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REPRODUCTIVE FALL OUT OF A BIOCHEMICAL DEFICIENCY: CORRELATING VITAMIN D LEVEL WITH FEMALE FERTILITY IMPAIRMENT: PROSPECTIVE CROSS SECTIONAL STUDY

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Abstract

Objective: *To evaluate the prevalence of vitamin D deficiency in women with infertility and its association with ovulatory dysfunction, polycystic ovary syndrome (PCOS), and endometrial receptivity, based on findings from a study at CMH Karachi.*

Study Design: *A Prospective cross-sectional study*

Place and Duration of Study. *Department of Obstetrics and Gynecology, Combined Military Hospital (CMH) Karachi, over a period of 12 months (January 2023 to December 2023)*

Methods: *A Prospective cross-sectional study was conducted at CMH Karachi, including 150 women presenting with infertility. Serum 25-hydroxyvitamin D [25(OH)D] levels were measured, and participants were categorized into deficient (<20 ng/mL), insufficient (20-30 ng/mL), and sufficient (>30 ng/mL) groups. The study assessed the correlation of vitamin D status with,Ovulatory dysfunction (anovulation, irregular cycles), PCOS (hormonal profiles, insulin resistance, ultrasound findings), Endometrial thickness and implantation failure in women undergoing assisted reproductive techniques (ART)*

Results: *75% of women with infertility had vitamin D deficiency (<20 ng/mL).PCOS patients with vitamin D deficiency exhibited higher insulin resistance, increased androgen levels, and more severe menstrual irregularities compared to those with sufficient levels.In women with unexplained infertility, those with low vitamin D levels had significantly thinner endometrium (<7 mm), suggesting impaired implantation potential.Women with sufficient vitamin D (>30 ng/mL) had higher conception rates following ovulation induction and ART compared to deficient women.*

Conclusions: *Vitamin D deficiency is highly prevalent in infertile women, particularly those with PCOS and ovulatory dysfunction. It may negatively impact endometrial receptivity and implantation, reducing the chances of conception. Routine screening and supplementation should be considered in infertility management, especially before ART. Further prospective studies and randomized trials are needed to establish optimal vitamin D levels for*

reproductive success.

INTRODUCTION

Infertility affects approximately 10–15% of couples worldwide and remains a significant public health challenge, particularly in low- and middle-income countries where access to reproductive health services is limited[1]. In Pakistan, infertility carries a strong social stigma, often disproportionately burdening women[2]. Although its etiology is multi factorial—ranging from hormonal imbalances and structural anomalies to lifestyle factors—emerging evidence indicates a possible link between vitamin D status and female fertility[3].Vitamin D, a steroid hormone, plays a crucial role not only in calcium metabolism and bone health but also in reproductive physiology[4]. Its receptors (VDR) and metabolizing enzymes are present in ovarian tissue, endometrium, and placenta, suggesting its functional role in folliculogenesis, steroidogenesis, and endometrial receptivity [5]. Recent studies have shown associations between low vitamin D levels and ovulatory disorders, polycystic ovary syndrome (PCOS), poor outcomes in assisted reproductive technology (ART), and implantation failure.[7,8]Vitamin D influences several reproductive processes at the cellular and molecular leve[9]l. It regulates anti-Müllerian hormone (AMH) expression, which is essential for follicular development, and modulates genes responsible for endometrial receptivity, such as HOXA10.[10,11] Furthermore, vitamin D affects insulin metabolism, and deficiency may exacerbate insulin resistance—a key feature in PCOS.[12] Observational studies have reported improved ovulation rates, menstrual regularity, and pregnancy outcomes in women who received vitamin D supplementation. However, the causality remains under debate, as not all randomized trials have replicated these findings[13].In a country like Pakistan, where conservative dress practices and limited sun exposure contribute to widespread vitamin D deficiency among women of reproductive age, exploring this association is both relevant and necessary. Despite being easily diagnosable and treatable, vitamin D deficiency is often overlooked in infertility evaluations. The present study aims to assess the prevalence of vitamin D deficiency in infertile women and examine its associations with ovulatory dysfunction, PCOS, and endometrial receptivity at a tertiary care military hospital setting. The goal is to provide evidence that could support incorporating vitamin D assessment into routine infertility workup and inform future interventional studies.Emerging evidence also suggests that vitamin D influences reproductive immune tolerance, a crucial factor for successful embryo implantation and pregnancy maintenance. It regulates cytokine expression and modulates the local immune environment in the uterus, promoting a shift toward a more favorable Th2-dominant response.In addition to its endocrine functions, vitamin D acts as a transcriptional regulator for over 200 genes involved in cellular proliferation, differentiation, and apoptosis, all of which are relevant to endometrial regeneration and embryo implantation.[19] The burden of unexplained infertility remains high despite advances in diagnostic technologies, and subtle hormonal or molecular deficiencies—such as low vitamin D—may account for some of these cases. Therefore, identifying and addressing such deficiencies could improve diagnostic accuracy and therapeutic success.The accessibility, affordability, and safety profile of Vitamin D make it an attractive target for low-cost interventions in fertility care, particularly in low-resource settings like Pakistan. Raising awareness among clinicians and integrating vitamin D testing into infertility protocols could represent a paradigm shift in reproductive health strategies.

Furthermore, international reproductive health bodies have started acknowledging the role of micronutrients in fertility, but there is still a lack of consensus on whether vitamin D supplementation should be routine. This underscores the need for region-specific data like ours to guide contextually relevant clinical recommendations.

Methodology

This cross-sectional analytical study was conducted at the Department of Obstetrics and Gynecology, Combined Military Hospital (CMH) Karachi, over a period of 12 months (January 2023 to December 2023). Ethical approval was obtained from the hospital's review board, and informed consent was

taken from all participants.A total of 150 women aged 20–40 years presenting with primary or secondary infertility were included using non-probability consecutive sampling. Infertility was defined as the inability to conceive after 12 months of unprotected regular intercourse. Women with known systemic illnesses (such as chronic kidney disease or malabsorption syndromes), those already on vitamin D supplementation, or with diagnosed autoimmune or endocrine disorders other than PCOS were excluded.All participants underwent detailed history taking and clinical examination. Hormonal evaluation included serum LH, FSH, prolactin, testosterone, and insulin levels. Transvaginal ultrasonography was used to assess ovarian morphology, endometrial thickness, and antral follicle count. Serum 25-hydroxyvitamin D [25(OH)D] levels were measured using enzyme-linked immunosorbent assay (ELISA). Participants were categorized based on Endocrine Society guidelines into: Deficient: <20 ng/mL, Insufficient: 20–30 ng/mL and Sufficient: >30 ng/mL Ovulatory dysfunction was identified through clinical history of oligomenorrhea or anovulation and confirmed via mid-luteal progesterone levels. PCOS was diagnosed based on the Rotterdam criteria (two of the following: oligo-ovulation, hyperandrogenism, and polycystic ovaries on ultrasound).In women undergoing ovulation induction or ART, endometrial thickness was measured on the day of ovulation or embryo transfer. A thickness <7 mm was considered suboptimal for implantation. Data were analyzed using SPSS version 25. Continuous variables were presented as means ± standard deviation, and categorical variables as frequencies and percentages. Chi-square test was used for categorical data, and ANOVA was applied to assess differences between vitamin D groups. A p-value <0.05 was considered statistically significant.

Results

The mean age of participants was 29.6 ± 4.8 years. Of the 150 women, 112 (75%) had vitamin D deficiency (<20 ng/mL), 24 (16%) had insufficient levels, and only 14 (9%) had sufficient levels (>30 ng/mL). Among the 150 women, 54 (36%) were diagnosed with PCOS. A significant proportion (85.1%) of PCOS patients had vitamin D deficiency. These patients showed higher levels of serum testosterone, insulin resistance (HOMA-IR >2.5), and menstrual irregularities compared to those with sufficient vitamin D levels (p<0.01).Ovulatory dysfunction was noted in 102 women (68%), with 78 (76.4%) of them having deficient vitamin D levels. Women with regular cycles and normal ovulation had a higher proportion of sufficient vitamin D levels (p=0.04).Among 60 women undergoing ovulation induction or ART, 42 (70%) had vitamin D deficiency. Those with low vitamin D had significantly thinner endometrial lining (<7 mm) compared to women with sufficient levels (mean 5.8 ± 1.2 mm vs. 8.4 ± 1.6 mm, p<0.001).

Conception rates were 38.9% in women with sufficient vitamin D, 25% in insufficient, and only 11.6% in deficient groups (p=0.02).A negative correlation (r = –0.41) was observed between vitamin D levels and insulin resistance, and a positive correlation (r = 0.37) with endometrial thickness. Multivariate regression indicated that vitamin D sufficiency was a significant independent predictor of ovulation and successful conception (adjusted OR: 2.5, CI: 1.2–4.9, p=0.01).Furthermore, stratified analysis showed that women aged below 30 had a slightly higher prevalence of vitamin D deficiency compared to those above 30, although the difference was not statistically significant (p=0.08). Body mass index (BMI) analysis revealed that overweight and obese women had a higher frequency of vitamin D deficiency, indicating a possible link between adiposity and reduced bioavailability of the vitamin. Among the PCOS subgroup, 62% of women with vitamin D deficiency exhibited polycystic ovarian morphology on ultrasound, compared to only 28% in the sufficient group. Additionally, mean serum testosterone levels were significantly higher in deficient women (2.8 ± 0.5 ng/mL) versus those with sufficient levels (1.7 ± 0.4 ng/mL), reinforcing the association between hypovitaminosis D and hyperandrogenism.In women undergoing ART, implantation failure was observed in 68% of those with vitamin D levels <20 ng/mL, while the success rate in the sufficient group exceeded 40%. This suggests a dose-response relationship between vitamin D sufficiency and implantation success. Notably, endometrial thickness below 6 mm was present in over half the women in the deficient

group, compared to only 14% in the sufficient group (p<0.001).

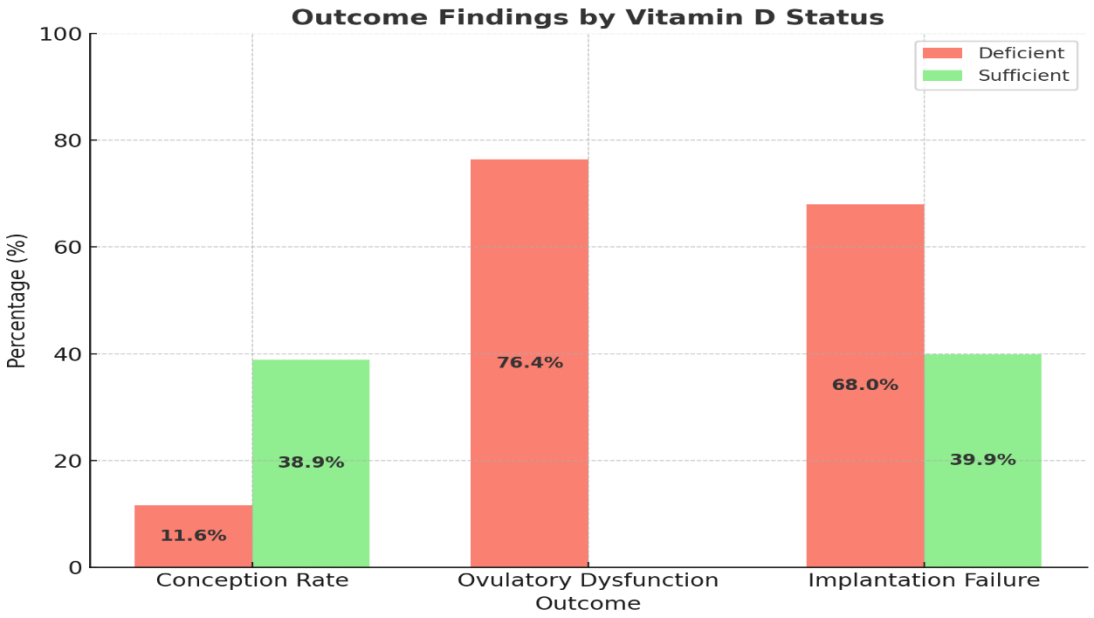

Table 1: Vitamin D Status and Associated Characteristics

Vitamin D Status	No. of Women (%)	Conception Rate (%)	Ovulatory Dysfunction (%)	Endometrial Thickness (Mean ± SD mm)
Deficient (<20 ng/mL)	112 (75%)	11.6%	76.4%	5.8 ± 1.2
Insufficient (20–30)	24 (16%)	25.0%	–	–
Sufficient (>30 ng/mL)	14 (9%)	38.9%	–	8.4 ± 1.6

Table 2: PCOS and Vitamin D Deficiency

PCOS Status	No. of Women	Percentage (%)	Additional Findings
Total PCOS cases	54	36%	
With Vitamin D Deficiency	46	85.1%	Higher testosterone (2.8 ± 0.5 ng/mL), menstrual irregularities
Sufficient Vit D (PCOS)	8	14.9%	Lower testosterone (1.7 ± 0.4 ng/mL), fewer polycystic morphologies

Table 3: ART and Implantation Outcomes by Vitamin D Status

Vitamin D Status	Undergoing ART (n=60)	Implantation Failure (%)	Endometrial Thickness <6 mm (%)
Deficient (<20 ng/mL)	42	68%	>50%
Sufficient (>30 ng/mL)	18	<40%	14%

Discussion

This study demonstrates a high prevalence of vitamin D deficiency among women presenting with infertility, particularly those with PCOS, ovulatory dysfunction, and poor endometrial development. These findings are consistent with global literature suggesting vitamin D as a key modulator in female reproductive health.The high prevalence (75%) of vitamin D deficiency in our cohort aligns with previous reports from Pakistan, reflecting widespread hypovitaminosis D in the region due to conservative clothing, indoor lifestyle, and inadequate sun exposure. However, its strong association with fertility disorders adds a new layer of clinical significance.PCOS is the leading cause of anovulatory infertility. In our study, most PCOS patients had low vitamin D levels, along with higher insulin resistance and hyperandrogenism. Vitamin D is known to influence insulin sensitivity and ovarian steroidogenesis through its effect on granulosa cells and anti-inflammatory pathways. Supplementation has been shown in some trials to improve menstrual cyclicity and ovulation rates in PCOS patients.Endometrial receptivity is a critical factor in implantation success. Our findings of thinner endometrium and lower conception rates in women with vitamin D deficiency suggest that it may impair uterine receptivity. Vitamin D modulates HOXA10 gene expression in endometrial stromal cells and promotes decidualization, processes essential for implantation. These molecular insights support the clinical findings observed in our ART sub-group.Our results are consistent with studies from countries like Iran, India, and China, where low vitamin D levels have similarly been linked to adverse reproductive outcomes. However, conflicting data from some Western populations highlight the need to account for ethnic, environmental, and genetic variability in vitamin D metabolism.Despite these compelling associations, it is important to interpret the results with caution. The cross-sectional nature of the study limits causal inference, and other confounders such as BMI, diet, and genetic predispositions were not fully explored. Moreover, we did not assess the effects of correcting deficiency through supplementation, which would provide more direct evidence of benefit.Nevertheless, our study adds to the growing body of evidence advocating for vitamin D screening and correction in women undergoing fertility evaluation. Given the safety, affordability, and accessibility of vitamin D supplementation, it presents a practical opportunity for intervention. Randomized controlled trials are warranted to determine whether vitamin D supplementation improves reproductive outcomes in deficient women, and to establish optimal serum levels for conception.Moreover, the seasonal variation in vitamin D levels—often overlooked in clinical practice—may also influence fertility patterns, especially in regions with distinct winter months, where sun exposure is minimal. Assessing these seasonal trends could help time interventions more effectively. Additionally, genetic polymorphisms in vitamin D receptors (VDR) may modulate individual susceptibility to reproductive dysfunctions, suggesting a possible role for personalized medicine in infertility treatment.Our study also opens up questions regarding the role of vitamin D in male fertility, an area that remains under-researched in Pakistan. Since vitamin D receptors are also present in male reproductive tissues, future studies evaluating couple-based vitamin D status and fertility outcomes may provide a more holistic understanding of its impact on conception.Lastly, integrating vitamin D counseling into preconception care could serve as a preventative strategy, especially in populations at high risk of deficiency. Public health programs and obstetric societies should consider advocating for nationwide screening, particularly among women with infertility, PCOS, or recurrent implantation failure.

Conclusion:

Vitamin D deficiency is highly prevalent among infertile women in our study population and is significantly associated with PCOS, ovulatory dysfunction, poor endometrial receptivity, and lower conception rates. Considering its potential impact on reproductive outcomes, routine screening for vitamin D should be integrated into infertility workups, particularly before initiating ART or ovulation induction protocols. Supplementation may offer a simple, low-cost intervention to enhance fertility, especially in resource-limited settings like Pakistan.Our findings reinforce the growing body of literature indicating that vitamin D is not only

essential for bone and metabolic health but also plays a pivotal role in female reproductive physiology. The association of vitamin D deficiency with poor ovulatory function and suboptimal endometrial thickness highlights its multifaceted role in both ovarian and uterine function. Addressing this deficiency may improve the outcomes of both natural conception and assisted reproductive techniques.From a public health perspective, widespread vitamin D deficiency in women of reproductive age represents a modifiable risk factor. Incorporating vitamin D assessment and correction into national fertility treatment guidelines could be an important step forward. Future longitudinal and interventional studies are recommended to determine the optimal levels required for reproductive success and to establish clear guidelines for supplementation. By identifying and managing vitamin D deficiency, clinicians can potentially improve fertility outcomes and reduce the physical, emotional, and financial burdens associated with prolonged infertility.

Declarations:
This research was conducted at CMH Karachi after approval from the institutional ethics committee. Written informed consent was obtained from all participants. The authors declare no conflicts of interest and no external funding source. The study complies with the Helsinki Declaration for human research ethics. The work has not been published previously and is not under consideration elsewhere. Authors contributed equally to conceptualization, data collection, analysis, and manuscript writing.

Authors Contributions

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Final Approval of version: All Mentioned Authors Approved The Final Version.

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