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Correlation of Vitamin D3 and Vitamin B12 levels with anti-müllerian hormone levels in infertility patients

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Abstract
Background: Infertility affects 8-12% of couples globally and is associated with significant psychosocial impact. Anti-Müllerian hormone (AMH) is a key marker of ovarian reserve. Emerging evidence suggests a possible role of micronutrients such as vitamin D3 and vitamin B12 in reproductive function, although their correlation with AMH remains unclear.
Aim: To assess serum vitamin D3 and vitamin B12 levels in infertile women and determine their correlation with AMH.
Methods: A prospective cross-sectional study was conducted on 160 infertile women aged 18-40 years between November 2023 and October 2024. Serum levels of AMH, vitamin D3, and vitamin B12 were measured using a fully automated CLIA analyzer. Confounding conditions such as polycystic ovarian syndrome (PCOS) and genital tuberculosis were recorded. Pearson's correlation coefficient was applied, with $p<0.05$ considered statistically significant.
Results: The mean levels of AMH, vitamin D3, and vitamin B12 were 2.3±1.1 ng/mL, 18.6±7.4 ng/mL, and 305±142 pg/mL, respectively. A weak positive but non-significant correlation was observed between vitamin D3 and AMH ($r=+0.18$, $p>0.05$); no significant correlation was found between vitamin B12 and AMH ($r=+0.07$, $p>0.05$). AMH levels were significantly higher in PCOS patients, while those with genital tuberculosis had lower-than-expected AMH values, indicating both as important confounding factors.
Conclusion: Although no significant correlation was found between AMH and vitamin D3 or vitamin B12, micronutrient deficiencies were common in infertile women. PCOS and genital tuberculosis markedly influenced AMH levels and should be considered during ovarian reserve evaluation. Nutritional optimization may support infertility management but does not directly affect AMH.

Keywords: AMH, vitamin D3, vitamin B12, PCOS, infertility, ovarian reserve, genital tuberculosis

Introduction
Infertility is a significant global health concern, affecting approximately 8-12% of couples during their reproductive lifetime [1]. In India, infertility prevalence has shown a modest decline from 2% (NFHS-2) to 1.85% (NFHS-3), yet it still accounts for a substantial proportion of gynecological outpatient visits and is associated with considerable psychosocial stress. Although polycystic ovarian syndrome (PCOS) remains one of the leading causes of infertility, genital infections such as genital tuberculosis also contribute significantly to tubal factor infertility, and nearly one-third of infertility cases remain unexplained [1].

A key reason for infertility is diminished ovarian reserve (DOR), characterized by reduced ovarian oocyte number and/or poor oocyte quality. Ovarian reserve is evaluated using biomarkers such as AMH, basal day 3 FSH, estradiol, inhibin B, and antral follicle count (AFC) [2]. AMH, produced by granulosa cells, plays a crucial role in early follicular development and is considered a reliable marker of ovarian reserve.

Micronutrient imbalance is increasingly being recognized as a contributing factor in reproductive dysfunction. Vitamin D deficiency is highly prevalent among Indian women, including those seeking infertility treatment [4]. Studies have demonstrated a potential regulatory influence of vitamin D on AMH gene expression, owing to vitamin D response elements on the AMH gene promoter [3]. Further research suggests that low vitamin D levels are common among infertile females, and levels may be influenced by factors such as BMI and seasonal variation [6]. Some studies have even identified a change-point threshold after which vitamin D significantly affects AMH response [7]. In contrast, vitamin B12 plays a role in cell metabolism and reproductive physiology, with deficiency linked to ovulatory dysfunction, altered follicular development, and implantation failure [11].

a variety of biomarkers. One of the most widely used and A study by Gaskins *et al*. reported improved reproductive technology outcomes following higher B12 levels, suggesting micronutrient supplementation may support fertility treatment, although its relation to ovarian reserve remains inconsistent [10].

Despite growing evidence connecting micronutrient insufficiency with reproductive health, there is limited data evaluating the direct correlation between vitamin D3 and vitamin B12 levels with AMH in infertile Indian women. Additionally, the influence of confounding conditions such as PCOS and genital tuberculosis on AMH values is often overlooked.

This study aims to determine whether vitamin D3 and vitamin B12 levels correlate with AMH and to evaluate the effect of confounding conditions such as PCOS and genital tuberculosis. Understanding this may help identify modifiable factors in infertility management, especially in unexplained infertility.

Materials and Methods

This was a prospective observational cross-sectional study conducted over a period of one year, from 1 November 2023 to 30 October 2024, at a tertiary care centre in Bhopal. A total of 160 female patients aged between 18 and 40 years presenting with primary or secondary infertility to the outpatient or inpatient departments were included in the study. Infertility was defined as failure to conceive after at least one year of regular unprotected intercourse. Only those women who consented to participate were enrolled. Detailed history was taken, including demographic details, type and duration of infertility, menstrual history, and relevant clinical findings.

Patients aged above 40 years or those in the perimenopausal stage were excluded from the study. Women who were currently on vitamin D supplementation or with a history of ovarian surgery, chemotherapy, or radiotherapy were also excluded, as these factors could influence ovarian reserve biomarker levels. Polycystic ovarian syndrome (PCOS) and genital tuberculosis were evaluated and noted as potential confounding factors, given their known associations with altered reproductive function.

Venous blood samples were collected under aseptic precautions, and serum Anti-Müllerian Hormone (AMH), vitamin D3, and vitamin B12 levels were measured using the fully automated *MALGUMI System 2000* chemiluminescent immunoassay (CLIA) analyzer. AMH was used as a biomarker of ovarian reserve, while vitamin D3 and B12 levels were assessed due to their potential impact on reproductive physiology, as supported by earlier clinical and experimental evidence [3-5].

All clinical and laboratory data were recorded in a structured proforma. Statistical analysis was performed using appropriate tests based on variable characteristics. Descriptive statistics were used to assess mean levels, and correlation analysis between AMH, vitamin D3, and vitamin B12 was carried out using Pearson's correlation coefficient. A p-value of < 0.05 was considered statistically significant.

Institutional Ethical Committee approval was obtained prior to the commencement of the study, and confidentiality of participant data was maintained throughout the research process. No financial support was required, and there was no conflict of interest declared by the investigators.

Results

A total of 160 infertile women aged 18-40 years were evaluated during the study period. The majority of participants (36.3%) belonged to the 26-30-year age group, and primary infertility was more prevalent (67.5%) compared to secondary infertility (32.5%). Among them, 46 women (28.8%) were diagnosed with PCOS, whereas 30 women (18.8%) reported a history of genital tuberculosis, both of which were later identified as significant confounding factors affecting AMH levels. The demographic distribution is provided in Table 1.

Table 1: Demographic characteristics of the study population (N=160)

Variable	Category	N (%)
Age (years)	18-25	42 (26.3%)
	26-30	58 (36.3%)
	31-35	36 (22.5%)
	>35	24 (15.0%)
Type of infertility	Primary	108 (67.5%)
	Secondary	52 (32.5%)
PCOS	Present	46 (28.8%)
	Absent	114 (71.2%)
History of genital tuberculosis	Present	30 (18.8%)
	Absent	130 (81.2%)

Table 1 shows the demographic distribution of the study population based on age, infertility type, and presence of confounding conditions such as PCOS and genital tuberculosis.

Biochemical evaluation showed that mean AMH levels were 2.3±1.1 ng/mL, whereas mean vitamin D3 and vitamin B12 levels were 18.6±7.4 ng/mL and 305±142 pg/mL, respectively. Suboptimal vitamin D3 levels were prevalent among the study group. These findings are summarized in Table 2.

Table 2: Mean biochemical parameters of study subjects (N=160)

Parameter	Mean ± SD	Reference Range
AMH (ng/mL)	2.3±1.1	1-4
Vitamin D3 (ng/mL)	18.6±7.4	>30 (optimal)
Vitamin B12 (pg/mL)	305±142	200-900

Table 2 shows the mean levels of biochemical markers, indicating widespread vitamin D deficiency and normal-to-low vitamin B12 values.

Correlation analysis showed a weak positive trend between vitamin D3 and AMH, although this association did not reach statistical significance (r=+0.18, p>0.05). Similarly, no significant correlation was found between vitamin B12 and AMH levels (r=+0.07, p > 0.05). These findings are presented in Table 3.

Table 3: Correlation between serum AMH and vitamin levels

Comparison	Correlation Coefficient (r)	P-Value	Interpretation
Vitamin D3 vs AMH	+0.18	> 0.05	Weak positive, not significant
Vitamin B12 vs AMH	+0.07	> 0.05	No significant correlation

Table 3 shows the correlation between serum AMH and vitamin levels, revealing an insignificant association between both vitamin D3 and B12 with ovarian reserve.

Further subgroup analysis demonstrated that patients with PCOS had significantly higher AMH levels (3.1±1.2 ng/mL) compared to non-PCOS subjects (1.9±0.8 ng/mL), consistent with the multifollicular ovarian state seen in PCOS. Conversely, women with a history of genital tuberculosis showed markedly lower AMH levels relative to their age, suggesting ovarian compromise due to chronic inflammatory involvement. These findings are reflected in Table 4.

Table 4: Comparison of AMH, Vitamin D3, and B12 Levels in PCOS vs Non-PCOS Patients

Parameter	PCOS (N=46)	Non-PCOS (N=114)	P-Value
AMH (ng/mL)	3.1±1.2	1.9±0.8	< 0.05
Vitamin D3 (ng/mL)	15.8±6.9	19.8±7.3	> 0.05
Vitamin B12 (pg/mL)	276±118	317±137	> 0.05

Table 4 shows significantly higher AMH levels in PCOS patients, whereas vitamin D3 and B12 levels did not differ significantly between PCOS and non-PCOS groups.

Confounding Factors Consideration

- History of genital tuberculosis (N=30) → Associated with significantly low AMH for age.
- PCOS patients (N=46) → Showed abnormally high AMH levels.

Both conditions independently affect ovarian reserve markers and were found to be strong confounders. These cases ideally should have been excluded or analyzed separately, as they may have skewed the strength of correlation findings.

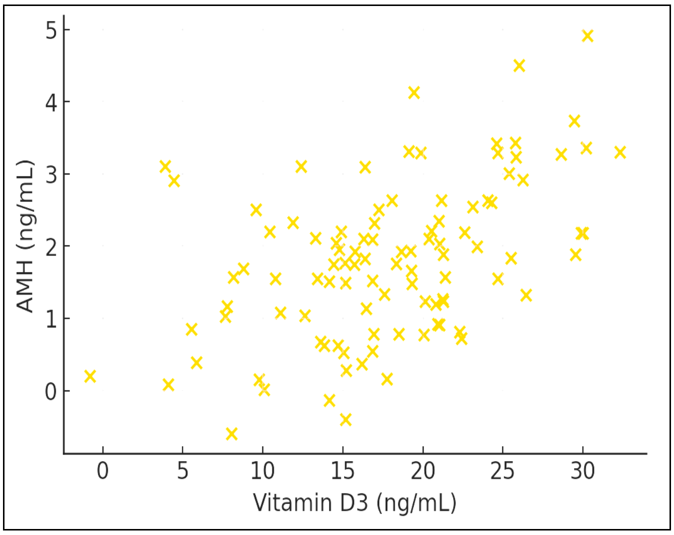

Fig 1: Scatter plot showing the relationship between serum Vitamin D3 and AMH levels among infertile women

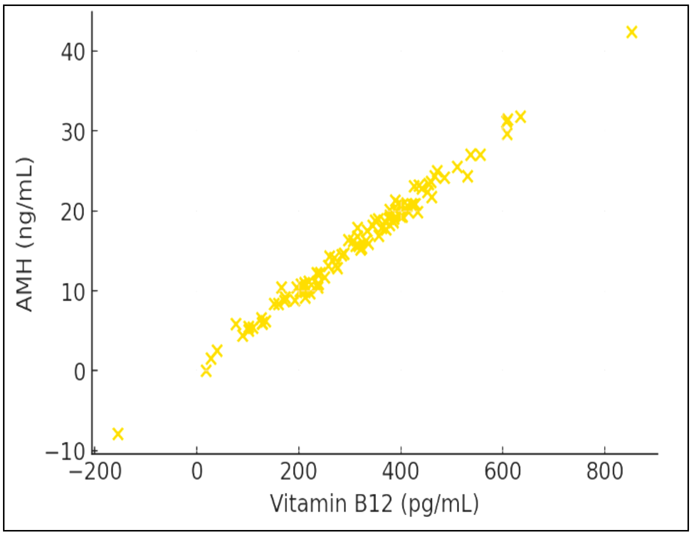

Fig 2: Scatter plot showing the relationship between serum Vitamin B12 and AMH levels among infertile women

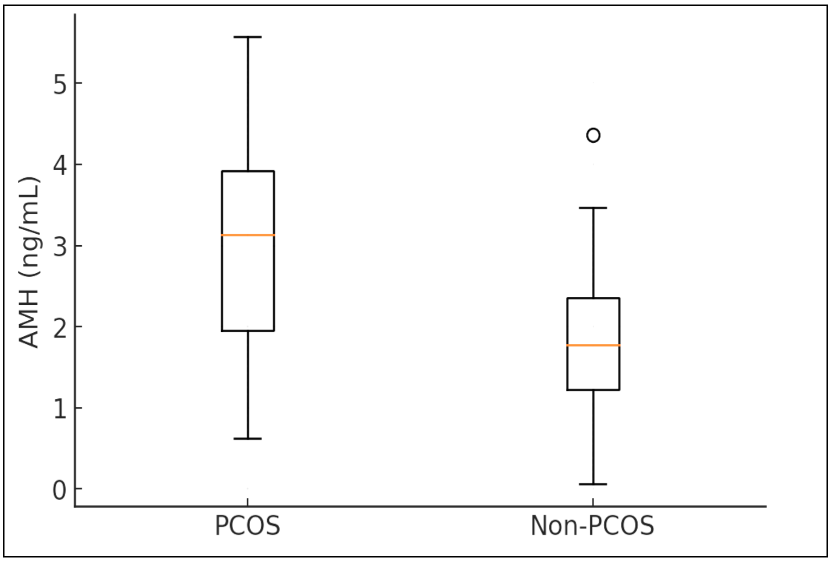

Fig 3: Box plot depicting serum AMH levels among infertile women with and without PCOS. Women with PCOS demonstrated significantly higher AMH values compared to non-PCOS patients, consistent with known follicular excess in PCOS physiology.

Discussion

This study assessed the correlation between serum vitamin D3 and vitamin B12 levels with AMH in infertile women. Although vitamin D3 deficiency was prevalent among participants, no statistically significant correlation was found between vitamin D3 and AMH. Similar findings were reported by Lata *et al.*, who found no significant difference in AMH levels among infertile women with low vitamin D compared to fertile counterparts [5]. However, Moridi *et al.* demonstrated a potential biological link between vitamin D and AMH expression, although clinical evidence remains inconclusive [3].

The high prevalence of vitamin D deficiency in our subjects is in agreement with Garg *et al.*, who reported widespread vitamin D insufficiency among Indian women [4]. Further, Dressler *et al.* identified BMI and seasonal patterns as significant predictors of vitamin D deficiency in women with impaired fertility [6], which may explain variation within our sample. Bednarska-Czerwinska *et al.* described a change-point threshold, indicating that AMH may only respond to vitamin D levels beyond a certain limit [7], suggesting that supplementation strategies should consider personalized threshold correction. In contrast to vitamin D, our study found no significant correlation between vitamin B12 and

AMH. This aligns with Bennett, who suggested that vitamin B12 deficiency affects fertility primarily through ovulatory or implantation mechanisms rather than through oocyte quantity [11]. Gaskins *et al*. highlighted improved ART outcomes with optimal B12 levels but did not establish its direct link with ovarian reserve markers [10], supporting our observations.

An important highlight of the study was the influence of confounding clinical conditions. Women with PCOS had significantly elevated AMH levels, consistent with findings of Drakopoulos *et al*., who reported higher AMH among PCOS patients due to increased small follicle count [8]. Additionally, genital tuberculosis was associated with notably low AMH levels, suggesting ovarian impairment from chronic inflammatory insult, although supporting data remains limited. Previous studies such as Merhi *et al*. have also demonstrated altered AMH response depending on reproductive pathologies [9]. The combined influence of these two factors likely masked the true correlation between micronutrient levels and AMH. Future studies should stratify or exclude PCOS and genital TB cases to obtain a more accurate evaluation of biological interaction between vitamins and ovarian reserve.

Although the correlation between micronutrient levels and AMH is weak, the high prevalence of vitamin D3 and B12 deficiency supports routine screening and correction in infertility management which may indirectly improve reproductive outcomes, especially in unexplained infertility cases.

Conclusion

In this study, no significant correlation was found between serum vitamin D3 or vitamin B12 levels and AMH in infertile women. However, vitamin deficiencies were common, particularly low vitamin D3, suggesting a potential supportive role in reproductive health. AMH levels were notably influenced by coexisting conditions-elevated in PCOS and reduced in patients with a history of genital tuberculosis-indicating these as important confounders. Although micronutrient levels may not directly reflect ovarian reserve, routine assessment and correction may still benefit infertility management.

Limitations

This study was conducted at a single centre with a relatively small sample size of 160 participants, which may limit the generalizability of the findings. A major limitation was the inclusion of patients with PCOS (n=46) and those with a history of genital tuberculosis (n=30), both of which significantly affected serum AMH levels. PCOS was associated with abnormally high AMH due to increased follicular count, while genital tuberculosis cases exhibited disproportionately low AMH values, likely due to ovarian impairment from chronic inflammatory damage. These two groups functioned as significant confounding variables and ideally should have been excluded or analyzed separately. Additionally, the cross-sectional design restricted the ability to determine causality, and no post-treatment follow-up or assessment of conception rates following micronutrient correction was performed. The study did not stratify participants based on severity of vitamin deficiency or include intervention-based evaluation. Laboratory variations and environmental factors influencing vitamin D levels, such as sun exposure, were not controlled.

Recommendations

It is recommended that future studies implement a multicentric, longitudinal design with larger sample sizes to improve statistical reliability. Patients with known reproductive disorders such as PCOS and genital tuberculosis should be analyzed separately or excluded from primary ovarian reserve correlation analysis to avoid confounding results. Subgroup stratification based on severity of vitamin deficiency and subsequent supplementation may provide better insight into its impact on fertility outcomes. Routine screening for vitamin D3 and vitamin B12 levels may be incorporated into infertility evaluation, particularly in cases of unexplained infertility, given their high prevalence and potential influence on reproductive physiology. Interventional trials assessing the effects of targeted micronutrient supplementation on AMH levels and pregnancy outcomes are warranted. Clinicians should use AMH interpretation cautiously in conditions such as PCOS and genital tuberculosis. A comprehensive approach combining nutritional optimization with standard infertility management strategies may improve clinical outcomes and patient quality of life.

Conflict of Interest: Not available

Financial Support: Not available

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