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The Effect of Vitamin D Supplementation on Serum Levels of Anti-Müllerian Hormone

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ABSTRACT

Background and Objective: Anti-Müllerian hormone is used as an indicator of ovarian reserve in the diagnosis of infertility. Vitamin D regulates the serum levels of this hormone. Considering the importance of nutritional factors in the reproductive process, the aim of the present study is to summarize the available evidence on the effect of vitamin D supplementation on serum levels of Anti-Müllerian hormone.

Methods: In the present systematic review and meta-analysis, Medline, Scopus and Web of Science databases were searched until December 2023 without time limit using the keywords "vitamin D", "Anti-Müllerian hormone" and related synonyms. Interventional studies aimed at investigating the effect of vitamin D on the serum levels of Anti-Müllerian hormone were included in the study. Observational, laboratory or combined intervention studies (combination of vitamin D and other supplements) were excluded.

Findings: From a total of 82 retrieved studies, 75 studies were excluded due to various reasons (lack of sufficient data, combined intervention, Anti-Müllerian hormone evaluation, laboratory study) and finally seven studies with a total of 282 participants were included in meta-analysis. The standardized mean difference between the two intervention groups and the control group was -0.09 (95% CI: -0.73 to 0.56), which indicated the ineffectiveness of vitamin D on increasing the level of Anti-Müllerian hormone. After aggregating the standardized mean difference of women with polycystic ovary syndrome, no statistically significant difference was seen between the intervention and control groups (standardized mean difference: -0.63, 95% CI: -1.74 to 0.47). Also, the integrated standardized mean difference among women without polycystic ovary syndrome was reported to be non-significant (standardized mean difference: 0.41, 95% CI: -0.01 to 0.84).

Conclusion: According to the available evidence, vitamin D supplementation is not effective on the serum level of Anti-Müllerian hormone. Conducting multiple clinical trials with a large sample size is recommended.

Keywords: *Anti-Müllerian Hormone, Vitamin D, Interventional Study.*

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Introduction

Vitamin D is a steroid hormone that plays an important role in calcium homeostasis and bone metabolism (1). Vitamin D is synthesized endogenously with the proximity of the skin to sunlight and also absorbed through food consumption. Cholecalciferol (D3) is produced in the skin from 7-dehydrocholesterol by ultraviolet rays, and after two hydroxylation processes in the liver and kidney, it is converted into 25-hydroxyvitamin D, the active form of 1,25-dihydroxyvitamin D. The level of 25-hydroxyvitamin D in blood can be easily measured and is considered the best indicator of vitamin D status in humans (2). The serum level of 30 ng/ml of vitamin D is necessary for human physical health and the daily limit is 400 units. Although in elderly people and in the conditions of insufficient intake of vitamin D, it is necessary to consume 800 to 1000 units of its supplement per day (3).

Most of the actions of vitamin D are mediated by the nuclear vitamin D receptor in more than 30 different tissues, such as the skeleton, brain, breast, pancreas, parathyroid glands, and ovaries (4). Therefore, the lack of this vitamin may cause a wide range of extra-skeletal effects such as cardiovascular diseases, breast and ovarian cancer, autoimmune diseases and mental disorders such as Alzheimer's and Parkinson's disease (5-10). Evidence has shown the association between low vitamin D concentrations and various pathological conditions including metabolic disorders (11), hypogonadism (12), polycystic ovary syndrome (PCOS) (13) and reduced female fertility, and several clinical studies have shown its potential benefits on different aspects of human reproduction (14). There is a hypothesis that vitamin D plays a role in ovarian follicular growth and luteinization by acting on Anti-Müllerian hormone (15, 16).

Anti-Müllerian hormone is a 140 kDa glycoprotein hormone belonging to the transforming growth factor beta (TGF-β) family, which is also known as Müllerian inhibitory substance. This hormone is essential for normal sexual differentiation in males and is responsible for the regression of the Müllerian duct during the first trimester of the fetal development of a male (17). The level of this hormone in girls is low until the age of puberty. In the pre-puberty period, its production begins in the ovaries and its level increases (18, 19). The serum levels of Anti-Müllerian hormone in women decreases with age and reaches undetectable levels after menopause (20). Follicle-stimulating hormone (FSH) is a known stimulus for the secretion of this hormone, and the increase in testosterone during puberty also indirectly reduces serum Anti-Müllerian hormone by facilitating Sertoli cell development (21). Furthermore, serum levels of Anti-Müllerian hormone are associated with follicle-stimulating hormone, Inhibin B, testis size, and high risk of ovarian hyperstimulation syndrome (22, 23). The measurement of this hormone in serum is used as an indicator of ovarian reserve in the diagnosis of infertility and diseases such as polycystic ovary syndrome (24).

The results of laboratory studies and animal models show that vitamin D regulates Anti-Müllerian hormone levels in vitro, both directly through the Anti-Müllerian hormone promoter and indirectly by regulating the number of granulosa cells and Anti-Müllerian hormone signaling, and altering follicle-stimulating hormone sensitivity as well as progesterone production and release in ovarian follicle cultures (25, 26). Also, the results of a study conducted by Kinuta et al. show that female mice lacking the vitamin D receptor suffer from ovarian failure, which is characterized by impaired follicular development (27). Despite the consistency of laboratory data, the effect of vitamin D on the levels of Anti-Müllerian hormone production in women is controversial. The results of several studies show that the vitamin D supplements improve the menstrual cycle and hyperandrogenism by affecting various aspects of women's fertility (28, 29) and its deficiency is associated with various clinical manifestations such as polycystic ovary syndrome, anovulation and insulin resistance (30-32). However, the mechanism of action of vitamin D on various aspects of fertility in women remains unknown. Recently, several interventional studies have been conducted with the aim of evaluating the effect of vitamin D levels and serum Anti-Müllerian hormone

levels, which have yielded very contradictory results. Several studies show a positive and significant relationship between vitamin D and markers of ovarian reserve, especially Anti-Müllerian hormone (33). In a study in 2021, it has been found that vitamin D consumption for three months is associated with an increase in serum levels of Anti-Müllerian hormone (34). Meanwhile, other studies have reported negative findings or no effect of vitamin D on the serum levels of Anti-Müllerian hormone (35).

Considering the importance of the role of environmental factors including the consumption of vitamin supplements on the factors involved in fertility and on the other hand, considering the increase in diseases involved in fertility and the importance of fertility and infertility in today's societies, the present systematic review was conducted to evaluate the effect of vitamin D on the level of Anti-Müllerian hormone as a marker of ovarian reserve in interventional studies.

Methods

The present systematic review and meta-analysis was performed in accordance with the published guidelines for systematic review articles and meta-analysis of randomized and controlled trials (PRISMA) and was registered in International Prospective Register of Systematic Reviews (PROSPERO) with the code CRD42023430406.

Two researchers independently searched Medline, Scopus, and Web of Science international databases for three months to retrieve randomized and non-randomized or quasi-experimental clinical trials since the establishment of these databases until December 2023 without language and time limit. Persian websites and published and unpublished theses were also examined. The search terms used in this study included vitamin D and Anti-Müllerian hormone and their synonyms. The search details and keywords used are shown in Appendix 1. Then, using OR and AND operators, these words were combined with each other and the search strategy was prepared. For each database, this search strategy was adapted according to the guidelines of each database. Also, a manual search of the references of the entered studies was also done through the Google Scholar search engine to find similar studies.

All articles were independently reviewed by two researchers. In order to screen the studies, first the title of the article was reviewed, and after removing the irrelevant articles, the abstract and the full text of the studies were reviewed. In case of contradiction, the researchers reviewed the studies by receiving consultation and guidance from the expert researcher. Studies that met the inclusion criteria were selected for further review. The inclusion criteria were as follows: interventional studies, including randomized and non-randomized controlled trials published in English or Persian, that investigated the effect of vitamin D or other forms of vitamin D, either vitamin D2 or D3, on the serum levels of Anti-Müllerian hormone in fertile or infertile women, and had the defined keywords (Appendix 1) in their title or keywords section. Combination intervention studies (combination of vitamin D with other supplements and vitamins), animal studies, studies that measured Anti-Müllerian hormone in seminal fluid or follicular fluid, and observational studies that investigated the relationship between vitamin D and Anti-Müllerian hormone levels were excluded from the study. Articles presented in conferences, review articles and letters to the editor, case reports and articles whose results were incompletely reported were excluded from the study.

The information of studies that were included in the present systematic review and meta-analysis, including basic information (first author, country, type of study, sample size), details regarding the therapeutic intervention including dose and duration of use, relevant outcome indicators and study results were extracted. The quality of included studies was assessed by two researchers using the Risk of Bias 2 (RoB 2) tool for randomized clinical trials and JBI for quasi-experimental studies. Finally, each study was rated as "low risk" of bias, "high risk" of bias, or "unknown risk".

Considering the inclusion of studies with a quasi-experimental design (4 studies; before and after), the effect size of each study was calculated as a standardized mean difference (the difference between the mean levels of Anti-Müllerian hormone before and after in the intervention group) with 95% confidence interval. The effect size indices of the studies (standardized mean difference) were integrated and reported using the random effects model. Based on this model, it is assumed that the parameter of each study is different and the difference between the effect size indices is due to the sampling error and the real difference (τ^2 index) between the parameters of the studies. Due to the low number of studies and the high dispersion of the effect size indices, the Restricted Maximum Likelihood Estimation (RMLE) method was used to calculate the real difference between the studies. Heterogeneity between studies was calculated and reported using the I^2 index. I^2 index values less than 25% indicate low heterogeneity, 25-75% indicate heterogeneity and above 75% indicate high heterogeneity. In order to explain the heterogeneity between studies, subgroup analysis was performed based on the type of population (with and without polycystic ovary syndrome) and the type of study (randomized clinical trial and quasi-experimental). Due to the low number of retrieved studies, publication bias was assessed using a funnel plot. Data analysis was performed using Stata 17 (StataCorp LLC., College Station, TX), and $p<0.05$ was considered significant.

Results

In the initial search, 599 studies were retrieved. After removing 254 duplicate cases, 145 review articles, 28 cohort studies, 87 cross-sectional studies and three case-report studies were also excluded by screening. Finally, from a total of 82 interventional studies, seven studies were included in the meta-analysis. Of these seven studies, three were experimental and four were quasi-experimental. Figure 1 summarizes the studies that were selected for inclusion in the initial search, studies that were excluded and studies that were finally included.

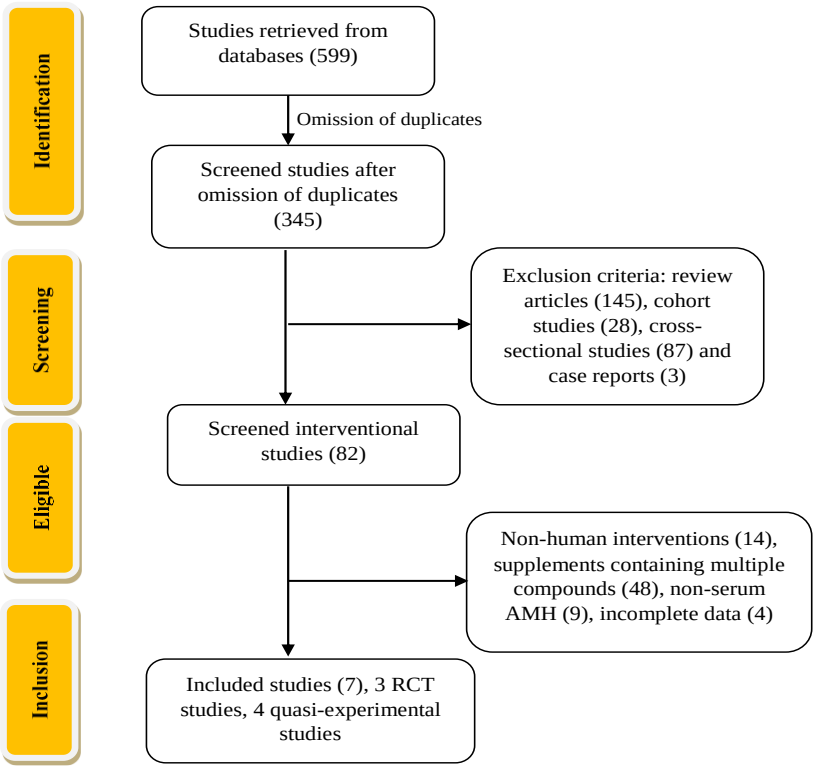

Figure 1. The process of the inclusion of studies in the systematic review and meta-analysis

In the seven selected studies, a total of 282 participants including 92 infertile women, 139 women with polycystic ovary syndrome and 51 healthy women were examined. The age range of the participants was between 18 and 35 years. All studies, except for one study, measured the level of Anti-Müllerian hormone by ELISA method. Of the seven mentioned studies, four studies prescribed vitamin D in a dose of 50,000 units and three studies prescribed a dose of 20,000 or 30,000 international units (33, 35-40) (Table 1). The quality assessment of the studies selected for meta-analysis is also shown in Appendixes 2 and 3.

Table 1. Characteristics of the initial studies included in the systematic review and meta-analysis (33, 35-40)

Author/year/ reference	Country	Type of study	Study population	Age based on years (mean)	Details of the intervention	Anti-Müllerian hormone evaluation method
Bacanakgil, 2022, 33	Turkey	quasi-experimental	Infertile women (n=62)	18-41	30,000 IU of vitamin D once a month for 2 months	ELISA
Cappy, 2016, 35	France	quasi-experimental	healthy women (n=23), Women with polycystic ovary syndrome (n=27)	Healthy women: 30.8, Women with polycystic ovary syndrome: 27.1	2 or 3 vials containing 10,000 IU of vitamin D once a week for 2 to 6 weeks	ELISA
Dastorani, 2018, 36	Iran	Randomized clinical trial	Infertile women (n=20)	Women in the intervention group: 29.9 Women in the placebo group: 30.1	50000 IU of vitamin D or placebo once a week for 8 weeks	ELISA
Dennis, 2017, 37	New Zealand	Randomized clinical trial	Women in the intervention group (n=27) Women in the placebo group (n=22)	Women in the intervention group: 21.7 Women in the placebo group: 21.7	50000 IU of vitamin D or placebo once a week for 1 week	ELISA
Irani, 2014, 38	USA	quasi-experimental	Healthy women (n=45) Women with polycystic ovary syndrome (n=22)	Women in the intervention group: 28 and 28.7 Women in the placebo group: 31.3 and 28.5	16 people with polycystic ovary syndrome and 35 people from the control group received 50,000 IU of vitamin D3 orally once a week for 8 weeks.	ELISA
Lerchbaum, 2021, 39	Austria	Randomized clinical trial	Women with polycystic ovary syndrome (n=80)	Women in the intervention group: 25.4 Women in the placebo group: 27.2	20000 IU of vitamin D per week for 24 weeks	ELISA
Naderi, 2018, 40	Iran	quasi-experimental	Infertile women (n=30)	Over 35 years old	50000 IU of vitamin D orally once a week for 3 months	Automated Elecsys assay (Roche. Diagnostics)

Randomization was done correctly in all three studies. Allocation concealment was not properly performed in the study of Dastorani et al. (36) and was properly performed and reported in the other two studies. Blinding of participants and personnel in one study and blinding of the outcome evaluator were incorrect in one study. The data analysis in the study of Dastorani et al. (36) was based on the available data without considering the samples excluded from the study. In the study of Dennis et al. (37), data analysis was ambiguous, and in the study of Lerchbaum et al. (39), data analysis was based on intention-to-treat analysis. All the results recorded in the study protocol were reflected in the article and there was no so-called reporting error. In general, the clinical trials had an acceptable methodological quality. Quasi-experimental studies were generally acceptable in terms of methodology and in terms of distortion, selection and measurement errors were at an acceptable level. The outcome of the study (Anti-Müllerian hormone level) was measured correctly and with the same method before and after the intervention in all four studies. The reliability of measuring the level of Anti-Müllerian hormone before and after the intervention was favorable and error-free. Data analysis in all four studies was done based on a standard and acceptable method. However, in two studies, the number of samples analyzed before and after the study was different, and some samples were excluded from the study, but this exclusion was small and did not lead to a selection error or decrease in the power of the study.

The effect of vitamin D intervention on Anti-Müllerian hormone levels: The findings of seven studies were combined in the meta-analysis. The standardized mean difference between the two intervention groups and the control group was -0.09 (95% CI: -0.73 to 0.56) and there was no statistically significant difference between the two groups, indicating that vitamin D has no effect on the level of Anti-Müllerian hormone (p=0.79). The heterogeneity between the studies was reported to be high (I^2=92.6), which was statistically significant (p<0.001) (Figure 2).

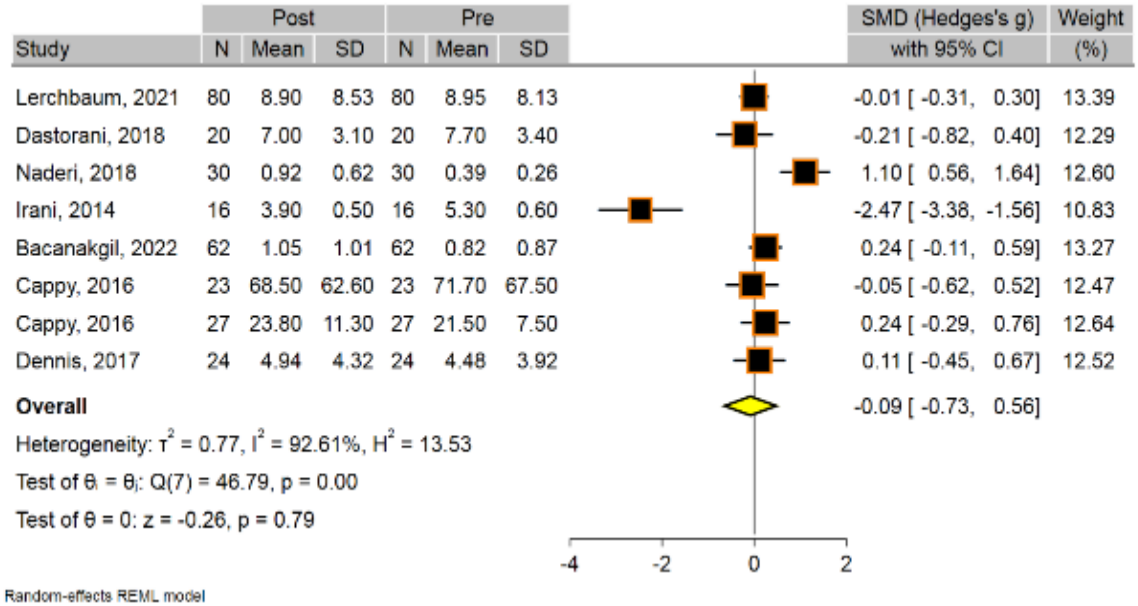

Figure 2. The effect of vitamin D supplementation on the serum level of Anti-Müllerian hormone

Subgroup analysis showed similar and non-significant findings. After aggregating the standardized mean difference of four studies on women with polycystic ovary syndrome, no statistically significant difference was seen between the intervention and control groups (standardized mean difference: -0.63, 95% CI: -1.74 to 47.0, $I^2 = 93.8$). Furthermore, the integrated standardized mean difference in studies conducted on women not suffering from polycystic ovary syndrome was also reported to be non-significant (standardized mean difference: 0.41, 95% CI: -0.01 to 0.84, $I^2 = 67.5$) (Figure 3).

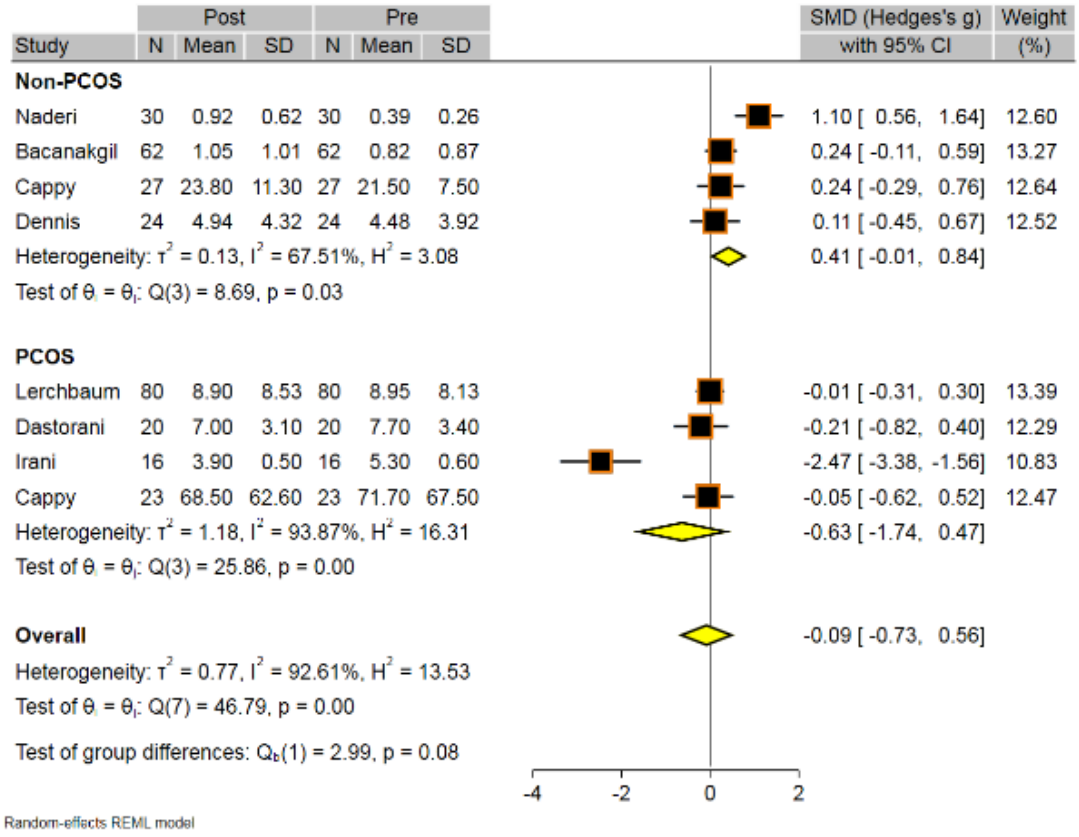

Figure 3. Subgroup analysis showing the effect of vitamin D supplementation on the serum level of Anti-Müllerian hormone in women with polycystic ovary syndrome and women without polycystic ovary syndrome

Three clinical trials with a sample size of 150 people investigated the effect of vitamin D on the level of Anti-Müllerian hormone, and after aggregating the standardized mean difference, vitamin D supplementation showed no significant effect (-0.02, 95% CI: -0.26 to 0.23, $I^2 = 0$). This finding was also seen in four quasi-experimental studies in a similar and non-significant way (-0.15, 95% CI: -1.28 to 0.98, $I^2 = 95.5$). (Figure 4).

Although the number of studies was less than ten, and according to the Cochrane guideline, it is not recommended to check the publication bias in these cases, but in general, a symmetrical graph distribution was seen (regardless of one study) (Figure 5).

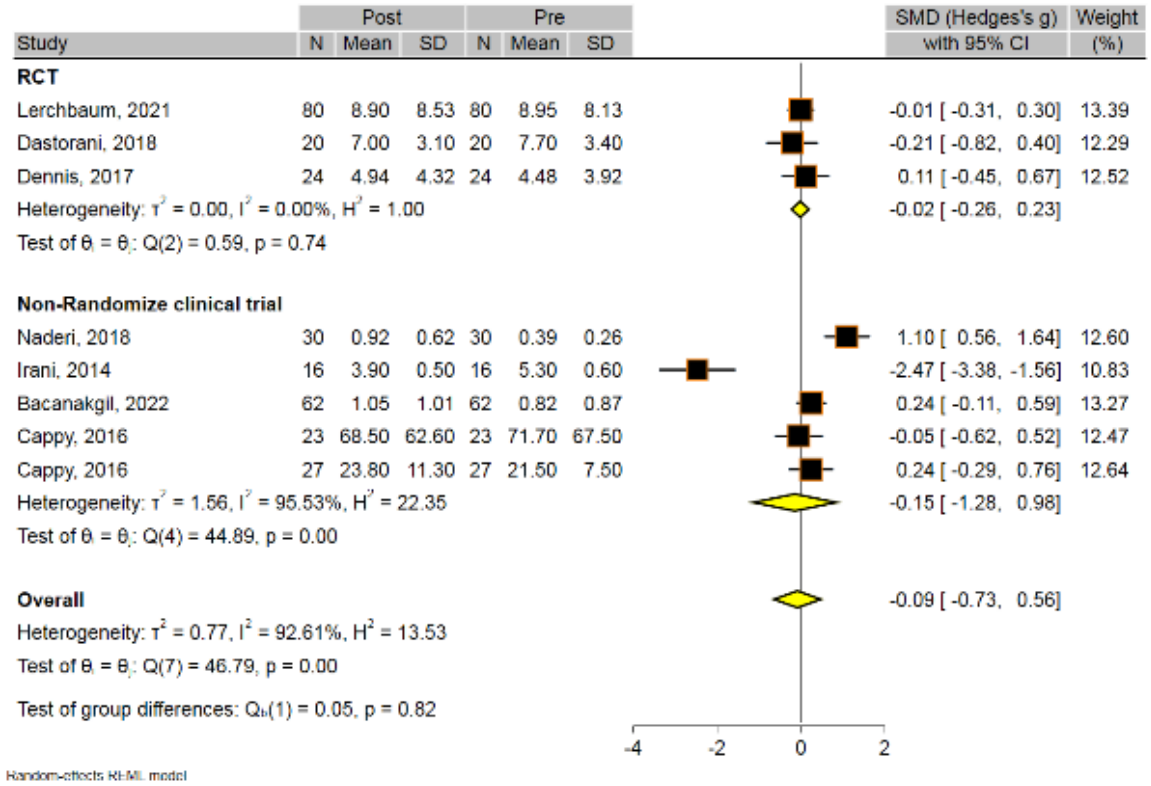

Figure 4. Subgroup analysis of the effect of vitamin D supplementation on the serum level of Anti-Müllerian hormone based on the type of study

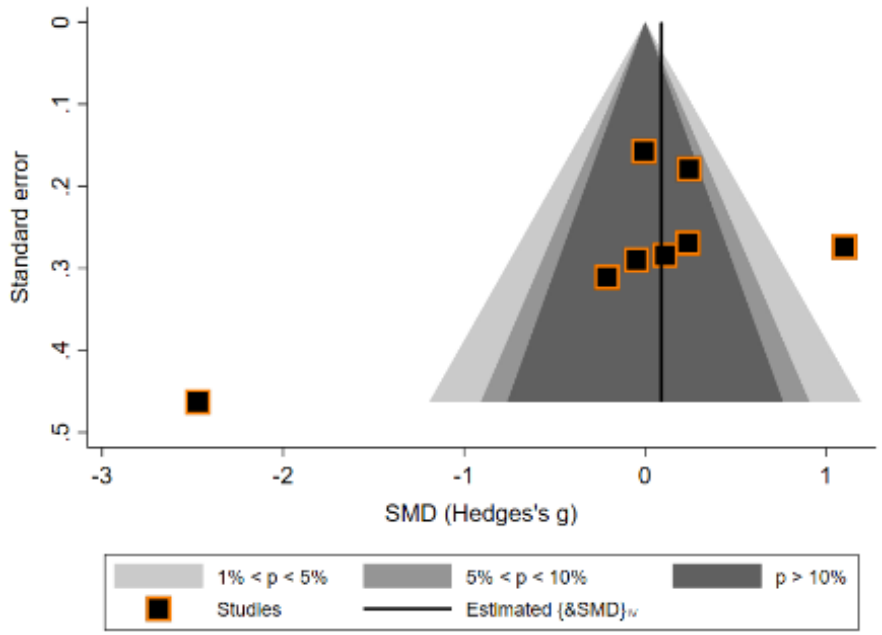

Figure 5. Funnel plot of the effect of vitamin D supplementation on the serum level of Anti-Müllerian hormone

Discussion

The results of the present study showed that the consumption of vitamin D supplements does not affect the serum levels of Anti-Müllerian hormone. Among the seven interventional studies included in this systematic review, three studies reported an increase in serum levels of Anti-Müllerian hormone following vitamin D supplementation. In a study by Dennis et al. (37) which examined the effect of vitamin D supplementation on the circulating levels of Anti-Müllerian hormone in women aged 19 to 24 years, with a steady increase in vitamin D supplementation, serum levels of Anti-Müllerian hormone gradually increased. Moreover, the results of a study by Naderi et al. showed that the serum level of Anti-Müllerian hormone increases significantly after treatment with vitamin D (40). It was also found that the average level of this hormone in women with sufficient vitamin D was higher than in women with insufficient vitamin D. However, in another study by Irani et al. (38), the serum level of Anti-Müllerian hormone in women with polycystic syndrome was significantly reduced with vitamin D supplementation. Nevertheless, in four out of seven studies, no change was observed in the level of Anti-Müllerian hormone following the consumption of D supplements. The results of examining the effect of vitamin D on Anti-Müllerian hormone and other endocrine markers in women with polycystic ovary syndrome by Lerchbaum et al. showed that vitamin D treatment for 24 weeks has no significant effect on Anti-Müllerian hormone level (39). In another study by Dastorani et al. (36) and Cappy et al. (35), no difference was observed in serum levels of Anti-Müllerian hormone before and after treatment. Therefore, even though both vitamin D and Anti-Müllerian hormone play a role in reproductive health, their mutual effects and specific effects on each other are not fully known. Studies that have investigated this effect have presented contradictory results. Therefore, the evidence is still not enough to make a definitive conclusion.

A new meta-analysis with the two objectives of examining the relationship between vitamin D levels and Anti-Müllerian hormone and the effectiveness of vitamin D supplementation on Anti-Müllerian hormone was conducted in 2020 by Moridi et al. (41). A total of 18 observational studies and six interventional studies were retrieved, with conflicting results. But in general, most studies (more than 90%) did not observe a relationship between vitamin D levels and Anti-Müllerian hormone. In some studies, even after controlling the effect of confounding variables such as age and body mass index, they still reported the relationship as non-significant. By aggregating six interventional studies, the authors of the above-mentioned meta-analysis reported the overall effectiveness of vitamin D on Anti-Müllerian hormone as non-significant (standardized mean difference: -0.16, 95% CI: -0.90 to 0.58, $I^2 = 89.1$). However, with subgroup analysis and based on the presence or absence of polycystic ovary syndrome, they observed different results. They discovered that the use of vitamin D supplements in women without polycystic ovary syndrome was associated with an increase in the serum level of Anti-Müllerian hormone (standardized mean difference: 0.49, 95% CI: 0.17 to 0.80, $I^2 = 73.9$) and in women with polycystic ovary syndrome, it was associated with a decrease in the serum level of Anti-Müllerian hormone (standardized mean difference: -0.53, 95% CI: -0.15 to -0.91, $I^2 = 90.7$) (41). It is worth noting that this effect was not observed in our meta-analysis; there was no significant difference in the serum level of Anti-Müllerian hormone between women without polycystic ovary syndrome and women with polycystic ovary syndrome.

The main reason for the difference between the results of our study and the aforementioned meta-analysis is the number of studies (we recovered two more studies) and also the lack of attention to the effect size index (standardized mean difference) in the studies of Irani et al. and Naderi et al. in the aforementioned meta-analysis. In general, the number of interventional studies is small in this area, and the effect of a particular study (with a large effect size index) on the integrated effect size index is certain. In the study of Irani et al., the standardized mean difference is very large and negative. This study was in the subgroup of women with polycystic ovary syndrome, so the integrated standardized mean difference affected by this

study has become negative and significant. In the study of Naderi et al., the standardized mean difference is very large and positive. This study related to the subgroup of women without polycystic ovary syndrome, so the integrated standardized mean difference affected by this study has become positive and significant. In the present meta-analysis, there was one clinical trial study and one quasi-experimental study more than the meta-analysis of Moridi et al., and by adding the standardized mean difference of these two studies to the overall results, we found non-significant results both in the subgroup of women with and without polycystic ovary syndrome.

The recommendation of experts in the field of meta-analysis is to perform sensitivity analysis with different scenarios. This approach is especially necessary in structured review articles with few initial studies. After the exclusion of studies by Irani et al. and Naderi et al. (they were excluded from the meta-analysis once individually and once together), the findings were seen as completely different and non-significant. In the meta-analysis of Moridi et al. (41), sensitivity analysis was not performed, and it is an obvious weakness in its data analysis.

It seems that several factors can play a role in noneffectiveness of vitamin D consumption on serum levels of Anti-Müllerian hormone. The regulation of reproductive hormones and markers, including Anti-Müllerian hormone, is a complex process that is influenced by various factors (42). This hormone is regulated at different levels of gene expression, transcription, translation and multiple post-translational processes (43). Also, factors such as aging and seasonal changes are associated with changes in the levels of this hormone (44). Vitamin D affects various aspects of pregnancy health (45). However, its exact mechanisms and effects on specific reproductive markers are still under investigation. Accordingly, Grzechocinska et al. showed that vitamin D has a direct effect on the production of Anti-Müllerian hormone. Therefore, patients with higher concentrations of vitamin D can maintain their ovarian reserves for a longer period of time (46).

The results of a recent meta-analysis that examined the fertility rate among 2700 infertile women show that there is a significant relationship between the levels of vitamin D and higher chances of fertility and live birth. In this regard, women who have sufficient levels of vitamin D, compared to women who are faced with vitamin D deficiency, have a higher probability of live birth (odds ratio: 1.33, 95% CI: 1.08 to 1.65) and a higher probability to get a positive pregnancy test (odds ratio: 1.34, 95% CI: 1.04 to 1.72) (47). However, it is necessary to mention that vitamin D is only one of many factors affecting reproductive health and its effect may vary among people (48). The role of various factors, including nutritional factors, insulin resistance, and various diseases, such as polycystic ovary syndrome, on reproductive health has been previously proven (49, 50). Moreover, people differ in the basic levels of vitamin D, genetics and other factors that can affect how the body responds to vitamin D supplementation (51). In this regard, Blum et al. showed that serum vitamin D response to vitamin D supplementation is inversely proportional to body mass index (52). It has also been found that vitamin D serum levels differ after receiving vitamin D supplements in people with different genetic changes in DBP, CYP2R1, CYP27B14 and CYP24A1 genes (53). Therefore, this individual variation can be the basis of different results in studies.

Although many observational studies have investigated the relationship between vitamin D levels and Anti-Müllerian hormone (54-56), interventional studies that have investigated the effect of vitamin D supplementation on Anti-Müllerian hormone levels are limited. These studies often face challenges in terms of study duration, dose and participant compliance. Thus, in the current meta-analysis study, the duration of vitamin D supplementation varies from 2 weeks to 24 weeks. The results of the studies show that the changes caused by taking vitamin D supplements do not work in the short term. It has been found that the

highest increase in vitamin D level was between three and nine months after starting to take the supplements (57). Therefore, new studies may provide more information about the effect of vitamin D on Anti-Müllerian hormone levels by controlling conditions such as optimal duration of vitamin D supplementation, appropriate dosage, and participants' compliance.

One of the strengths of the present study is the comprehensive review of studies conducted to investigate the effect of vitamin D supplementation on the serum levels of Anti-Müllerian hormone. In a recent meta-analysis, five studies with a total of 140 women were examined, but the current study with a sample size of 282 provides more reliable results. However, in general, the number of clinical trials conducted in this field is very small. The small number of studies makes it impossible to analyze multiple subgroups such as dose or duration of intervention. In this study, subgroup analysis was only performed based on the type of study and presence or absence of polycystic ovary syndrome. Even though all valid public and specialized databases were searched with suitable and extensive keywords, distortion of meaning is still possible. Since the number of studies included in the meta-analysis was less than 10, the relative symmetry of the funnel plot cannot be regarded as a definite absence of publication bias, and it is possible that small and newer studies were not retrieved.

Based on the results of the present meta-analysis, the effect of vitamin D supplementation on the serum levels of Anti-Müllerian hormone is not significant. Due to the small number of studies, there is not enough clinical evidence for a definite effect of vitamin D on the serum level of Anti-Müllerian hormone. Therefore, new studies may provide more insight into the effect of vitamin D on reproductive health, including its effect on levels of Anti-Müllerian hormone.

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