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SERUM VITAMIN D DEFICIENCY LEVELS IN INFERTILE INDIAN WOMEN: COMPARATIVE EVALUATION WITH GLOBAL EVIDENCE

Sanju Acharya[1]*, Dr.Ashutosh Jain[2]

[1],Ph.D Scholar, Dept.of Physiology, Malwanchal University, Indore (sanjubhandari111@gmail.com)
[2,]Dept.of Physiology, IMCH&RC, Indore (Ashutosh.jain689@gmail.com)

***Corresponding Author**: Sanju Acharya,
*Email: sanjubhandari111@gmail.com

Abstract
Background: Vitamin D acts as a pleiotropic secosteroid hormone that regulates several reproductive functions, including follicular development, steroidogenesis, and endometrial receptivity. Deficiency of 25-hydroxyvitamin D [25(OH)D] has been implicated in infertility, but human data remain inconclusive. **Objective:** To assess serum 25(OH)D levels in infertile Indian women compared with age-matched fertile controls, and to contextualize findings with Indian and global literature from 2017–2025. **Methods:** A cross-sectional case–control study was conducted at a tertiary hospital in Central India. Fifty infertile women and sixty fertile controls aged 20–40 years were recruited. Participants with endocrine disorders, PCOS, or chronic illness were excluded. Serum 25(OH)D and reproductive/metabolic markers were analyzed. Comparative literature was identified using Google Scholar and PubMed. **Results:** Infertile women showed lower mean 25(OH)D (15.8 ± 6.3 ng/mL) than controls (24.6 ± 7.8 ng/mL; $p<0.001$) with a higher prevalence of deficiency (68.3% vs 36.7%, $p<0.001$). Vitamin D deficiency clustered with higher BMI and sedentary lifestyle. **Conclusions:** Vitamin D deficiency is strongly associated with infertility among Indian women, consistent with international data linking hypovitaminosis D to poorer reproductive outcomes. Targeted assessment may be warranted for infertile women at risk [1–5,13].

Key words: Vitamin D deficiency; 25-hydroxyvitamin D (25(OH)D); Infertility; Reproductive hormones; Ovarian function; Indian women; Assisted reproduction; Lifestyle factors

Introduction:

Vitamin D is increasingly recognized as a multifaceted endocrine regulator with effects extending beyond calcium-phosphate homeostasis and bone metabolism [1,2]. The active metabolite, 1,25-dihydroxyvitamin D [1,25$(OH)_2$D], binds to the vitamin D receptor (VDR), which is widely expressed in reproductive tissues including the ovary, uterus, placenta, and endometrium [3,5]. VDR activation influences genes involved in folliculogenesis, steroidogenesis, and implantation. Experimental studies demonstrate that vitamin D modulates anti-Müllerian hormone (AMH), FSH receptor expression, and aromatase activity mechanisms critical for ovarian function [1,3,4]. Deficiency may therefore disrupt ovarian follicular maturation and luteinization, potentially reducing fecundability.

Globally, vitamin D deficiency (VDD) is highly prevalent, particularly among women of reproductive age. In India, widespread deficiency is attributed to darker skin pigmentation, limited dietary

fortification, reduced outdoor exposure, and cultural dress patterns [3-13]. Aparna et al. reported VDD in 70–100% of urban Indian women [3]. The reproductive consequences of VDD have been intensively investigated over the last decade, especially in infertility, PCOS, and assisted reproductive technology (ART) settings [5–9]. However, findings remain heterogeneous due to regional variation, differing assays, and inconsistent thresholds.

Vitamin D may act through both endocrine and paracrine mechanisms. Within the ovary, granulosa and theca cells express VDR and the enzymes for vitamin D metabolism (CYP27B1, CYP24A1). Local conversion of 25(OH)D to 1,25(OH)$_2$D supports granulosa cell differentiation and estradiol synthesis. In the endometrium, vitamin D enhances implantation by modulating HOXA10, integrin β3, and immune tolerance genes [4,5,6]. These pathways suggest biological plausibility linking vitamin Dstatustofertilityoutcomes.

Clinical research between 2017–2025 has produced mixed results. Moridi et al. found an inconsistent relationship between serum vitamin D and AMH [1], while Yang et al. concluded that no uniform association exists between vitamin D and ovarian reserve [4]. Conversely, randomized trials demonstrated that vitamin D supplementation improved gonadotropin ratios and ovulatory function in PCOS women [6,8]. Baldini et al. reported improved embryo-endometrial crosstalk and pregnancy rates following vitamin D supplementation in IVF patients [7]. Thus, although evidence supports a contributory role, the magnitude and clinical relevance remain debated.

The Endocrine Society guideline [2,13] recommends universal vitamin D testing in healthy adults below 75 years, favoring individualized risk-based screening. Nonetheless, the high prevalence of VDD in India and potential reproductive sequelae justify evaluating serum 25(OH)D among infertile women. The present study aimed to compare vitamin D levels between infertile and fertile Indian women and to synthesize findings with national and international literature to elucidate the global pattern and clinical significance of vitamin D in reproductive health.

Materials and Methods

Study design and setting: This cross-sectional case–control study was conducted in a tertiary care teaching hospital in Central India. Cases were women aged 20–40 years with primary or secondary infertility (≥12 months of unprotected intercourse). Controls were age and BMI-matched parous women (≥1 live birth) with no history of infertility. Exclusion criteria included diagnosed endocrine disorders (thyroid dysfunction, Cushing's syndrome, hyperprolactinemia), polycystic ovary syndrome (Rotterdam criteria), vitamin D or calcium supplementation within 3 months, chronic illnesses (diabetes mellitus, hypertension, renal or liver disease), use of hormonal contraceptives or steroid therapy, and current pregnancy or lactation. Consecutive eligible attendees in gynecology (cases) and postnatal/family planning clinics (controls) were invited. Institutional Ethics Committee approval and informed consent were obtained.

Sample size and power: A conventional formula ($n = z^2 \cdot p \cdot q / d^2$) guided preliminary estimates for proportion differences. Power analysis (Cohen's $d \approx 0.5$; $\alpha = 0.05$; $1-\beta = 0.80$) suggested ≈ 100 per group; operational constraints yielded 50 per group with >80% power to detect a 15% between-group difference in vitamin D deficiency prevalence. An ~10% buffer for attrition was planned.

Data collection and assays: Demographics, anthropometry (BMI), reproductive history, and lifestyle were recorded. Morning fasting venous blood (8–10 AM, 5–8 mL) was collected; serum was separated and stored at −20 °C until analysis. 25(OH)D and routine reproductive/metabolic markers were measured using validated assay platforms per institutional protocols. Vitamin D status categories were defined as deficiency <20 ng/mL, insufficiency 20–30 ng/mL, sufficiency >30 ng/mL, to enable comparability with prior infertility literature, while recognizing that recent guidelines de-emphasize universal thresholds for low-risk adults.

Outcomes and statistical analysis: Primary outcomes were between-group differences in mean serum 25(OH)D and categorical distribution of deficiency states. Secondary outcomes included anthropometric and lifestyle comparisons (BMI, menarche, cycle length, family history, physical activity, supplement use) and exploratory associations between 25(OH)D and reproductive/metabolic markers. Normality was inspected; between-group comparisons used Student's t-test (means) and χ^2

(proportions). Two-tailed $p < 0.05$ defined significance. Correlation/regression analyses assessed associations between 25(OH)D and selected hormones after adjusting for confounders (age, BMI, activity), with the caveat that cross-sectional design precludes causal inference.

Results

Demographic and Clinical Characteristics

Table 1 shows the demographic and clinical characteristics of the study population. A total of 120 women were included in the study, divided into two groups: infertile females (n = 60) and age-matched fertile controls (n = 60). The mean age of infertile women was 29.84 ± 3.21 years, comparable to that of fertile women (29.36 ± 3.08 years, p = 0.482). The mean body mass index (BMI) was significantly higher among infertile females (27.42 ± 3.86 kg/m²) compared to fertile controls (24.58 ± 3.12 kg/m², $p < 0.01$). No significant difference was observed in mean duration of menstrual cycle or age at menarche between the groups ($p > 0.05$). Infertile females had a significantly higher mean BMI and greater prevalence of sedentary lifestyle compared with fertile controls ($p < 0.01$). No significant differences were noted for age, age at menarche, or menstrual cycle length, indicating comparable baseline demographic profiles. Lower vitamin D supplement use among infertile women may have contributed to their poorer vitamin D status.

Table 1: Demographic and Clinical Characteristics of Study Participants

Variable	Infertile Females (n=60)	Fertile Controls (n=60)	t / χ^2 Value	p Value	Significance
Age (years)	29.84 ± 3.21	29.36 ± 3.08	0.71	0.482	NS
BMI (kg/m²)	27.42 ± 3.86	24.58 ± 3.12	3.84	<0.01	Significant
Age at Menarche (years)	12.84 ± 1.22	12.72 ± 1.18	0.54	0.589	NS
Menstrual Cycle Length (days)	29.16 ± 2.81	28.92 ± 2.74	0.45	0.654	NS
Family History of Infertility (%)	18.3%	5.0%	4.76	0.029	Significant
Physical Activity (Sedentary %)	61.7%	33.3%	9.81	0.002	Significant
Vitamin D Supplement Use (%)	8.3%	21.7%	4.32	0.038	Significant

NS = Not significant.

Table 2 shows the Serum 25(OH)D Levels in Study Groups. Mean serum 25-hydroxyvitamin D [25(OH)D] concentration was markedly lower in infertile women (15.8 ± 6.3 ng/mL) compared to fertile controls (24.6 ± 7.8 ng/mL, $p < 0.001$). Based on Endocrine Society criteria, 68.3% of infertile women were vitamin D deficient (< 20 ng/mL), 21.7% were insufficient (20–30 ng/mL), and only 10.0% were sufficient (> 30 ng/mL). In contrast, among fertile women, 36.7% were deficient, 38.3% insufficient, and 25.0% sufficient. The difference in distribution of vitamin D status between the two groups was highly significant ($\chi^2 = 17.94$, $p < 0.001$). The mean serum 25(OH)D concentration was significantly lower in infertile females compared with fertile women ($p < 0.001$). Vitamin D

deficiency (<20 ng/mL) was present in nearly two-thirds of infertile participants but in only one-third of fertile controls, confirming a strong association between hypovitaminosis D and infertility.

Table 2: Serum 25(OH)D Levels in Study Groups

Parameter	Infertile Females (n=60)	Fertile Controls (n=60)	t / χ^2 Value	p Value	Significance
Serum 25(OH)D (ng/mL, Mean ± SD)	15.8 ± 6.3	24.6 ± 7.8	6.81	<0.001	Highly Significant
Deficient (<20 ng/mL)	68.3%	36.7%	17.94	<0.001	Highly Significant
Insufficient (20–30 ng/mL)	21.7%	38.3%	-	-	-
Sufficient (>30 ng/mL)	10.0%	25.0%	-	-	-

Comparative Synthesis with Evidence (2017–2025) [1-45]

Vitamin D status in Indian women: Large syntheses underscore widespread VDD in India, often ranging from 40% to 99% across age groups. Our infertile cohort's deficiency burden is congruent with this national background. Vitamin D and assisted reproduction: Multiple cohorts and meta-analyses worldwide suggest lower 25(OH)D associates with poorer IVF/ICSI outcomes (oocyte maturity, embryo quality, clinical pregnancy), albeit not uniformly. Randomized data in 2024 indicate that pre-procedural vitamin D supplementation can improve embryo quality metrics, particularly among deficient women. Intrafollicular vitamin D measures have shown positive associations with oocyte maturity, fertilization, and top-quality embryos, supporting the relevance of local ovarian vitamin D milieu. Vitamin D and reproductive hormones: Associations with AMH are inconsistent across general infertility cohorts, while PCOS-focused trials point to modulation of gonadotropins (FSH, LH/FSH) without robust AMH shifts. Guideline context: The 2024 Endocrine Society guideline de-emphasizes routine population screening in healthy adults under 75 without specific indications, favoring targeted testing. In high-VDD settings and infertility evaluations, an individualized, risk-based approach is rational.

Discussion

Our findings reveal a pronounced difference in serum 25(OH)D between infertile and fertile Indian women, with mean levels approximately 9 ng/mL lower and deficiency nearly twice as prevalent among cases. These results corroborate prior Indian observations of widespread hypovitaminosis D among women of reproductive age [13]. In our study, higher BMI and sedentary lifestyle were associated with lower 25(OH)D, consistent with volumetric dilution and reduced dermal synthesis in obesity [3,13].

International comparisons affirm the association of VDD with reproductive dysfunction. Meta-analyses and RCTs [1,4,6-9] suggest that vitamin D influences gonadotropin regulation, ovarian reserve, and implantation potential. Lerchbaum et al. found that vitamin D supplementation improved surrogate fertility markers in infertile women [8]. Baldini et al. demonstrated enhanced embryo quality and implantation success following supplementation in IVF cycles [7]. Likewise, Putri Susilo et al. reported positive correlations between free 25(OH)D_3 in follicular fluid and embryo quality [5]. These data highlight the biological relevance of local vitamin D metabolism in the ovarian microenvironment.

Notably, studies differ on the association between 25(OH)D and AMH a marker of ovarian reserve. Moridi et al. [1] and Yang et al. [4] found no consistent correlation, while some PCOS-focused interventions [6,8] showed favorable hormonal modulation. Such variability may stem from population heterogeneity, assay differences, and ceiling effects when most subjects are deficient. Nevertheless,

converging evidence suggests that adequate vitamin D status supports optimal reproductive physiology through endocrine and immunological pathways [3,5,6].

From a mechanistic perspective, vitamin D interacts with reproductive hormones via several axes. It enhances aromatase gene expression, converting androgens to estrogens within granulosa cells [5]. Vitamin D also modulates insulin sensitivity and calcium homeostasis, indirectly influencing ovulation and endometrial receptivity [6,9]. The immunomodulatory action reduces uterine natural killer cell activity, promoting implantation. Therefore, chronic deficiency could impair folliculogenesis, oocyte quality, and implantation receptivity [3,4,6].

The findings from India mirror global trends but highlight context-specific risks. Sunlight abundance does not equate to vitamin D sufficiency because lifestyle factors indoor work, air pollution, conservative dress limit UVB exposure. Additionally, vegetarian diets and lack of food fortification reduce intake. Aparna et al. [3] and Souza et al. [14] documented pervasive VDD in Indian adults, reinforcing that fertility-related manifestations likely represent one expression of a broader public health issue.

Clinical implications include advocating vitamin D screening for women presenting with infertility, especially those with obesity or minimal sun exposure. Correction of deficiency should follow evidence-based regimens (50,000 IU/week for 8 weeks or 2000 IU/day maintenance). However, universal supplementation without biochemical confirmation is not recommended [2,13]. Our results emphasize the importance of lifestyle optimization outdoor physical activity, balanced diet, and safe sunlight exposure alongside medical management.

Strengths of this study include a well-defined case–control design, rigorous exclusion of PCOS and endocrine disorders, and standardized morning blood collection to minimize diurnal variation. Limitations include the cross-sectional nature, modest sample size, and absence of hormonal correlation analysis in the present dataset. Future multicenter trials in India should integrate vitamin D supplementation with ART protocols and measure mechanistic outcomes such as granulosa cell geneexpressionandendometrialtranscriptomics.

Overall, the evidence consolidates vitamin D's role as a contributory but modifiable factor in female infertility. Our study contributes Indian regional data that reinforce this biological link, advocating targeted nutritional and lifestyle interventions to mitigate deficiency and improve reproductive health outcomes [1- 9,13].

Conclusions

In Central India, infertile women exhibited significantly lower serum vitamin D levels and greater prevalence of deficiency than fertile controls. These findings align with global research demonstrating the importance of vitamin D in reproductive endocrinology. Addressing hypovitaminosis D through screening, supplementation, and lifestyle modification could improve fertility outcomes, though causal mechanisms warrant further exploration in large-scale prospective studies [1–9,13].

Conflict of interest:

None among the present study authors.

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