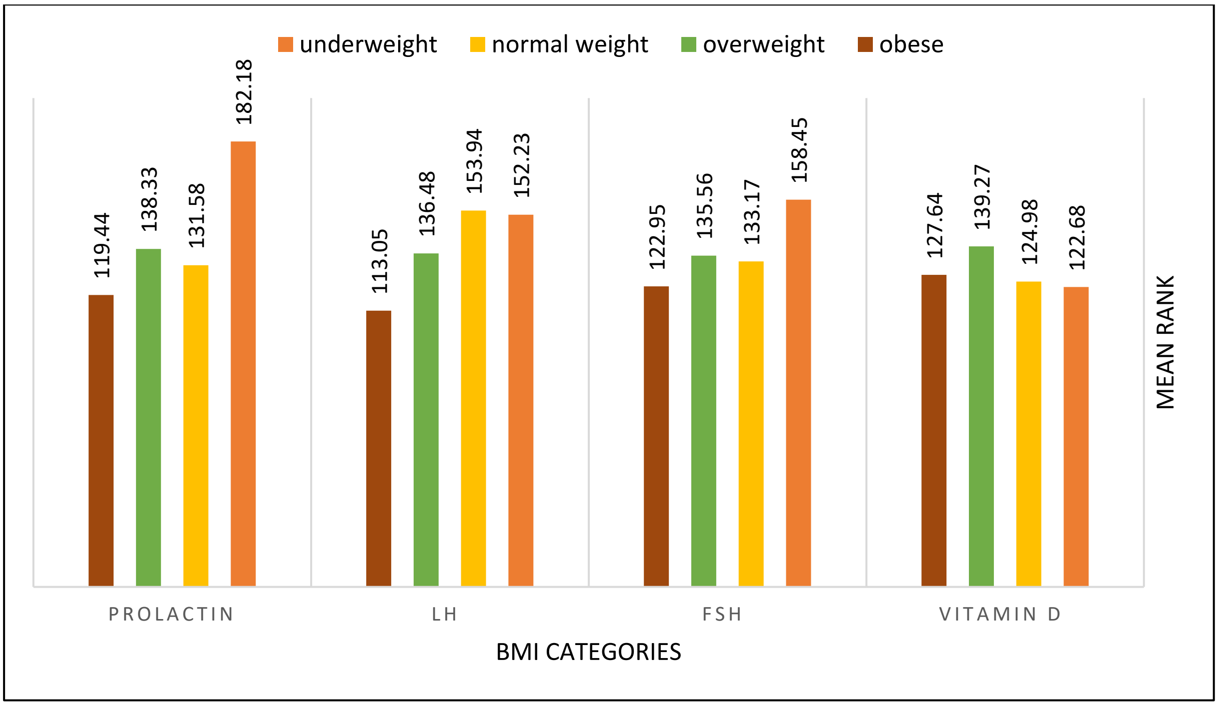

Figure 6: Differences on the mean based on BMI categories (infertile group)**.**

Spearman's Correlation between Vitamin D and the Other variables

A Spearman's rank-order correlation was computed to assess the relationship between serum vitamin D levels (ng/mL) and body mass index (BMI), also with FSH, LH, Prolactin and the study groups in an overall sample of 380 participants.

The analysis revealed a statistically significant, negative correlation between vitamin D levels and the study groups, correlation coefficient = −0.177, p = **0.001**. Conversely, there was statistically no significance, very weak negative correlation between vitamin D level and the BMI, correlation coefficient = - 0.078, p = 0.131.

There was no significance correlation exist between vitamin D level and the hormonal profile in the selected study participants as shown in the Table 7.

Table 7: The spearman's correlation coefficient between vitamin D and the other variables.

Variable	Correlation Coefficient (rs)	P value
Study group	-0.177	**0.001**
BMI	-0.078	0.131
FSH	-0.036	0.48
LH	-0.016	0.763
Prolactin	0.009	0.861

Subgroup Analysis of Study Variables According to BMI (Obese Category)

The Kruskal-Walli's test was conducted to compare the study groups and reproductive hormones across three vitamin D level categories within the obese women as subgroup analysis. As represented by the Table 8 and figure 7, there was a statistically significant difference in study group distribution (H = 8.220, p = 0.016) and FSH levels (H = 8.720, p = 0.013) across vitamin D levels.

In contrast, there was no significant differences were found for prolactin (H = 1.029, p = 0.598) or LH levels (H = 0.875, p = 0.646), showing that these hormones may not differ significantly by vitamin D status in this subgroup.

Table 8: Differences in the study groups and hormone profiles based on level of vitamin D on obese women (n = 130)

variables	VitD deficient < 20 (mean)	VitD insufficient 20-29.9 (mean)	VitD sufficient 30-100 (mean)	H (df)	p-value
Study group	68.74	63.65	51.83	8.220 (2)	**0.016**
FSH	72.65	56.38	46.47	8.720 (2)	**0.013**
LH	67.78	60.7	63.6	0.875 (2)	0.646
Prolactin	66.41	60.47	71.6	1.029 (2)	0.598

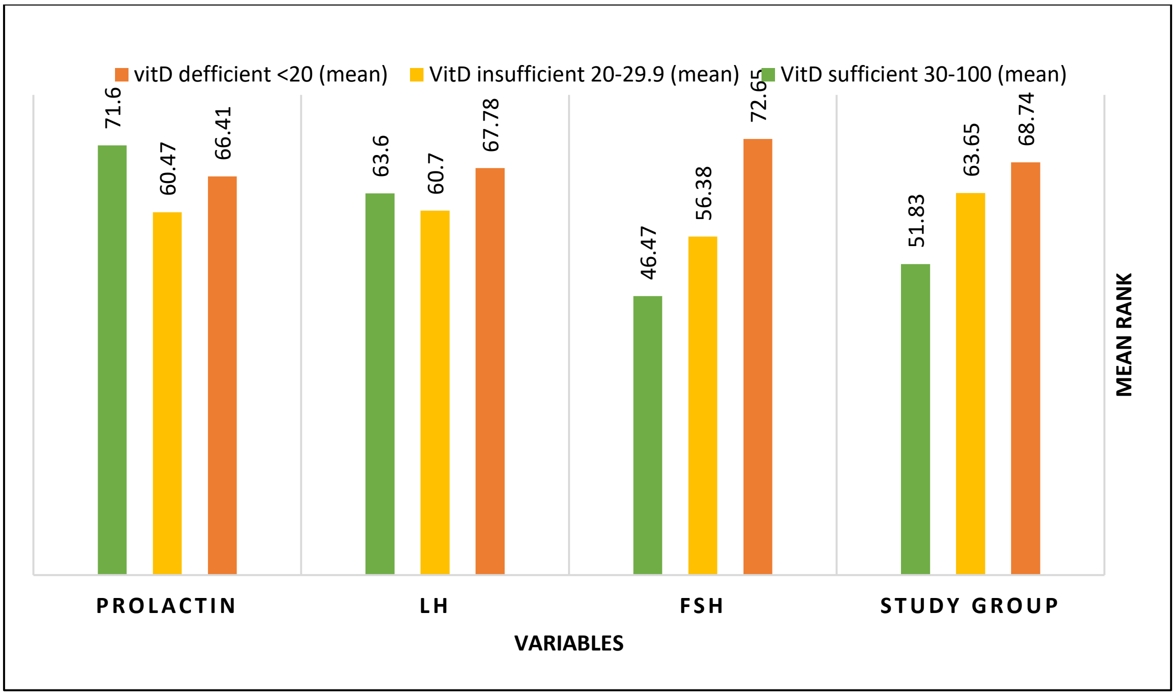

Figure 7: The differences in the means of the variables on obese women

DISCUSSION

The frequency of vitamin D insufficiency among women of reproductive age in both the fertile control group and the infertile group—a total of 380 women—was alarmingly high, according to this research. Just 17% of individuals had adequate levels of vitamin D, whereas 60% of people had vitamin D deficiencies. There are similar studies in Libya have stated significant association for prevalence of vitamin D deficiency among women at the reproductive age (18-45). As reported by Ahmed A. et al. (2023) in Tripoli, Libya, revealed a significant prevalence of vitamin D deficiency, particularly among females aged 20-25. In addition to a study in Tobruk, Libya by Amal Rajab Agila (2020), stated that majority of women in the age group (30-35) experienced deficiency in vitamin D (Ahmed A. et al., 2013). 65% of the infertile women in this research had low vitamin D levels (less than 20 ng/mL). Of these, just 12.3% had adequate vitamin D levels, while 22.7% did not. In contrast, the control group had a greater percentage of sufficiency (26.7%) and a lower incidence of deficit (49.2%). However, 24.2% had an inadequate level. A significant difference between the two groups was discovered using the Mann-Whitney test (p = 0.001).

The majority of infertile women fell into the inadequate and insufficient level of vitamin D with a p-value of 0.001, and the Kruskal-Walli's test also revealed significant disparities in the distribution of participants among the research groups and vitamin D categories. Additionally, the Chi-Square test revealed statistically significant differences (p=0.001), indicating that the infertile women had lower levels of vitamin D. In addition, the Spearman's correlation coefficient indicated a negative association (p = 0.001, rs = -0.177) between the research groups and vitamin D. which suggests that a lack of vitamin D may be linked to infertility. These results are coherent with previous studies showing lower serum vitamin D levels among women with infertility compared to fertile controls. According to Thomson, R. L., et al., vitamin D insufficiency is a worldwide trend that affects people of all ages and is often seen in reproductive clinic patients (Thomson, R. L., et al.,2012). Furthermore, a cross-sectional

research in Shaanxi, China by Wang, L., et al. found that women with PCOS had significantly lower serum 25(OH)D concentrations than the study's control group (Wang, L., et al., 2020). Additionally, a research conducted at Ain Shamus University in Egypt found that the group with unexplained infertility had considerably lower serum 25(OH)D concentrations than the control group. (Ramy, A., Rafaat, T., Haji, I., & Abdel Hameed, A. (2023)).
According to the Chi-Square test, the majority of women across the BMI categories had low level of vitamin D, as the highest deficiency observed among the obese group, as 66.7 % of obese women had a vitamin D deficiency. And about 61.7% of overweight women had a vitamin D deficiency. On the other hand, only 8.8% of obese women had a sufficient level of vitamin D. Regardless the variation in the distribution of vitamin D level across BMI categories, the Chi-Square test showed no statistically significant result ($p = 0.541$).
Kruskal-Walli's test was conducted for the infertile group only to assess the differences in the reproductive hormones based on the categories of BMI and it's revealed to inverse relationship between the BMI and the level of LH ($p = 0.005$), in addition to that, the finding show the link between the higher BMI with lower level of prolactin ($p = 0.036$). However, there is no statistically significant differences had shown in the level of FSH and BMI in the infertility women ($p = 0.376$). In the obese women, a Kruskal-Wallis was conducted to assess the association between the vitamin D and other risk factors, the result exhibits that vitamin D level have potential association with both of infertility group and FSH. As demonstrated by the result the infertile group had the lower level of vitamin D ($p = 0.016$) as well as FSH ($p = 0.013$). In contrast, the normal-weight group did not show the same pattern, indicating that BMI may act as a confounding factor in the relationship of vitamin D with women infertility.
Beyond the interaction observed with vitamin D and BMI, this study also demonstrated fluctuations in reproductive hormones (FSH, LH, and prolactin) across various level of vitamin D and the classification of BMI. Nevertheless, not all these showed a statistically significant difference. Supporting this finding, Wehr et al. (2010) who demonstrated the lower levels of vitamin D connected with impaired gonadotropin function, particularly disrupted FSH/LH ratio in women with reproductive issues. Consistent with the study finding that demonstrate the moderate negative correlation between the level of vitamin D and prolactin in some subgroup, Kinuta et al. (2000) documented that lower level of vitamin D impact on the pituitary secretion of prolactin and gonadotropins.
Numerous factors may perhaps justify the lack of significant correlation between vitamin D and the reproductive hormones across the overall sample, could be attributed to the multifactorial nature of infertility, where vitamin D is just one aspect among many. Furthermore, the prevalence of obesity among infertile women may partly explain the vitamin D – infertility relationship, as the bioavailability of vitamin D reduced and absorbed by the fat tissue.

CONCLUSION

A significant finding was the association between the deficiency of vitamin D and the infertile participants, demonstrating that the reproductive outcome could be impaired by vitamin D deficiency as a contributing factor. Moreover, the vitamin D level showed very weak negative correlation with the body mass index indicate that the bioavailability of vitamin D could be reduced by increasing of adiposity.

One of the findings was the significant association between the infertility status and BMI. These finding demonstrated the obesity as strong contributing factor to impact on the reproductive outcomes. Conversely, the total sample didn’t show significant correlation between the level of vitamin D and the reproductive hormones (FSH, LH and prolactin). Majority of infertile women were found to be obese or overweight. Emphasizing the interaction between the level of vitamin D and the body weight in reproductive health. The result highlighted the significant association of the vitamin D with both FSH and infertility in obese women.

Overall, these finding highlight the importance of assessing vitamin D status in the management and assessment of infertility. The reproductive outcomes could be improved by the routine screening for vitamin D deficiency and addressing modifiable factors such as BMI.

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ETHICS

After receiving a thorough explanation of the study's goals and procedures, participants gave their informed permission.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that they have no conflict of interest.

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