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ASSOCIATION BETWEEN SERUM VITAMIN D LEVELS AND OVULATORY FUNCTION IN WOMEN OF REPRODUCTIVE AGE

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ABSTRACT

Background: Vitamin D deficiency is a global health concern and has been increasingly recognized as a modifiable risk factor for reproductive disorders. This study investigates the association between serum Vitamin D (25(OH)D) levels and ovulatory function, as measured by mid-luteal progesterone, in women of reproductive age.

Methods: A cross-sectional study was conducted with 40 women (aged 18–40 years) over a 12-month period (January 2025 – December 2025). Anthropometric data (BMI) and biochemical markers, including serum 25(OH)D and mid-luteal progesterone, were collected. Participants were categorized into Ovulatory (n=24) and Anovulatory (n=16) groups based on a progesterone threshold of 3.0 ng/ml. Statistical analyses included independent samples t-tests and Pearson correlation.

Results: The mean Vitamin D level for the cohort was 20.29 ±8.96 ng/ml, with 57.5% of participants being deficient (<20 ng/ml). Women in the ovulatory group had significantly higher Vitamin D levels compared to the anovulatory group (23.69 ±8.86 ng/ml vs. 15.18 ±6.46 ng/ml; $p = 0.0021$). A moderate positive correlation was observed between Vitamin D levels and mid-luteal progesterone ($r = 0.446$). Within the anovulatory group, 81.25% of women were Vitamin D deficient. In the subgroup of women with Polycystic Ovary Syndrome (PCOS, n=6), all were anovulatory and 83.3% were deficient. No significant correlation was found between BMI and Vitamin D levels in this cohort ($r = 0.018$).

Conclusion: Our findings suggest that Vitamin D deficiency is strongly associated with ovulatory dysfunction. The significant disparity in Vitamin D levels between ovulatory and anovulatory women underscores its role as a critical modifiable factor in female reproductive health. Screening and correction of Vitamin D deficiency may serve as a valuable intervention for women experiencing ovulatory disorders.

KEYWORDS: Vitamin D, Ovulation, Progesterone, Infertility, Reproductive Health, PCOS.

INTRODUCTION

Vitamin D deficiency affects over a billion people worldwide and has emerged as a modifiable risk factor for reproductive disorders, including ovulatory dysfunction in women of reproductive age [1-2]. Traditionally known for its role in calcium homeostasis and bone health, Vitamin D-a steroid hormone precursor is now recognized for its significant pleiotropic effects, particularly within the female reproductive system [3]. The Vitamin D receptor (VDR) and the enzymes responsible for its metabolism are expressed in key reproductive tissues, including the ovaries, endometrium, and pituitary gland, suggesting a direct role in follicular development and steroidogenesis [4-5].

Ovulatory dysfunction remains one of the leading causes of female infertility, often characterized by irregular menstrual cycles and subtherapeutic levels of mid-luteal progesterone [6]. While many factors contribute to ovulatory failure, including Body Mass Index (BMI) and conditions such as Polycystic Ovary Syndrome (PCOS), the specific contribution of Vitamin D status is of increasing clinical interest [7]. Emerging evidence suggests that Vitamin D may influence the expression of genes involved in estrogen synthesis and the signaling of anti-Müllerian hormone (AMH), potentially serving as a regulator of ovarian reserve and ovulation [8-9].

Despite these biological links, Vitamin D deficiency remains under-diagnosed in reproductive-aged women. Given its status as a "modifiable" risk factor, understanding the threshold at which Vitamin D levels impact hormonal markers like progesterone is crucial for developing targeted nutritional interventions [10].

This study aims to evaluate the association between serum Vitamin D levels and ovulatory status in a cohort of 40 women. By analyzing the correlation between 25-hydroxyvitamin D [25(OH)D] concentrations and mid-luteal progesterone, as well as accounting for confounding factors such as age and BMI, this research seeks to clarify the role of Vitamin D sufficiency in maintaining healthy ovulatory cycles.

MATERIALS AND METHODS

Study Design and Participants

This cross-sectional study involved a cohort of 40 women (N=40) of reproductive age, ranging from 18 to 40 years over a 12-month period (January 2025 – December 2025) at Obstetrics & Gynaecology Department at Medinirai Medical College and Hospital (MMCH) in Palamu, Jharkhand. Participants were recruited to evaluate the relationship between serum Vitamin D levels and ovulatory function. Women with a known diagnosis of Polycystic Ovary Syndrome (PCOS) were identified within the cohort (n=6) to assess their specific Vitamin D and ovulatory profiles.

Clinical and Anthropometric Measurements

Height and weight were measured for all participants to calculate Body Mass Index (BMI), defined as weight in kilograms divided by the square of height in meters (kg/m^2). The cohort represented a diverse range of body compositions, with BMIs ranging from 16.3 to 34.4 kg/m^2.

Biochemical Analysis

Venous blood samples were collected from all participants for biochemical assessment:

1. **Serum Vitamin D:** Total 25-hydroxyvitamin D [25(OH)D] levels were measured in ng/ml. Vitamin D status was categorized based on clinical guidelines[11]:
   - **Deficient:** < 20 ng/ml
   - **Insufficient:** 20–30 ng/ml
   - **Sufficient:** > 30 ng/ml
2. **Mid-luteal Progesterone:** Serum progesterone levels were measured during the mid-luteal phase (approximately day 21 of a 28-day cycle). Progesterone concentrations were used as the primary biochemical marker to determine ovulatory status.
3. **Definitions of Ovulatory Status**

Participants were categorized into two groups based on their mid-luteal progesterone levels [12]:

- **Ovulatory:** Defined by serum progesterone levels ≥ 3.0 ng/ml, indicative of successful ovulation and corpus luteum formation.
- **Anovulatory:** Defined by serum progesterone levels < 3.0 ng/ml.

Statistical Analysis

Data were analyzed using **SPSS Statistics (Version 28)**. Descriptive statistics, including means, standard deviations, and frequencies, were generated for all variables. An **independent samples t-test** compared Vitamin D levels between groups, while **Pearson's r** was used for correlation. **Chi-square tests** (or cross-tabulations) assessed the distribution of Vitamin D deficiency. Significance was set at $p < 0.05$.

RESULTS

Participant Characteristics and Vitamin D Status

A total of 40 women of reproductive age were included in the study. The mean age of the cohort was 30.1 ± 7.0 years, and the mean Body Mass Index (BMI) was 24.78 ± 4.04 kg/m^2. The mean serum Vitamin D (25(OH)D) level for the entire group was 20.29 ± 8.96 ng/ml.

Vitamin D deficiency was highly prevalent among the participants. Specifically, 57.5% (n=23) were classified as deficient (<20 ng/ml), 25% (n=10) were insufficient (20-30 ng/ml), and only 17.5 (n=7) reached sufficient levels (>30 ng/ml).

Association between Vitamin D and Ovulatory Status

Participants were categorized into two groups based on their mid-luteal progesterone levels: Ovulatory (n=24) and Anovulatory (n=16).

Women in the ovulatory group exhibited significantly higher Vitamin D levels (23.69 ± 8.86 ng/ml) compared to those in the anovulatory group (15.18 ±6.46 ng/ml). An independent samples t-test confirmed that this difference was statistically significant ($t(38) = 3.296$, $p = 0.0021$), suggesting a strong link between Vitamin D status and ovulatory function.

The prevalence of Vitamin D deficiency was markedly higher in the anovulatory group. Among anovulatory women, 81.25% (13/16) were Vitamin D deficient, whereas only 41.67 (10/24) of the ovulatory group were deficient. Furthermore, 85.7(6/7) of the women with sufficient Vitamin D levels were found to be ovulatory.

Correlation with Mid-Luteal Progesterone

Mid-luteal progesterone levels, a marker of ovulatory quality, showed a moderate positive correlation with serum Vitamin D levels ($r = 0.446$, $p < 0.01$). This indicates that higher Vitamin D concentrations are associated with robust progesterone production during the luteal phase.

In contrast, BMI showed a weak negative correlation with mid-luteal progesterone ($r = -0.275$), while no significant correlation was observed between BMI and Vitamin D levels ($r = 0.018$) in this specific cohort.

Subgroup Observation: PCOS

Within the cohort, 6 participants were diagnosed with Polycystic Ovary Syndrome (PCOS). All 6 women with PCOS were found to be anovulatory, and 83.3% (5/6) of these individuals were Vitamin D deficient, aligning with the broader trend of Vitamin D-linked reproductive dysfunction.

Parameter	Anovulatory (n=16)	Ovulatory (n=24)	p-value
Vitamin D (ng/ml)	15.18 ±6.46	23.69 ± 8.86	0.0021
Mid-luteal Progesterone (ng/ml)	1.73 ± 0.77	7.88 ± 3.40	<0.0001
Age (years)	28.1 ± 7.5	31.4 ± 6.4	0.141
BMI (kg/m^2)	26.12± 3.86	23.88± 3.96	0.083

DISCUSSION

The findings of the present study are consistent with a growing body of literature that links Vitamin D deficiency to ovulatory dysfunction and impaired reproductive outcomes [13-14]**.** Several observational and interventional studies have reported significantly lower serum 25(OH)D levels in anovulatory women compared to those with normal ovulation, supporting our observation of markedly reduced Vitamin D levels in the anovulatory group [15-16]**.**

Similar to our results, **Lerchbaum et al.** reported that women with sufficient Vitamin D levels had higher ovulation and pregnancy rates compared to Vitamin D–deficient counterparts, suggesting a threshold effect of Vitamin D on ovarian function [17]**.** The mean Vitamin D level observed in ovulatory women in our study, although still within the insufficient range, was significantly higher than that of anovulatory women—aligning with reports by **Merhi et al.**, who demonstrated that even modest increases in Vitamin D levels may positively influence follicular development and ovulatory success [18]**.**

The moderate positive correlation observed between serum 25(OH)D and mid-luteal progesterone levels in our cohort ($r = 0.446$) corroborates findings from **Wehr et al.**, who documented a positive association between Vitamin D status and luteal progesterone secretion [19]**.** Experimental studies have shown that Vitamin D, via the Vitamin D receptor (VDR), regulates steroidogenic enzymes in granulosa cells, enhancing progesterone synthesis [20-21]**.** This mechanistic evidence supports our clinical observation that improved Vitamin D status may enhance luteal phase adequacy [22]**.**

In contrast to several studies reporting an inverse association between BMI and Vitamin D levels, such as those by **Wortsman et al.** [23]**,** our study demonstrated a negligible correlation between BMI and Vitamin D status. This discrepancy may be attributable to the relatively small sample size and narrower BMI distribution in our cohort. However, the observed weak negative correlation between BMI and progesterone levels is consistent with prior studies suggesting that excess adiposity adversely affects luteal hormone production independent of Vitamin D status [24-25]**.**

The findings in the PCOS subgroup further reinforce existing evidence. Studies by **Thomson et al.** and **Pal et al.** have shown that Vitamin D deficiency is highly prevalent in women with PCOS and is associated with anovulation, insulin resistance, and follicular arrest [26-27]**.** The high prevalence of Vitamin D deficiency (83%) and universal anovulation among PCOS participants in our study mirrors these observations, suggesting that Vitamin D deficiency may aggravate the endocrine and metabolic disturbances characteristic of PCOS [28]**.**

Overall, the present study supports prior research identifying Vitamin D deficiency as a common and potentially modifiable contributor to ovulatory dysfunction [29-30]**.** While earlier studies have primarily focused on infertility outcomes or PCOS populations, our findings extend this association to a broader group of women of reproductive age, emphasizing the relevance of Vitamin D assessment in routine gynecological and infertility evaluations [31]**.**

LIMITATIONS

This study is limited by its small sample size ($N = 40$), which may reduce the generalizability of the results to larger, more diverse populations**.** Additionally, as a cross-sectional analysis, we can establish an association but not a direct causal relationship. Future longitudinal studies or randomized controlled trials (RCTs) are required to determine if Vitamin D supplementation alone can restore ovulatory function in deficient women.

CONCLUSION

In conclusion, our data supports the article's premise that Vitamin D deficiency is a significant risk factor for reproductive disorders. The clear disparity in Vitamin D levels between ovulatory and anovulatory women, coupled with the positive correlation between Vitamin D and progesterone, underscores the importance of maintaining Vitamin D sufficiency for optimal ovarian function. Screening and correcting Vitamin D deficiency should be considered a fundamental component of preconception care and the management of ovulatory dysfunction.

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