

MALE FACTOR INFERTILITY AND ITS ASSOCIATION WITH VITAMIN D3 DEFICIENCY IN KASHMIR, NORTH INDIA: AN OBSERVATIONAL ANALYSIS OF CLINICAL AND SOCIODEMOGRAPHIC FACTORS

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ABSTRACT

Introduction: Male factor infertility is a rising concern regarding health and contributes to global infertility. Vitamin D3 is significant for spermatogenesis, the regulation of hormones, and the motility of the sperm. The study aims to analyse the relationship between the deficiency of vitamin D3, features of semen, hormonal profile and the socio-demographic parameters among infertile males.

Method: This hospital-based cross-sectional analytical study included 150 infertile men. Clinical evaluation, semen analysis, serum Vitamin D3 estimation, and testosterone assessment were performed for all participants. Semen parameters including sperm count, motility, morphology, and seminal white blood cell count were evaluated according to standard WHO guidelines. Participants were categorised based on Vitamin D3 status. Data were analyzed using descriptive statistics, Chi-square tests, correlation analysis, one-way ANOVA, and post hoc multiple comparison tests, with $p < 0.05$ considered statistically significant.

Result: Among 150 infertile men, 34.0% were Vitamin D deficient and 17.3% were Vitamin D insufficient. Vitamin D sufficiency was associated with significantly higher total sperm count, sperm motility, actively motile sperm percentage, and testosterone levels compared with Vitamin D deficiency ($p < 0.05$). Vitamin D levels showed significant positive correlations with total sperm count ($r = 0.303$), motility ($r = 0.336$), active motility ($r = 0.355$), and testosterone ($r = 0.321$), while demonstrating negative correlations with immotile sperm percentage ($r = -0.354$) and seminal white blood cell count ($r = -0.326$) (all $p < 0.05$).

Conclusion: The study has concluded that Vitamin D3 deficiency is highly prevalent among men with infertility and is associated with poorer semen quality. Lower Vitamin D3 levels were significantly associated with reduced total sperm count, sperm motility, actively motile sperm percentage, and testosterone levels, while showing significant negative associations with immotile sperm percentage and seminal white blood cell count.

KEYWORDS: Male infertility, Vitamin D3 deficiency, Sperm motility, Testosterone, Kashmir population

INTRODUCTION

Infertility in males is a condition in which males cannot father a pregnancy in a fertile female after one year or longer of frequent, unprotected sexual intercourse. In this case, it is also associated with pathological alterations in semen composition such as low sperm count, low motility or abnormal sperm morphology due to any type of: endocrine, genetic or anatomical alterations. The most current GBD report indicates that male infertility has become increasingly prevalent worldwide, rising from 2752.5 per 100,000 population to 3218.9 per 100,000 population, with low- and middle-income countries experiencing the largest increases in prevalence. According to reports, India is the nation with the most total cases and disability-adjusted-year (DALY) worldwide as of 2021 due to about 0.5% per year estimated change rate and its population of more than one billion people [1, 2]. Various multisite studies have been conducted to substantiate these findings; for example, in the IM-FaST multi-site study which enrolled 3165 infertile couples, 27.3% of all couples assessed experienced male factor subfertility while the primary cause of male factor subfertility was asthenozoospermia (23.4% of total) [3]. In addition, urban living, lower socioeconomic status, heat exposure, severe varicoceles, and a history of COVID-19 were identified as independent predictors of poor semen quality [3]. In addition, longitudinal data from a cohort of males living in rural areas demonstrate that, after the age of 35 years, sperm volume, sperm count, and sperm motility all have significantly declined due to the advancing age of males [4].

Vitamin D in its active form (Vitamin D3) acts as an important reproductive steroid-like signalling molecule through the vitamin D receptor (VDR) present on Leydig, Sertoli, and germ cells to modify the testicular microenvironment [5]. Active

VDR signalling regulates genomic pathways required for germ cell development, sperm maturation and the integrity of the germinal epithelium in experimental animals in India [5, 6]. VDR expression in Leydig cells is a direct mechanistic pathway that regulates testosterone biosynthesis [5]. Vitamin D3 supplementation alters steroidogenic markers and sexual modulators (androgens/estrogens), increases CYP19A1 expression and blood levels of androgens. Thus, this autocrine-paracrine system maintains spermatogenesis and mitigates age-related declines in reproduction [6].

The vitamin D deficiency endemic in all areas of Kashmiri population living in rural and urban locations is an incongruity where we have theoretical availability of sunlight, but due to being at high altitudes, multiple months with temperatures below freezing and limited exposure to sunlight prevent any cutaneous production from happening [7]. This nationwide vitamin D deficiency is compounded by the Kashmiri tradition of layered clothing, sedentary living during cold months, and very little to no dietary intake of fortified foods or fatty fish [7]. In contrast to this widespread problem, we see localized reports of life-threatening complications associated with vitamin D overdose and acute renal failure due to use of injectable cholecalciferol in very large doses as a means of addressing vitamin D deficiency [8].

Male infertility in Kashmir is due in part to genetics, lifestyle factors, and exposure to toxins that produce oxidative stress and disrupt the endocrine system [9]. The data from Jammu and Kashmir also indicates that variations in the genes for antioxidant enzymes superoxide dismutase 1 (SOD1) and superoxide dismutase 2 (SOD2) (Ala16Val) appear to produce an altered baseline redox balance that increases the chance of producing abnormally shaped sperm in males with a genetic disposition to infertility [9]. The genetic predisposition of male infertility (along with exposure to globally occurring environmental xenobiotics, high levels of pesticide exposure from agriculture, and substance use) will introduce pathways for the depletion of glutathione (an antioxidant). This ultimately leads to oxidative stress and extensive damage to semen nucleic acids from oxidative stress or oxidants; ultimately shifting the redox balance of males predisposed to infertility toward poor sperm production and reproductive potential [9, 10]. Ultimately, chronic psychosocial stressors and limited access to healthcare have a significant negative effect on the ability of males in this region to cope with their infertility and will further lead to an already genetically predisposed redox state being dramatically altered towards extremely poor sperm viability and fertility potential [9, 10].

This observational community-based study will assess the regional prevalence of male factor infertility in North/Central Kashmir; identify independent sociodemographic/clinical risk factors for male factor infertility, and examine how male factor infertility directly relates to vitamin D3 deficiency.

METHOD

Research design

The present study was a hospital-based cross-sectional analytical study conducted among 150 infertile men attending a tertiary care center. The study was carried out in a Tertiary Care Hospital for a period of one year. Eligible participants underwent detailed clinical evaluation, semen analysis, and biochemical assessment of serum Vitamin D3 and testosterone levels. Semen samples were analyzed according to standard WHO guidelines to determine sperm count, motility, morphology, and seminal white blood cell count. Based on serum Vitamin D3 levels, participants were categorized into deficient, insufficient, and sufficient groups. The study aimed to evaluate the association of Vitamin D3 status with semen quality, sperm morphology, testosterone levels, and genital tract infection. Statistical analysis included descriptive statistics, Chi-square tests, correlation analysis, one-way ANOVA, and post hoc multiple comparisons. Relationships between Vitamin D3, testosterone, and semen parameters were assessed using Pearson's and Spearman's correlation coefficients. The findings were used to determine the potential role of Vitamin D3 deficiency in male factor infertility.

Inclusion Criteria

- Patients of age between 20 to 50 years were selected for the study.
- Those who were diagnosed with the primary or secondary infertility for ≥ 1 year were included.
- All patients were taken with the informed consent.
- Analysis of the semen with the evaluation of the Vitamin D3 was collected and analysed.

Exclusion Criteria

- Patients with previous history of genetic or congenital causes of infertility or any other urogenital surgery, chemotherapy, or radiotherapy were excluded.
- Patients with Chronic systemic diseases excluded
- Supplements of Vitamin D or any undergoing with fertility treatment were not included for the study.
- Irrelevant data or missed data were not included.

Procedure

A total of 150 males presenting with infertility were enrolled in the study after obtaining informed consent and fulfilling the eligibility criteria. Detailed demographic and clinical information was collected using a structured case record form. Clinical assessment included evaluation of infertility history, duration of infertility, previous reproductive outcomes, lifestyle factors, and evidence of genital tract infection. All participants underwent laboratory investigations including semen analysis, serum Vitamin D3 estimation, and serum testosterone measurement.

Semen samples were collected by masturbation following a recommended period of 2–7 days of sexual abstinence. Samples were obtained in sterile, wide-mouthed containers and allowed to liquefy at room temperature before analysis. Semen analysis was performed according to World Health Organization (WHO) laboratory guidelines. Parameters assessed included total sperm count (TSC), total motility, actively motile sperm (AM), sluggishly motile sperm (SM), immotile sperm percentage, sperm morphology, and seminal white blood cell (WBC) count. Based on semen characteristics, participants were categorized into normozoospermia, azoospermia, oligozoospermia,

oligoasthenozoospermia, and teratozoospermia groups. Seminal WBC count was used as an indicator of genital tract inflammation or infection.

Venous blood samples were collected under aseptic precautions for estimation of serum Vitamin D3 and testosterone levels. Serum 25-hydroxyvitamin D [25(OH)D] concentration was measured using a standardized laboratory assay. Based on the measured values, participants were classified into Vitamin D-deficient, Vitamin D-insufficient, and Vitamin D-sufficient groups according to established reference ranges. Serum total testosterone levels were estimated using a standardized immunoassay technique and were categorized as normal or low according to laboratory reference values.

Data were entered into Microsoft Excel and analyzed using Statistical Package for the Social Sciences (SPSS) software. Continuous variables such as sperm count, motility parameters, seminal WBC count, Vitamin D3 levels, and testosterone levels were summarized using mean and standard deviation, whereas categorical variables were presented as frequencies and percentages. The association between sperm morphology categories and genital tract infection status, as well as the association between sperm morphology and testosterone status, was assessed using the Pearson Chi-square test. The relationship between Vitamin D3 deficiency status and categorical reproductive variables was also evaluated using Chi-square analysis.

To further explore the association between sperm morphology and Vitamin D3 status, Pearson Chi-square, Likelihood Ratio, and Linear-by-Linear Association tests were performed. Pearson's and Spearman's correlation coefficients were calculated to assess the strength and direction of associations between Vitamin D3 levels, testosterone levels, and semen analysis parameters. Bivariate correlation analysis was used to determine relationships between Vitamin D3 concentration, testosterone levels, total sperm count, sperm motility, actively motile sperm percentage, sluggishly motile sperm percentage, immotile sperm percentage, and seminal white blood cell count.

Statistical analysis

Data analysis was performed by the SPSS version 27. For comparison of semen analysis parameters among Vitamin D-deficient, insufficient, and sufficient groups, one-way analysis of variance (ANOVA) was performed. Variables demonstrating significant overall differences were subjected to post hoc multiple comparison testing to identify specific intergroup differences. Mean differences and corresponding p-values were calculated for each pairwise comparison. Throughout the analysis, a p-value less than 0.05 was considered statistically significant. A p-value <0.05 was maintained for statistical significance.

RESULT

Table 1 shows the descriptive statistics of semen analysis parameters among the study participants. The mean total sperm count was 53.3 ± 18.65 million, with values ranging from 0 to 95 million, indicating substantial variability in sperm concentration across the study population. The mean total motility was $69.98 \pm 16.94\%$, while the proportions of actively motile and sluggishly motile sperm were $53.33 \pm 15.1\%$ and $16.89 \pm 7.28\%$, respectively. The mean percentage of immotile sperm was $29.82 \pm 16.93\%$, with values extending up to 100%, reflecting severe motility impairment in some individuals. The mean seminal white blood cell count was 6.35 ± 3.58 , ranging from 0 to 20, suggesting varying degrees of inflammatory activity within the reproductive tract. Overall, the findings demonstrate marked heterogeneity in semen quality among the participants.

Table 1: Findings related to Semen analysis of the patients

Semen Analysis Parameter	N	Mean $\pm$ SD	Minimum	Maximum
TSC	149	53.3 ± 18.65	0	95
Motility	149	69.98 ± 16.94	0	90
AM	149	53.33 ± 15.1	0	85
SM	149	16.89 ± 7.28	0	45
Immotile	149	29.82 ± 16.93	10	100
WBC	149	6.35 ± 3.58	0	20

Table 2 shows the distribution of serum Vitamin D3 and testosterone levels among the study participants. The mean Vitamin D3 concentration was 29.47 ± 13.06 ng/mL, with values ranging from 8.2 to 61.2 ng/mL, indicating considerable variation in Vitamin D status and the presence of both deficient and sufficient individuals within the cohort. The mean serum testosterone level was 3.91 ± 0.96 ng/mL, with a minimum value of 2.35 ng/mL and a maximum value of 6.5 ng/mL. Compared with testosterone, Vitamin D3 levels exhibited greater variability, suggesting differences in nutritional and metabolic status among the participants. These findings provide a foundation for examining the relationship of these biochemical parameters with semen quality and reproductive health.

Table 2: Vitamin D3 and Testosterone level of the patients

Semen Analysis	N	Mean $\pm$ SD	Minimum	Maximum
VitaminD3	150	29.47 ± 13.06	8.2	61.2
Testosterone	150	3.91 ± 0.96	2.35	6.5

Table 3 shows the association between sperm morphology, Vitamin D3 deficiency status, and genital tract infection among the study participants. A statistically significant association was observed between sperm morphology and genital tract infection (Pearson $\chi^2 = 24.748$, $p < 0.001$). Genital tract infection was present in all patients with oligoasthenozoospermia (100%) and oligozoospermia (100%), whereas no infection was detected among patients with azoospermia or teratozoospermia. Among participants with normozoospermia, 17.9% had genital tract infection while 82.1% were free from infection. These findings indicate that abnormalities in sperm morphology, particularly those involving reduced sperm count and impaired motility, are significantly associated with a higher prevalence of genital tract infection ($p < 0.001$).

Furthermore, genital tract infection was observed in 31.4% of participants with Vitamin D deficiency, compared with 19.2% among those with Vitamin D insufficiency and 13.7% among those with sufficient Vitamin D levels. Although the prevalence of infection decreased progressively with improving Vitamin D status, the association did not attain statistical significance (Pearson $\chi^2 = 5.76$, $p = 0.056$). Nevertheless, the results demonstrate a notable trend suggesting that lower Vitamin D3 levels may be associated with an increased occurrence of genital tract infection, warranting further investigation in larger cohorts.

Table 3: Association between Sperm Morphology and Vitamin D3 Deficiency Status with Genital Tract Infection of the patients

Parameter	Category	Genital Tract Infection		Pearson χ^2	P-value
		No n (%)	Yes n (%)		
Sperm Morphology	Azoospermia (n=3)	3 (100.0)	0 (0.0)	24.748	<0.001
	Normozoospermia (n=140)	115 (82.1)	25 (17.9)		
	Oligoasthenozoospermia (n=5)	0 (0.0)	5 (100.0)		
	Oligozoospermia (n=1)	0 (0.0)	1 (100.0)		
	Teratozoospermia (n=1)	1 (100.0)	0 (0.0)		
Vitamin D3 Deficiency Status	Deficient (n=51)	35 (68.6)	16 (31.4)	5.76	0.056
	Insufficient (n=26)	21 (80.8)	5 (19.2)		
	Sufficient (n=73)	63 (86.3)	10 (13.7)		

Table 4 shows the association between sperm morphology, Vitamin D3 deficiency status, and testosterone levels among the study participants. A statistically significant association was observed between sperm morphology and testosterone status (Pearson $\chi^2 = 24.748$, $p < 0.001$). Low testosterone levels were most frequently observed among patients with oligozoospermia (100.0%), oligoasthenozoospermia (80.0%), and azoospermia (66.7%), whereas the majority of participants with normozoospermia (85.0%) had normal testosterone levels. The only patient with teratozoospermia exhibited a normal testosterone level. These findings indicate that abnormal sperm morphology, particularly conditions characterized by reduced sperm count and impaired motility, is significantly associated with lower testosterone levels ($p < 0.001$). Furthermore, low testosterone levels were observed in 33.3% of participants with Vitamin D deficiency, compared with 19.2% among those with Vitamin D insufficiency and 8.2% among those with sufficient Vitamin D levels. Although the prevalence of low testosterone decreased progressively with improving Vitamin D status, the association did not reach statistical significance (Pearson $\chi^2 = 5.76$, $p = 0.056$). Nevertheless, the observed trend suggests a potential relationship between Vitamin D deficiency and reduced testosterone levels, which may warrant further investigation in larger populations.

Table 4: Association between Sperm Morphology and Vitamin D3 Deficiency Status with the status of Testosterone level of the patients

Parameter	Category	Testosterone		Pearson χ^2	P-value
		Low n (%)	Normal n (%)		
Sperm Morphology	Azoospermia (n=3)	2 (66.7)	1 (33.3)	24.748	<0.001
	Normozoospermia (n=140)	21 (15.0)	119 (85.0)		
	Oligoasthenozoospermia (n=5)	4 (80.0)	1 (20.0)		
	Oligozoospermia (n=1)	1 (100.0)	0 (0.0)		
	Teratozoospermia (n=1)	0 (0.0)	1 (100.0)		

Vitamin D3 Deficiency Status	Deficient (n=51)	17 (33.3)	34 (66.7)	5.76	0.056
	Insufficient (n=26)	5 (19.2)	21 (80.8)		
	Sufficient (n=73)	6 (8.2)	67 (91.8)		

Table 5 shows the distribution of Vitamin D3 deficiency status across different sperm morphology categories. Among patients with normozoospermia, 52.1% had sufficient Vitamin D levels, while 30.7% and 17.1% were Vitamin D deficient and insufficient, respectively. In contrast, Vitamin D deficiency was highly prevalent among participants with abnormal sperm morphology, affecting 80.0% of those with oligoasthenozoospermia and 100.0% of those with oligozoospermia and teratozoospermia. Similarly, 66.7% of patients with azoospermia were Vitamin D deficient. Overall, 34.0% of the study population was Vitamin D deficient, 17.3% was insufficient, and 48.7% had sufficient Vitamin D levels. These findings suggest a greater burden of Vitamin D deficiency among individuals with abnormal sperm morphology compared with those exhibiting normal semen characteristics.

Table 5: Association Between Sperm Morphology and Vitamin D3 Deficiency Status (n = 150)

Sperm Morphology	Deficient n (%)	Insufficient n (%)	Sufficient n (%)	Total n (%)
Azoospermia	2 (66.7)	1 (33.3)	0 (0.0)	3 (100.0)
Normozoospermia	43 (30.7)	24 (17.1)	73 (52.1)	140 (100.0)
Oligoasthenozoospermia	4 (80.0)	1 (20.0)	0 (0.0)	5 (100.0)
Oligozoospermia	1 (100.0)	0 (0.0)	0 (0.0)	1 (100.0)
Teratozoospermia	1 (100.0)	0 (0.0)	0 (0.0)	1 (100.0)
Total	51 (34.0)	26 (17.3)	73 (48.7)	150 (100.0)

Table 6 shows the statistical association between sperm morphology and Vitamin D3 deficiency status. The Pearson Chi-square test demonstrated no statistically significant association between the two variables ($\chi^2 = 13.088$, $df = 8$, $p = 0.109$). However, the Likelihood Ratio test indicated a statistically significant association (Likelihood Ratio = 16.25, $df = 8$, $p = 0.039$), while the Linear-by-Linear Association test also revealed a significant linear trend ($\chi^2 = 3.845$, $p = 0.050$). Correlation analysis demonstrated a weak negative relationship between sperm morphology and Vitamin D3 deficiency status, as reflected by Pearson's correlation coefficient ($r = -0.161$, $p = 0.050$) and Spearman's correlation coefficient ($\rho = -0.133$, $p = 0.105$). Collectively, these findings suggest the presence of a weak inverse relationship between worsening Vitamin D status and sperm morphology, although the evidence for a definitive association remains limited and should be interpreted with caution.

Table 6: Statistical Association Between Sperm Morphology and Vitamin D3 Deficiency Status

Statistical Test	Value	df	p-value
Pearson Chi-square	13.088	8	0.109
Likelihood Ratio	16.25	8	0.039*
Linear-by-Linear Association	3.845	1	0.050*
Pearson's Correlation (r)	-0.161	—	0.05
Spearman Correlation (ρ)	-0.133	—	0.105

Table 7 shows the post hoc pairwise comparisons of male factor infertility parameters according to Vitamin D3 deficiency status. Participants with sufficient Vitamin D3 levels demonstrated significantly higher total sperm count (TSC) compared with those who were Vitamin D deficient (mean difference = 10.72, $p = 0.005$), whereas no significant difference was observed between the deficient and insufficient groups ($p = 0.393$) or between the insufficient and sufficient groups ($p = 0.984$). Similarly, motility and actively motile sperm (AM) were significantly higher in the sufficient group than in the deficient group (mean differences = 11.50 and 10.81, respectively; both $p \leq 0.001$), while no significant differences were identified in the remaining pairwise comparisons. In contrast, the proportion of immotile sperm was significantly greater among Vitamin D-deficient participants than among those with sufficient Vitamin D levels (mean difference = 11.88, $p < 0.001$), indicating poorer semen quality in the deficient group. Seminal white blood cell (WBC) count was also significantly higher in Vitamin D-deficient individuals compared with Vitamin D-sufficient individuals (mean difference = 2.61, $p < 0.001$), suggesting a greater degree of genital tract inflammation. As expected, serum Vitamin D3 concentrations differed significantly between all three groups (all $p < 0.001$), confirming the validity of the deficiency categorization. Regarding hormonal status, participants with sufficient Vitamin D3 levels exhibited significantly higher testosterone concentrations than those with Vitamin D deficiency (mean difference = 0.63, $p = 0.001$), whereas no significant differences were observed between the deficient and insufficient groups ($p = 0.156$) or between the insufficient

and sufficient groups ($p = 1.000$). Overall, these findings indicate that Vitamin D sufficiency is associated with significantly better sperm count, motility, testosterone levels, and lower inflammatory burden compared with Vitamin D deficiency.

Table 7: Post hoc analysis between male factor infertility parameters and vitamin D3 deficiency

Male Factor Infertility Parameters	(I) VitaminD3 deficiency status	(J) VitaminD3 deficiency status	Mean Difference (I-J)	P-value
TSC	Deficient	Insufficient	-6.65846	0.393
		Sufficient	-10.72274*	0.005
	Insufficient	Deficient	6.65846	0.393
		Sufficient	-4.06428	0.984
	Sufficient	Deficient	10.72274*	0.005
		Insufficient	4.06428	0.984
Motility	Deficient	Insufficient	-4.28462	0.828
		Sufficient	-11.49589*	0.001
	Insufficient	Deficient	4.28462	0.828
		Sufficient	-7.21128	0.16
	Sufficient	Deficient	11.49589*	0.001
		Insufficient	7.21128	0.16
AM	Deficient	Insufficient	-3.96769	0.766
		Sufficient	-10.81068*	0
	Insufficient	Deficient	3.96769	0.766
		Sufficient	-6.84299	0.116
	Sufficient	Deficient	10.81068*	0
		Insufficient	6.84299	0.116
SM	Deficient	Insufficient	-0.31692	1
		Sufficient	-1.16466	1
	Insufficient	Deficient	0.31692	1
		Sufficient	-0.84773	1
	Sufficient	Deficient	1.16466	1
		Insufficient	0.84773	1
Immotile	Deficient	Insufficient	3.8	0.994
		Sufficient	11.87534*	0
	Insufficient	Deficient	-3.8	0.994
		Sufficient	8.07534	0.09
	Sufficient	Deficient	-11.87534*	0
		Insufficient	-8.07534	0.09
WBC	Deficient	Insufficient	1.12769	0.514
		Sufficient	2.61452*	0
	Insufficient	Deficient	-1.12769	0.514
		Sufficient	1.48683	0.171
	Sufficient	Deficient	-2.61452*	0
		Insufficient	-1.48683	0.171
VitaminD3	Deficient	Insufficient	-10.09032*	0
		Sufficient	-26.09864*	0
	Insufficient	Deficient	10.09032*	0
		Sufficient	-16.00832*	0
	Sufficient	Deficient	26.09864*	0
		Insufficient	16.00832*	0

Testosterone	Deficient	Insufficient	-0.43257	0.156
		Sufficient	-.62784*	0.001
	Insufficient	Deficient	0.43257	0.156
		Sufficient	-0.19526	1
	Sufficient	Deficient	.62784*	0.001
		Insufficient	0.19526	1

The mean difference is significant at the 0.05 level.

Table 8 shows the bivariate correlation between serum Vitamin D3 levels, testosterone levels, and semen analysis parameters. Serum Vitamin D3 concentration demonstrated significant positive correlations with total sperm count ($r = 0.303$, $p < 0.001$), sperm motility ($r = 0.336$, $p < 0.001$), and actively motile sperm ($r = 0.355$, $p < 0.001$), indicating that higher Vitamin D3 levels were associated with improved semen quality. In contrast, Vitamin D3 levels exhibited significant negative correlations with the proportion of immotile sperm ($r = -0.354$, $p < 0.001$) and seminal white blood cell count ($r = -0.326$, $p < 0.001$), suggesting that higher Vitamin D3 concentrations were associated with reduced sperm immobility and lower inflammatory activity. No significant correlation was observed between Vitamin D3 levels and sluggishly motile sperm (SM) ($r = 0.073$, $p = 0.378$). Similarly, testosterone levels demonstrated significant positive correlations with total sperm count ($r = 0.434$, $p < 0.001$), sperm motility ($r = 0.306$, $p < 0.001$), and actively motile sperm ($r = 0.274$, $p = 0.001$), indicating that higher testosterone concentrations were associated with better sperm production and motility. Significant negative correlations were observed between testosterone and immotile sperm percentage ($r = -0.305$, $p < 0.001$) as well as seminal white blood cell count ($r = -0.197$, $p = 0.016$), suggesting a relationship between higher testosterone levels and reduced sperm dysfunction and inflammation. However, no statistically significant correlation was identified between testosterone and sluggishly motile sperm ($r = 0.126$, $p = 0.125$). Furthermore, a significant positive correlation was observed between Vitamin D3 and testosterone levels ($r = 0.321$, $p < 0.001$), indicating that participants with higher Vitamin D3 concentrations tended to have higher serum testosterone levels. Overall, the findings suggest that both Vitamin D3 and testosterone are positively associated with favorable semen parameters and negatively associated with sperm immobility and inflammatory markers, highlighting their potential role in male reproductive function.

Table 8: Bivariate Correlation between the Vitamin D level and Testosterone with that of Parameters of Semen Analysis

Male Factor Infertility Parameters	Correlations	VitaminD3	Testosterone
TSC	Pearson Correlation	.303**	.434**
	P-value	0	0
Motility	Pearson Correlation	.336**	.306**
	P-value	0	0
AM	Pearson Correlation	.355**	.274**
	P-value	0	0.001
SM	Pearson Correlation	0.073	0.126
	P-value	0.378	0.125
Immotile	Pearson Correlation	-.354**	-.305**
	P-value	0	0
WBC	Pearson Correlation	-.326**	-.197*
	P-value	0	0.016
VitaminD3	Pearson Correlation	1	.321**
	P-value		0
Testosterone	Pearson Correlation	.321**	1
	P-value	0	

DISCUSSION

South Asia is disproportionately affected, with DALYs increasing by 45.66% (EAPC 1.40%) and incidence rates increasing by 47.19% (EAPC 1.50%). Both DALYs and incidence rate increases were particularly pronounced in India, which has seen increases in DALYs and incidence rates of 55.87% and 58.82%, respectively [11]. The highest overall burden of male infertility is found in the 25–34-year age group, where there exists a positive relationship with middle and upper middle sociodemographic index regions [11, 12]. Continued increases in estimated incidence rates of male infertility will require the development of public health interventions targeted at specific geographic regions [11, 12]. A multi-centre Indian study of 16,714 semen samples showed significant regional differences in male subfertility. There were high rates of azoospermia found in Kurnool and Jodhpur, 38.3% and 37.4%, respectively. In Kurnool, the rate for

oligozoospermic was also very high at 51% [13]. No significant difference was observed in mean sperm counts among continent populations of normospermics [13]. In addition to these findings, a 10-year retrospective study in 1,219 males at a rural Central Indian tertiary center showed that advanced paternal age has the greatest negative impact on semen quality [4]. This longitudinal study showed progressive age-related declines in ejaculate volume, total number of sperm, forward motility, and normal morphology, with statistically significant decreases occurring after the age of 35 years [4]. A study conducted on healthy adults in the Kashmir Valley found that 83% had low levels of Vitamin D (hypovitaminosis D) in either a mild (25%), moderate (33%) or severe (25%) capacity [7]. The condition was seen to be very common in both rural and urban populations, affecting 76.6% of males and 96.4% of women, mostly in relationship to receiving insufficient amounts of sun exposure each week [7]. Moreover, factors associated with male infertility account for around 50% of all cases of infertility globally; with there being a physiological role for Vitamin D during sperm maturation, causing the sperm to gain an ability to penetrate an egg by executing acrosomal reactions [14].

As vitamin D deficiency levels throughout India range from 50% - 90%, many studies have shown that the use of Vitamin D and calcium supplements for 6 months on subfertile males having Oligoasthenozoospermia significantly enhance total number of sperm produced (sperm Concentration) / sperm with the ability to move forward (progressive motility): ($P < 0.001$) [14]. A seasonal evaluation of ultraviolet B index (UVBI) across multiple zones demonstrates clear seasonal changes to the level of UV light available, with the peak level of UVBI occurring from 11 am through 1 pm across all zones [15]. Children with normal health status do not have sufficient levels of cholecalciferol from winter sun exposure. This causes an average decline in serum 25(OH)D levels from 6.3 to 5.1 ng/mL ($p < 0.001$), which highlights the need for supplemental vitamin D during this time of year [15]. In a concurrent cross-sectional study of the indigenous population of the Kashmir Valley, there was a 66% prevalence of vitamin D deficiency and a 14.71% prevalence of vitamin D insufficiency [16]. This population experienced the same rates of hypovitaminosis D in both males and females and has a negative correlation to the serum alkaline phosphatase level. The factors that affected both the sexes also did not affect the risk of developing hypovitaminosis D depending on altitude, body mass index (BMI), or physical activity [16].

The problem of male infertility in Kashmir is worsened by the high prevalence of vitamin D3 deficiency due to sunless lifestyles combined with limited seasonal sunlight, which may account in part for the biological mechanisms that may inhibit semen cell function through receptor-mediated pathways [7]. While many studies in India have demonstrated the benefit of supplementing oligoasthenozoospermic males with cholecalciferol, thereby improving both sperm count and motility [14], the reproductive healthcare system in the Kashmir region must include population-based testing of 25-hydroxyvitamin D levels to determine vitamin D status alongside conventional semen analysis [7,14]. Creating and providing standardised seasonal supplementation programmes and dietary fortification at local urology clinics may provide an effective and evidence-based means of improving male fertility parameters [7, 14].

CONCLUSION

The study has concluded that Vitamin D3 deficiency is highly prevalent among men with infertility and is associated with poorer semen quality. Lower Vitamin D3 levels were significantly associated with reduced total sperm count, sperm motility, actively motile sperm percentage, and testosterone levels, while showing significant negative associations with immotile sperm percentage and seminal white blood cell count. Men with Vitamin D sufficiency demonstrated significantly better reproductive parameters and lower inflammatory burden compared with Vitamin D-deficient individuals. Furthermore, Vitamin D3 levels exhibited a positive correlation with serum testosterone, suggesting a potential role of Vitamin D in hormonal regulation and spermatogenesis. These findings indicate that Vitamin D3 deficiency may contribute to male factor infertility and highlight the importance of assessing and correcting Vitamin D deficiency in infertile men.

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Conflict of Interest

The authors declare that they have no known financial or non-financial competing interests that could have influenced the work reported in this manuscript.

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