SYSTEMATIC REVIEW/META ANALYSIS

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A Symbiotic Relation Between Anti-Mullerian Hormone (AMH) and Vitamin D in Polycystic Ovary Syndrome (PCOS): A Meta-Analysis and Systematic Review

Kandarp J Patel[1], Haiya J Sheth[2], Nidhi Y Mangrola[3], Neeti A Patel[4*]

[1,2]Department of Pharmacology, Kiran Medical College, Surat, India
[3]Department of Community Medicine, Kiran Medical College, Surat, India
[4]Department of Biochemistry, Parul Institute of Medical Sciences and Research, Parul University, Vadodara, India

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***Corresponding author**:
Neeti Patel
Email: neetipatel44@gmail.com

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ABSTRACT

Background: The association of vitamin D levels with AMH and other hormones is still under exploration as mentioned in the existing literature. The capacity of vitamin D to regulate ovarian reserve, measured by the AMH level, is a subject of much discussion, especially in PCOS women. Thus, the present study was planned with a primary aim to assess and summarize the available evidence about the interrelationship between vitamin D and AMH levels in PCOS.

Methods: A detailed literature search was done across various databases based on PRISMA guidelines till December 2024. Meta-analysis was done on 10 articles from the 37 articles selected for systematic review.

Results: Serum AMH levels in PCOS subjects significantly decreased (SMD=-0.57; CI: -1.1 to 0.04; p=0.03) on vitamin D supplementation, while, it increased (SMD=-0.67; CI: 0.12 to 1.23; p=0.02) in non-PCOS women.

Conclusion: This indicates a complex interplay of metabolic and hormonal parameters and makes vitamin D a potential factor for PCOS management.

Key-Words: Anti-Mullerian Hormone, Meta analysis, Ovarian Reserve, Polycystic Ovary Syndrome, Vitamin D

INTRODUCTION

The endocrine disorder that presents with ovaries that are polycystic and with impaired function, increased androgen levels, and menstrual cycle irregularities is known as Polycystic Ovary Syndrome (PCOS).[1] It is heterogeneous in nature, where the females of the reproductive age group show metabolic, hormonal, and inflammatory disturbances.[2] Granulosa cells of antral follicles (small and pre) secrete AMH, which is known to indicate ovarian reserve and follicular process.[3] When AMH levels in PCOS women are higher, it shows higher follicular mass along with weak folliculogenesis. This means that AMH is a potential biomarker for the diagno-

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sis as well as critical analysis of PCOS.[4]

Out of all the fat-soluble vitamins, vitamin D plays a crucial role in various endocrine and reproductive physiologies, including PCOS.[5] The mere presence of receptors and metabolizing enzymes for vitamin D in the ovarian tissue has pointed towards the fact that vitamin D may have some influence on the ovary's functioning.[6] One of the studies has even mentioned its relation with insulin resistance, inflammation, and hormonal imbalance amongst patients with PCOS.[7] Thus, the relationship between vitamin D and AMH in PCOS patients should be explored, as there can be an underlying mechanism of vitamin D modulating AMH levels along with ovarian functions in these PCOS patients. Furthermore, the positive influence of vitamin D supplementation on AMH status may thereby improve the ovarian reserve in these PCOS patients.

Hence, this systematic review and meta-analysis was envisaged with the aim to evaluate and gather all current evidence by integrating findings from different studies conducted on similar subjects exploring the interrelation of vitamin D supplementation and AMH status in PCOS subjects. Thus, the aspiration of this study was to clarify the effects and nature of this correlation as well as explore underlying pathophysiology, thereby providing valuable insight on clinical implications related to vitamin D supplementation in PCOS. This will also highlight the gaps in existing research and the need for further research studies in the field of metabolic disorders and reproductive endocrinology.

MATERIALS AND METHODS

Search Blueprint and Study Preference: An immense literature search was carried out in English language from inception to December 2024 across various databases like PubMed, Scopus, Web of Science, and Embase in order to investigate an association between levels of AMH and vitamin D supplementation in PCOS patients, after obtaining waiver for ethical considerations. Searches included MESH and non-MESH prompts like "vitamin D", "AMH", "anti-mullerian hormone", "PCOS", and "polycystic ovarian syndrome". Cross-referencing of bibliographies was done to identify additional studies. Only those studies fulfilling the eligibility criteria were included in the systematic review.

Data Extraction: Evaluation of articles and collection of information were done by two independent researchers. Any inconsistencies noted were discussed with other senior authors. The PRISMA guidelines were followed for the meta-analysis and narrative review description. Interventional studies with vitamin D supplements, having pre- and post- treatment quantitative data of AMH and vitamin D, were selected and included in the present meta-analysis. The final data, drawn out from the article's tables and text, included first author's last name, country, study population, sample size, intervention data, and serum levels of vitamin D and AMH along with their interrelationship.

Eligibility Benchmark: Those studies adhering to the following standards were included in the present systematic review: (a) study population having female subjects with and without PCOS (b) measurement of serum AMH and vitamin D levels should be done in all the study participants at one point in time (c) vitamin D supplementation administered to all the study participants, (d) a description of the association between vitamin D supplementation and serum AMH levels, and (e) any study design except a case report. In addition, studies carried out on prepubertal, menopausal, and postmenopausal women were excluded.

The selection of studies for meta-analysis was based on specific methodological criteria. Only those studies that (i) evaluated vitamin D supplementation as an intervention, (ii) reported pre- and post-treatment quantitative data for serum AMH and vitamin D levels, and (iii) provided sufficient statistical detail (mean ± SD or equivalent) suitable for pooling were included.

The studies in which either presented data was in non-extractable formats or some of the data was not available in the article text were excluded from the meta-analysis. They provided broader evidence base and hence synthesized narratively in the systematic review.

Study Quality Analysis: Each included in the review was evaluated for the following quality characteristics by the present study investigators using the Newcastle-Ottawa scale (NOS): (a) Embodiment of exposed cohort, (b) Choice of non-exposed cohort, (c) Cohort comparability, (d) Absence of aftermath of interest study initiation, (e) Exposure evaluation, (f) Outcome analysis, (g) Sufficiency of time length before follow-up and (h) Cohort follow-up suitability.

Data Analysis: Mean difference (MD) was applied for continuous data (vitamin D levels, AMH levels, etc.). A continuous measure meta-analysis was done using IBM SPSS Statistics 25.0 software. Standardized mean difference (SMD) was used for comparison of various studies. Heterogeneity quantification of included studies was done using the I-squared measure and Cochran's Q test, where $I^2 > 50\%$ and $p < 0.10$ suggested statistical heterogeneity. A fixed-effect model was used for $I^2 < 50\%$. Otherwise, the random-effect model was conducted to calculate the effect of vitamin D supplements on AMH status in PCOS females (all 7 cohorts) and non-PCOS females (all 4 cohorts). This was based on a postulation that these studies shared a common true effect of increasing *vs.* decreasing levels of AMH in non-PCOS *vs.* PCOS females, respectively.

RESULTS

A total of 185 retrieved articles, comprises of 163 and 22 from the database and external sources respectively. After screening, 34 duplicate articles were removed, and 151 articles were selected. Almost 73 articles were eligi-

ble for the study after excluding 39 articles that did not meet the inclusion criteria. Thirty-six records that did not contain any relevant data for the review, lacked specific data on AMH, and were published in different languages were excluded. A total of 10 studies were selected for meta-analysis out of the 37 studies included in the systematic review. (figure 1)

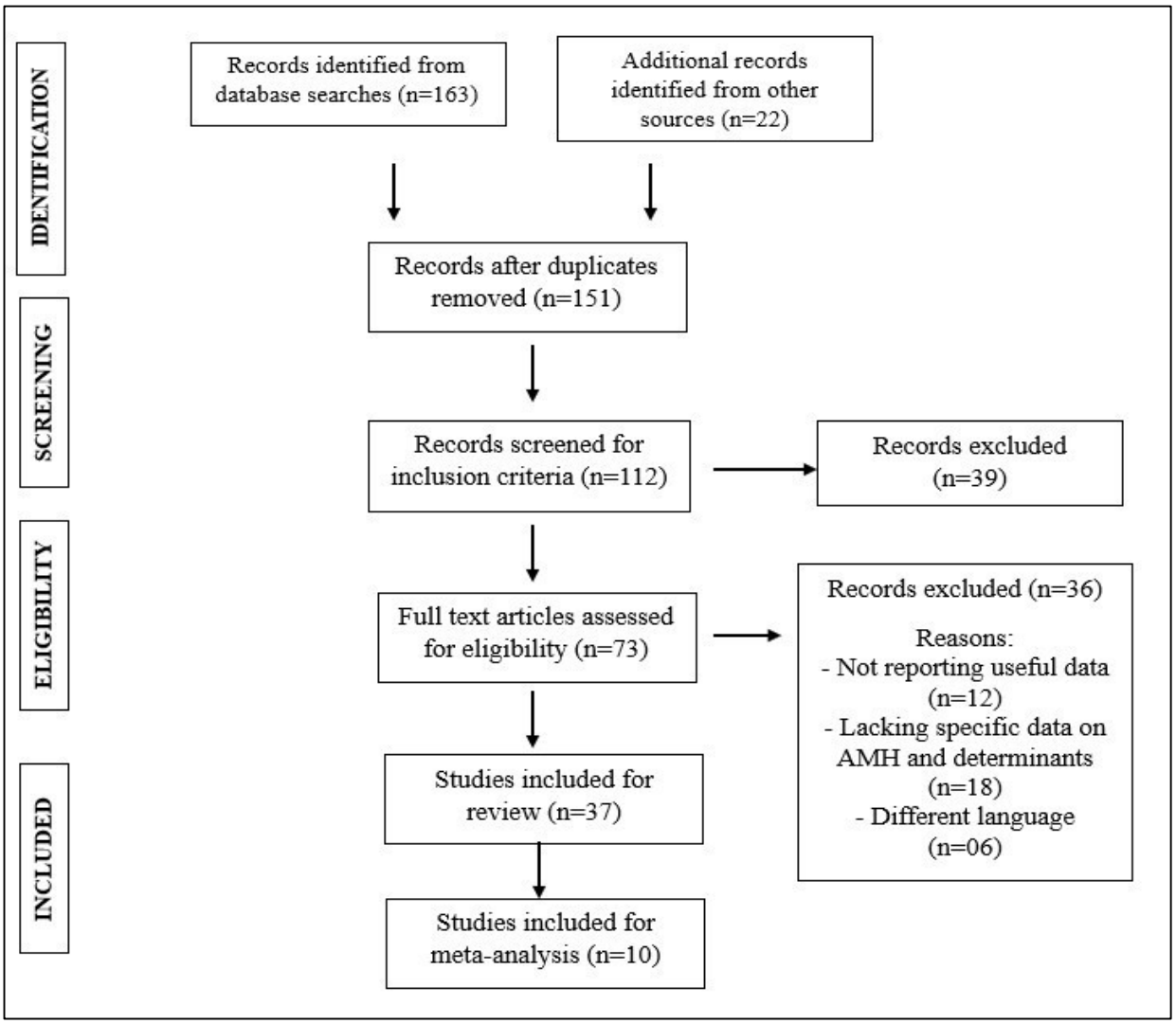

Figure 1: Prisma flow chart for study selection

AMH levels in PCOS (figure 2): AMH, a glycoprotein hormone belongs to the transforming growth factor-beta superfamily and is secreted in Sertoli cells and granulosa cells.[8] In women, AMH levels slightly fluctuate during different menstrual cycle phases, but it is not significant enough to affect its measurement. After menopause, the levels are undetectable as they keep declining with age.[9] The major role of AMH is during embryogenesis for inhibiting the Mullerian ducts development and preventing the formation of the uterus, fallopian tubes and upper third of the vagina in the absence of AMH.[10] AMH expression begins at the primary follicle stage, increases in pre- and small antral follicles, and reduces in large follicles during the process of folliculogenesis.[11] The oestrogen production, follicular selection, and ovulation are caused by AMH, which inhibits premature follicle maturation and downregulation in larger follicles that increases sensitivity to FSH.[12] Higher numbers of small and pre-antral follicles are found in the ovaries of women with PCOS, suggesting a halt in follicular development at a stage when AMH production peaks. Additionally, AMH concentration in the follicular fluid was found to be 5 times greater in anovulatory women as compared to ovulatory women. In PCOS subjects with anovulation, the production of AMH per granulosa cell increased 60-fold as compared to subjects with healthy ovaries due to higher AMH-mRNA expression.[13] The elevated number of follicles and increased granulosa cell mass in ovaries, along with greater production by individual granulosa cells, underlie the overproduction of AMH in PCOS.

Women with anovulatory PCOS, increased AMH levels, and higher numbers of small antral follicles are prone to ovulatory dysfunction because the negative effect of high AMH levels affects the FSH, and the development of the follicles gets impaired.[14] Higher LH, testosterone, and insulin resistance are major factors contributing to elevated AMH levels in PCOS. Elevated LH af-

fects the production of testosterone in theca cells positively, and the enzyme aromatase then converts it to oestrogen in granulosa cells by the enzyme aromatase. Higher levels of AMH and decreased FSH sensitivity may reduce the activity of aromatase in granulosa cells, leading to impaired oestrogen formation and higher testosterone levels.[15]

The metabolic syndrome, including insulin resistance and obesity, is a common issue in anovulatory PCOS individuals, where IR is increased by the action of increased leptin and decreased adiponectin.[16] Higher testosterone levels due to decreased sex hormone-binding globulin (SHBG) lead to symptoms like menstrual cycle irregularities, acne, and hirsutism. Elevated AMH levels are correlated with IR, yet factors, such as BMI and WHR, cause variations. Increased advanced glycation end products can modify the effects of AMH, leading to metabolic and hormonal dysfunctions.[17] AMH shows correlation with the manifestations like hyperandrogenism, oligo/amenorrhea, and polycystic ovaries seen in PCOS, suggesting that AMH may serve as a biomarker for PCOS.

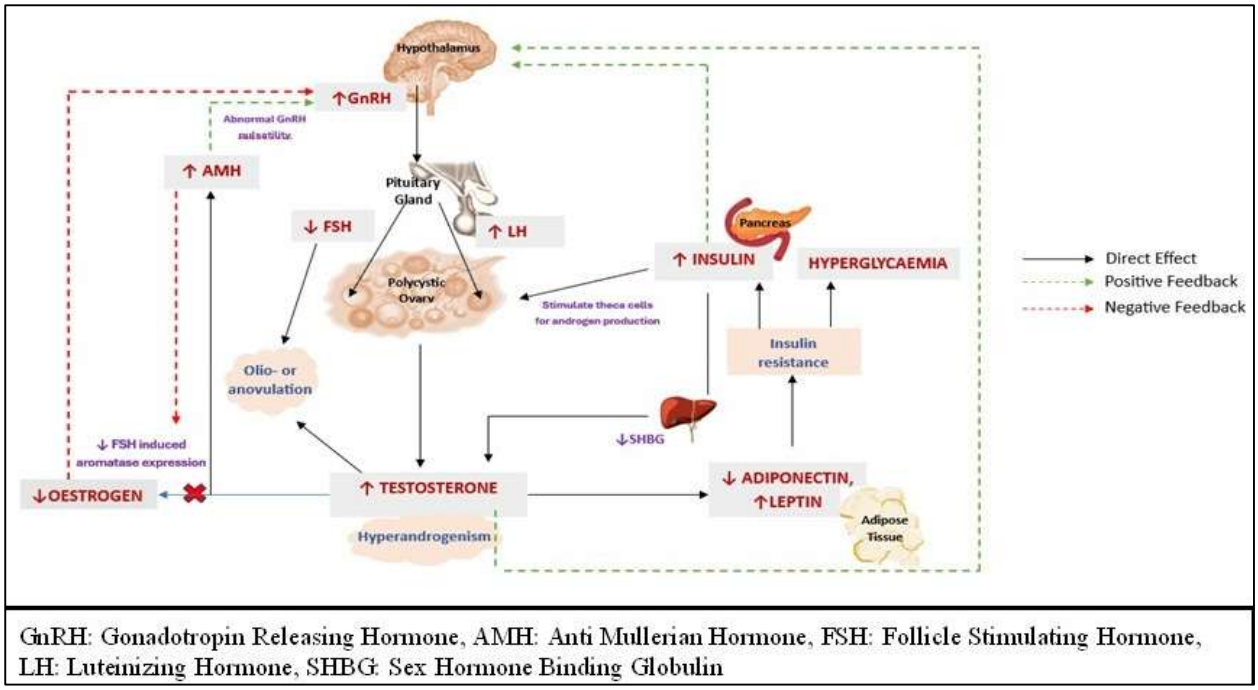

Figure 2: Understanding the role of AMH in pathogenesis of PCOS

Relationship between serum Vitamin D and AMH levels (table 1): The sensitivity of antral follicles to FSH might be reduced by the increased AMH levels in PCOS women, leading to follicular arrest. Vitamin D supplements can help in lowering the elevated levels of serum AMH in vitamin D- deficient PCOS women, thus playing an important role in overcoming infertility and ovulatory dysfunction by reducing intrafollicular androgens and enhancing follicular response to FSH in individuals with PCOS.[18]

Very few studies have examined the association between AMH and 25(OH)D levels. In a case-control study by Gungor et al.,[19] 60 patients with PCOS having BMI in the normal range were having significantly higher AMH levels than 30 women with unexplained infertility in controls with normal BMI. AMH levels did not show significant correlation with 25(OH)D in the women with PCOS or control group. Szafarowska et al.,[20] Drakopoulos et al.,[21] and by Arslan et al.[22] obtained findings that were comparable. On applying the Pearson correlation coefficient between two random quantitative variables (AMH and Vitamin D status), Fabris et al.[23] found no significant correlation observed by the p-value. The potential confounders, such as age and BMI index, were adjusted by using a regression model, showed no significant differences.

In a study by Kokanali et al.,[24] a negative correlation was seen between the levels of 25(OH)D3 and AMH in PCOS subjects ($r=-0.356$, $p=0.016$). Bakeer et al.[25] found similar results among 53 PCOS females with primary or secondary infertility, but no statistical significance was found ($p=0.343$). 64.28% of females with infertility had vitamin D deficiency in study of Lata et al.[26], the mean vitamin D levels in deficient cases was 6.18±2.09 ng/ml, while for AMH it was 1.94±1.30 ng/ml. Compared to the infertile females, the vitamin D levels in the fertile females were considerably lower with p value 0.04.

Pearce et al.[27] observed no correlation of AMH levels with vitamin D ($r^2=0.04$, $P=0.4$), even when adjusted for the confounders that are known, like age and BMI ($r^2=0.04$, $P=0.5$), in women <40 y. Merhi et al.[28] in a study conducted on 388 premenopausal women observed a contrasting correlation between AMH and vita-

min D in women <35 years (negative) and in women >40 years (positive). The study by Dennis et al.[29] reported a positive correlation between AMH levels and vitamin D in 33 women (R=0.36, P=0.004) by administering vitamin D3, D2 and a placebo in 16,7 and 10 women respectively.

Effect of Vitamin D Supplementation on AMH Levels (table 2): Variable responses to vitamin D intervention were observed in the nine studies shown in the table, including significant increases to no substantial change in AMH levels. The doses of vitamin D ranged from 50,000 to 100,000 IU weekly for variable durations from 8 to 24 weeks, as observed in most of the studies. Subgroup analyses in women with PCOS showed a more pronounced impact of vitamin D on AMH, while in healthy reproductive-aged women, the effect remained inconsistent. In most of the studies reviewed regarding Vitamin D supplementation affecting the levels of AMH, Vitamin D dosage given to the study subjects was 50,000 IU/ week for 2 months, out of which few studies showed decrease in AMH levels after supplementation,[18,30-32] few showed a slight increase,[33,34] while one study found no effect.[35] While in a retrospective study conducted in Bulgaria by Yanachkova et al.,[36] 70 female patients showed a sharp reduction of AMH levels (5.6 ng/ml vs. 6.2 ng/ml, p=0.010) after supplementation with microencapsulated liposomal form of vitamin D3 (2000 IU daily for 3 months). While a double-blinded RCT conducted by Lerchbaum et al.,[37] showed a slight decrease in AMH levels after Vitamin D3 supplement at dose 20,000 IU/week for 6 months.

In a prospective randomized-controlled study conducted in 41 PCOS women with clomiphene citrate resistance by Abdulameer et al.,[30] after 2 months of vitamin D treatment, AMH levels reduced significantly (p<0.01). Dastorani et al.[31] observed similar findings after an 8-week intervention with vitamin D supplementation (50,000 IU vitamin D/week); the serum AMH levels showed a significant reduction (-0.7±1.2 vs. -0.1±0.5 ng/ml, p=0.02). Sharma et al.[32] observed a significant change in the mean AMH levels (4.88 ± 2.06 ng/ml to 3.79 ± 2.00 ng/ml). Following intervention, the group with deficiency of vitamin D showed a weak correlation between the levels of AMH and vitamin D, yet not statistically significant. While statistically significant, higher levels of AMH were observed by Naderi et al.[33] in the women with sufficient Vitamin D levels, in comparison to those with Vitamin D insufficiency (p=0.043).

In a randomised control trial by Dennis et al.,[34] after supplementation of women with a dose of 50,000 IU of vitamin D3, no significant correlation was seen compared to baseline serum 25(OH)D levels (r=0.20). In another study by Cappy et al.[35] conducted on vitamin D deficient patients (23 PCOS patients and 27 controls), vitamin D supplementation was given according to the levels of their insufficiency. But no change was observed in the serum AMH levels in patients and controls before and after the supplementation. Irani et al.[18] in his study observed that after providing replacement with vitamin D3, the concentrations of AMH reduced significantly in PCOS women (P=0.003) compared to the women who did not have PCOS (P=0.6).

Vitamin D supplementation in PCOS women was found to significantly reduce AMH levels in majority of the studies reviewed, suggesting a potential role in alleviating follicular arrest and enhancing FSH sensitivity. In contrast, non-PCOS women demonstrated an increase in AMH levels following supplementation, indicating a possible beneficial effect on ovarian reserve in vitamin D–deficient but otherwise healthy populations. These findings highlight a contrasting effect of vitamin D on AMH depending on the reproductive phenotype, with a decline observed in hyperandrogenic, follicle-rich ovaries of PCOS women and an enhancement of ovarian reserve in non-PCOS women. Together, these findings suggest that while vitamin D supplementation may modulate AMH differently in PCOS and non-PCOS women, the interpretation of its effect requires careful consideration of clinical, metabolic, and methodological contexts.

Meta-analysis showing the effect of vitamin D supplementation on serum AMH levels: In PCOS Women: Seven studies were analysed, with a total of 222 subjects in the pretreatment group and posttreatment group.[18,30-32,35-37] In the PCOS groups, the baseline mean serum AMH levels ranged from 4.47 to 10.04 ng/ml, while the mean serum levels of AMH after vitamin D supplements ranged from 3.79 to 9.59 ng/ml. On performing meta-analysis using a random effects model to compare the standardized mean difference (SMD), five out of six studies show a negative SMD, indicating a reduction in AMH levels following Vitamin D supplementation. The pooled SMD is -0.57 [95% CI: -1.10 to -0.04], suggesting a statistically significant overall reduction in AMH levels (p = 0.03).

The analysed studies showed a significant amount of diversity (p<0.01), suggesting varying effects in magnitude. The I2 value was 78%, which suggests the level of variability found across studies was not due to random chance but heterogeneity. (figure 3)

In Non-PCOS Women: Meta-analysis was carried out in 4 studies with a total of 132 subjects in the pre-treatment group and post-treatment group [33,34,37,38] Random effects model was utilized for the analysis to compare the SMD. While two studies[33,38] show a strong positive effect, the other two studies[34,37] show modest or no significant effects. The overall effect is moderate and statistically significant with the summarized standardized mean difference (SMD) as 0.67 and a 95% CI of 0.12 to 1.23 (p=0.02). In comparison to the meta-analysis of PCOS, the serum AMH levels significantly increased in non-PCOS women with vitamin D supplementation.

There was a significant amount of heterogeneity in the studies analysed (p<0.01), suggesting varying effects in magnitude. The I2 value was 79%, which suggests the level of variability found across studies was not due to random chance but heterogeneity. (figure 4)

Table 1: Studies showing the relationship between serum Vitamin D and AMH levels

Author	Country	Population	Study Design	Age (Mean, Median or Range)	Vitamin D status	AMH Levels*	Relationship between AMH levels and Vitamin D
Gungor et al.[19] 2023	Turkey	Infertile women with PCOS=60 Women with unexplained infertility (UEI)=30	Retrospective case-control study	PCOS - 24.6 ± 3.22 y UEI - 25.1 ± 2.09 y	PCOS - 23.9 ± 3.44 ng/ml UEI - 28.4 ± 5.07 ng/ml	PCOS - 4.28 ± 1.46 ng/ml UEI - 3.57 ± 0.80 ng/ml	With rise in BMI, a significant reduction in levels of AMH and vitamin D was seen in PCOS women, but no significant change was seen in AMH levels of all three BMI groups
Szafarowska et al.[20] 2019	Poland	PCOS women= 75 Control women=23	Prospective case-control study	25–43 y (33.9±4.0)	PCOS - 14.2 ng/ml Control - 19.60 ng/ml	PCOS- 8.4 ng/ml Control - 4.3 ng/ml	Significantly lower Vitamin D levels (p =0.008) and higher AMH levels (p = 0.001) were seen in the PCOS group compared with the control group but no statistically significant associations were identified between them.
Drakopoulos et al.[21] 2016	Belgium	Infertile women=283	Prospective cross-sectional study	32.2±5.19 y	Vitamin D deficient, n=87- <20ng/ml Normal vitamin D levels, n=196- ≥20ng/ml	Vitamin D deficient - 3.9±3.8 ng/ml Normal Vitamin D - 4.3±4.8 ng/ml	Levels of Vitamin D and AMH showed no correlation (r=0.02, P value=0.7)
Arslan et al.[22] 2019	Turkey	146 infertile women NOR=86 PCOS=60	Prospective Cross-sectional study	NOR= 28.1±6.4 y PCOS=27.7±4.8 y	NOR - 9.0±6.0 ng/ml PCOS - 8.5±6.7 ng/ml	NOR- 0.031±0.019 ng/ml PCOS- 0.075±0.045 ng/ml	25(OH)D levels and AMH levels showed no correlation in either PCOS (r=−0.112, p=0.307) or NOR (r =0.027, p=0.836).
Fabris et al.[23] 2017	Spain	851 oocyte donors	Non-interventional retrospective study	18-35 y	Vitamin D replete (n=251) - ≥ 30 ng/ml Vitamin D insufficiency (n = 443) - 20-30 ng/ml Vitamin D deficiency (n = 157) - ≤ 20ng/ml	Vitamin D replete - 2.4 ± 0.2 ng/ml Vitamin D insufficiency - 2.3 ± 0.1 ng/ml Vitamin D deficiency - 2.3 ± 0.3 ng/ml	The value between vitamin D levels and AMH r = 0.059, but P-value showed no significant correlation between the two parameters.
Kokanalı et al.[24] 2018	Turkey	PCOS women Infertile=274 and Fertile=111	Retrospective case-control study	Infertile - 25.01±3.58 y Fertile women - 25.47±2.96 y	Infertile women- 11.63±5.61 ng/ml Fertile women- 15.45±6.89 ng/ml	Infertile women - 7.92±4.39 ng/ml Fertile women- 7.54±4.59 ng/ml	25(OH)D3 levels in all women included in the study correlated negatively with AMH levels (r=−0.356, p=0.016)
Bakeer et al.[25] 2018	Egypt	53 PCOS females with primary or secondary infertility and 17 Controls	Cross-sectional study	17 to 39 y PCOS- 25.96 ± 5.70 y Control - 26.24 ± 4.90 y	PCOS - 7.14±4.53 ng/ml Control - 4.24± 2.68 ng/ml	PCOS - 12.53±5.94 ng/ml Control - 19.46 ± 10.92 ng/ml	Significantly lower serum Vitamin D levels in the PCOS group vs. control. Multivariate backward regression showed no significant relation between AMH levels and 25(OH)D status.
Lata et al.[26]	India	Cases - 35 infertile fe-	Prospective study	18–40 y	Cases - 6.18±2.09 ng/ml	Cases - 1.94±1.30	No correlation between AMH and vitamin D in

Author	Country	Population	Study Design	Age (Mean, Median or Range)	Vitamin D status	AMH Levels*	Relationship between AMH levels and Vitamin D
2017		males Controls – 35 normal fertile females			Controls - 4.85±3.02 ng/ml	ng/ml Controls - 3.47±2.59 ng/ml	both the infertile and fertile groups.
Pearce et al.[27] 2015	South Australia	Women aged less than 40 y = 340	Retrospective Cohort Study	32.2±0.2 y	23±0.44 ng/ml	5.54±0.23 ng/ml	No correlation between AMH levels and vitamin D levels (r=0.04, P=0.4)
Merhi et al.[28] 2012	United States	388 premenopausal women	Prospective cross-sectional study	25-45 y (37.82 [33.11–41.4] y)	14 [9–21] ng/ml	1.12 [0.4–2.4] ng/ml	Serum levels of Vitamin D and AMH showed a weak positive correlation in late reproductive-aged women +0.011 (SE=0.005, p=0.021)
Dennis et al.[29] 2012	New Zealand	Men =113 Premenopausal women =33 Boys=4	Correlative & intervention study	Men 54-93 y Premenopausal women 19-39 y Boys 5-6 y	Median levels in Men 39.6 ng/ml	Median level in Men 2.92 ng/ml	AMH levels were correlated with the levels of 25(OH)D positively in men(r= 0.22, P=0.02) and women (r=0.60, p=000.2); while it was absent in boys (r=0.07, P=0.54)

*Cut-off values of Vit D status: Deficient (<20 ng/ml), insufficient (20–30 ng/ml), sufficient (> 30 ng/ml); NOR: Normal Ovulatory Reserve, PCOS: Polycystic Ovarian Syndrome, UEI: Unidentified Infertility, VDD: Vit D Deficiency

Table 2: Characteristic of the studies showing the relationship between Vitamin D Supplementation and AMH levels

Author	Country	Population	Study Design	Age (Range, Mean ± SD or Median)	Intervention	Vitamin D Supplementation	Effect of intervention on AMH Levels
Abdulameer et al.[30] 2019	Iraq	41 PCOS patient	Prospective interventional randomized- controlled, open-label study	18-34 y Group 1 - 24.71 ± 4.07 y Group 2 - 22.76 ± 3.8 y	Group 1 =24 Vitamin D, Group 2 =17 Co-Enzyme Q10 Supplements	10000 IU daily for 2 months	Significant decrease in AMH (P=0.002)
Dastorani et al.[31] 2018	Iran	PCOS women =40	Randomized double-blinded trial	18–40 y Vitamin D group: 29.9±4.4 y Placebo group: 30.1±3.4 y	Vitamin D (n = 17) or placebo (n = 17) *6 loss to follow up	50,000 IU alternate week for eight weeks	Serum AMH levels reduced significantly (P = 0.02) in vitamin D group (−0.7 ± 1.2 ng/ml) vs. in the placebo group (−0.1 ± 0.5 ng/ml),
Sharma et al.[32] 2018	India	PCOS females=60	Observational, comparative and interventional study	16-40 y	Vitamin D3- (n=30) no vitamin D deficiency, Control (n=30) vitamin D deficiency	60,000 IU once a week for eight weeks	Statistically significant change in mean AMH levels from baseline (4.88±2.06 ng/ml) to post treatment (3.79±2.00 ng/ml).
Naderi et al.[33] 2018	Iran	Infertile women with diminished ovarian reserve=30	Nonrandomized clinical trial	37.6 ± 2.65 y	Vitamin D3 for 3 months (n=30)	50,000 IU once a week for 3 months	AMH levels showed significant increase from 0.39 ±0.26 to 0.92 ±0.62 ng/ml
Dennis et	New Zea-	Women with regu-	Randomized	Vitamin D3	Vitamin D = 24) and placebo	50,000 IU once a week	AMH levels showed significant changes

Author	Country	Population	Study Design	Age (Range, Mean ± SD or Median)	Intervention	Vitamin D Supplementation	Effect of intervention on AMH Levels
al.[34] 2017	land	lar menstrual cy-cle=49	double-blinded trial	21.7 ± 1.1 y (19.6–24.5) Control 21.7 ± 1.4 y (19.4–25.2)	= 22		with mean rise as 1.46±0.53 a range 5.12 to 7.69 ng/ml in Vit D group.
Cappy et al.[35] 2016	France	PCOS women = 23 NOR women = 27	Prospective	18 - 43 y PCOS 27.1±4.4 NOR 30.8±5.4	According to severity of deficiency from 2 vials over 2 weeks in women with Vit D 20–29 ng/ml to 4 vials over six weeks in Vit D < 10 ng/ml	100,000 IU according to the depth of insufficiency (From 2 weeks for light deficiency to 6 weeks for deeper deficiency)	No change in the serum levels of AMH after treatment in either of the groups (PCOS or NOR)
Yanachkova et al.[36] 2021	Bulgaria	PCOS women with vitamin D deficiency =70	Monocentric, retrospective study	Vitamin D3- 26.4 (3.9) y Control- 26.1 (3.4) y	vitamin D3- (n=35), Control (n=35)	Microencapsulated liposomal form of Vitamin D3 2000 IU/ml	Significant decrease in serum AMH levels (p=0.010)
Lerchbaum et al.[37] 2021	Austria	PCOS women=180 Non-PCOS women=150	Single-centre, double-blind RCT	PCOS - 26.0 ± 5.0 y Non PCOS - 35.8 ±8.7 y	PCOS Vitamin D3 n=119, Placebo n=61 Non PCOS Vitamin D3 n=99, Placebo n=51	20,000 IU per week for 24 weeks	Decrease in serum AMH levels in PCOS women with Vitamin D supplementation, but it was not statistically significant
Irani et al.[18] 2014	USA	PCOS women = 22 Non PCOS women = 45	Prospective	PCOS women: Non-treated group 31.3±3.1 y Treated group 27.0±0.9 y Non-PCOS women: Non-treated 28.5±1.5 y Treated 28.7±1.3 y	PCOS women-16 out of 22 Controls 35 out of 45 were treated with vitamin D	50,000 IU once a week for eight weeks	Decrease in AMH levels in women with PCOS after vitamin D3 supplementation

*Cut-off values of Vitamin D status: Deficient (< 20 ng/ml), insufficient (20–30 ng/ml), sufficient (> 30 ng/ml); NOR: Normal Ovulatory Reserve, PCOS: Polycystic Ovarian Syndrome

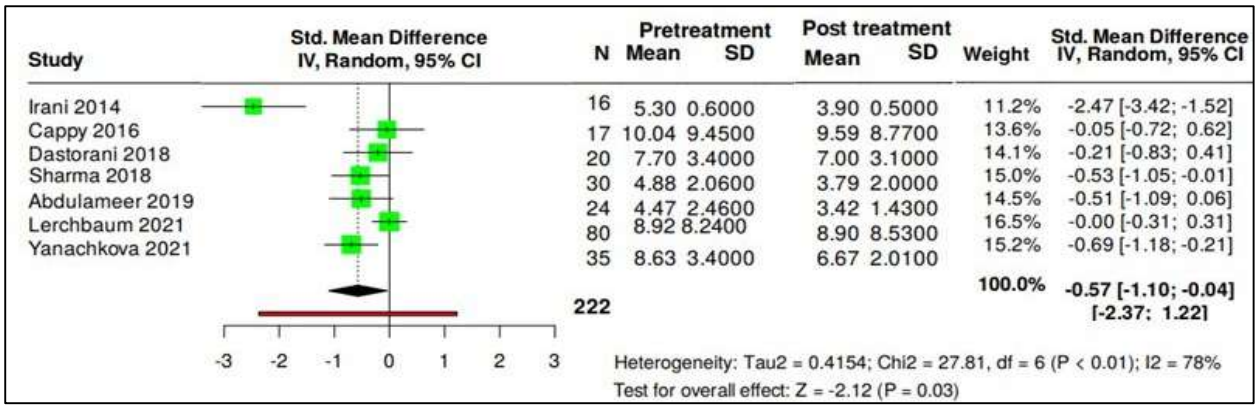

Figure 3: Forest plot showing effects of vitamin D supplements on serum levels of AMH in PCOS women

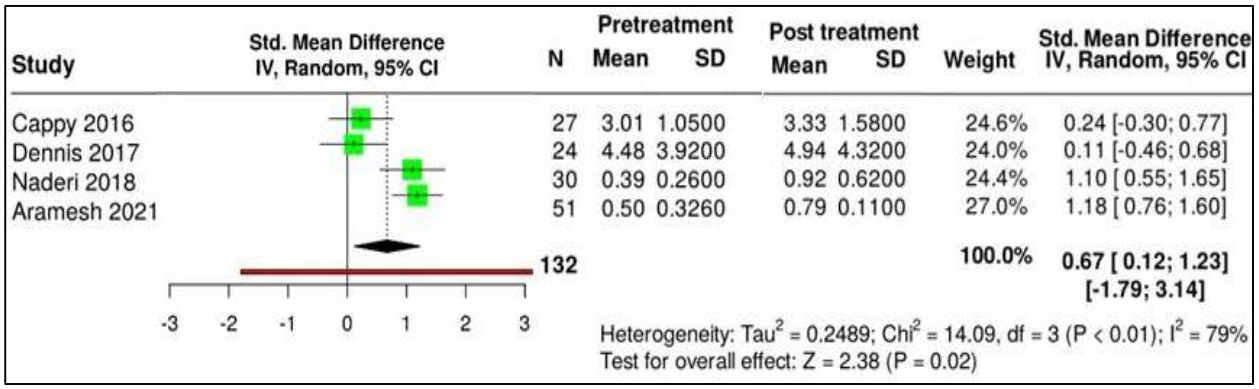

Figure 4: Forest plot showing effects of vitamin D supplements on serum levels of AMH in Non-PCOS women

DISCUSSION

The present systematic review and meta-analysis provided valuable insights into the relationship between vitamin D levels and AMH levels, particularly in women with polycystic ovary syndrome (PCOS). Several studies report a decrease in AMH levels following vitamin D supplementation in PCOS women and modest increases in non-PCOS populations. Although the studies reviewed showed mixed results, certain trends and patterns emerged, underscoring the complexity of the interrelationship of vitamin D supplementation with AMH levels in various populations. While these findings suggest a biological plausibility of vitamin D as a modulator of ovarian function, the inconsistency suggests that the association between AMH and vitamin D is affected by multiple biological and methodological factors.

The connection between AMH and vitamin D is stronger in PCOS subjects as compared to non-PCOS women. Abdulameer et al.,[30] Dastorani et al.,[31] and Yanachkova et al.,[36] have showed that after giving vitamin D supplements, there was a significant reduction in the levels of AMH, suggesting its role in hormonal dysregulations of the disease. It is hypothesised that decreased sensitivity of FSH for antral follicles causing follicular arrest can be due to elevated AMH levels in PCOS, so vitamin D supplements can decrease AMH levels, improving FSH sensitivity and better ovulatory functions.[39]

Some contrasting findings were also noted by Merhi et al.[28] and Lerchbaum et al.,[37] having slight reductions in AMH levels or no significant correlation with vitamin D. A few other studies conducted in PCOS women also found similar results regarding AMH levels.[20,40] Differences in the study design, patient population, and vitamin D supplementation - dosage, form, frequency and duration can lead to intervention bias and variability of the results. Moreover, variations in the baseline vitamin D levels may also affect the outcomes. Some studies included only vitamin D-deficient women, while others included mixed groups, leading to non-comparable populations and thus, selection bias. Measurement bias also remains a concern, as different assays for AMH and vitamin D can yield variable results. Thus, the lack of evidence-based supplementation guidelines, especially in PCOS women, further emphasized the negligible correlation between AMH and vitamin D, indicating their mixed effects on ovarian as well as follicular functions and development. Similar observations were made by Karimi et al.[40] and Cochrane et al.[41] in their systematic reviews. Thus, clinical indications of vitamin D supplements on AMH levels are still under research, but to date it is hypothesized that vitamin D can provide therapeutic benefits in PCOS and health of women regarding ovarian functions.[42]

Naderi et al.[33] and Cappy et al.[35] found variations regarding the association of AMH and vitamin D in non-PCOS women. A positive effect on ovarian function, fol-

licular growth, and reproductive health in non-PCOS groups was found after vitamin D supplementation influenced AMH levels to rise, which was in line with the hypothesis.[6,43,44] There are also some studies in women without PCOS with contraindicatory results in the area of change in AMH levels by vitamin D supplementation.[45] Thus, the advantageous influence of vitamin D supplementation on ovarian reserve in non-PCOS females is still in a grey area. This unpredictability leads to the hypothesis that the effects of supplementation with vitamin D can be based on multiple factors like age, primary reproductive health, and baseline vitamin D levels.

Insulin resistance and obesity are significant metabolic problems in PCOS, which may be associated with increased AMH levels.[46] These metabolic imbalances can result in various clinical symptoms such as acne, hirsutism, and menstrual irregularities, making AMH a crucial biomarker for diagnosing and predicting PCOS. In such cases, vitamin D supplementation may drastically produce improved metabolic and hormonal outcomes along with reduced cardiovascular risk as well.[5,47,48] It was also noted in literature published recently that glucose metabolism and insulin resistance are improved with vitamin D supplementation given continuously at doses less than 4000 IU/day, along with beneficial effects on menstrual frequency and hyperandrogenism. [5,49-51]

Studies reviewed in the present exploration have underscored the key challenges for better comprehension of the association of vitamin D status and AMH. A variability of study design, study population, smaller sample sizes in the selected studies with considerable variation in baseline vitamin D status, diagnostic criteria for PCOS, metabolic profile, subject demographics, measurement techniques for AMH, and difference in duration and dosage of vitamin D supplementation may be the grounds for the diversity of the results and cause difficulties in drawing inferences about the causality.

From a clinical perspective, although vitamin D supplementation shows improvements in menstrual regularity, insulin sensitivity, and hyperandrogenism in PCOS, the evidence for a direct role in modulating AMH remains inconclusive. Current findings do not justify routine use of vitamin D supplementation solely for the purpose of altering AMH levels or improving ovarian reserve. Instead, supplementation should continue to be guided by established recommendations for bone and metabolic health, with potential reproductive benefits considered secondary. Personalised approaches like factoring in age, baseline vitamin D levels, metabolic profile, and genetic predispositions may eventually help identify subgroups of women most likely to benefit from supplementation.

Future research should prioritise well-designed, adequately powered randomised controlled trials with standardised AMH and vitamin D assays, consistent supplementation protocols, and adjustment for metabolic and lifestyle confounders. Longitudinal studies are also needed to determine whether modulation of AMH by vitamin D translates into clinically meaningful improvements in ovulation, fertility, or long-term reproductive health.

CONCLUSION

The present systematic review, along with meta-analysis, determines the budding use of vitamin D supplementation in modulating AMH levels and improving ovarian function, particularly in vitamin D- deficient women with PCOS. Genetic variability in the AMH signalling pathways and vitamin D receptor polymorphism also points towards the fact that personalized treatment approaches with dose modulations may positively impact the outcome responses in women with PCOS.

While the evidence is promising, the variability in results, combined with the challenges, further demands high-quality studies that can help in better understanding mechanisms, optimizing supplementation strategies, and determining long-term impact on reproductive health outcomes. Until stronger evidence emerges, the therapeutic role of vitamin D in modifying AMH levels should be regarded as promising but unproven.

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