

Impact of Magnesium and Vitamin D Supplementation on Serum Antimüllerian Hormone and Follicle Stimulating Hormone in Women with Infertility

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ARTICLE INFO	ABSTRACT
*Correspondence to Rabea Ejaz Department of Zoology, Rawalpindi Women University-46300, Pakistan. Email: rabea.ejaz@rwu.edu.pk Article History Received: 21-07-2026 Revised: 13-09-2026 Accepted: 19-09-2026 Published: 30-09-2026 How to Cite Sagheer F, Ejaz R, Ahmad S, Qazi S, Qamer S, Ambreen F, Gul T, Iqbal M. Impact of Magnesium and Vitamin D Supplementation on Serum Antimüllerian Hormone and Follicle Stimulating Hormone in Women with Infertility. Pak J Clin Res. 2026;3(9):32-38. doi:10.65761/pjcr.2026.336 DOI: https://doi.org/10.65761/pjcr.2026.336	Background: Infertility is influenced by multiple reproductive, endocrine, metabolic, and nutritional factors. Anti-Müllerian hormone (AMH) and follicle-stimulating hormone (FSH) are established biochemical markers used in the assessment of ovarian function. Evidence regarding the effects of combined magnesium and vitamin D supplementation on these markers in women with infertility remains limited. Objective: To evaluate the association of 60 days of combined magnesium and vitamin D supplementation with changes in serum AMH and FSH concentrations among infertile women aged 25–35 years. Methods: This single-arm, quasi-experimental pre-post intervention study included 40 infertile women aged 25–35 years. Participants received magnesium 150 mg/day and cholecalciferol 2000 IU/day for 60 consecutive days. Serum AMH and FSH were measured before supplementation and after completion of the intervention during the early follicular phase. Paired-samples t-tests were used to compare baseline and post-intervention concentrations. Results: Mean serum AMH increased from 0.898 ± 0.226 to 1.137 ± 0.235 ng/mL, representing a mean increase of 0.239 ng/mL (95% CI: 0.164–0.315; $t(39) = -6.392$; $p < 0.001$). Mean FSH decreased from 11.925 ± 0.987 to 11.720 ± 1.000 mIU/mL, with a mean reduction of 0.205 mIU/mL (95% CI: 0.174–0.236; $t(39) = 13.514$; $p < 0.001$). Significant AMH increases and FSH reductions were observed in both age groups. Conclusion: Sixty days of combined magnesium and vitamin D supplementation was associated with increased AMH and modestly reduced FSH concentrations in infertile women aged 25–35 years. Controlled randomized trials are required to determine causality and clinical fertility relevance. Keywords: Infertility, Anti-Müllerian Hormone, Follicle-Stimulating Hormone, Magnesium, Vitamin D Supplements

INTRODUCTION

Infertility is a condition of the male or female reproductive system characterized by the inability to achieve pregnancy after 12 months or more of regular, unprotected sexual intercourse. Globally, approximately 17.5% of adults, or about one in six people, experience infertility during their reproductive lives.¹ Nutritional status and micronutrient intake have been increasingly investigated in relation to reproductive health and fertility, with dietary and nutritional factors potentially influencing reproductive outcomes.^{2,3} Ovarian reserve refers primarily to the remaining quantity of ovarian follicles and is an important component of reproductive potential. Ovarian reserve declines with advancing age and is associated with progressive reductions in the number and quality of available oocytes.⁴ Anti-Müllerian hormone (AMH) is produced predominantly by granulosa cells of preantral and small antral follicles and is considered one of the established biochemical markers of ovarian reserve.^{5,6} Serum AMH concentrations vary across the reproductive lifespan, increasing during childhood and adolescence, reaching relatively high levels during early

adulthood, and subsequently declining with age.⁷ AMH also has an important role in folliculogenesis by regulating follicular recruitment and development and modulating follicular sensitivity to follicle-stimulating hormone (FSH).⁸ FSH is secreted by the anterior pituitary gland and promotes follicular growth and maturation. Elevated basal FSH, particularly when measured during the early follicular phase, may reflect reduced negative feedback from ovarian hormones and diminished ovarian function.⁹ FSH contributes to follicular steroidogenesis and maturation through its actions on granulosa cells, while differential FSH and luteinizing hormone actions are important throughout folliculogenesis.¹⁰ Accordingly, AMH and FSH should not be regarded as equivalent measures of ovarian reserve. The AMH-to-FSH relationship and its association with ovarian dysfunction may also be influenced by metabolic and adipose-related factors.¹¹ AMH has additionally been investigated as a predictive marker in assisted reproductive technology and as a potential indicator of ovarian function across different reproductive conditions.^{12,13} Micronutrients are essential for numerous biochemical and



metabolic processes and may contribute to reproductive health through their effects on cellular function, oxidative balance, endocrine regulation, and follicular physiology.^{14,15} Magnesium is an essential mineral involved in enzymatic reactions, cellular metabolism, nucleic acid synthesis, and normal neuromuscular and reproductive physiology.¹⁶ Magnesium status has also been investigated in relation to reproductive and pregnancy-related outcomes. Evidence from pregnancy research suggests that magnesium supplementation may influence maternal and fetal outcomes in selected populations, although evidence regarding its specific effects on ovarian endocrine markers in infertile women remains limited.¹⁷ Magnesium has also been examined in relation to premature ovarian insufficiency, in which AMH, FSH, luteinizing hormone, thyroid-stimulating hormone, progesterone, estrogen, vitamin D, calcium, and magnesium concentrations may be altered.¹⁸

Experimental and nutritional research has further demonstrated the importance of adequate micronutrient availability during pregnancy, fertility, and reproductive development.¹⁹ Magnesium has been investigated in relation to women's health and well-being, although the evidence remains heterogeneous with respect to specific reproductive endpoints.²⁰ Observational research has also examined dietary magnesium intake in relation to broader health outcomes, supporting the biological importance of magnesium while not establishing a direct causal relationship with ovarian reserve.²¹

Vitamin D has recognized endocrine, paracrine, and autocrine functions within the female reproductive system. Vitamin D receptors and metabolizing enzymes are expressed in reproductive tissues, including the ovary and endometrium, suggesting a potential role in follicular development, steroidogenesis, and endometrial function.²² Vitamin D status has been associated with AMH concentrations in women with infertility, and systematic review evidence has examined the overall relationship between vitamin D and AMH.^{23,24} Studies in infertile women have reported associations between vitamin D status and ovarian reserve, including relationships between vitamin D deficiency and AMH concentrations.^{25,26} Recent intervention research has further evaluated vitamin D supplementation in relation to ovarian reserve and oxidative stress among infertile women.²⁷

Vitamin D has also been investigated in women with PCOS. Evidence from meta-analysis suggests that vitamin D supplementation may influence hormonal and glycaemic profiles in women with PCOS, although improvements in biochemical parameters should not automatically be interpreted as improvements in fertility or live-birth outcomes.²⁸ Pakistani research has similarly explored vitamin D as a predictor of fertility-related parameters, highlighting the relevance of vitamin D status in the local reproductive-health context.²⁹ The broader nutritional approach to female fertility emphasizes the potential importance of adequate intake of vitamins, minerals, and other nutrients while recognizing that nutritional interventions require appropriate clinical evaluation.³⁰

Female reproductive function is additionally influenced by metabolic status, nutritional adequacy, and endocrine regulation. Obesity-related endocrine disturbances may affect reproductive function through alterations in hypothalamic-pituitary-gonadal signaling and ovarian physiology.³¹ Nutritional and metabolic disturbances during critical reproductive periods may also influence later reproductive and metabolic outcomes.³² These findings reinforce the importance of investigating potentially modifiable nutritional factors in women with infertility while avoiding direct extrapolation from pregnancy, metabolic, or experimental models to ovarian reserve.

The effects of nutritional interventions may depend on the

population, dose, duration, baseline nutritional status, and outcome assessed. A randomized controlled trial of preconceptional dietary intervention in women undergoing in vitro fertilization demonstrated the feasibility of investigating nutritional modification in relation to reproductive treatment outcomes.³³ Magnesium supplementation has also been specifically evaluated in relation to serum AMH in premenopausal women, with reported increases in AMH following supplementation.³⁴ These findings provide a rationale for further investigation of magnesium as a potentially modifiable nutritional factor affecting ovarian reserve.

Laboratory methodology is also important when evaluating biochemical ovarian markers because preanalytical factors can influence the reliability of measured concentrations. Venepuncture-related complications and sample-handling procedures should therefore be appropriately controlled in studies involving biochemical measurements.³⁵ Centrifugation conditions can affect laboratory measurements and represent an important source of preanalytical variation.^{36,37} The timing and conditions of centrifugation may influence plasma or serum preparation and should consequently be standardized when assessing endocrine biomarkers.^{36,37} Previous experimental and laboratory research has also used controlled biological and biochemical measurement approaches in nutritional and reproductive investigations.³⁸

Nevertheless, evidence concerning the effects of combined magnesium and vitamin D supplementation on biochemical markers of ovarian function in women with infertility remains limited. The present single-arm, quasi-experimental pre-post intervention study was designed to evaluate the association of combined magnesium and vitamin D supplementation with changes in serum AMH and FSH concentrations among women with infertility. The study focused specifically on these biochemical markers and did not assess pregnancy, live-birth, or other clinical fertility outcomes.

METHODOLOGY

A This single-arm, quasi-experimental pre-post intervention study evaluated the effects of combined magnesium and vitamin D supplementation on serum anti-Müllerian hormone (AMH) and follicle-stimulating hormone (FSH) concentrations in infertile women. Forty women aged 25–35 years were enrolled. The study was approved by the ethical review committee of Rawalpindi Women University. Infertility was defined according to World Health Organization criteria as failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse.¹ Eligible participants were women aged 25–35 years with diagnosed infertility, not receiving hormonal medication at enrollment, and willing to provide informed consent. Women with polycystic ovary syndrome, premature ovarian insufficiency, thyroid disorders, hyperprolactinemia, endometriosis, diabetes mellitus, current hormonal treatment, or other endocrine or reproductive disorders were excluded. Participants were categorized into two age groups for secondary analyses: Group I comprised women aged 25–30 years ($n = 17$), and Group II comprised women aged 31–35 years ($n = 23$).

All participants received magnesium supplementation at a dose of 150 mg daily together with vitamin D supplementation for 60 consecutive days. Vitamin D was administered as cholecalciferol at a daily dose of 2000 IU. Participants received the same supplementation regimen throughout the intervention and were instructed to avoid additional hormonal preparations, fertility medications, vitamins, minerals, or other supplements during the study period. Blood samples were collected before supplementation on day 0 and following completion of the intervention on day 61. To minimize menstrual-cycle-related

variation in reproductive hormone concentrations, both samples were collected during the early follicular phase (cycle days 2–5). Venous blood was obtained from the antecubital vein using standard aseptic procedures and collected into yellow-capped serum separator tubes containing clot activator and gel. Samples were allowed to clot before centrifugation.

Blood samples were centrifuged at 3000 rpm for 5–10 minutes at 15 °C. The separated serum was carefully aspirated without disturbing the cellular layer and transferred into appropriately labelled tubes. Serum samples were stored at –20 °C until analysis and were thawed and gently mixed before testing. Serum AMH and FSH concentrations were determined using an automated immunoassay platform based on enzyme-linked fluorescent assay methodology. The Solid Phase Receptacle served as the reaction vessel, with assay-specific antibodies mediating the antigen–antibody reaction. Following automated incubation and washing steps, the resulting fluorescent signal was measured by the analyzer and converted into quantitative hormone concentrations using the assay calibration system. All analytical procedures were performed according to the manufacturer's instructions, with appropriate calibration and internal quality-control procedures. AMH concentrations were expressed in ng/mL and FSH concentrations in mIU/mL.

Statistical analysis was performed using SPSS version 26.0. Continuous variables were summarized as mean $\pm$ standard deviation, and paired baseline and post-intervention hormone concentrations were compared using paired-samples t-tests. Normality of the paired differences was assessed using the Shapiro–Wilk test before inferential analysis. Mean paired differences, standard errors, 95% confidence intervals (CIs), t statistics, and exact p-values were calculated. Pearson correlation coefficients were used to evaluate associations between baseline and post-intervention hormone concentrations. The same paired analytical approach was applied separately to the two age groups. All tests were two-tailed, and $p < 0.05$ was considered statistically significant. Because the study lacked a concurrent control group, statistically significant pre–post changes were interpreted as associations with the intervention rather than definitive evidence of causality.

RESULTS

The A total of 40 infertile women aged 25–35 years completed the study and were included in the pre–post analysis. The participants were categorized into two age groups: 17 women (42.5%) were aged 25–30 years and 23 women (57.5%) were aged 31–35 years. Baseline and post-intervention serum AMH and FSH concentrations were compared following 60 days of combined magnesium and vitamin D supplementation.

Serum AMH demonstrated a significant increase following the intervention. The mean AMH concentration increased from 0.898 ± 0.226 ng/mL at baseline to 1.137 ± 0.235 ng/mL after supplementation, corresponding to a mean increase of 0.239 ng/mL. The 95% CI for the paired mean difference was 0.164–0.315 ng/mL. This change was statistically significant ($t(39) = -6.392$, $p < 0.001$). The corresponding effect represents an approximately 26.6% increase in the mean AMH concentration from baseline.

In contrast, serum FSH showed a statistically significant but comparatively small reduction. Mean FSH concentration decreased from 11.925 ± 0.987 mIU/mL before supplementation to 11.720 ± 1.000 mIU/mL after supplementation. The mean reduction was 0.205 mIU/mL, with a 95% CI of 0.174–0.236 mIU/mL. The paired-samples t-test demonstrated a statistically significant difference ($t(39) = 13.514$, $p < 0.001$), representing an approximately 1.7% reduction from the baseline mean.

Table 1. Overall serum AMH levels following magnesium and vitamin D supplementation in infertile women.

Hormonal outcome	Baseline, mean $\pm$ SD	Post-intervention, mean $\pm$ SD	Mean change	95% CI	t(39)	p-value
AMH (ng/mL)	0.898 ± 0.226	1.137 ± 0.235	+0.239	0.164 to 0.315	-6.392	<0.001
FSH (mIU/mL)	11.925 ± 0.987	11.720 ± 1.000	-0.205	0.174 to 0.236	13.514	<0.001

Age-Stratified Changes in Serum AMH

Among women aged 25–30 years ($n = 17$), mean AMH increased from 0.994 ± 0.168 ng/mL at baseline to 1.147 ± 0.170 ng/mL following supplementation. The mean paired difference was -0.153 ng/mL when calculated as baseline minus post-intervention concentration. The 95% CI ranged from -0.179 to -0.127 ng/mL. The increase was statistically significant ($t(16) = -12.257$, $p < 0.001$). The corresponding Pearson correlation between baseline and post-intervention AMH was strong and statistically significant ($r = 0.954$, $p < 0.001$).

Among women aged 31–35 years ($n = 23$), mean AMH increased from 0.826 ± 0.240 ng/mL to 1.128 ± 0.281 ng/mL after the intervention. The mean increase was 0.302 ng/mL, with a 95% CI of 0.171–0.434 ng/mL. This change was statistically significant ($t(22) = -4.760$, $p < 0.001$). The correlation between baseline and post-intervention AMH was weak to moderate and did not reach statistical significance ($r = 0.323$, $p = 0.133$).

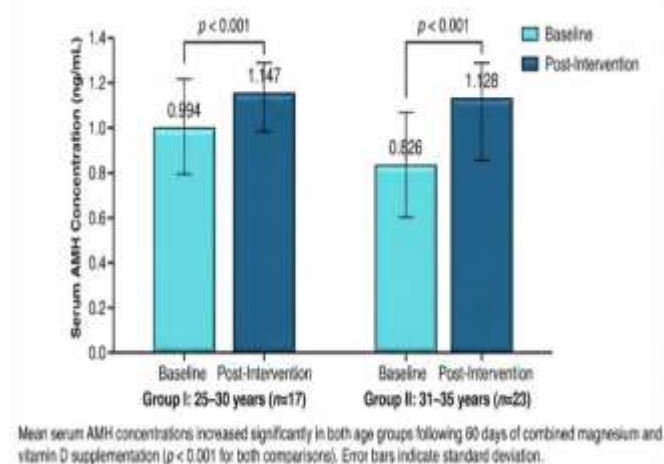
Table 2. Serum AMH levels among infertile women aged 25–30 years.

Age group	n	Baseline AMH, mean $\pm$ SD (ng/mL)	Post-intervention AMH, mean $\pm$ SD (ng/mL)	Mean change	t	p-value
25–30 years	17	0.994 ± 0.168	1.147 ± 0.170	+0.153	-12.257	<0.001
31–35 years	23	0.826 ± 0.240	1.128 ± 0.281	+0.302	-4.760	<0.001
Overall	40	0.898 ± 0.226	1.137 ± 0.235	+0.239	-6.392	<0.001

The magnitude of the observed AMH increase was greater among women aged 31–35 years in absolute terms, although the available analyses were not designed to formally test whether the magnitude of change differed significantly between age groups.

Age-Stratified Changes in Serum FSH

In women aged 25–30 years, mean FSH concentration decreased from 11.888 ± 0.925 mIU/mL at baseline to 11.700 ± 0.949 mIU/mL after supplementation. The mean reduction was 0.188 mIU/mL, and the paired-samples t-test demonstrated a statistically significant difference ($t(16) = 9.935$, $p < 0.001$). The 95% CI for the reduction was 0.148–0.228 mIU/mL. Baseline and post-intervention FSH concentrations demonstrated a very strong positive correlation ($r = 0.997$, $p < 0.001$).

Figure 1: Age-Stratified Pre- vs. Post-Intervention AMH Concentration Comparison

Among women aged 31–35 years, mean FSH decreased from 11.952 ± 1.051 mIU/mL to 11.735 ± 1.058 mIU/mL. The mean reduction was 0.217 mIU/mL, with a 95% CI of 0.171–0.264 mIU/mL. This difference was statistically significant ($t(22) = 9.721$, $p < 0.001$). A very strong positive correlation was also observed between baseline and post-intervention FSH concentrations ($r = 0.995$, $p < 0.001$).

Table 3. Baseline and post-intervention serum FSH concentrations by age group in infertile women receiving magnesium and vitamin D supplementation.

Age group	n	Baseline FSH, mean $\pm$ SD (mIU/mL)	Post-intervention FSH, mean $\pm$ SD (mIU/mL)	Mean reduction	95% CI	t	p-value
25–30 years	17	11.888 $\pm$ 0.925	11.700 $\pm$ 0.949	0.188	0.148–0.228	9.935	<0.001
31–35 years	23	11.952 $\pm$ 1.051	11.735 $\pm$ 1.058	0.217	0.171–0.264	9.721	<0.001
Overall	40	11.925 $\pm$ 0.987	11.720 $\pm$ 1.000	0.205	0.174–0.236	13.514	<0.001

Correlation Between Baseline and Post-intervention Hormone Concentrations

Baseline and post-intervention measurements demonstrated different correlation patterns for the two hormones. For AMH, a moderate positive correlation was observed across the complete cohort ($r = 0.472$, $p = 0.002$), indicating that participants with relatively higher baseline AMH concentrations tended to retain relatively higher concentrations after supplementation. The correlation was particularly strong among women aged 25–30 years ($r = 0.954$, $p < 0.001$), whereas the association was not statistically significant among women aged 31–35 years ($r = 0.323$, $p = 0.133$).

For FSH, baseline and post-intervention concentrations showed very strong positive correlations in the overall cohort ($r = 0.995$, $p < 0.001$), as well as among women aged 25–30 years ($r = 0.997$, $p < 0.001$) and 31–35 years ($r = 0.995$, $p < 0.001$). These correlations indicate substantial preservation of the relative ranking of participants' hormone concentrations despite the

observed reduction in mean FSH.

Table 4. Correlation between baseline and post-intervention serum AMH and FSH levels in infertile women overall and according to age group.

Outcome	Overall r	p-value	25–30 years r	p-value	31–35 years r	p-value
AMH	0.472	0.002	0.954	<0.001	0.323	0.133
FSH	0.995	<0.001	0.997	<0.001	0.995	<0.001

Overall, the 60-day supplementation period was associated with a statistically significant increase in serum AMH and a statistically significant reduction in serum FSH. The direction of these changes was consistent across both age groups, although the magnitude of the AMH increase was greater in absolute terms among women aged 31–35 years. Given the single-arm pre-post design, these findings represent within-participant changes observed following supplementation and should not be interpreted as definitive evidence of a causal treatment effect.

DISCUSSION

The present study evaluated the association of 60 days of combined magnesium and vitamin D supplementation with serum AMH and FSH concentrations in infertile women aged 25–35 years. Following the intervention, AMH increased significantly from 0.898 ± 0.226 to 1.137 ± 0.235 ng/mL, representing a mean increase of 0.239 ng/mL, whereas FSH decreased from 11.925 ± 0.987 to 11.720 ± 1.000 mIU/mL, representing a mean reduction of 0.205 mIU/mL. Both changes were statistically significant ($p < 0.001$). The direction of change was consistent across the two age groups, with significant increases in AMH and reductions in FSH among women aged 25–30 years and 31–35 years. These findings suggest that the supplementation period was associated with measurable changes in reproductive hormone concentrations. However, because the study used a single-arm pre-post design, these findings should be interpreted as temporal associations rather than definitive evidence of a causal treatment effect.

The increase in AMH observed in the present study is consistent with findings from interventional studies examining vitamin D supplementation in women with infertility. Bacanakgil et al. reported significant increases in AMH and antral follicle count, together with a significant reduction in FSH, after vitamin D supplementation among infertile women with diminished ovarian reserve and vitamin D deficiency.¹ The direction of these changes closely resembles the present findings, despite differences in participant selection, baseline ovarian reserve, supplementation protocol, and treatment duration. In that study, participants received a high-dose vitamin D regimen, whereas the present investigation evaluated combined magnesium and vitamin D supplementation. Therefore, the similarity in hormonal responses provides supportive but not definitive evidence for a potential relationship between nutritional supplementation and reproductive endocrine function.

The present findings are also compatible with the nonrandomized clinical trial reported by Naderi et al., in which administration of 50,000 IU vitamin D weekly for up to three months significantly increased serum AMH and 25-hydroxyvitamin D concentrations in infertile women with low baseline concentrations of both biomarkers.² The authors additionally observed higher post-treatment AMH among women who achieved vitamin D sufficiency.² Together with the present results, these observations provide biological plausibility for an association between correction of vitamin D insufficiency and AMH dynamics. Nevertheless, the evidence remains heterogeneous because not

all observational studies have demonstrated a relationship between vitamin D status and ovarian reserve markers.

For example, Lata et al. reported that vitamin D deficiency was common among infertile women but did not identify a significant correlation between vitamin D deficiency and AMH.³ Similarly, Moridi et al., in a systematic review and meta-analysis, found that the association between vitamin D and AMH varied across available studies, highlighting substantial heterogeneity in the evidence.⁴ These discrepancies indicate that vitamin D status alone may not adequately explain variation in ovarian reserve markers and that treatment effects may differ from cross-sectional associations. Differences in baseline vitamin D status, ovarian reserve, infertility etiology, age distribution, body composition, assay methodology, and study design may contribute to these inconsistent findings.

Evidence from Pakistan further demonstrates the complexity of the relationship between vitamin D and reproductive hormones. Salahuddin et al. reported that vitamin D deficiency was present among infertile women and examined its relationship with fertility-related hormonal parameters.⁵ In contrast, the present study demonstrated a significant increase in AMH and a modest reduction in FSH following supplementation. The difference may partly reflect the longitudinal nature of the present study and the fact that participants received combined magnesium and vitamin D rather than vitamin D alone. Nevertheless, the present results should not be interpreted as establishing an independent effect of either nutrient because the study did not include separate magnesium-only and vitamin D-only intervention groups.

The possible contribution of magnesium is of particular interest. Sundas et al. reported a significant increase in serum AMH following magnesium supplementation in premenopausal women, providing preliminary human evidence that magnesium status may be relevant to ovarian endocrine function.⁶ Magnesium participates in numerous cellular processes, including enzymatic activation, cellular metabolism, nucleic acid synthesis, and other physiological processes relevant to reproductive function.⁷ Magnesium has also been investigated in relation to women's health and well-being, although evidence concerning its specific effects on ovarian reserve remains limited.⁸ Thus, the present findings may support further investigation of magnesium as a potentially modifiable nutritional factor affecting ovarian endocrine function.

The potential interaction between vitamin D status and oxidative stress also warrants consideration. Azhar et al. reported that vitamin D supplementation in infertile women was associated with changes in AMH and oxidative-stress-related biomarkers, providing a possible biological framework through which nutritional supplementation may influence ovarian function.⁹ Oxidative stress may adversely affect granulosa-cell function, follicular development, and ovarian cellular integrity. However, oxidative-stress biomarkers were not measured in the present study; therefore, an antioxidant mechanism cannot be established from the current data and should be regarded as a biologically plausible hypothesis requiring direct investigation.

Observational evidence involving magnesium and vitamin D status also provides contextual support. Khudhair et al. evaluated AMH, FSH, vitamin D, magnesium, and other reproductive hormones in women with premature ovarian insufficiency and reported differences in several of these biochemical parameters compared with healthy women.¹⁰ Although that study was observational and involved a clinically distinct population, its findings are compatible with the possibility that micronutrient status may interact with ovarian endocrine function. Albahlol et al. similarly investigated vitamin D-related biomarkers in infertile women and reported differences according to infertility-related clinical characteristics, including PCOS and unexplained

infertility.¹¹ These studies establish an important biological and clinical context for examining nutritional factors in infertility but do not, by themselves, demonstrate that supplementation improves ovarian reserve.

The relationship between nutritional status and female fertility is likely multifactorial. Skoracka et al. emphasized the importance of adequate nutritional intake for female reproductive health while noting that individual nutrients should be interpreted within the broader nutritional and metabolic context.¹² Similarly, the PREPARE trial demonstrated the feasibility of investigating preconceptional dietary interventions among women undergoing IVF, although nutritional intervention effects cannot necessarily be generalized to ovarian biochemical markers.¹³

The magnitude of the observed changes is also clinically relevant to interpretation. Although the increase in AMH was statistically significant, the baseline AMH concentration remained relatively low, and AMH is primarily a marker of the ovarian follicular pool rather than a direct measure of oocyte quality or spontaneous fertility. Likewise, the reduction in FSH was statistically significant but numerically modest. Therefore, statistical significance should not be equated with clinically meaningful improvement in reproductive potential. Importantly, the present study did not assess antral follicle count, oocyte yield, embryo quality, conception, clinical pregnancy, miscarriage, or live birth. Consequently, it cannot establish whether the observed biochemical changes translated into improved fertility outcomes. The strong correlations between baseline and post-intervention FSH concentrations indicate that participants largely retained their relative hormonal ranking despite the reduction in mean FSH. The corresponding AMH correlation was moderate in the overall cohort and particularly strong in the younger age group. These correlations should not be interpreted as evidence of treatment efficacy; rather, they indicate the degree to which individual baseline concentrations were associated with their subsequent measurements. The statistically significant within-participant changes provide the more relevant evidence for the pre-post comparison.

The age-stratified findings are noteworthy because both age groups demonstrated significant hormonal changes. AMH increased from 0.994 to 1.147 ng/mL in women aged 25–30 years and from 0.826 to 1.128 ng/mL in those aged 31–35 years. FSH decreased in both groups by approximately 0.19–0.22 mIU/mL. The larger absolute AMH increase among women aged 31–35 years may indicate that baseline reproductive characteristics modify the response to supplementation; however, no formal between-group interaction test was performed. Therefore, it would be inappropriate to conclude that supplementation was more effective in one age group than the other.

The current findings should also be interpreted in light of the broader evidence concerning vitamin D and AMH. Although individual supplementation studies have reported increases in AMH, systematic evidence indicates that findings are inconsistent across populations and intervention protocols.⁴ Therefore, the apparent improvement observed in the present study should not be generalized as proof that vitamin D supplementation consistently increases AMH. Differences in baseline AMH, vitamin D status, treatment protocols, assay methods, and study populations may explain part of the heterogeneity. The present study therefore contributes preliminary longitudinal evidence but does not resolve the broader controversy surrounding vitamin D and ovarian reserve.

Several limitations should be considered. First, the sample size was small and the study was conducted without a parallel control group. Second, participants were not randomly assigned, preventing adjustment for unmeasured confounding and regression toward the mean. Third, magnesium and vitamin D

were administered together, preventing estimation of their individual or synergistic effects. Fourth, the study did not evaluate antral follicle count or clinical reproductive outcomes. Fifth, biochemical confirmation of changes in vitamin D and magnesium status was not incorporated into the primary outcome analysis. Finally, the 60-day follow-up period was relatively short for determining whether changes in ovarian biomarkers translate into meaningful fertility outcomes.

Overall, the findings indicate that combined magnesium and vitamin D supplementation was associated with a significant increase in serum AMH and a modest reduction in FSH among infertile women aged 25–35 years. The consistency of the direction of change across age groups and the agreement with selected supplementation studies support further investigation of nutritional interventions in reproductive medicine. However, the contradictory observational evidence and the limitations of the present single-arm design require cautious interpretation. Future adequately powered randomized controlled trials should include placebo or comparator groups, separate magnesium and vitamin D intervention arms, biochemical assessment of nutrient status, antral follicle count, standardized ovarian-reserve assessment, and clinically meaningful outcomes including pregnancy and live birth rates.

Limitations

This study has several limitations. First, the sample size was relatively small, comprising only 40 participants, which may limit the statistical power and generalizability of the findings. Second, the single-arm quasi-experimental pre-post design lacked a concurrent control group, making it difficult to distinguish intervention-related changes from temporal variation, regression to the mean, or other confounding factors. Third, magnesium and vitamin D were administered simultaneously; therefore, their independent and potentially synergistic effects could not be determined. Fourth, baseline and post-intervention vitamin D and magnesium concentrations were not assessed. Fifth, the intervention duration was limited to 60 days. Finally, clinical fertility outcomes, including pregnancy, oocyte yield, embryo quality, and live birth, were not evaluated.

CONCLUSION

In conclusion, 60 days of combined magnesium and vitamin D supplementation was associated with a significant increase in serum AMH and a modest reduction in FSH among infertile women aged 25–35 years. Similar directions of hormonal change were observed in both age groups, suggesting a consistent within-participant biochemical response during the intervention period. However, AMH and FSH are biochemical markers and do not directly establish improvement in oocyte quality, conception, pregnancy, or live-birth outcomes. Because this study used a single-arm pre-post design without a control group, the observed changes cannot be attributed definitively to supplementation. Larger randomized controlled trials with separate nutrient groups, longer follow-up, biochemical assessment of nutrient status, and clinical fertility outcomes are warranted.

Ethical Approval

Ethical approval was obtained from the relevant institutional research ethics board Ref. No. MIHS/03-14/27-01 before commencement of the study. The study was conducted in accordance with applicable ethical principles and institutional requirements.

Data Availability

The data generated and analyzed during the current study are available from the corresponding author upon reasonable

request, subject to applicable ethical and institutional restrictions.

Author Contributions

Fiza Sagheer, Rabea Ejaz, Sohail Ahmad, Sana Qazi, Samina Qamer, Fareeha Ambreen, Tasmia Gul, and Maria Iqbal contributed to the conception and design of the study, data acquisition, analysis and interpretation of data, and drafting of the manuscript. All authors critically revised the manuscript for important intellectual content, approved the final version for publication, and agreed to be accountable for all aspects of the work, ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Informed Consent

Written informed consent was obtained from all participants before enrollment.

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Conflict of Interest

The authors declare no conflict of interest.

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Declaration of AI-Assisted Language Editing

Grammarly AI tool was used solely for language and grammar correction. The authors reviewed and approved the final manuscript and remain responsible for its content.

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