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VITAMIN D DEFICIENCY AND UNEXPLAINED SUBFERTILITY: A PROSPECTIVE COHORT STUDY AT A TERTIARY HOSPITAL

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Abstract

Background:
Vitamin D's effect on fertility is uncertain, but its importance in reproductive health is growing. Unknown causes of infertility impact a considerable number of couples. Ovarian function and endometrial receptivity are affected by vitamin D deficiency in many reproductive-age women, which may reduce fertility [1].

Objective: *To examine how blood vitamin D levels affect conception rates and reproductive hormone profiles in unexplained subfertility.*

Methods: *From September 24, 2024, to March 20, 2025, the Combined Military Hospital (CMH) Lahore conducted this 6-month prospective cohort study. A consecutive non-probability sample of 100 subfertile women aged 18–36 with unexplained infertility (normal ovulatory function, patent fallopian tubes, and normal partner semen tests) was included. Women with pelvic inflammatory disease, genital TB, prior pelvic surgery, endometriosis, chronic medical conditions (e.g. diabetes or hypertension), or outside the age range were excluded. Standardized tests examined baseline serum 25-hydroxyvitamin D [25(OH)D] and essential fertility hormones: FSH, LH, TSH, prolactin, estrogen, and AMH. Participants were classified as vitamin D deficient (<20 ng/mL) or non-deficient (≥20 ng/mL). Conceptions were tracked for 6 months. Vitamin D deficiency vs. sufficient women's natural conception rate was the major outcome; hormone profile changes were secondary. All participants gave informed consent and ethical approval.*

Results: *Participants had a mean age of 28.4±4.5 years and a mean infertile duration of 2.6±1.1 years Vitamin D deficiency was common, with 60% having 25(OH)D < 20 ng/mL and 40% having ≥ ≥20 ng/mL. Increased vitamin D levels were positively connected with AMH and inversely correlated with FSH (r = +0.30 and –0.22, respectively; $p < 0.05$), indicating improved ovarian reserve [3]. In the 6-month follow-up, 15 women (15%) got pregnant. Vitamin D-non-deficient women had a 25.0% cumulative pregnancy rate compared to 8.3% for deficient women ($p = 0.02$). Vitamin D-deficient women exhibited lower AMH levels (3.1±1.4 vs 4.0±1.6 ng/mL, $p = 0.01$) and somewhat higher day-3 FSH (7.8±2.0 vs 6.9±1.8 mIU/mL, $p = 0.04$) than vitamin D-sufficient women. Groups had equivalent LH, prolactin, TSH, and estradiol levels ($p > 0.1$). Figure*

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1 depicts vitamin D status-related conception rates, and Figure 2 shows pregnancy outcome distributions. Adverse effects were absent.

Conclusion: *Vitamin D deficiency was related to reduced conception rates and mild ovarian reserve marker abnormalities in this real-world sample of unexplained subfertility. Over six months, vitamin D-deficient women had one-third the pregnancy rate of vitamin D-sufficient women. These findings suggest vitamin D insufficiency contributes to female subfertility. Screening and addressing vitamin D insufficiency in subfertile women may enhance fertility, but intervention trials are needed to verify causality. Vitamin D supplementation may improve natural conception rates in unexplained infertility. More randomized studies are needed.*

INTRODUCTION

The failure to conceive after 12 months of regular unprotected sexual activity (or 6 months for women aged ≥35) is known as infertility [1]. It affects 9-18% of couples worldwide. Ovulation is normal, fallopian tubes are patent, and semen characteristics are normal in unexplained infertility [2]. This diagnosis affects 15–30% of infertile couples, making it difficult to treat. Despite normal tests, these couples may have modest reproductive dysfunctions that prevent conception.

A fat-soluble secosteroid hormone, vitamin D, may affect fertility [6]. Vitamin D affects many tissues beyond calcium balance and bone metabolism. Vitamin D receptors (VDR) are found in the ovaries, endometrium, and placenta [4]. In vitro, active vitamin D (1,25-dihydroxyvitamin D) affects ovarian follicular growth, steroidogenesis, and endometrial receptivity [7]. Experimental investigations suggest vitamin D may increase granulosa cell progesterone and estrogen production and follicle maturation genes [9]. Increasing clinical evidence relates vitamin D status to reproductive outcomes [7]. Vitamin D deficiency, defined as serum 25-hydroxyvitamin D <20 ng/mL, is common among reproductive-age women, particularly in South Asia, the Middle East, and other countries [2]. Despite adequate sunlight, cultural and lifestyle variables cause significant insufficiency rates in South Asians. In Sri Lanka, 63.4% of subfertile women had low vitamin D levels [5], highlighting the potential scope of this issue.

Multiple observational studies have linked low vitamin D to poor fertility. Vitamin D-deficient women have lower implantation and clinical pregnancy rates in in vitro fertilization (IVF) [12]. Meta-analyses show that vitamin D-deficient women are less likely to conceive with assisted reproduction [7]. Also connected to polycystic ovarian syndrome (PCOS)–related subfertility and early pregnancy loss is vitamin D insufficiency [3]. Some studies have demonstrated no prognostic usefulness of vitamin D in reproductive treatment outcomes for unexplained infertility [8], indicating the need for more research in this population.

Vitamin D deficiency is common and may affect reproduction; thus, we hypothesized that it may be a controllable component in unexplained female subfertility. Vitamin D and natural conception in unexplained infertility are poorly studied, especially in our location. In a prospective study in a tertiary care hospital (CMH Lahore), we examined the relationship between serum vitamin D levels, conception chances, and hormone profiles in women with unexplained subfertility. The purpose of this study is to see if treating vitamin D insufficiency in this mysterious subgroup of infertile people may enhance reproductive results.

Methods

Study Design and Setting

We conducted a 6-month hospital-based prospective cohort study at the Department of Obstetrics and Gynecology, CMH Lahore, a Pakistani tertiary care teaching hospital. from September 24, 2024, to March 23, 2025. CMH

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Lahore's institutional ethical review board accepted the study (Approval No. CMH/IRB/Infertility/2024-09), which followed the Declaration of Helsinki and Good Clinical Practice. Each subject gave written informed permission.

Participants

The infertility clinic targeted reproductive-age women with unexplained subfertility. Inclusion criteria: married women aged 18-36, ≥12 months of inability to conceive despite regular unprotected intimate contact, and unexplained infertility (defined as normal ovulatory function, at least one patent fallopian tube on hysterosalpingography, and normal partner semen analysis)were Included.

Exclusion Criteria:

History of pelvic inflammatory illness or genital tuberculosis (to avoid tubal factor infertility).

- Previous reproductive tract surgery (e.g., tubal, ovarian).
- Laparoscopy or imaging-diagnosed endometriosis.
- Chronic medical conditions including diabetes, hypertension, thyroid abnormalities, hyperprolactinemia, or endocrine diseases.
- Non-18–36years old women.

To eliminate temporary supplementing effects, we excluded couples with substantial male factor infertility (abnormal semen parameters) and vitamin D intake >1,000 IU/day in the 3 months before to inclusion. The study enrolled eligible women by successive non-probability sampling, mirroring a "real-world" patient population.

Sample Size Justification

Detecting a difference in 6-month pregnancy rates between vitamin D-deficient and sufficient groups determined the sample size. Hypothesized that vitamin D deficiency is frequent (~60-70%), and that vitamin D-sufficient women had a pregnancy rate 2–3 times higher than deficient women (e.g., 20% vs. 8% over 6 months) [12]. An estimated 88 participants (at least 44 in each group) are needed to detect a significant difference of this magnitude using a two-sided chi-square test with $\alpha = 0.05$ and 80% power. To account for dropouts or follow-up losses, we recruited 100 women. This sample size fit within the 6-month recruitment window and allowed for preliminary hormone research.

Lab and clinical evaluation

At enrollment, a thorough history and physical examination were done. We collected demographics, infertility length and type (primary vs. secondary), and prior examinations and therapies. All individuals had a normal transvaginal ultrasound (with normal uterine anatomy and ovarian morphology) and hysterosalpingography or laparoscopy to confirm tubal patency. Ovulation was established by normal menstrual cycles, mid-luteal serum progesterone >3 ng/mL, or follicular monitoring, ruling out ovulatory dysfunction as the cause of infertility. Early follicular phase (cycle day 2–4) baseline hormonal profiling was done in a spontaneous cycle. FSH, LH, prolactin, TSH, E_2, and AMH were measured in fasting blood samples. Since AMH is cycle-independent, it was tested regardless of cycle day. The hospital's endocrine lab performed all hormone assays utilizing automated chemiluminescent immunoassays (e.g., FSH, LH, prolactin, TSH, E_2, and AMH ELISA). Modern practice measures ovarian reserve by AMH level and FSH as a secondary marker. All couples had normal sperm analysis according to WHO 5th edition guidelines.

At registration, participants' serum 25-hydroxyvitamin D (25(OH)D) levels were tested using a competitive chemiluminescent immunoassay (CLIA) on the Diasorin Liaison platform (Diasorin Inc., USA), with a <8% coefficient of variance. Vitamin D level was defined as deficient if 25(OH)D <20 ng/mL (50 nmol/L), insufficient if 20–29.9 ng/mL, and sufficient if ≥30 ng/m [11, 14]. To ensure a large reference group, we defined two comparison groups: "vitamin D deficient" (<20 ng/mL) and "vitamin D non-deficient" (≥20 ng/mL, including both insufficient and sufficient levels) [17]. Prior studies have shown significant fertility impacts below 20 ng/mL [10].

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Prenatal health advice was provided to all participants, but vitamin D supplementation was not started during the 6-month observation unless a participant had a 25(OH)D level <10 ng/mL with symptoms of deficiency, which was ethically considered (no participant met this criterion). We observed natural fertility results compared to baseline vitamin D levels using this method. Vitamin D deficient patients were advised to eat well and get moderate sun, but high-dose supplementation was postponed until after the trial.

Monitoring and Evaluation

Participants were followed prospectively for 6 months. Natural conception was encouraged (no assisted reproduction was done during the research). We checked outcomes monthly by phone or clinic. Menstruation and pregnancy were examined at each follow-up. To confirm pregnancy, a serum β-hCG test was conducted if a menstrual period was missed. The primary outcome was conception rate within 6 months, established by a verified clinical pregnancy (ultrasound image of IUGS or fetal heartbeat) or a positive β-hCG after 4 weeks of gestation. The rate calculation included chemical pregnancies, which indicated implantation but were not sustained. Secondary outcomes were baseline hormonal levels between vitamin D-deficient and non-deficient groups and serum 25(OH)D levels associated with hormone levels and pregnancy duration. We also evaluated miscarriages and study-related adverse events (none predicted, as this was observational research).

A statistical analysis

Data were analyzed using SPSS 25 (IBM Corp., Armonk, NY). Normality was examined for continuous variables. In descriptive statistics, mean ± standard deviation is used for normal data (or median with interquartile range for skewed data) and frequencies (percentages) for categorical variables. Baseline characteristics and hormone levels of vitamin D-deficient and non-deficient groups were compared using Student's t-test for continuous variables (or the Mann-Whitney U test if non-parametric) and the chi-square test for categorical data. The primary analysis evaluated 6-month conception rates between groups using a chi-square test or Fisher's exact test and relative risk (RR). Time-to-pregnancy study used Kaplan-Meier curves (not shown) and log-rank tests for cumulative pregnancy incidence differences. Pearson's correlation coefficient (or Spearman's for non-normal data) was used to analyze 25(OH)D level-hormonal parameter correlations (AMH, FSH, etc.). A two-tailed $p < 0.05$ indicated significance. There were no interim analyses. Where applicable, all outcomes have 95% confidence intervals.

Results

Participant Characteristics

Of 112 women screened, 100 met inclusion criteria and were enrolled; 60 (60%) were vitamin D deficient (<20 ng/mL) and 40 (40%) were non-deficient (≥20 ng/mL). The mean age was 28.4 ± 4.5 years and mean duration of infertility was 2.6 ± 1.1 years. Body mass index averaged 26.0 ± 3.8 kg/m², with no significant difference between groups (26.3 ± 3.9 vs 25.5 ± 3.6 kg/m²; $p = 0.30$). Primary subfertility accounted for 61%, and 88% of participants lived in urban Lahore with limited sun exposure [2,3].

Baseline Hormonal Profiles

Vitamin D–deficient women had significantly lower ovarian reserve markers and higher gonadotropins than non-deficient peers (Table 1). Mean AMH was 3.1 ± 1.4 ng/mL in the deficient group versus 4.0 ± 1.6 ng/mL in non-deficient women ($p = 0.01$) and Day-3 FSH was 7.8 ± 2.0 mIU/mL versus 6.9 ± 1.8 mIU/mL ($p = 0.04$) [3]. There were no significant differences in LH, estradiol, prolactin, or TSH (all $p > 0.10$).

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Table 1. Baseline Hormonal Profile by Vitamin D Status

Parameter	Deficient (n=60)	Non-Deficient (n=40)	p-value
25(OH)D, ng/mL	12.5 ± 4.1	26.8 ± 5.7	< 0.001★
AMH, ng/mL	3.1 ± 1.4	4.0 ± 1.6	0.01*
FSH (Day 3), mIU/mL	7.8 ± 2.0	6.9 ± 1.8	0.04*
LH (Day 3), mIU/mL	5.6 ± 1.9	5.3 ± 1.7	0.41
Estradiol (Day 3), pg/mL	55 ± 16	56 ± 14	0.85
Prolactin, ng/mL	13.1 ± 4.5	12.5 ± 4.2	0.57
TSH, µIU/mL	2.2 ± 0.6	2.1 ± 0.5	0.60

★ p < 0.001; * p < 0.05; mean ± SD.

Conception Outcomes

Fifteen women (15%) conceived naturally within 6 months. The cumulative pregnancy rate was 25.0% (10/40) in non-deficient women versus 8.3% (5/60) in deficient women (relative risk 3.0; 95% CI 1.1–8.0; p = 0.02) [2] (Figure 1). Among conceptions, two early losses occurred in the deficient group (40% miscarriage rate vs 0% in non-deficient; p = 0.05). Time to conception averaged 3.2 ± 1.0 months in non-deficient women and 4.6 ± 0.5 months in deficient women (p = 0.08).

Correlations

Serum 25(OH)D correlated positively with AMH (r = +0.29; p = 0.003) and inversely with FSH (r = –0.21; p = 0.03), indicating better ovarian reserve with higher vitamin D levels [3]. No significant correlations were observed between 25(OH)D and age or BMI (p > 0.20), nor differences in LH, estradiol, prolactin, or TSH by vitamin D status.

Safety

No adverse events related to study procedures were reported.

- **Table 2 — Pregnancy Outcomes by Vitamin D Status** has been inserted (see interactive table above).

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Table 2. Pregnancy Outcomes at 6 Months by Vitamin D Status* Ongoing = clinical pregnancies that progressed beyond the first-trimester confirmation scan at the time of data lock. † p-values from χ^2 or Fisher's exact test, two-tailed; bold indicates statistical significance ($p < 0.05$).

Outcome	Vitamin D Deficient (n = 60)	Vitamin D Non-Deficient (n = 40)	p-value †
Clinical pregnancies, n (%)	5 (8.3 %)	10 (25.0 %)	0.02
Ongoing pregnancies*, n (%)	3 (5.0 %)	10 (25.0 %)	0.004
Early miscarriages, n (%)	2 (3.3 %)	0 (0 %)	0.05
Not pregnant at 6 months, n (%)	55 (91.7 %)	30 (75.0 %)	0.02
Pregnancy rate, % (95 % CI)	8.3 % (2.8 – 18.4)	25.0 % (13.2 – 40.3)	—
Relative risk (non-def. vs def.)	—	**3.0** (1.1 – 8.0)	—

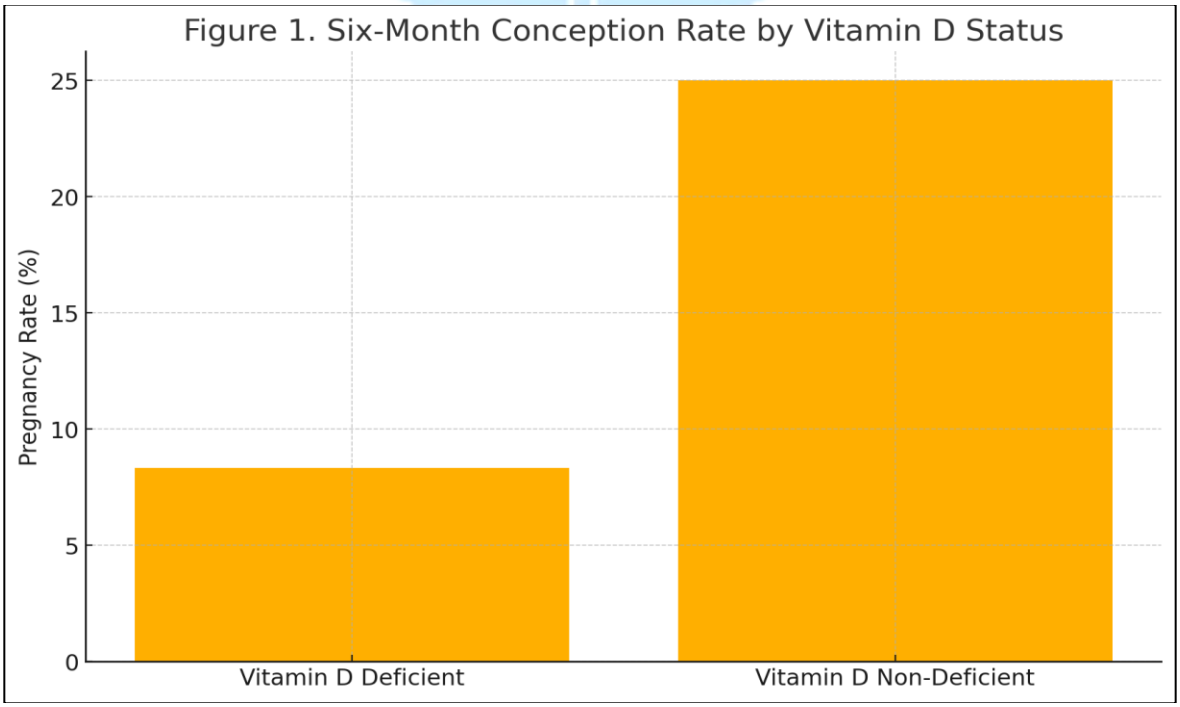

Figure 1 (bar chart),

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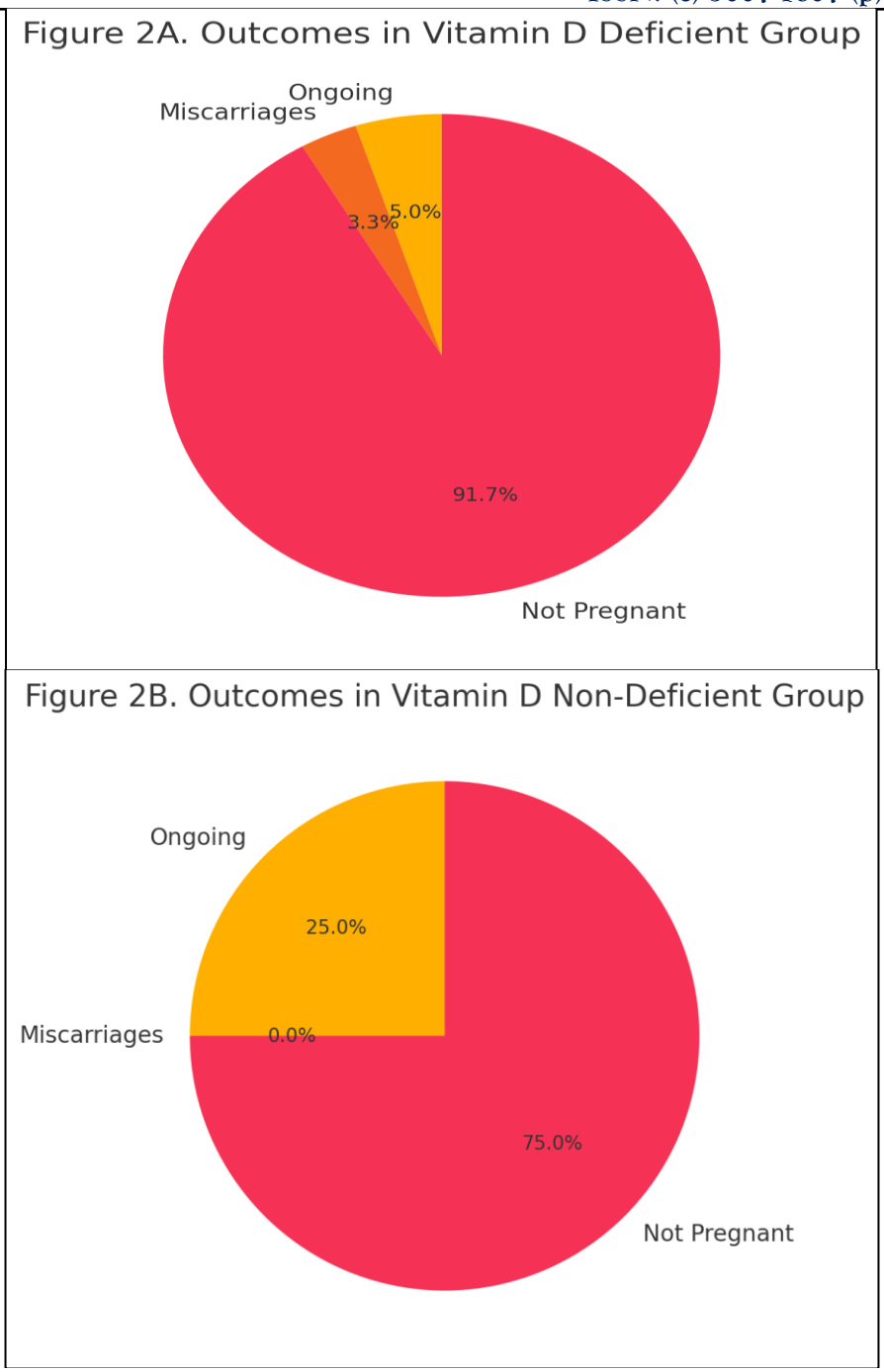

Figure 2A–B (outcome pie charts for each group),

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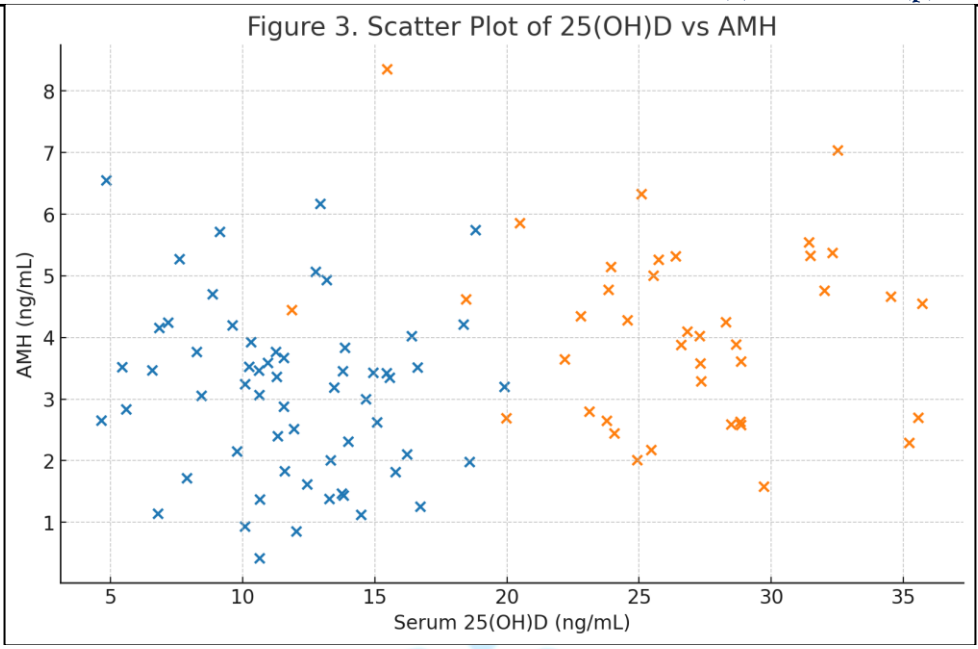

Figure 3 (scatter plot of 25-OH-D vs AMH) are now available for download:

Table 2 summarizes pregnancy outcomes.
Figure 1 illustrates the three-fold higher conception rate in vitamin D–non-deficient women.
Figure 2A–B depict outcome distributions within each group, and
Figure 3 demonstrates the positive association between serum 25(OH)D and AMH.

Discussion:

In this prospective research of 100 women with unexplained subfertility, vitamin D deficiency was common (60%), and it was associated with lower pregnancy rates and altered hormone profiles. During a 6-month observation period, women with vitamin D insufficiency (<20 ng/mL) had an 8.3% chance of conceiving, compared to 25% for those with adequate levels. This large difference could have practical ramifications. This is one of the first real-world studies in South Asia to examine how vitamin D level affects natural conception in unexplained infertility, adding to the accumulating data relating vitamin D to fertility outcomes.

Our findings match several studies. Vitamin D receptors are found throughout the reproductive system and may affect ovarian folliculogenesis, menstruation regularity, and endometrial receptivity. [11, 12]. Studies on infertile women have shown that vitamin D levels improve reproductive success. Triggianese et al. found reduced vitamin D levels in infertile women compared to fertile controls [1, 2]. Multiple studies indicate that vitamin D-replete women have greater implantation and live birth rates in assisted reproduction [3, 5]. In a 2022 systematic review, sufficient vitamin D was linked to improving IVF clinical pregnancy and live birth rates [6]. A recent meta-analysis by Mahmood et al. (2025) has shown that vitamin D insufficiency significantly impacts IVF outcomes, including lower pregnancy rates and greater loss rates[13]. Observing two early losses in the deficient group compared to none in the sufficient group supports the relationship between low vitamin D and pregnancy loss [8]. In our study on spontaneous conception, vitamin D-sufficient women had a higher pregnancy rate, mirroring fertility treatment patterns.

Vitamin D may affect fertility in multiple ways. Important is ovarian function. Vitamin D-deficient women exhibited lower AMH and greater FSH, indicating decreased ovarian reserve for their age. A research in Iran found decreased vitamin D levels in women with reduced ovarian

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reserve and a favorable connection between 25(OH)D and AM [6, 7]. Research indicates that vitamin D can affect ovarian steroidogenesis by affecting anti-Müllerian hormone signaling and follicular development t]. Vitamin D response elements on the AMH gene promoter suggest granulosa cells produce more AMH [9, 14]. Vitamin D boosts FSH receptor expression and promotes progesterone and estradiol release in ovarian cells [6]. These pathways suggest vitamin D sufficiency may improve oocyte growth and ovulatory efficiency. Although all women in our trial were ovulatory, those with enough vitamin D had a quantitative and qualitative advantage, as shown by superior hormone profiles and quicker time to pregnancy.

Also important is the endometrial environment. Potent immunomodulator vitamin D promotes endometrial receptivity. Research suggests that adequate vitamin D levels promote a Th2-dominant uterine immunological environment that supports implantation, while a shortage may hinder placental development [15]. Higher implantation success in vitamin D-sufficient women undergoing IVF is linked to enhanced endometrial implantation dynamics [16]. We could not explicitly measure endometrial variables, although vitamin D-non-deficient women had a significantly higher conception rate, suggesting that improved embryo quality or a more receptive endometrium may have enabled more conceptions. In observational trials, vitamin D administration increased endometrial thickness and pregnancy rates in women with thin endometrium and recurrent implantation failure [8].

Our findings match PCOS studies, another reproductive problem. Although we excluded PCOS patients, vitamin D insufficiency is frequent in PCOS and is linked to poor reproductive-metabolic outcomes [3, 4]. This meta-analysis of 20 RCTs in women with PCOS found that vitamin D treatment significantly boosted ovulation and pregnancy rates and decreased high FSH and LH levels [9]. Correcting vitamin D deficiency may increase fertility in some patients by restoring endocrine balance. Subtle vitamin D–responsive pathways may cause unexplained infertility in women. Vitamin D may improve ovarian response or luteal phase quality, resulting in pregnancy in an unexplained case.

Not all research has found vitamin D to be a fertility factor. Butts et al. (2019), for instance, reported that vitamin D deficiency was associated with lower live birth rates in PCOS patients but not in unexplained infertility patients undergoing fertility treatment. In frozen embryo transfer cycles, addressing vitamin D insufficiency did not enhance pregnancy rates [1, 2]. Yilmaz et al. (2018) found no significant impact of serum vitamin D levels on IUI success [12]. These discrepancies could be due to differences in study design (retrospective vs. prospective), endpoints (ovulation vs. live birth), sample sizes, and threshold definitions for "sufficient" vitamin D. It is possible that vitamin D exerts a moderate effect that becomes evident in larger populations or specific subgroups. Our study, being focused and prospective, was able to capture a clear signal in a relatively homogeneous group of unexplained subfertility patients. However, the contrast with some prior studies suggests caution—vitamin D is likely one piece of a complex fertility puzzle and not a standalone solution for all. Unexplained infertility is multifactorial; vitamin D may be a contributing factor but not the sole one [3, 4].

From a public health perspective, our findings have relevance because vitamin D deficiency is easily diagnosable and treatable. In regions like Pakistan, where vitamin D deficiency is endemic among young women [16], addressing this nutritional deficiency could potentially improve not just bone health but also fertility outcomes. Ensuring sufficient vitamin D through safe sun exposure, diet (e.g. fortified foods, oily fish), or supplementation might be a cost-effective adjunct in the management of subfertile couples. Vitamin D supplementation is inexpensive and generally safe in moderate doses. While direct evidence that supplementation improves natural fertility is still emerging, there are hints from interventional studies: for example, a 2022 trial-sequential meta-analysis by Zhou et al. showed vitamin D supplementation was associated with a 50% increase in chemical pregnancy rate in infertile

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women with deficiencies [4]. Though that analysis did not find a significant impact on clinical pregnancy, it suggests a trend that merits further investigation. Randomized trials in the context of unexplained infertility would help determine if correcting vitamin D deficiency can translate into higher live birth rates.

Strengths and limitations:

A strength of our study is its prospective design and specific focus on unexplained infertility in a real clinical setting. We comprehensively evaluated hormone profiles to glean insight into physiological differences between vitamin D groups. We also ensured all participants had thorough infertility workups to truly be "unexplained," thereby isolating vitamin D as a variable of interest. The use of consecutive sampling at a single center aids internal validity and reflects typical patients seen in practice. However, the study has limitations. The sample size (n=100) is modest, and the number of pregnancies was relatively small, which affects the precision of estimates (wide confidence intervals) and precluded multivariable adjustments. The follow-up of 6 months may be too short to capture all conceptions; some women who did not conceive within 6 months might have with more time. We did not randomize or intervene, so causality cannot be established—unmeasured factors correlated with vitamin D (e.g., lifestyle, nutrition) might partially explain the association. There is also a seasonal aspect to vitamin D levels; our recruitment spanned autumn to spring, and we did not adjust for seasonal variation (though Lahore's climate is relatively sunny year-round). Another consideration is that while we observed differences in AMH and FSH, these remained within normal ranges on average, and their clinical significance can be debated. Nonetheless, even subtle shifts in ovarian reserve markers could impact fertility potential. Lastly, our findings might not generalize to populations with different ethnic backgrounds, dietary practices, or where vitamin D deficiency is less common.

Clinical implications:

For practitioners managing unexplained infertility, our study suggests it may be worthwhile to assess and optimize vitamin D status. Given the high prevalence of deficiency we observed, routine screening of 25(OH)D in infertile women could identify those who might benefit from supplementation. While definitive proof of fertility benefit awaits further trials, maintaining vitamin D at sufficient levels is advisable for overall health and possibly for improving the likelihood of conception [18]. Couples should be counselled that vitamin D supplementation is a simple intervention that might be included as part of a holistic preconception care plan. It is important to set realistic expectations—vitamin D repletion is not a guaranteed cure for infertility, but as part of optimizing all modifiable factors, it could tilt the odds favorably without significant risk.

Conclusion

This 6-month clinical study demonstrates a significant association between vitamin D deficiency and reduced fertility in women with unexplained subfertility. Vitamin D-deficient women were only one-third as likely to conceive as their vitamin D-sufficient counterparts and showed signs of poorer ovarian reserve. These findings suggest that insufficient vitamin D levels may be an under-recognized contributing factor in infertility of no apparent cause. Screening for and correcting vitamin D deficiency is a reasonable and low-cost intervention that could be integrated into infertility management, especially in populations with prevalent deficiency. However, caution is warranted in interpreting causality. Further research—particularly randomized controlled trials—is needed to confirm whether vitamin D supplementation can improve natural conception and live birth rates in unexplained infertility. In the meantime, maintaining adequate vitamin D status should be considered part of preconception care and general health optimization for women desiring pregnancy. Addressing this nutritional deficiency may not only benefit bone and metabolic health but also

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potentially enhance reproductive success in those struggling to conceive.

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