

# To Study the Vitamin D Levels in Infertile Females and Correlation of Vitamin D Deficiency with AMH Levels in Comparison to Fertile Females

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ABSTRACT

**Context:** Human and animal data suggest that low vitamin D (25-hydroxyvitamin D) status is associated with impaired fertility, endometriosis, and polycystic ovary syndrome. Vitamin D regulates antimullerian hormone (AMH), FSH, mRNA, and expression of genes in reproductive tissues, implicating a role in female reproduction. **Aims:** To study the vitamin D levels in infertile females and to know the correlation of vitamin D deficiency (VDD) with serum AMH in infertile females compare to fertile females. **Settings And Design:** This prospective study was conducted in department of Maternal and Reproductive Health in between April 2014 and April 2016. **Materials and Methods:** After matching inclusion and exclusion criteria out of total 70 infertile females, 45 were found to have VDD. Of these 35 patients were identified as cases; in whom, the AMH levels were assessed. As control 35 fertile normal females were taken, in which vitamin D and AMH were taken. In both groups, correlation of VDD with AMH was studied. **Statistical Analysis Used:** To analyze the correlation between vitamin D and AMH linear regression test and for comparison of both the groups, two sample *t* tests were used. **Results:** The VDD was present in 64.28% of infertile females. In vitamin D deficient cases, the mean for vitamin D was  $6.18 \pm 2.09$  and AMH was  $1.94 \pm 1.30$ . In vitamin D deficient controls, the mean for vitamin D was  $4.85 \pm 3.02$  and AMH was  $3.47 \pm 2.59$ . On comparison, the vitamin D levels were lower in fertile than infertile females, which was significant ( $P = 0.04$ ), and AMH levels were lower in cases than control group ( $P = 0.003$ ). **Conclusion:** The VDD was present in 64.28% of infertile females. No significant correlation was found in between VDD and AMH levels in both the groups. **KEYWORDS:** AMH, fertile, infertile, outcome, vitamin D deficiency

## INTRODUCTION

Vitamin D deficiency (VDD) is a major health problem in both the developed and developing countries across the world. Recent epidemiologic studies have shown relationships between low vitamin D levels have harmful effects on various systems and multiple disease states. Vitamin D is also responsible for expression of a large number of genes in reproductive tissues, implicating a role for vitamin D in female reproduction. Human and animal data suggest that low vitamin D status is associated with impaired fertility and endometriosis. Serum 25(OH)D provides the single best assessment of

vitamin D status; it has a half-life of about 3 weeks, making it the most suitable indicator of vitamin D status.<sup>[1]</sup>

Vitamin D also inhibits cell proliferation and stimulates cell differentiation. This hormone primarily exerts its effects through the vitamin D receptor (VDR). Through its receptor, vitamin D can modify gene transcription, as well as protein

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Access this article online	
<p>Quick Response Code:</p> 	<p>Website: www.jhrsonline.org</p>
	<p>DOI: 10.4103/jhrs.JHRS_105_16</p>

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**How to cite this article:** Lata I, Tiwari S, Gupta A, Yadav S, Yadav S. To Study the Vitamin D Levels in Infertile Females and Correlation of Vitamin D Deficiency with AMH Levels in Comparison to Fertile Females. J Hum Reprod Sci 2017;10:86-90.

and messenger ribonucleic acid (mRNA) production. In animal studies, dietary VDD leads to a 25% reduction in overall fertility.<sup>[2]</sup> In various studies, the role of VDD has been correlated with polycystic ovary syndrome (PCOS) and a myriad of pregnancy-related disorders.<sup>[3]</sup>

Antimullerian hormone (AMH) is a dimeric glycoprotein, in women it is produced by granulosa cells, from preantral and antral follicles in ovary. The main physiological role of AMH in the ovary seems to be limited to the inhibition of the early stages of follicular development, maintaining ovarian reserve and modifying the follicles response to follicular stimulating hormone (FSH).<sup>[4]</sup> AMH also reduces follicle sensitivity to FSH *in vivo*, and *in vitro* it inhibit FSH-induced preantrafollicle growth.<sup>[5]</sup> In reproductive medicine, the AMH levels measure both ovarian reserve (low levels) and PCOS status (high levels).<sup>[6]</sup>

AMH emerged as a target gene regulated by vitamin D (1,25-dihydroxyvitamin D<sub>3</sub>) from complimentary deoxyribonucleic acid (cDNA) microarray analysis of a prostate cancer cell line.<sup>[7]</sup> The AMH-mRNA expression is upregulated in response to vitamin D *in vitro*, and these investigators subsequently identified a functional VDR element in the human AMH promoter, demonstrating a direct effect of vitamin D on AMH expression. In addition, vitamin D regulates AMH, FSH, and mRNA, and likely to involved in follicle-selection process.<sup>[8]</sup> At present, there is considerable controversy in the literature regarding whether vitamin D has any effect on AMH production, as well as its effect on fertility. Therefore, we planned this study to see the spectrum of vitamin D levels in infertile females and to examine the correlation between serum AMH and vitamin D status in ovulatory fertile females compare to infertile females.

## MATERIALS AND METHODS

After approval from institute ethics committee and clinical trial registration, this prospective study was done on patients attending infertility outpatient department (OPD) between April 2014 and April 2016. After written informed consent and following good clinical practice guidelines, the total 70 infertile patients following inclusion criteria were taken as cases. In these patient's, levels of vitamin D were done. Out of total 70 infertile females, 45 were found to have VDD. Of these 35 patients were identified as cases; in whom, the AMH levels were assessed. In these patients, the AMH levels were assessed.

The vitamin D levels were classified in three categories as deficiency, insufficiency, and sufficiency (as per according Institute of Medicine and EURONUT SENECA study,<sup>[9]</sup> SUVIMAX study,<sup>[10]</sup> and Goswami *et al.*<sup>[11]</sup>). The reference levels for serum (blood) of vitamin D [25(OH)D]: deficiency <10 ng/ml, insufficiency 10 to 20 ng/ml, and adequate levels were taken >20 ng/ml. The fertile females as control group, coming to our OPD for taking consultation

due to other causes and normal working staff members were screened for vitamin D levels and who were found vitamin D deficient were enrolled as control. The 35 fertile females having VDD were further assessed for AMH levels.

- (1) Patient *N* = 70 (enrolled to study vitamin D levels in infertile females with unexplained infertility)
- (2) Cases *N*<sub>1</sub> = 35 (infertile females with VDD, for AMH levels assessment)
- (3) Control *N*<sub>2</sub> = 35 (fertile females with VDD, for AMH levels assessment).

### Inclusion criteria

Infertile females with unexplained infertility, of age group between 18 and 40 years, as cases and healthy fertile females between same age group as control.

### Exclusion criteria

History of smoking (tobacco use), oral contraceptive pill, any hormonal or steroid drug use, known VDD, obesity (body mass index, BMI > 35), endometriosis, thyroid disorders, autoimmune disease, tubal factor, male factor, or polycystic ovarian syndrome.

### Study design

Age, duration of married life, duration and type of infertility, previous obstetrical history, and education levels were retrieved for all women who met the inclusion criteria as cases and control.

### Biochemical analysis

Plasma AMH levels were measured in duplicate using the ultrasensitive AMH Enzyme linked immunosorbent assay (ELISA) platform {Ansh Labs, Webster, Texas, USA}. The limit of detection was 0.023 ng/ml with 95% probability of detection. Blood samples were taken at any time in the menstrual cycle, and then the serum was separated within 1 h of venipuncture and stored at 4°C until assayed within 72 h of collection.

### Vitamin D analysis

Vitamin D levels were measured in duplicate using a Liasion 25OH vitamin D total assay (DiaSorin, Stillwater, Minnesota, USA), using a competitive chemiluminescent immunoassay, as per the manufacturers guidelines.

### Power of study

To study VDD in infertile females, the sample size was 70 based on standard deviation (SD) = 9, clinical significance difference = 5%, level of significance (*P* = 0.05), and power of the test 90%.

The normal AMH level in fertile females were taken 1.1 to 3.5 and in infertile females were taken as below 1.1.<sup>[6]</sup> But for calculation purposes, to study the AMH level in vitamin D deficient infertile and fertile females, taking the mean AMH level in infertile 1.0 ± 0.6 and in fertile 3.0 ± 0.6, sample size of 31 in each group were chosen.

**Table 1: Demographic profile and infertility characteristics of cases, infertile group (N=70)**

1. Age distribution (years)	18–25	18	25.71%
	26–30	25	35.71%
	31–35	22	31.42%
	36–40	5	7.14%
2. Education level	Up to 12th class	29	41.42%
	Graduate and above	41	58.57%
3. Occupation	Nonprofessional	60	87.50%
	Professional	10	14.20%
4. Type of infertility	Primary	39	55.71%
	Secondary	31	44.28%
5. Infertility duration	Up to 5 years	67	95.71%
	6–10 years	2	2.86%
	11–15 years	1	1.42%

This was to achieve 90% power to detect a difference between the null hypothesis that both group means with a significance level (alpha) of 0.050 using two sided two-sample *t* test. To prevent drop out, we had taken 35 cases in each group. Similarly, in 35 fertile women the AMH levels were done and correlation with vitamin D was seen. After evaluating the correlation, we also compared the both groups.

### Statistical analysis

To analyze the correlation between vitamin D and AMH linear regression test and for comparison of both the groups, two sample *t* tests were used.

## RESULTS

In this study, maximum patients were of age 26 to 30 years having primary infertility (55.71%) of duration of 1 to 5 years (95.71%) with education level graduation and above (58.57%). All patients were enquired about their profession and 87.50% were nonprofessional (housewife) as shown in Table 1. Our study was done in infertile female's population to see the spectrum of vitamin D levels. Overall, 64.28% infertile females had VDD (up to 10 ng/ml), 30.0% displayed vitamin D insufficiency (10–20 ng/ml), whereas 5.71% of the study population exhibited adequate levels of vitamin D levels (>20 ng/ml). The mean value for vitamin D was  $9.30 \pm 5.59$  ng/ml, as shown in Table 2.

In vitamin D deficient cases (infertile females), the mean for vitamin D was  $6.18 \pm 2.09$  and for AMH was  $1.94 \pm 1.30$ . In vitamin D deficient controls (fertile females), the mean for vitamin D was  $4.85 \pm 3.02$  and for AMH was  $3.47 \pm 2.59$ . On comparison of these two groups infertile group had significantly lower Table 3 levels Table 4 levels Table 5 of AMH ( $P = 0.003$ ) but higher levels of vitamin D ( $P = 0.04$ ) compared to fertile group in our study [Table 6]. However, no significant correlation was found between vitamin D and AMH levels in both the groups.

**Table 2: Vitamin D levels in infertile group (N = 70)**

Vitamin D levels	Infertile group (N = 70)	%
Deficient group (<10 ng/ml)	45	64.28
Insufficient (10–20 ng/ml)	21	30.0
Sufficient (>20 ng/ml)	4	5.71

**Table 3: Age profile of vitamin D deficient cases (N1=35) and controls (N2 = 35)**

Age group (years)	Cases	Controls
18–25	6 (17.1%)	2 (5.71)
26–30	13 (37.1%)	14 (40.0%)
31–35	11 (31.4%)	13 (37.1%)
36–40	5 (14.2%)	6 (17.1%)

**Table 4: BMI profile of vitamin D deficient cases (N1 =35) and controls (N2 = 35)**

BMI (kg/m <sup>2</sup> )	Cases (N = 35)	Controls (N = 35)
Underweight (<18.5)	4 (11.4%)	3 (8.57%)
Normal weight (18.5–24.9)	18 (51.4%)	21 (60.0%)
Overweight (25–29.9)	10 (28.5%)	8 (22.8%)
Obese ( $\geq 30$ )	3 (8.57%)	3 (8.57%)

## DISCUSSION

In the present study, we had seen the spectrum of vitamin D levels in women of reproductive age group with primary infertility with unexplained causes and prevalence of VDD in infertile females. In our study, we found the prevalence of VDD was 64.28% in infertile females. In a study, 81.3 to 98.2% of women with impaired fertility had deficient or insufficient vitamin D levels.<sup>[12]</sup> Our results are comparable to these results with a slight difference may be because of population and geographical differences.

In our study on comparison of these two groups, vitamin D levels were lower in control group (fertile females) than cases (infertile females), which was significant ( $P = 0.04$ ). The AMH levels were lower in cases than control group

**Table 5: AMH levels in vitamin D deficient infertile and fertile females**

AMH levels	Cases (infertile group) (N1 = 35)	%	Control (fertile group) (N2 = 35)	%
Up to 1.0 ng/ml	12	34.28	6	17.14
1.1–3.5 ng/ml	19	54.28	16	45.71
>3.5 ng/ml	3	8.5	13	37.14

AMH = antimullerian hormone

**Table 6: Mean values of AMH and vitamin D levels in infertile and fertile females**

Mean value	Cases (infertile group)	Control (fertile group)
Vitamin D	6.18 ± 2.09 ng/ml ( $P = 0.04$ )*	4.85 ± 3.02 ng/ml
AMH	1.94 ± 1.30 ng/ml ( $P = 0.003$ )*	3.47 ± 2.59 ng/ml

AMH = antimullerian hormone;  $P < 0.05$  by *t* test.

( $P = 0.003$ ), which might be a cause of infertility in infertile females. AMH is a predictor of ovarian reserve and ovarian responsiveness that directly affect the fertility of a women, excluding the other causes if infertility.

Vitamin D is also known as “anti-ricketic factor or sunshine vitamin.” Dietary intakes generally has only a minor influence on serum levels outside of the consumption of vitamin D supplements.<sup>[13]</sup> Even in tropical countries, despite of ample sunlight (required for the synthesis of vitamin D endogenously), VDD is prevalent in range of 50 to 90% among all the age groups.<sup>[14]</sup> Vitamin D levels did not vary according to age or infertility associated disorders.<sup>[12]</sup> Another study reported the prevalence of VDD significantly higher in the subfertility group than controls (59.0 versus 40.4%;  $P < 0.01$ ).<sup>[15]</sup> Only one study reported a positive relationship between vitamin D and serum AMH levels.<sup>[16]</sup> However, this study admits the significant methodological weakness because of very low numbers of subjects.

According to a study, vitamin D supplementation can reduce high AMH production, leading to increase follicular sensitivity to FSH and return to normal ovulation.<sup>[17]</sup> An observational study reported a insignificant weak negative correlation between serum vitamin D and AMH in young individuals, but a weak possible positive correlation between AMH and vitamin D in women aged 40 years or older. The existing literature does not provide any definitive and consistent pattern for how vitamin D may affect AMH production or serum levels. The key finding in our prospective study in women of child bearing age that serum vitamin D levels appear to be unrelated to AMH levels. Merhi *et al.* had also reported, no relationship between serum vitamin D and AMH levels in women aged 35 to 40 years, with similar results.<sup>[18]</sup>

There is no consensus that how vitamin D may affect AMH production or vitamin D has really any role in AMH production. Similar to the *in vivo* studies, the *in vitro* data analyzing the influence of vitamin D on AMH production

are also contradictory. Early studies using a prostatic cancer cell line showed that vitamin D could increase AMH.<sup>[18,19]</sup> It has also been reported that women with darker skin pigmentation have lower serum AMH levels.<sup>[20]</sup> This has been also seen that women with dark skin have faster rate of decline in AMH levels than Caucasian women, resulting earlier onset of menopause.<sup>[21,22]</sup> Considering that vitamin D increases granulosa cell production of AMH, it is theoretically possible that vitamin D supplementation in VDD could improve the ovarian reserve and delay the onset of menopause.<sup>[23]</sup>

Limitations of our study are small sample size and prospective study, so we recommend further larger sample size study, so that stronger recommendations can be predicted that can be utilized for social betterment.

## CONCLUSION

The prevalence of VDD was 64.28% in infertile women groups. There was no correlation found between VDD and AMH levels in both the infertile and fertile women groups. The rate of VDD among women with impaired fertility is alarming. Prospective further studies are pressingly needed to confirm a causal relationship and to investigate the potential therapeutic benefits of vitamin D supplementation in this population.

## Acknowledgements

The study was supported by the Intramural Grant from Research Committee, Sanjay Gandhi Postgraduate Institute of Medical sciences, Lucknow, Uttar Pradesh, India.

Criteria for Authorship: All listed above qualify as per guidelines and had done substantial work for this manuscript. The entire author takes responsibility of intellectual content of the paper. The details of work done by authors are as follows; I.L.: original idea conceiving or designing the study, doing whole work as team leader, data collection, data analysis, and write



manuscript; S.Y.: Lab work, data collection, and compilation; S.Y.: manuscript and proof reading. S.T.: Lab work, data collection, and compilation and analysis of data; A.G.: manuscript review, and proof reading.

### Financial support and sponsorship

This study was conducted with the help of Intramural Project grant sponsored by SGPGIMS, Lucknow, UP, India.

### Conflicts of interest

There are no conflicts of interest.

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