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An investigation into the relationship between serum vitamin D levels and the success rate of pregnancy in a cycle of in vitro fertilization

Nurgül Ulusoy[1], Ayşe Şeyma Küçükakça[2]

[1]Private Gynecology and Obstetric Clinic, Istanbul, Turkiye
[2]Department Gynecology and Obstetric, Istanbul Medipol University Çamlıca Hospital, İstanbul, Turkiye

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Corresponding Author: Nurgül Ulusoy, nculusoy17@gmail.com

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ABSTRACT

Aims: The significance of vitamin D in women's health is well-established, and numerous studies have explored its role in reproductive physiology. This examination aimed to delineate the association between serum vitamin D levels and the success rate of pregnancy in in vitro fertilization (IVF) cycles among infertile women aged 20-45.

Methods: This retrospective cohort study, conducted between March 2020 and August 2023, included 134 infertile women with insufficient vitamin D levels and 152 infertile women with sufficient vitamin D levels. The study aimed to investigate the impact of adequate vitamin D serum levels on the success of the IVF method. The threshold for determining insufficient vitamin D level was set at 30 ng/mL, measured using the ELISA method seven days before embryo transfer.

Results: The results revealed that gestational sac and fetal heart rate were significantly higher in women with sufficient vitamin D levels ($p<0.05$). Additionally, a statistically significant association was observed between the case and control groups regarding live births ($p<0.05$; 13.4% vs. 75.7%). Multiple logistic regression, adjusting for confounding variables, demonstrated a significant difference between the groups in terms of pregnancy success rate ($p<0.05$).

Conclusion: It is inferred that sufficient vitamin D levels increase the likelihood of success in IVF among Turkish infertile women.

Keywords: Infertile women, in vitro fertilization, IVF, vitamin D, pregnancy success

INTRODUCTION

Vitamin D deficiency represents a notable global health concern across various age groups, with a particular emphasis on pregnant women, adult females, and girls.[1] High rates of vitamin D deficiency have been demonstrated among scholars in Turkiye.[2] This deficiency has implications for immune system dysfunction, as well as its association with conditions such as cancer, psoriasis, diabetes, leukemia, and osteoporosis.[3] Considerable research has focused on exploring the impact of this vitamin on the reproductive system of females.[4]

Vitamin D comprises two principal isoforms: vitamin D2 (ergocalciferol) and vitamin D3 (cholecalciferol), with 1 and 25-hydroxyvitamin D3 representing the active form of the vitamin.[5,6] In this study, the focus is on 25-hydroxyvitamin (OH) D3. Vitamin D is present in various foods, including but not limited to fish, reindeer lichen, mushrooms, fish liver oils, cheese, beef liver, dark chocolate, fat spreads, fish fat, yogurt, milk, orange juice, breakfast grains, and eggs.[7] The concentration of 25 (OH) D in blood serum serves as a key determinant for evaluating vitamin D deficiency.[8,9] Numerous studies have proposed varying thresholds for the appropriate and acceptable level of vitamin D concentration in blood serum, with most research indicating adequacy at 30 nanograms per milliliter (ng/mL) or higher.[10]

Infertility poses numerous challenges for both couples and the healthcare system. Defined as the inability to achieve a successful pregnancy after 12 months of regular unprotected sexual intercourse, infertility affects approximately 8-12% of couples worldwide.[11] Among the various causes, factors contributing to infertility include issues related to ovulation, the uterine-tubal system, and male factors.[12] Over the past decades, the increasing age of marriage and the postponement of childbearing have emerged as significant contributors to infertility.[13] In vitro fertilization (IVF), an assisted reproduction treatment (ART) method, involves the combination of sperm and eggs in a laboratory setting. Many

couples have successfully addressed their infertility concerns through the application of this technique. Numerous researchers have explored factors influencing the success of IVF.[14] This study investigates the impact of vitamin D on the likelihood of IVF success.

Vitamin D deficiency represents a notable public health concern in Turkiye, with reported prevalence ranging from 58.9% to 66.6%.[2] Testing for vitamin D serum levels is relatively inexpensive and widely accessible, and the associated treatment is cost-effective.[15] Consequently, the diagnosis and treatment of vitamin D deficiency in women contemplating assisted reproductive methods may prove beneficial. The impact of vitamin D deficiency on women's reproductive health underscores the necessity for extensive and diverse studies addressing the increasing prevalence of this deficiency and its adverse consequences on the female reproductive system. This study aims to explore the correlation between vitamin D levels and IVF outcomes, marking what we believe to be the first investigation into this subject among Turkish women.

METHODS

This retrospective cohort study was conducted on patients between March 2020 and August 2023. Ethical approval was granted by the İstanbul Medipol University Non-invasive Researches Ethics Committee (Date: 26.10.2023, Decision No: 879). All procedures adhered strictly to ethical guidelines and the principles outlined in the Declaration of Helsinki.

Inclusion criteria encompassed infertile women within the reproductive age range (20-45 years) experiencing either primary or secondary infertility. The study focused on women undergoing their initial cycle of in vitro fertilization (IVF). Exclusion criteria comprised cases involving repeated pregnancy loss, ovarian hyperstimulation syndrome, autoimmune disease, chronic illness, cardiovascular problems, endocrine disorders, infertility attributed to severe endometriosis, and uterine abnormalities.

Infertile women meeting the study criteria received detailed information about the study's purpose and methodology. Subsequently, the researcher conducted interviews to complete a demographic profile questionnaire, and blood samples were collected from all participants seven days prior to embryo transfer to measure the serum level of vitamin D. The laboratory employed the ELISA method to measure vitamin D serum levels, ensuring uniformity by conducting all assessments in a single laboratory.

Following embryo transfer, luteal phase support for all participants involved intramuscular progesterone injections of 50 mg every other day and vaginal progesterone suppositories of 400 mg every 12 hours.

Participants were categorized into two groups based on their serum vitamin D levels: insufficient (<30 ng/mL) constituted the case group, while sufficient (≥30 ng/mL) formed the control group. Sampling continued until the number of participants reached 134 in the insufficient vitamin levels group and 154 in the sufficient vitamin levels group.

Subsequently, participants were closely monitored, and assessments for intrauterine gestational sac and fetal heart rate were conducted at seven weeks of gestation.

Statistical Analyses

The study utilized mean (M) and standard deviation (SD) to present descriptive statistics for the data. Categorical variables were delineated using number and percentage and analyzed through the Chi-Squared test. The normality of quantitative data was assessed using the Kolmogorov-Smirnov test, revealing a normal distribution for all the data. Group comparisons were conducted using the Independent t-test, deemed appropriate for datasets with normal distributions. Exploring the significant relationship between vitamin D and IVF success involved employing multivariate logistic regression analysis. Statistical analysis was performed using SAS statistical software, and the threshold for statistical significance was set at a p-value less than 0.05.

The sample size for the study was determined using the G-Power 3.2 program. The calculation, based on Pearson's Chi-Square Test of Association, aimed for a power of 80%, an effect size of 40%, and a type 1 error of 0.25, resulting in a minimum requirement of 278 patients.[16]

RESULTS

The study encompassed two hundred and eighty-six infertile women, carefully matched for age (31.25±5.21) and body mass index (BMI) (26.62±4.45). **Table 1** provides a comprehensive statistical overview of maternal characteristics, gestational sac, fetal heart rate (FHR), vitamin D supplement usage, vitamin D levels, pregnancy test outcomes, and pregnancy results. Maternal characteristics, comprising age, BMI, husband's age, duration of infertility, type of infertility, smoking habits, and employment status, were presented for detailed analysis.

As indicated in **Table 2**, an Independent t-test revealed no statistically significant differences between the insufficient vitamin D group and the sufficient vitamin D group concerning age, husband's age, BMI, and duration of infertility ($p>0.05$). However, a statistically significant difference was observed between the case and control groups in terms of vitamin D levels ($p<0.05$). The mean and standard deviation (SD) of vitamin D levels in the case and control groups were 14.37±6.47 and 35.69±12.5, respectively.

As presented in **Table 2**, a chi-squared test identified a statistically significant difference between the insufficient vitamin D and sufficient vitamin D groups in terms of the presence of a gestational sac ($p<0.05$). Additionally, a statistically significant difference was observed between the case and control groups concerning fetal heart rate (FHR) ($p<0.05$). However, no statistically significant difference was found between the case and control groups regarding vitamin D supplements ($p>0.05$).

Furthermore, **Table 2** reports that the chi-squared test revealed a statistically significant difference between the insufficient vitamin D and sufficient vitamin D groups in

relation to the pregnancy test results (p<0.05). All infertile women in the control group exhibited positive results. Similarly, a statistically significant difference was noted between the case and control groups concerning pregnancy outcomes (p<0.05). The number and percentage of live births in the insufficient vitamin D and sufficient vitamin D groups were 18 (13.4) and 115 (75.7), respectively.

Table 3 provides insight into a logistic regression test conducted to explore factors influencing live births in pregnant women. Binary logistic regression was utilized to predict pregnancy outcomes using variables such as vitamin D, women's age, husband's age, and BMI level. Vitamin D emerged as a significant predictor of pregnancy outcomes (Exp(B)=1.083, p=<0.001, 95% CI [1.059, 1.107]). Additionally, the age of pregnant women proved to be a significant predictor of pregnancy outcomes (Exp(B)= 0.939, p=0.021, 95% CI [0.891, 0.991]). However, husband's age and BMI did not emerge as significant predictors of pregnancy outcomes (p>0.05).

Table 1. Statistical description of study parameters in infertile women (n=286)

Study parameters	mean ± SD (range) or n (%)
Maternal characteristics	
Age (years)	31.25±5.21 (20-44)
BMI (kg/m²)	26.62±4.45 (17.30-40.80)
Husband's age	35.59±4.69 (24-47)
Duration of infertility	3.08±1.25 (1-6)
Type of infertility	
Primary	236 (82.5)
Secondary	50 (17.5)
Smoking	
Yes	32 (11.2)
No	254 (88.8)
Employment status	
Employed	213 (74.5)
Unemployed	73 (25.5)
Gestational sac	
Zero	130 (45.5)
One	111 (38.8)
Two	45 (15.7)
Fetal heart rate (FHR)	
Yes	156 (54.5)
No	130 (45.5)
Use of Vitamin D supplement	
Yes	184 (64.3)
No	102 (35.7)
Vitamin D	
Insufficient	134 (46.9)
Sufficient	152 (53.1)
Pregnancy test results	
Positive	177 (61.9)
Negative	109 (38.1)
Pregnancy results	
Birth	133 (46.5)
Negative	109 (38.1)
Abort	44 (15.4)

"BMI: Body mass index, FHR: Fetal heart rate, SD: standard deviation, n: Number."

Table 2. Comparison of insufficient vitamin D and sufficient vitamin D groups

Study parameters	Insufficient Vitamin D (n=134) mean ± SD (range)	Sufficient Vitamin D (n=152) mean ± SD (range)	p value
Age (years)	31.53±5.29 (21-42)	31±5.14 (20-44)	0.392*
Husband's age	35.86±4.9 (24-47)	35.36±4.5 (27-46)	0.396*
BMI (kg/m²)	27.01±4.63 (17.3-40.8)	26.28±4.27 (18.9-36.9)	0.170*
Duration of infertility	3.19±1.24 (1-6)	2.98±1.26 (1-6)	0.150
The serum vitamin D (ng/ml)	14.37±6.47 (2.3-31)	35.69±12.5 (5.3-95.2)	<0.001*
Gestational sac			<0.001**
Zero	113 (84.3)	17 (11.2)	
One	15 (11.2)	96 (63.2)	
Two	6 (4.5)	39 (25.7)	
FHR			<0.001**
No	113 (84.3)	17 (11.2)	
Yes	17 (12.7)	97 (63.8)	
Twin	4 (3)	38 (25)	
Use of Vitamin D supplement			0.409**
Yes	89 (66.4)	95 (62.5)	
No	45 (33.6)	57 (37.5)	
Pregnancy test results			<0.001**
Positive	25 (18.7)	152 (100)	
Negative	109 (81.3)	0 (0)	
Pregnancy results			<0.001**
Live birth	18 (13.4)	115 (75.7)	
Negative	109 (81.3)	0 (0)	
Abort	7 (5.2)	37 (24.3)	

*An Independent t-test. **A chi-squared test.

Table 3. Logistic regression test to investigate factors affecting pregnancy results

Study parameters	95% C.I.for EXP(B)		Exp(B)	p-value
	Lower	Upper		
Vitamin D n(ng/ml)	1.059	1.107	1.083	<0.001
Age	0.891	0.991	.939	0.021
Husband's age	0.931	1.047	.987	0.667
BMI	0.959	1.067	1.012	0.669

DISCUSSION

The primary objective of this investigation was to establish a connection between serum vitamin D levels and the success rate of pregnancy in an IVF cycle. The study encompassed 134 infertile women with insufficient and 152 infertile women with sufficient vitamin D serum levels. The research findings demonstrated a significant impact of serum vitamin D levels on the success of the IVF method in achieving pregnancy for infertile women. Over the past decades, numerous studies have delved into the physiological role of vitamin D in influencing outcomes in assisted reproductive technology. However, the results of these prior studies exhibit inconsistency and contradiction.[17] This disparity may be attributed to various factors influencing vitamin D, including sunlight exposure and dietary habits.[18]

In a cross-sectional study involving 848 Chinese infertile women, Liu et al.[19] discovered that vitamin D levels were not associated with the live birth rate and clinical pregnancy following IVF. Similarly, Cozzolino et al.[20] conducted a meta-analysis and systematic review, encompassing multiple articles, and concluded that serum

vitamin D levels do not impact clinical pregnancy, live birth, and ongoing pregnancy rates in conventional IVF or intracytoplasmic sperm injection outcomes. Franasiak et al.[21] in a retrospective cohort study involving 517 infertile women, found no significant relationship between serum vitamin D levels on the day of ovulation and implantation rate and clinical pregnancy in IVF.

The influence of race on the association between serum vitamin D levels and pregnancy rate after IVF was explored by a study conducted by.[22] They observed that in most studies involving Asians, the effect of adequate vitamin D levels on pregnancy outcomes was not evident.[23] Limited research has been conducted on Turkish women regarding the impact of vitamin D on assisted reproductive technology (ART) success. Yilmaz et al.[24] reported that serum vitamin D levels do not affect intrauterine insemination success in Turkish infertile women.

Contrary to the findings of the studies mentioned above, the present study revealed a significant relationship between vitamin D levels in Turkish infertile women and IVF outcomes, differing from the results reported in these studies.

The findings of this study align with those of several other studies. Garbedian et al.[25] reported that adequate levels of vitamin D increase the likelihood of clinical pregnancy following IVF and suggested vitamin D supplementation to enhance pregnancy rates in infertile women. Zhou et al.[26] echoed this recommendation for vitamin D supplementation to improve success in IVF. Previous studies have indicated that elevated follicular vitamin D levels are associated with higher pregnancy rates in IVF.[17,27] In a meta-analysis and systematic review, Iliuta et al.[28] found favorable outcomes in IVF for women with sufficient vitamin D status, while another systematic review by Shen et al.[29] suggested that vitamin D deficiency tended to reduce IVF pregnancy outcomes.

Vitamin D emerges as a significant factor in women's fertility, whether achieved spontaneously or with the assistance of assisted reproductive technology (ART) such as IVF. The ongoing discourse on the role of vitamin D in reproductive health underscores the need for further investigation. Conflicting research results on vitamin D and infertility emphasize the necessity for additional studies. Studies with larger sample sizes, involving thousands of women, are warranted to establish the vitamin D threshold influencing the reproductive process, which may differ from recommended amounts for bone health. The role of vitamin D in ART processes across diverse ethnic groups is another crucial area requiring further exploration. The primary contribution of this study lies in demonstrating a significant relationship between vitamin D and IVF outcomes in Turkish women. Discrepancies among studies conducted in different countries may be attributed to factors such as lifestyle, seasonal influences, or the involvement of other ovarian factors in the reproductive process. Vitamin D is linked to various factors, including geographic, demographic, and clinical parameters, which can influence its effects on women's fertility. Conducting randomized trials could yield more reliable answers.

Limitations

This study has both limitations and advantages. One limitation is its single-center nature, and future studies are recommended to utilize data from multiple centers. Additionally, exploring the role of vitamin D levels in men and their impact on IVF success is suggested for future research. Another limitation is the absence of data related to men. The study's substantial sample size stands out as one of its key strengths, enhancing the reliability of its results.

CONCLUSION

This study underscores a significant correlation between serum vitamin D levels in infertile women and successful pregnancy outcomes in the IVF method. Women with elevated vitamin D levels exhibited a heightened probability of achieving live births and positive pregnancy test results. Given that vitamin D supplementation is both affordable and safe, it stands as a viable recommendation for infertile women undergoing IVF, offering potential benefits. The results, derived from large-scale studies conducted in Turkiye, contribute to shedding light on the precise mechanisms of vitamin D in the realm of fertility. To further enhance our understanding, future research endeavors should explore this relationship through comprehensive cellular and clinical studies for more accurate and nuanced results.

ETHICAL DECLARATIONS

Ethics Committee Approval

The study was carried out with the permission of the Balıkesir University Faculty of Medicine Non-invasive Clinical Research Ethics Committee (Date: 23.08.2023 Decision No: 2023/114).

Informed Consent

Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process

Externally peer-reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

The authors declared that this study had no financial support.

Author Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version.

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Nurgül Ulusoy

I graduated from Istanbul University, Istanbul Medicine Faculty in 1997. I'm specialist in gynecology and obstetrics. I completed my specialization at Bakırköy Women and Children's Diseases Training and Research Hospital in 2002. I worked at Gynecology and Obstetrics Department of Bakırköy Women and Children's Diseases Training and Research Hospital between 2002-2006 as chief assistant. Then I worked as a Gynecology and Obstetrics specialist at Private Avicenna Esenler Ensar Hospital between 2006-2016 and at Private Incirli Ethica Hospital between 2016-2017. I served at VM Medicalpark Florya Hospital as a Physician Lecturer at İstanbul Aydın University Gynecology and Obstetrics Department between March 2017-2020. As of 2023, I have been continuing my proffesional studies at my own private clinic (Bakirkoy, İstanbul) since January 2021.