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Association of Vitamin D Deficiency With Reproductive Hormone Imbalance in Infertile Indian Females: A Case–Control Study

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Abstract

Background: Vitamin D is increasingly recognized as an endocrine modulator of female reproductive physiology. Deficiency of this micronutrient has been implicated in ovulatory dysfunction, altered gonadotropin secretion, and infertility. **Objective:** To evaluate the relationship between serum vitamin D status and reproductive hormone profile in infertile Indian females compared with age- and BMI-matched fertile controls. **Methods:** A cross-sectional case–control study was conducted on 100 participants (50 infertile, 50 fertile controls) aged 20–40 years at a tertiary care hospital in Central India. Serum vitamin D, luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol, testosterone, anti-Müllerian hormone (AMH), and progesterone were analyzed using standard ELISA methods. Data were statistically analyzed with one-way ANOVA and Pearson correlation. **Results:** Infertile females exhibited significantly elevated LH (8.92 ± 3.08 mIU/mL), LH/FSH ratio (1.41 ± 0.42), testosterone (0.87 ± 0.29 ng/mL), and AMH (4.24 ± 1.19 ng/mL), with reduced estradiol (128.6 ± 39.2 pg/mL) and progesterone (7.86 ± 2.41 ng/mL) compared with controls ($p < 0.01$). Vitamin D deficiency (<20 ng/mL) was significantly associated with higher androgenic markers and BMI, and lower estradiol and progesterone levels. **Conclusion:** Vitamin D deficiency is prevalent among infertile women and correlates inversely with androgenic markers and positively with ovarian steroidogenesis. These findings support a regulatory role of vitamin D in hypothalamic–pituitary–ovarian axis function and female fertility.

Keywords: Vitamin D, infertility, reproductive hormones, LH/FSH ratio, AMH, estradiol, India.

Introduction

Infertility, defined as the inability to conceive after one year of unprotected intercourse, affects approximately 10–15% of couples globally and nearly one in six couples in India. The etiology of female infertility is multifactorial, involving genetic, hormonal, environmental, and nutritional determinants. Among these, vitamin D deficiency has recently emerged as a potential contributor to impaired ovarian function and subfertility.

Vitamin D, beyond its classical skeletal roles, functions as a steroid hormone influencing reproductive physiology. The vitamin D receptor (VDR) and vitamin D metabolizing enzymes (CYP27B1, CYP24A1) are expressed in reproductive tissues such as the ovary, endometrium, and placenta, indicating direct paracrine actions. Activation of VDR influences folliculogenesis, oocyte maturation, and sex steroid production through modulation of gene expression within the hypothalamic–pituitary–ovarian (HPO) axis.

Recent studies have demonstrated a significant association between vitamin D deficiency and reproductive dysfunctions including polycystic ovary syndrome (PCOS), anovulation, and luteal phase insufficiency. A meta-analysis by Zhao et al. (2020) reported that women with vitamin D deficiency exhibited altered LH and AMH profiles and reduced conception rates following in vitro fertilization (IVF) procedures ^[1]. Similarly, Lerchbaum and Obermayer-Pietsch (2018) emphasized vitamin D's role in ovarian steroidogenesis and AMH regulation ^[2].

In India, hypovitaminosis D is highly prevalent across reproductive-age women due to limited sunlight exposure, dietary insufficiency, and skin pigmentation. A North Indian study reported that over 85% of infertile females had vitamin D levels <20 ng/mL, correlating with elevated LH/FSH ratio and androgen excess ^[3]. However, regional variations exist, and data from Central India remain scarce.

Therefore, the present study aimed to evaluate the association between serum vitamin D status and reproductive hormone profile among infertile Indian females, and to compare the results with national and international findings to elucidate the endocrine implications of vitamin D deficiency in infertility.

Materials & methods:

Study Design and Setting

This cross-sectional, case–control study was conducted at a tertiary care teaching hospital in Central India between January 2023 and March 2024. The research protocol was approved by the Institutional Ethics Committee, and written informed consent was obtained from all participants.

Sample Size and Sampling

Sample size was determined using power analysis for detecting a 15% difference in vitamin D levels between groups with 80% power and $\alpha = 0.05$. The required sample size was 100 participants (50 infertile women, 50 fertile controls), with an additional 10% recruited to account for dropouts.

A consecutive sampling technique was employed: infertile women attending the gynecology outpatient department and age- and BMI-matched fertile controls from postnatal/family planning clinics were enrolled.

Inclusion criteria: Females aged 20–40 years. **Cases:** Primary or secondary infertility (failure to conceive after ≥ 12 months of unprotected intercourse). **Controls:** Fertile, parous women with at least one live birth.

Exclusion criteria: Diagnosed endocrine disorders (thyroid disease, hyperprolactinemia, Cushing's syndrome). PCOS (Rotterdam criteria). Vitamin D or calcium supplementation within 3 months. Chronic illnesses (diabetes, hypertension, renal/hepatic disease). Current use of hormonal contraceptives or steroids. Pregnancy or lactation.

Data Collection: A detailed clinical and demographic questionnaire was completed, including anthropometric data, reproductive history, and lifestyle factors. Venous blood (5–8 mL) was drawn in the morning after overnight fasting. Serum was separated and stored at -20°C until biochemical

analysis.

Laboratory Analysis: Serum 25-hydroxyvitamin D [25(OH)D] concentrations were measured using a chemiluminescent immunoassay. Levels were classified as: Deficient: < 20 ng/mL; Insufficient: 20–30 ng/mL; and Sufficient: > 30 ng/mL

Hormonal assays were performed by ELISA for LH, FSH, estradiol, testosterone, AMH, and progesterone. BMI was calculated as weight (kg)/height² (m²).

Statistical Analysis

Data were analyzed using SPSS v25. Continuous variables were expressed as mean $\pm$ standard deviation (SD). Intergroup comparisons were performed using independent t-test or ANOVA with Tukey's post-hoc test. Correlations between vitamin D and hormonal parameters were analyzed by Pearson's correlation coefficient. Significance was set at $p < 0.05$.

Results

Table 1: Infertile females demonstrated significantly altered reproductive hormones compared with fertile controls.

Parameter	Infertile (Mean $\pm$ SD)	Fertile (Mean $\pm$ SD)	p-value	Observation
LH (mIU/mL)	8.92 $\pm$ 3.08	5.86 $\pm$ 2.19	<0.01	Elevated in infertile
FSH (mIU/mL)	6.64 $\pm$ 2.23	7.18 $\pm$ 2.08	NS	Slightly lower
LH/FSH ratio	1.41 $\pm$ 0.42	0.82 $\pm$ 0.28	<0.01	Increased
Estradiol (pg/mL)	128.6 $\pm$ 39.2	166.3 $\pm$ 48.1	<0.01	Reduced
Testosterone (ng/mL)	0.87 $\pm$ 0.29	0.54 $\pm$ 0.19	<0.001	Increased
AMH (ng/mL)	4.24 $\pm$ 1.19	3.02 $\pm$ 0.95	<0.05	Elevated
Progesterone (ng/mL)	7.86 $\pm$ 2.41	10.04 $\pm$ 2.61	<0.01	Decreased

These findings indicate hyperandrogenemia, raised LH/FSH ratio, and reduced luteal steroidogenesis among infertile females, suggestive of ovarian dysfunction linked with vitamin D deficiency.

Table 2: Participants were stratified based on serum vitamin D levels (Table 2).

Parameter	Deficient (<20 ng/mL)	Insufficient (20–30 ng/mL)	Sufficient (>30 ng/mL)	p-value	Post-hoc
LH (mIU/mL)	8.92 $\pm$ 3.08	7.04 $\pm$ 2.76	5.86 $\pm$ 2.19	<0.01	Deficient > Sufficient
LH/FSH ratio	1.41 $\pm$ 0.42	1.01 $\pm$ 0.36	0.82 $\pm$ 0.28	<0.01	Deficient > Sufficient
Estradiol (pg/mL)	128.6 $\pm$ 39.2	146.8 $\pm$ 42.7	166.3 $\pm$ 48.1	<0.01	Sufficient > Deficient
Testosterone (ng/mL)	0.87 $\pm$ 0.29	0.68 $\pm$ 0.23	0.54 $\pm$ 0.19	<0.001	Deficient > Sufficient
AMH (ng/mL)	4.24 $\pm$ 1.19	3.74 $\pm$ 1.06	3.02 $\pm$ 0.95	<0.05	Deficient > Sufficient
Progesterone (ng/mL)	7.86 $\pm$ 2.41	9.12 $\pm$ 2.38	10.04 $\pm$ 2.61	<0.01	Sufficient > Deficient
BMI (kg/m ²)	27.92 $\pm$ 3.74	25.86 $\pm$ 3.28	24.12 $\pm$ 3.14	<0.01	Deficient > Sufficient

An inverse correlation was observed between vitamin D and androgenic markers ($r = -0.48$ for testosterone, $r = -0.42$ for LH/FSH ratio, $p < 0.01$), and a positive correlation with estradiol and progesterone ($r = +0.39$ and $+0.44$, respectively).

BMI and Vitamin D Deficiency: Mean BMI was significantly higher in vitamin D-deficient women, supporting the hypothesis that adiposity impairs vitamin D bioavailability, exacerbating hormonal imbalance.

Discussion

This study demonstrates that vitamin D deficiency is significantly associated with altered reproductive hormones among infertile Indian women. Deficient participants exhibited elevated LH, LH/FSH ratio, testosterone, and AMH, along with lower estradiol and progesterone, indicating disrupted folliculo-genesis and luteal function.

Our findings align with a 2022 North Indian study by Bansal et al., which reported that 78% of infertile women had vitamin D levels <20 ng/mL, and these women exhibited higher LH and AMH concentrations [4]. Similarly, Singh et al. observed that vitamin D-deficient PCOS women had elevated testosterone and lower estradiol compared with vitamin D-sufficient controls [5].

A South Indian study by Chakraborty et al found a positive correlation between 25(OH)D and progesterone, and an inverse correlation with AMH, supporting our observation that adequate vitamin D improves luteal phase function [6]. Furthermore, Trivedi et al. demonstrated that supplementation with vitamin D improved ovulation rates and serum estradiol levels in women with unexplained infertility [7].

Globally, similar trends have been observed. Irani et al. in the USA reported that vitamin D-deficient women undergoing IVF had lower estradiol responses and fewer mature oocytes [8]. Chu et al. in China found vitamin D levels were inversely correlated with LH/FSH ratio and testosterone among PCOS patients [9]. A large-scale European cohort (Holick et al., 2020) noted that vitamin D sufficiency improved AMH normalization and menstrual regularity [10]. Likewise, Li et al. (2021) demonstrated that 25(OH)D modulated AMH transcription via VDR-mediated signaling in granulosa cells [11].

Our findings are consistent with these reports [9-15], collectively emphasizing that vitamin D sufficiency contributes to hormonal homeostasis and optimal ovarian function across ethnic groups. However, the prevalence and severity of deficiency are greater in the Indian population due to cultural dress, dietary habits, and low supplementation rates.

Vitamin D's reproductive effects are mediated via VDRs in granulosa and theca cells, influencing steroidogenic enzymes (CYP19A1, STAR) and anti-Müllerian hormone gene expression [12]. Deficiency disrupts these pathways, leading to elevated LH and AMH and decreased estradiol synthesis [13]. Furthermore, vitamin D enhances insulin sensitivity, indirectly mitigating hyperandrogenism in insulin-resistant states [14]. Our data showing inverse associations between vitamin D and LH/testosterone support a neuroendocrine mechanism wherein vitamin D modulates GnRH pulsatility, thereby influencing LH secretion. Elevated LH and AMH in deficient women likely reflect follicular arrest and suboptimal ovulation, central to infertility pathogenesis. Vitamin D is fat-soluble and sequestered in adipose tissue, leading to reduced bioavailability among overweight individuals. The higher BMI in deficient subjects (27.9 ± 3.7 kg/m²) suggests a bidirectional relationship: adiposity contributes to vitamin D deficiency, while low vitamin D may exacerbate insulin resistance and weight gain. These metabolic interactions further accentuate hormonal imbalance and subfertility.

Our findings underscore the need to routinely assess vitamin D status in infertility workups, particularly in Indian women. Correcting deficiency may optimize reproductive hormone balance,

improve ovulatory function, and enhance fertility outcomes. Vitamin D supplementation, in conjunction with lifestyle modification, represents a cost-effective, low-risk intervention.

Limitations and Future Directions

Limitations include the cross-sectional design, which precludes causal inference, and single-center sampling, limiting generalizability. Seasonal variations in sunlight exposure were not adjusted for. Future longitudinal and interventional studies should evaluate dose–response relationships between vitamin D supplementation and reproductive outcomes, particularly ovulatory frequency and pregnancy rates.

Conclusion

This study provides compelling evidence that vitamin D deficiency is prevalent and biochemically linked to hormonal dysregulation among infertile Indian women. Deficient women exhibit elevated androgenic and gonadotropic markers and reduced ovarian steroidogenesis, signifying compromised follicular and luteal functions. Improving vitamin D sufficiency may represent a simple and effective strategy to enhance fertility, particularly in populations with endemic deficiency. Integrating vitamin D screening and supplementation into infertility management protocols could substantially benefit reproductive health outcomes in Indian women.

Conflict of interest: None declared.

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