

# Does the Serum Vitamin D Status and its Possible Effect on Serum Anti-Müllerian Hormone Levels Predict Fertility in Premenopausal Women?

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ABSTRACT

**Context:** Evidence suggests that serum Vitamin D level influences female reproduction. However, clinical studies have reported conflicting evidence on the effect of serum Vitamin D levels on serum Anti-Müllerian hormone (AMH), with little evidence in African women. **Aim:** The study aimed to compare the relationship between serum Vitamin D and serum AMH among infertile and fertile women. **Settings and Design:** This comparative cross-sectional study analyzed data from 170 premenopausal women; 81 infertile, and 89 fertile women attending a Nigerian tertiary hospital between March and June 2019. **Materials and Methods:** Serum AMH and 25-hydroxyvitamin D (25(OH) Vitamin D) concentrations were analysed using enzyme-linked immunosorbent assay. **Statistical Analysis:** Statistical analysis was done using SPSS version 25 for windows. Categorical variables were summarized in frequencies and proportions while continuous variables were summarized in means  $\pm$  standard deviation and median (interquartile range). The association was explored using linear regression. The level of significance was set at 0.05. **Results:** The prevalence of Vitamin D deficiency ( $<20$  ng/ml) in infertile women and fertile women was 16% and 18%, respectively. There was no difference in serum Vitamin D levels between infertile and fertile women in this study after controlling for age and body mass index (BMI) ( $P = 0.186$ ). There was no association between serum 25(OH) Vitamin D and serum AMH in infertile ( $B = 0.002$ ;  $P = 0.474$ ) and fertile women ( $B = -0.002$ ;  $P = 0.522$ ) after adjusting for age and BMI. **Conclusion:** Infertile and fertile women had similar serum Vitamin D levels and there was no relationship between serum Vitamin D and serum AMH in both infertile and fertile women.

**KEYWORDS:** 25-hydroxyvitamin D, anti-Müllerian hormone, infertility, ovarian reserve, premenopausal women, Vitamin D

## INTRODUCTION

Vitamin D is a steroid hormone produced by the skin on exposure to sunlight and is also derived from dietary sources.<sup>[1]</sup> Research suggests that Vitamin D deficiency is linked to reproductive failure.<sup>[2]</sup> Natural fertility and conception have been shown to have a seasonal variation and studies have reported a lower rate of oocyte maturation in winter and a seasonal variation in the success of assisted

reproductive technology.<sup>[2-8]</sup> These suggest that variation in exposure to sunlight and by extension serum Vitamin D levels in regions with significant seasonal variation in sunlight may influence fertility.<sup>[2-8]</sup> The influence of

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Vitamin D on fertility has been linked to its role in the regulation of anti-Müllerian hormone (AMH); a measure of ovarian reserve and function secreted by the granulosa cell of the ovaries.<sup>[9-16]</sup> However, the influence of Vitamin D on ovarian reserve and reproductive potential in women remains controversial.<sup>[17-22]</sup>

Few studies on this subject have been done in Africa, despite the high prevalence of Vitamin D deficiency among African women.<sup>[23]</sup> Therefore, this study compared serum Vitamin D status and the relationship between serum 25-hydroxyvitamin D (25(OH) Vitamin D) and serum AMH in infertile and fertile Nigerian women.

## MATERIALS AND METHODS

### Selection and description of participants

This comparative cross-sectional study was conducted using data and blood samples from infertile and fertile women recruited from the gynecology and immunization clinics of a Nigerian Teaching Hospital between March and July of 2019. The study aimed to compare serum Vitamin D status and determine the relationship between serum 25(OH) Vitamin D and serum AMH in these women. Data from infertile and fertile women within the previous study population that met the eligibility criteria were purposively selected and were analysed for this study. Infertile participants were women 18–45 years of age, who had an average cycle length ranging from 21 to 35 days and who had difficulty conceiving despite adequate, unprotected sexual intercourse for at least a year.

Fertile women 18–45 years old, with menstrual cycle length within 21–35 days and who had at least one term pregnancy in the preceding 2 years, with each pregnancy occurring spontaneously within a year of unprotected intercourse.

The study exempted all women with a history of hormonal contraceptives or medications use 3 months before presentation, thyroid disorders, diabetes mellitus, chemotherapy and or radiotherapy use, pelvic surgery (uterine or ovarian), and those who did not have a signed and documented informed consent.

### Data collection

Data from a total of 170 women; 81 infertile and 89 fertile women were retrieved from the previous study and analyzed for the study.

### Sample analysis

Serum samples frozen at  $-20^{\circ}\text{C}$  were thawed and analysed for both serum AMH and 25(OH) Vitamin D at the chemical pathology research laboratory by the same chemical pathologist. The concentration of AMH was analyzed using

Human AMH enzyme-linked immunosorbent assay (ELISA) kits following the manufacturer manual (Calbiotech, CA, USA). The absorbance was read using a microplate ELISA reader at 450 nm. The sensitivity of the Human AMH ELISA kits was 0.039 ng/ml.

Serum Vitamin D levels were quantified using human 25(OH) Vitamin D ELISA kits (Calbiotech, CA, USA). The absorbance was determined using a microplate ELISA reader at 450 nm. The sensitivity of the 25(OH) Vitamin D ELISA kits was 0.67 ng/ml.

### Ethical consideration

The study was conducted with ethical approval from the institutional ethics review committee (ERC/PAN/2019/01/1869) and the state Ministry of Health following the Helsinki Declaration of 1975 and later revisions. Informed consent was signed by each participant before participation.

### Statistical analysis

Data were analysed using SPSS version 25 for Windows (IBM Corp. Armonk, NY, USA). The dependent variable in the study was serum AMH level while the independent variable was serum Vitamin D. Descriptive statistics of frequencies and proportions were used to summarise categorical variables; whereas, continuous variables were summarised using means and standard deviation or median and interquartile range depending on the normality distribution of the data. Test of difference Mann–Whitney U and Kruskal–Wallis were used to test the difference between nonnormally distributed continuous variables. The difference in proportion was explored using Chi-square.

Values of serum AMH concentrations were log-transformed to meet the criteria for linear regression analysis. The relationship between Vitamin D and serum AMH (controlling for factors that were significantly different between the two groups) was explored using multiple regression. The level of significance was set as 0.05. Serum 25(OH) Vitamin D status was classified as deficient ( $<20$  ng/ml), insufficient ( $20$ – $<30$  ng/ml), and normal status ( $\geq 30$  ng/ml) based on the Endocrine Society Clinical Practice Guideline.<sup>[24]</sup>

The minimum sample size was calculated using a formula for comparing means given by formula;  $n = (Z_{\alpha/2} + Z_{\beta})^2 \sigma^2 / d^2$ .<sup>[25]</sup> A population variance ( $\sigma^2$ ) of 48.44 for Vitamin D was used from a previous study<sup>[26]</sup> and a mean difference of 5 ng/ml was assumed. The minimum sample size at, 80% power, 5% level of significance, and an assuming a 10% nonresponse rate was determined to be 34 participants per study group. Therefore, a minimum sample size of 68 women was needed for the study.

## RESULTS

Table 1 summarises the baseline study variables among the study participants. The age range of women in this study was 21–40 years. There were significant differences in age, body mass index (BMI) and serum Vitamin D levels between infertile and fertile women, with infertile women having higher age, BMI and Vitamin D levels than fertile women in the study. However, controlling for age and BMI in multivariate regression analysis, there was no significant difference in serum Vitamin D levels between the two groups ( $B = 0.327$ ;  $P = 0.186$ ).

All women in this study were nonsmokers; with 11 (12.4%) fertile women and six (7.4%), infertile women exposed to second-hand smoking. The majority of infertile women in the study have secondary infertility and the underlying cause of infertility was unexplained infertility at the end of the study. The majority of infertile women in the study had infertility duration between one to 3 years (40.5%) [Table 2].

There is no difference in the prevalence of Vitamin D deficiency or insufficiency between infertile and fertile women [Table 3].

There was no significant difference in serum AMH levels among infertile or fertile women with Vitamin D deficiency/insufficiency and normal levels [Table 4]. Correlation analysis found no significant relationship between serum AMH and Vitamin D in both infertile ( $r = 0.042$ ;  $P = 0.711$ ) and fertile ( $r = -0.055$ ;  $P = 0.611$ ) women [Figures 1 and 2]. Multivariable regression analysis adjusting for age and BMI – which were significantly different between the two groups and predetermined to be independent predictors of serum AMH – also showed no significant relationship between Vitamin D level and serum AMH in infertile ( $B = 0.002$ ;  $P = 0.474$ ) and fertile women ( $B = -0.002$ ;  $P = 0.522$ ) [Table 5].

## DISCUSSION

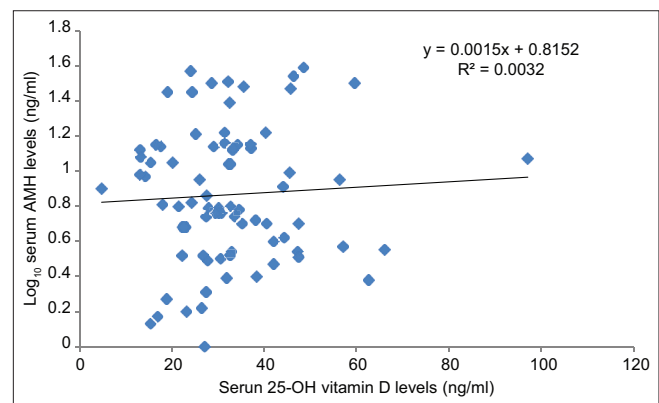
The findings showed that the prevalence of Vitamin D deficiency in infertile women and fertile women was 16% and 18%, respectively. This is comparable to findings by Makwe and Aliyu among premenopausal

women in southwest Nigeria in which authors found a prevalence of Vitamin D deficiency of 18.5%.<sup>[21]</sup> Similarly, Durazo-Arvizu *et al.*, in their study reported a lower prevalence of Vitamin D deficiency in Nigerian women (0% deficiency and 24% insufficiency) residing in Nigeria compared to African-American women (58.9%) residing in the United States of America.<sup>[26]</sup>

In India, 94.28% of infertile women were reported to have serum Vitamin D levels of 20 ng/ml or less.<sup>[27]</sup> In Germany, a two-centered study found a prevalence of 98.2% and 81.3% of combined Vitamin D insufficiency and deficiency among infertile women.<sup>[28]</sup> The contradiction may be attributable to the significant seasonal variation in the amount of sunlight in certain geographical locations and an increased tendency for Vitamin D deficiency in this region due to reduced sunlight exposure.

Although high skin melanin content which prevents adequate absorption of ultraviolet light reduces the synthesis of Vitamin D by the skin and predisposes black women to Vitamin D deficiency, residing in the tropics and increased exposure to sunlight mitigates the development of Vitamin D deficiency. To further buttress this, a meta-analysis reported that Immigrants of sub-Saharan African descent residing in temperate regions have a high prevalence (56%) of Vitamin D deficiency.<sup>[29]</sup>

There were no differences in serum 25(OH) Vitamin D levels between infertile and fertile women after

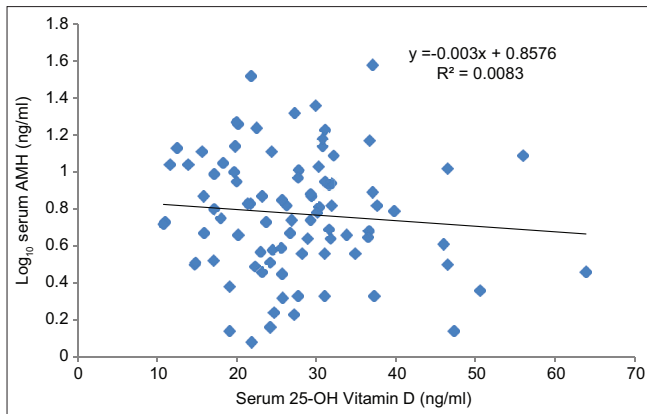


**Figure 1:** The correlation between serum 25-hydroxyvitamin D and serum anti-Müllerian hormone in infertile women

**Table 1: Baseline study variables**

Variables	Median (IQR)			P
	Total	Infertile	Fertile	
Age (years)	30 (7)	32 (7)	30 (6)	0.011*
BMI (kg/m <sup>2</sup> )	24.74 (6.57)	25.40 (5.87)	23.56 (6.01)	0.002*
Anti-Müllerian hormone (ng/ml)	5.29 (8.57)	5.50 (9.94)	5.00 (6.70)	0.125
25(OH) Vitamin D (ng/ml)	28.4 (12.43)	31.4 (14.65)	26.70 (11.50)	0.006*

\*Significant at 0.05 level of significance. BMI=Body mass index, IQR=Interquartile range, 25(OH) Vitamin D=25-hydroxyvitamin D



**Figure 2:** The correlation between serum 25-hydroxyvitamin D and serum anti-Müllerian hormone in fertile women

**Table 2: Baseline reproductive characteristics of infertile women**

	Frequency (n=81), n (%)
Type of infertility	
Primary infertility	30 (37.0)
Secondary infertility	51 (63.0)
Duration of fertility <sup>a</sup> (years)	3.93±2.36
1-3	40 (49.4)
4-6	28 (34.6)
7+	13 (16.0)
Cause of infertility	
Female	34 (42.0)
Male	5 (6.2)
Both	9 (11.1)
Unexplained	33 (40.7)
Clinical diagnosis	
Unexplained	33 (40.7)
Anovulation including PCOS	13 (16.0)
Tubal factor	13 (16.0)
Male factor	5 (6.2)
Uterine factor	7 (8.6)
More than one diagnosis	10 (12.5)

<sup>a</sup>Data summarized in mean±SD. SD=Standard deviation, PCOS=Polycystic ovarian syndrome

adjusting for age and BMI. This is similar to findings from a study by Franasiak *et al.* where researchers found no difference in serum 25(OH) Vitamin D levels between infertile and fertile women; although they found that infertile women had significantly higher bioavailable Vitamin D and free 25(OH) Vitamin D and significantly lower Vitamin D binding protein than regularly cycling fertile women.<sup>[30]</sup> Similarly, Al-Jaroudi *et al.* reported a significantly higher serum Vitamin D level in infertile women compared to pregnant women in Saudi Arabia.<sup>[31]</sup> Contrary to this study, researchers in Iraq found infertile women had a higher proportion of Vitamin D deficiency and insufficiency than fertile women.<sup>[32]</sup> The contradiction with the results of this study may be due to the inclusion of lactating women among the fertile group. This may affect serum Vitamin D levels in these women and subsequently obscure the difference.

There was no difference in serum AMH between women with Vitamin D deficiency and insufficiency and those with normal Vitamin D status in both groups. This is similar to the report by Makwe and Aliyu among premenopausal women in southwest Nigeria.<sup>[21]</sup> Furthermore, adjusting for age and BMI in this study population, there was no significant relationship between serum Vitamin D level and serum AMH. This is similar to findings from cross-sectional studies done among infertile women and premenopausal women outside of sub-Saharan Africa.<sup>[17,19,20]</sup>

Contrary to these, a cross-sectional study involving 388 women found no correlation in women ages 35–40 years but found a weak negative correlation among women of ages <35 years and a weak positive correlation in those >40.<sup>[22]</sup> Also, a randomized control trial done on women 18–25 years of age with Vitamin D deficiency found that Vitamin D supplementation was associated with a significant increase in serum AMH levels in the

**Table 3: Distribution of study participants by categories of serum 25-hydroxyvitamin D status**

Vitamin D status	Total, n (%)	Infertile, n (%)	Fertile, n (%)	$\chi^2$ , df (P)
Deficiency (<20 ng/ml)	29 (17.1)	13 (16)	16 (18)	4.21, 2 (0.122)
Insufficiency (20-<30 ng/ml)	60 (35.3)	23 (28.4)	37 (41.6)	
Normal (≥30 ng/ml)	81 (47.6)	45 (55.6)	36 (40.4)	

**Table 4: Test of difference in serum anti-Müllerian hormone levels between women with normal and insufficient/deficient serum Vitamin D levels**

Serum AMH levels (ng/ml)	Median (IQR)		Mann-Whitney U (P)
	Normal (≥30ng/ml)	Deficiency or insufficiency (<30ng/ml)	
Total	5.30 (9.25)	5.28 (7.75)	0.249
Infertile	5.30 (10.02)	5.56 (9.72)	0.377
Fertile	5.34 (6.81)	4.51 (6.85)	0.732

IQR=Interquartile range, AMH=Anti-Müllerian hormone



**Table 5: Multiple linear regression analysis for the relationship between serum 25-hydroxyvitamin D and serum anti-Müllerian hormone**

Independent variables	Model: Dependent variable: Log10 AMH					
	Infertile			Fertile		
	B	SE	P	B	SE	P
Constant	1.641	0.312	0.000	1.551	0.321	0.000
25(OH) Vitamin D (ng/ml)	0.002	0.003	0.474	-0.002	0.003	0.522
Age (years)	-0.042	0.008	0.000*	-0.023	0.008	0.007*
BMI (kg/m <sup>2</sup> )	0.019	0.007	0.009*	-0.001	0.008	0.859

\*Indicates significance at 0.05 level of significance. 25(OH) Vitamin D=25-hydroxyvitamin D, BMI=Body mass index, AMH=Anti-Müllerian hormone, SE=Standard error

treatment group when compared to placebo.<sup>[18]</sup> Similarly, a study involving 33 women of reproductive age found a seasonal variation in serum Vitamin D and AMH levels, with Vitamin D supplementation preventing the seasonal reduction in both Vitamin D and serum AMH.<sup>[33]</sup> Naderi *et al.* reported a significant increase in serum AMH following administration of 50,000 IU of supplemental 25(OH) Vitamin D weekly for 3 months in a nonrandomized clinical trial of 30 infertile women with low serum level of both 25(OH) Vitamin D and AMH.<sup>[34]</sup>

The complex relationship between Vitamin D and serum AMH was further elucidated in a systematic review and meta-analysis by Moridi *et al.* in which researchers found contradicting relationships between Vitamin D and AMH in 18 observational studies included. However, the meta-analysis of 6 interventional studies on the influence of vitamin D supplementation on serum AMH suggested a cause-effect relationship with the direction of effect depending on the ovulatory status of the women studied. The researchers reported an increase in serum AMH in ovulatory; non-polycystic ovarian syndrome (PCOS) women while serum AMH decreased in women with PCOS with Vitamin D supplementation.<sup>[35]</sup>

The contradictions between these studies and ours could be attributed to the study design, since most studies that depicted a relationship with serum AMH were prospective, interventional studies that are better at demonstrating causality. The relationship between serum AMH and Vitamin D may be obscured in the cross-sectional studies. Also, given the relatively small sample sizes of these interventional studies, their results may not be a good depiction of the general population. Perhaps, the influence of adequate sunlight exposure on Vitamin D status and a relatively low level of Vitamin D deficiency in our study obscured the relationship between serum AMH and Vitamin D.

### Strengths and limitation

The study design allowed for the comparison of the influence of Vitamin D on serum AMH in a sample of black

African women with clinically diagnosed infertility and those with demonstrable fertility. This aimed to adjust for the influence of infertility since some underlying etiologies of infertility can predispose to low serum AMH levels and can obscure the influence of Vitamin D on serum AMH.

However, this study is limited by its hospital-based nature and small sample size which prevents the generalization of study results to the population. However, the sample size had enough statistical power for all the analyses that were done within this study. Also, a prospective and interventional study design will be better at depicting the influence of Vitamin D, if any, on serum AMH. In addition, the lack of random selection of participants and the secondary analysis of data in the study could have introduced some relative selection bias of the women.

### CONCLUSION

There is no difference in Vitamin D levels between infertile and fertile women after controlling for age and BMI in this study population. In addition, the results suggest that there is no relationship between Vitamin D and serum AMH levels in both infertile and fertile women, implying that Vitamin D status does not depict fertility status and does not influence ovarian reserve. However, future research should involve a larger sample size with a prospective, interventional, and follow-up study design to better study the true influence of Vitamin D on serum AMH levels in women.

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### Conflicts of interest

There are no conflicts of interest.

## Data availability statement

Data on findings are available with corresponding author on reasonable request.

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