

Does Serum Vitamin D Level Have an Impact on Semen Parameters?

Priya M¹, Kundavi K. M², Rashmi G.V³, Geetha V⁴, Geovin Ranji⁵, Yamini Asokan⁶,
Hema Nivedha K.R⁷, Sandhya Devarajan⁸

¹ Clinicians, Institute of Reproductive Medicine and Women's Health, The Madras Medical Mission, Chennai, Tamilnadu
Corresponding Author Email: [mohanapriya1974\[at\]gmail.com](mailto:mohanapriya1974[at]gmail.com)

^{2, 3, 4, 5} Clinicians, Institute of Reproductive Medicine and Women's Health, The Madras Medical Mission, Chennai, Tamilnadu

^{6, 7, 8} Embryologists, Institute of Reproductive Medicine and Women's Health, The Madras Medical Mission, Chennai, Tamilnadu

Abstract: **Background:** Vitamin D receptors located on the head and mid piece of a sperm suggest a possible role of vitamin D in male fertility. This study evaluated the association between serum vitamin D levels and semen parameters in subfertile men. **Methods:** In this prospective observational study, after applying inclusion and exclusion criteria, 75 subfertile men were included and they underwent semen analysis according to WHO 2021 criteria and serum vitamin D assessment by ELISA assay. Participants were categorized as vitamin D deficient or sufficient and semen parameters were compared. The semen parameters of the males belonging to vitamin D sufficient versus vitamin D deficient group were expressed in terms of Mean \pm standard deviation. Student t-test was employed as appropriate, p-value < 0.05 was considered significant. **Results:** Vitamin D deficient men had significantly lower sperm concentration, total sperm count, progressive motility, morphology and total motile sperm count compared with vitamin D sufficient men, while age, BMI and semen volume showed no significant association. The p-value of total sperm motility, the primary outcome of this study was 0.002 and was statistically significant. The secondary outcomes like total motility of sperms and morphology were also clinically significant. **Conclusion:** Adequate serum vitamin D levels were associated with better semen quality. Larger prospective and interventional studies are needed to clarify causality.

Keywords: Vitamin D, Male infertility, Semen quality, Subfertility, Spermatogenesis, Sperm motility

1. Introduction

In recent days, due to its autocrine, paracrine, endocrine activity on a number of target organs and systems, vitamin D has been regarded as an intriguing research topic. Intestine, cardiovascular system, skeletal system, thyroid, kidneys, parathyroid glands, immunological system, pancreas, adipose tissue, central nervous system and the reproductive system are primary target organs of vitamin D.¹ Benefits of vitamin D depend on activation of cholecalciferol, or inactive vitamin D₃, that typically begins in skin when 7-OH cholesterol is converted to vitamin D₃ by Ultraviolet B radiations. Following its activation by renal 1 α -hydroxylase and hepatic 25-hydroxylases, active calcitriol (1,25(OH)₂D₃) binds to the vitamin D receptors with until CYP24A1 inactivates it.² Through binding to these receptors, calcitriol performs both genomic and non-genomic functions. By demonstrating presence of the Vitamin D receptors on human sperm and demonstrating effect on total number of motile sperm, significant reduction in sperm motility, effect on amount of sex hormone globulin in vitamin D deficient males than vitamin D sufficient males, the significance of vitamin D in human reproductive physiology was further investigated. In a group like Indian population where vitamin D deficiency is prevalent, and incidence of male factor infertility is about 20%, we have to ascertain whether serum vitamin D level has impact on semen parameters.³ Our research aims to correlate the vitamin D levels with the semen parameters in men so that in future we can correct vitamin D deficiency and hence improve semen parameters as well.

Aim:

To find the correlation between serum vitamin D levels and semen parameters in subfertile males

2. Methodology

Study design and setting

This was a prospective observational study conducted in the Institute of Reproductive Medicine and Women's Health at The Madras Medical Mission Hospital, Chennai over a period of 6 months.

Study population

All the male patients who attended the OPD for evaluation of subfertility during the period of study were recruited.

Inclusion criteria

Men aged 20-55 years who attended the OPD for evaluation of subfertility at our institution during the period of study of 6 months.

Exclusion criteria

Patients who were excluded from research includes, males who were already on vitamin D therapy, those who were receiving calcium supplementation, those with diabetes mellitus, parathyroid gland disease, malabsorption syndrome, those who had undergone gastric bypass surgery, those with celiac disease, history of varicocele repair in the last 6 months, those with inflammatory bowel disease, smokers and those with any malignancies.

Sample Size Calculation

Sample size was calculated using a two-sided confidence level of 95% and a statistical power of 80%, with an alpha error of 0.05 and beta error of 0.20. The standard deviation (SD) was taken as 23.3

The sample size was estimated using the formula

$$n = 2SD^2(z\alpha/2 + z\beta)^2/d^2$$

Where:

n = sample size

σ = standard deviation

Z $\alpha/2$ = standard normal deviate at 95% confidence level

Z β = standard normal deviate at 80% power

d = effect size

Based on this calculation, the minimum required sample size was 90 participants. After applying exclusion criteria cited above, 75 men were included in the study

Data Collection

After informed consent had been received from patients, a thorough history and clinical examination of the male patients was done. Data collected included age, BMI and duration of infertility and relevant medical history. Patients were asked to provide semen sample by masturbation followed by 3-5 days of abstinence in a sterile container. Samples were allowed to liquefy and analyzed within 1 hour of collection. Sperm concentration was assessed using a Makler's counting chamber. Motility and morphology was evaluated under phase-contrast microscopy. A minimum of 200 spermatozoa were evaluated per sample.

The fertility status of the participants is based on WHO criteria 2021 Cut off for normal semen analysis.⁴ The semen parameters are as follows:

- Total sperm count >39million/ejaculate
- Total motility (progressive + Non progressive): >40 % (observed in 1 hour of collection)
- Morphology: > or equal to 4%
- Total motile sperm concentration is calculated with the formula

= volume(ml) x concentration(million/ml) x (A+B)/ 100

'A' is rapid progressive motility, 'B' is slow progressive motility.

For vitamin D samples: 5 ml of venous blood is collected. The cut off points for serum vitamin D: Deficient: <20ng/ml. Serum vitamin D levels have been calculated employing Enzyme linked Immunosorbent Assay (ELISA) method.

After obtaining results of serum vitamin D levels and semen analysis, subjects are distinguished into 2 groups: one group with normal vitamin D and another group with vitamin D deficiency. The semen parameters were then correlated with vitamin D levels.

Primary endpoint of research has to determine correlation between serum vitamin D levels and sperm motility and secondary endpoint was to find correlation among serum vitamin D levels, sperm count, morphology.

Statistical Analysis:

Data were entered in Microsoft Excel software and analyzed employing IBM SPSS software windows V27. Normality of distribution was assessed using the Shapiro–Wilk test. Normally distributed variables were expressed as mean \pm standard deviation and analyzed using Student's t-test. Multivariable linear regression analysis was not performed since confounding variables such as age, BMI, smokers, males who were already on vitamin D therapy, those who were receiving calcium supplementation, those with diabetes mellitus, parathyroid gland disease, malabsorption syndrome, those who had undergone gastric bypass surgery, those with celiac disease, history of varicocele repair in the last 6 months, those with inflammatory bowel disease, and those with any malignancies were excluded from the study. A p-value less than 0.05 was considered statistically significant.

Ethical Considerations

The study was approved by the Institutional Ethics Committee, and informed consent was obtained from all participants.

(EC Reg no.: ECR/140/Inst/TN/2013/RR-25).

Reference number: MMM-IEC-NCT:039/2025

3. Results

In this study, 75 patients were included after applying the exclusion criteria. Most of patients belonged to age group 31-40 years (68.9%). The age distribution of the population is shown in figure1. The BMI distribution of research subjects is shown in Table 1. It shows that around 6.6% of the patients (5 patients) were overweight with BMI between 25-29.9 and around 12% of the patients were obese (9 patients) and remaining of 81.1% patients had normal BMI (61 patients). Out of 9 obese patients, only 3 had vitamin D deficiency and out of 5 overweight patients, two had vitamin D deficiency. Out of 75 patients, 43 patients had normal vitamin D levels (57.4%) and 32 patients (42.6%) were vitamin D deficient (table 2). In both groups, association between the semen parameters and vitamin D levels was expressed in terms of mean \pm standard deviation. The semen volume did not differ between two groups, with p-value of 0.94, which was not statistically significant. Mean sperm concentration (million/ml) value in vitamin D sufficient group was 37.86 \pm 20.06 million/ml whereas deficient group was 24.97 \pm 22.73 million/ml. The p-value was 0.011 and was statistically significant (Table 3). The mean value for total sperm count (million/ejaculate) in vitamin D sufficient group was 93.49 \pm 69.31 million/ejaculate, whereas deficient group was 57.00 \pm 54.38 million/ejaculate (Table 3 and Figure 3a). The p-value was 0.016 and was statistically significant. The mean value of total sperm motility was 49.63 \pm 18.52% in sufficient group versus 29.13 \pm 21.77% in deficient group, which was statistically significant with p-value of 0.002 (Table 3 and Figure 3b). Mean value of rapidly progressive motility has been 9.21 \pm 5.85 percentage versus 4.28 \pm 4.23% respectively in sufficient versus deficient group (table 3). The mean values of slow progressive and non-progressive sperm motility are shown in Table 3.

The mean value of morphology of sperms was 1.88 \pm .90 versus 0.97 \pm .78 % in sufficient versus deficient group with highly significant p-value of 0.001. Mean value of total motile

sperm concentration was 33.12 ± 30.80 versus 16.91 ± 19.70 in sufficient versus deficient group with p -value of 0.011 and was statistically significant (table 3)

4. Discussion

Vitamin D employs its biological impacts via the Vitamin D receptors, which are extensively present in male reproductive system. They were identified in prostate, epididymis, seminal vesicles, and testicular tissue including germ cells such as spermatogonia, spermatocytes, Sertoli cells. Additionally, localization of these receptors in human spermatozoa, particularly in the post-acrosomal region, mid-piece and neck, very well supports its vital role on sperm function.

Our study results proves that there is a statistically significant association between the serum vitamin D levels and semen parameters like sperm concentration total sperm count, motility and morphology. These findings propose potential role of vitamin D in male reproductive physiology and semen quality.

Our study was similar to the study done by Hammoud AO *et al.*, in which the sperm concentration, total progressive motile sperm count, rapid progressive motility were low in vitamin D-deficient individuals than in healthy individuals.

Morphology of the sperm also showed a similar pattern, where it was better in men having normal vitamin D levels. This was similar to the research conducted by Ramlau-Hansen *et al.*⁵

Vitamin D plays acts as a regulator for steroidogenesis. This steroidogenesis is important for production of sex hormones like Estrogen, Testosterone and progesterone. Hence, vitamin D deficiency in males lowers the testosterone levels, thereby affecting the spermatogenesis. Experimental investigations have shown that vitamin D deficiency may lead to down-regulation of testicular testosterone synthesis enzymes, thereby potentially impairing spermatogenesis and overall semen quality.⁶

Our outcomes are consistent with several studies which established a positive association between serum vitamin D levels and sperm parameters, mainly the sperm motility and total sperm count. Vitamin D deficiency was described to adversely affect sperm motility, possibly through its role in calcium homeostasis. Many in vitro studies demonstrated that the exposure of human spermatozoa to active vitamin D [$1,25(\text{OH})_2\text{D}_3$], it increases intra-cellular calcium levels via VDR-mediated mechanisms, thereby enhancing sperm motility and inducing acrosome reaction. Furthermore, increased adenosine triphosphate (ATP) production and improved sperm kinetics following vitamin D incubation highlight its role in energy metabolism and sperm function.

A recent cross-sectional study by Hajianfar *et al.* demonstrated that reduction in serum vitamin D levels, were significantly associated with impaired semen parameters like total motility, semen volume, sperm count and normal morphology. It was also observed that, normal vitamin D levels were independently associated with normal semen parameters after adjusting for confounding variables such as

smoking, age, BMI, alcohol intake, varicocele status.⁷ The Hajianfar *et al* study demonstrates a broader positive relationship across multiple semen parameters, thereby strengthening the argument for vitamin D's role in spermatogenesis.

While some studies demonstrate significant positive associations between vitamin D and semen quality, others report no such relationship.⁸ This inconsistency might be ascribed to differences in study design, population characteristics, assay variability and seasonal variation of vitamin D levels. One such study done by Thuesen *et al* showed that there was seasonal variation in serum vitamin D levels(vitamin D levels were higher in summer).⁹

Overall, the current body of evidence implies adequate vitamin D levels might have beneficial role in improving semen quality, particularly motility and possibly other parameters such as sperm count and morphology. Few studies have demonstrated that vitamin D increased the calcium ions in the head and neck of the sperms, which helped in motility of sperms.¹⁰ Future research is needed to determine whether greater Vitamin D levels can reduce the negative consequences of oxidative stress, lipid peroxidation, and DNA damage while also improving sperm quality and/or testosterone levels.¹¹

5. Limitations

Despite these findings, certain limitations must be considered. Study has been performed on comparatively small sample size, mainly in subgroup analyses, which might restrict generalization of outcomes. Additionally, potential confounding factors, namely lifestyle habits, smoking status, nutritional factors have not been assessed. Important biochemical parameters, including calcium, phosphorus, parathyroid hormone levels, and sperm DNA fragmentation index, have not been determined. Seasonal variation in serum vitamin D levels and its influence on semen parameters were not taken into account. Furthermore, genetic polymorphisms in VDR and vitamin D binding protein, that might influence serum vitamin D levels and reproductive outcomes, have not been studied.

6. Conclusion

Sufficient Serum vitamin D level was associated with improved semen parameters, particularly sperm count, motility and morphology, among subfertile men. These findings support a possible role of vitamin D in male reproductive physiology, but causal relationships and the effect of supplementation require confirmation through larger prospective and randomized studies.

7. Future Research

Further large- scale prospective studies must be done to prove whether vitamin D supplementation improves semen parameters. Future research should also explore the role of hormone interactions (testosterone, LH, FSH) and calcium mediated mechanisms on spermatogenesis.

Acknowledgements

We gratefully thank the department of embryology of “The Madras Medical Mission Hospital, Chennai” for helping in this article.

Declarations

Funding: NIL

Conflict of interest: NIL

Ethical approval: This research is approved by “Ethics committee of The Madras Medical Mission Hospital (EC Reg no.: ECR/140/Inst/TN/2013/RR-25).”

Reference number: MMM-IEC-NCT:039/2025

Table 3: Association of Semen parameters with Vitamin D level among the study subjects

| Semen parameters | Deficient (≤ 20) (Mean \pm SD) | Sufficient (> 21) (Mean \pm SD) | P value |
|---------------------------------------|---|---------------------------------------|---------|
| Semen volume(ml) | 2.34 \pm 1.25 | 2.363 \pm 1.21 | .947 |
| Sperm concentration (million/ml) | 24.97 \pm 22.73 | 37.86 \pm 20.06 | .011* |
| Total sperm count (million/ejaculate) | 57.00 \pm 54.38 | 93.49 \pm 69.31 | .016* |
| Total motility (%) | 29.13 \pm 21.77 | 49.63 \pm 18.52 | .002* |
| Rapid progressive motility (%) | 4.28 \pm 4.23 | 9.21 \pm 5.85 | .000* |
| Slow progressive (%) | 13.00 \pm 9.36 | 22.07 \pm 8.63 | .000* |
| Non progressive (%) | 12.13 \pm 8.81 | 17.98 \pm 6.65 | .002* |
| Morphology (%) | .97 \pm .78 | 1.88 \pm .90 | .001* |
| TMSC (million/ml) | 16.91 \pm 19.70 | 33.12 \pm 30.80 | .011* |

*p value<0.05-statistically significant

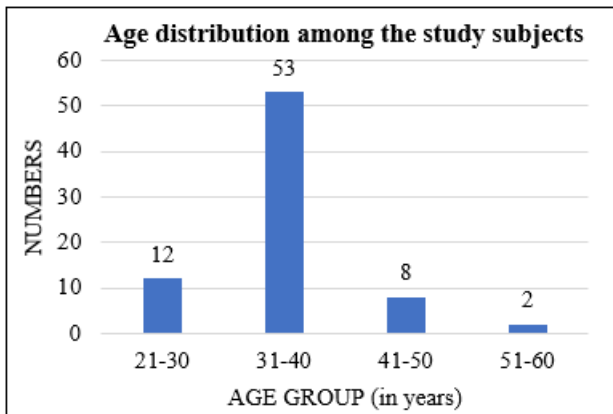


Figure 1: Age distribution among the study subjects

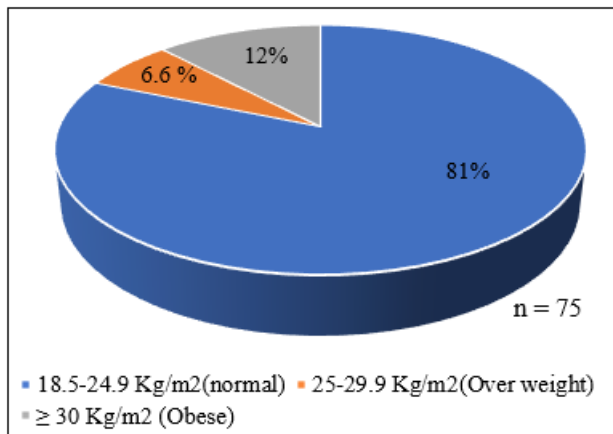


Figure 2: BMI distribution among the study subjects

Table 1: BMI distribution of study subjects

| BMI (kg/m ²) | Frequency (n=75) | Percentage (%) |
|--------------------------|------------------|----------------|
| 18.5-24.9 (normal) | 61 | 81.1 |
| 25-29.9 (Overweight) | 5 | 6.6 |
| ≥ 30 (Obese) | 9 | 12.0 |

Table 2: Vitamin D level among the study subjects

| Vitamin D | Frequency (n=75) | Percentage (%) |
|------------------|------------------|----------------|
| Deficient (<20) | 32 | 42.6 |
| Sufficient (>21) | 43 | 57.4 |

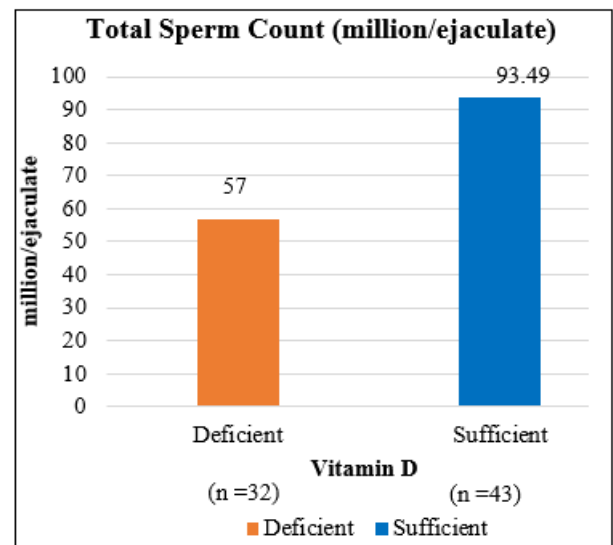


Figure 3a,3b: Semen Parameters in Vitamin-D deficient versus Sufficient Subjects (Mean +SD)

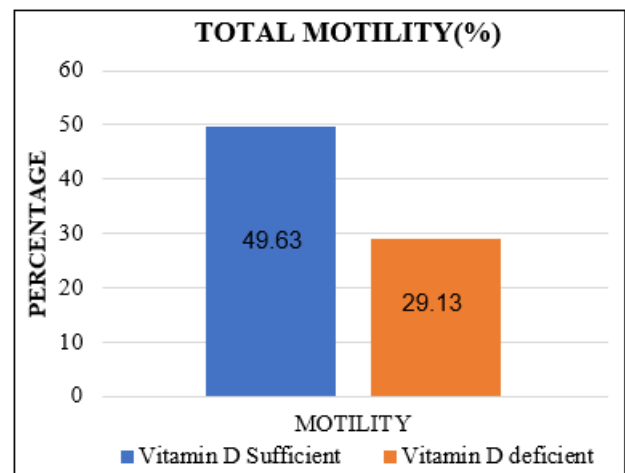


Figure 3b

References

[1] Cito G, Cocci A, Micelli E, Gabutti A, Russo GI, Coccia ME, et al. Vitamin D and male fertility: an updated review. World J Mens Health. 2020;38(2):164-177. doi:10.5534/wjmh.190057.

- [2] Blomberg Jensen M, Bjerrum PJ, Jessen TE, Nielsen JE, Joensen UN, Olesen IA, et al. Vitamin D is positively associated with sperm motility and increases intracellular calcium in human spermatozoa. *Hum Reprod.* 2011;26(6):1307-1317. doi:10.1093/humrep/der059.
- [3] Agarwal A, Srivastava A, Fathima F, Lodhi B. Insight into epidemiology of male infertility in central India. *Int J Reprod Contracept Obstet Gynecol.* 2022;12(1):215-220. doi:10.18203/2320-1770.ijrcog20224556.
- [4] World Health Organization. WHO laboratory manual for the examination of human semen and sperm-cervical mucus interactions. 6th ed. Cambridge, UK: Cambridge University Press;2021.
- [5] Hammoud AO, Meikle AW, Peterson CM, Stanford J, Gibson M, Carrell DT. Association of 25-hydroxyvitamin D levels with semen and hormonal parameters. *Asian J Androl.* 2012;14(6):855-859. doi:10.1038/aja.2012.77.
- [6] Ramlau-Hansen CH, Moeller UK, Bonde JP, Olsen J, Thulstrup AM. Are serum levels of vitamin D associated with semen quality? Results from a cross-sectional study in young healthy men. *Fertil Steril.* 2011;95(3):1000-1004. doi:10.1016/j.fertnstert.2010.11.002.
- [7] Hajianfar H, Karimi E, Mollaghasemi N, Rezaei S, Arab A. Is there a relationship between serum vitamin D and semen parameters? A cross-sectional study of Iranian infertile men. *Basic Clin Androl.* 2021;31(1):29. doi:10.1186/s12610-021-00147-3.
- [8] Arab A, Hadi A, Moosavian SP, Askari G, Nasirian M. The association between serum vitamin D, fertility and semen quality: a systematic review and meta-analysis. *Int J Surg.* 2019; 71:101-109. doi: 10.1016/j.ijssu.2019.09.025.
- [9] Thuesen B, Husemoen L, Fenger M, Jakbsen J, Schwarz P, Toft U et al. Determinants of vitamin D status in a general population of Danish adults. *Bone.* 2012; 50(3): 605-610. doi:10.1016/j.bone.2011.12.016
- [10] Yan TF, Qi JJ, Li LX, Li F. The association between serum vitamin D levels and male fertility: a systematic review and meta-analysis. *Andrologia.* 2023;55:e14789. doi:10.1155/2023/9002938.
- [11] Ciccone IM, Costa EM, Pariz JR, Teixeira TA, Drevet JR, Gharagozloo P, et al. Serum vitamin D content is associated with semen parameters and serum testosterone levels in men. *Asian J Androl.* 2021;23(1):52-58. doi:10.4103/aja.aja_9_20.