

ASSOCIATION OF VITAMIN D AND CERTAIN LIFESTYLE HABITS WITH SPERM QUALITY

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Abstract

The aim of our study was to investigate the individual and combined effects of 25-hydroxycholecalciferol, BMI, smoking, and alcohol consumption on sperm quality. Anthropometric data, blood and ejaculate samples were collected from 60 healthy men. Information about lifestyle habits was obtained by a questionnaire. 25-hydroxycholecalciferol levels were measured by ELISA method. Semen quality was evaluated by sperm analyses. Depending on their 25-hydroxycholecalciferol levels and lifestyle habits, the participants were stratified into different groups. Sperm parameters were compared between the groups. 25-hydroxycholecalciferol levels were highest in normozoospermic group compared with non-normozoospermic and oligoasthenoteratozoospermic groups, respectively. The percentage of normal sperm morphology was higher in the group with vitamin D sufficiency compared with vitamin D insufficiency. Obesity and heavy alcohol consumption affected negatively sperm morphology. Frequency of smoking negatively correlated with semen volume. The combined effect of variables contributed up to 16.5% to sperm quality. The strongest predictor of the normal morphology of the spermatozoa was 25-hydroxycholecalciferol. Men with the highest percentage of normal sperm morphology were those with higher vitamin D blood levels and lower BMI, moderate alcohol consumption and non-smokers.

Key words: vitamin D, man fertility, semen quality, lifestyle

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Introduction. In recent years, there had been an increasing number of cases of reduced fertility in men due to impaired spermatozoa (sperm) quality leading to reduced fertilization rate. Different factors may affect male fertility – genetic, hormonal, immunological, lifestyle, diet, and others. Vitamin D deficiency is common in developed societies. Recently it had been hypothesized that vitamin D might be involved in semen quality because of the presence of vitamin D receptor (VDR), and metabolizing enzymes such as 1- α -hydroxylase inside spermatozoa. This allows assuming that vitamin D deficiency may be associated with changes in sperm quality, however, actually this relationship remains unclear. On the other hand, many studies suggest a negative association between lifestyle related habits such as smoking, alcohol consumption, and obesity with male fertility. Smoking significantly worsens spermogram parameters. Similar effects were reported also for alcohol consumption [1,2]. Unfortunately, these harmful habits are quite common among young people already prior to reproduction. Although male infertility is the focus of a large amount of research, data about the effects of lifestyle factors including vitamin D on sperm quality are controversial. The aim of our study was to determine the individual and the combined effect of vitamin D status, BMI, smoking, and alcohol consumption on sperm quality in a sample from the general population in Bulgaria.

Materials and methods. Subjects. A total of 60 men (aged 25–45) among the patients at the Medical Center for Assisted Reproduction – Varna (Bulgaria) were interviewed for recruitment in the study during the period from July 2021 to July 2022. The activities for recruitment of volunteers were carried out in accordance with the Helsinki Declaration and started after approval from the Research Ethics Committee at the Medical University – Varna (Protocol No. 101/24.03.2021). Inclusion criteria were written consent for participation, appropriate age, and lack of disease. Exclusion criteria included patients submitted to medication, hormonal therapy, vitamin D supplementation; anatomical and infectious diseases of the genital system, renal failure, liver damage, thyroid disease, disorders of calcium metabolism, and others. All volunteers filled out a questionnaire for the assessment of their health, anthropometric status and lifestyle habits.

Sampling. Two biological samples were collected from each participant: venous blood and semen from ejaculate.

Analyses. The levels of 25OHD and total testosterone were measured in blood serum by an ELISA assay kit (Nordhorn, Germany). Semen quality was evaluated by spermogram analyses of the ejaculate. At least two replicates for each spermogram were performed.

Lifestyle variables and grouping. Depending on semen analysis, participants were classified into the following groups: a control group with normozoospermia (CG) and a target group (TG) without normozoospermia, with one or more abnormal seminal parameters. Inside the target group a subgroup with the worst semen parameters or oligoasthenoteratozoospermia (OG) was also identi-

fied. We analyzed and compared vitamin D levels, BMI, the frequency of smoking and the alcohol consumption between the CG and the TG and between the CG and the OG groups.

To assess the role of vitamin D and lifestyle related factors for sperm quality, we grouped participants as follows: depending on the BMI into two groups: a normal weight group ($\text{BMI} \leq 25 \text{ kg/m}^2$) and an overweight/obesity group ($\text{BMI} > 25 \text{ kg/m}^2$); regarding vitamin D status and according to the National Academy of Medicine (NAM), in two groups: a group with vitamin D deficiency (25OHD levels $< 20 \text{ ng/mL}$) and a group with vitamin D sufficiency (25OHD levels $> 20 \text{ ng/mL}$). Alcohol consumption was calculated in alcohol units per week, where one alcohol unit was equal to 8 g (10 mL) of pure alcohol [3]. Depending on the consumed alcohol units per week we divided the participants into a group that consumed above 14 alcohol units per week ($A > 14$) and a group that consumed below 14 alcohol units per week ($A < 14$). Regarding smoking, the participants were divided into smokers (S) and non-smokers (NS). Spermogram parameters were compared between the groups.

In order to rule out hypo- or hypergonadism as a determining factor in sperm parameters we examined the levels of total testosterone in each volunteer. In order to discard age as a possible factor affecting sperm parameters we also performed a correlation analysis between the age and the semen parameters to find associations and compared the semen parameters between men aged ≤ 35 years and men aged > 35 years.

Statistical analysis. The statistical methods used for the different analyses were descriptive statistics, Mann–Whitney U test and unpaired *t*-student for quantitative comparisons. Pearson and Spearman correlations as well as univariate linear regression analysis and multivariate regression analysis were used to find associations. Data were presented as mean $\pm$ SD and $p < 0.05$ was considered statistically significant. Statistical methods were performed using GraphPad Prism 7.0 and Jamovi statistical software.

Results. The mean values of the spermogram parameters, lifestyle related factors, and vitamin D status for CG, TG and OG are presented in Table 1.

In our study the TG had vitamin D deficiency ($16.10 \pm 7.32 \text{ ng/mL}$) with significantly lower mean levels of 25OHD than the CG ($20.39 \pm 8.20 \text{ ng/mL}$). 25OHD levels were lowest in OG, establishing a pronounced vitamin D deficiency ($13.37 \pm 6.7 \text{ ng/mL}$). The TG had the highest BMI (28.68 ± 4.52), followed by OG (27.45 ± 4.66). Regarding alcohol consumption, the results showed that volunteers in the TG consumed more alcohol compared to volunteers in the CG (27.40 ± 23.1 vs. 18.00 ± 16.14 , $p = 0.08$). Seasonal variations of vitamin D may not significantly differ between CG and TG due to the proportional distribution of participants between the two seasons (Table 1).

According to BMI (≤ 25 vs. > 25) no significant differences were observed in spermogram parameters between the groups. However, after regrouping the

Table 1

Mean values of spermogram parameters and comparison of lifestyle habits parameters including vitamin D levels and seasonal variation of 25OHD levels between the groups: control group, target group, and oligoasthenoteratozoospermic group

Spermogram analysis	CG (<i>n</i> = 24)	TG (<i>n</i> = 36)	OG (<i>n</i> = 9)	References range
pH	7.67 ± 0.13	7.70 ± 0.12	7.66 ± 0.15	7.2–7.8
Liquefaction (min)	24.13 ± 7.01	28.82 ± 11.01	29.38 ± 12.94	15–60 min
Sperm count ×10 ⁶ /mL	60.8 ± 30.7	26.3 ± 19.2	4.5 ± 3.8	≥ 15 × 10 ⁶ /mL
Total sperm count ×10 ⁶ in ejaculate	185.9 ± 82.8	83.4 ± 66.9	14.1 ± 10.5	≥ 39 × 10 ⁶
Semen volume, mL	3.76 ± 1.87	3.41 ± 1.46	3.38 ± 1.02	1.5–5.0 mL
Total sperm motility, %	65.0 ± 10.7	41.9 ± 16.4	22.7 ± 15.6	≥ 40%
Progressive sperm motility, %	47.8 ± 11.5	29.4 ± 14.3	14.1 ± 12.4	≥ 32%
Sperm non-progressive motility, %	17.1 ± 6.1	12.5 ± 6.5	8.6 ± 6.5	–
Normal sperm morphology (median, 25–75 percentile), %	6 (5.25–7)	2 (2–3)	1 (0.5–2)	≥ 4%
Total testosterone ng/mL	3.28 ± 0.71	3.63 ± 0.86	3.97 ± 0.94	2.0–6.90 ng/mL

Comparison of lifestyle habits parameters and vitamin D levels between the groups according to the baseline spermogram analyzes

Characteristics	CG	TG	<i>p</i> ^a	OG	<i>p</i> ^b
Age (Y)	37.13 ± 6.25	38.03 ± 5.50	ns	33.89 ± 5.60	ns
BMI (kg/m ²)	26.96 ± 4.32	28.68 ± 4.52	0.08	27.45 ± 4.66	ns
25OHD (ng/mL)	20.39 ± 8.20	16.10 ± 7.32	0.04	13.37 ± 6.70	0.02
Alcohol consumption, (units/week)	18.00 ± 16.14	27.40 ± 23.1	0.08	27.43 ± 29.25	ns
Smoking, (cigarettes per day)	5.83 ± 8.58	8.02 ± 11.69	ns	2.88 ± 6.71	ns

Seasonal variation of 25OHD levels (ng/mL)

Winter season (November–May)	CG (<i>n</i> = 13)	TG (<i>n</i> = 26)	<i>p</i>
	15.01 ± 3.88	13.70 ± 6.06	ns
Summer season (June–October)	CG (<i>n</i> = 11)	TG (<i>n</i> = 10)	
	26.26 ± 7.66	22.35 ± 6.82	ns

p^a-values when comparing the variables between the control and the target group (Student's *t*-test); b) *p*^b-values when comparing the variables between the control and the sample of the target group with oligoasthenoteratozoospermia (Student's *t*-test), *p* values < 0.05 were considered significant.

participants into a non-obesity group (BMI ≤ 30; average 25.63 ± 2.61) and an obesity group (BMI > 30; average 33.50 ± 2.74) we found that men with obesity had a significantly decreased percentage of normal sperm morphology (Table 2). Pearson correlation analyses showed an almost statistically significant association between BMI and the total sperm count in ejaculate (Pearson *r* =

-0.24; $p = 0.06$). Furthermore, a significant negative correlation between BMI and the percentage of normal sperm morphology was estimated (Pearson $r = -0.26$, $p = 0.04$).

The average level of vitamin D in the sufficiency group was 26.08 ± 5.99 and in the deficiency group it was 13.52 ± 4.72 (Table 2). Statistically difference between the groups was estimated in the percentage of sperm cells with normal morphology that was higher in the group with sufficient levels of vitamin D compared to the group with deficiency (4.5 (3–6) vs. 3 (2–6), $p = 0.01$). A clear trend for higher sperm motility (total and progressive) was reported in the vitamin D sufficiency group.

A tendency for decreased semen volume in smokers compared with non-smokers was observed (3.2 ± 1.5 vs. 3.8 ± 1.7 , respectively, Table 2) confirmed by the correlation analysis (Pearson $r = -0.31$; $p = 0.01$). On the other hand, smokers had significantly increased progressive sperm motility compared with nonsmokers ($p = 0.04$) as it is presented in Table 2. A significant positive correlation was estimated between the number of cigarettes smoked per day and the progressive sperm motility (Pearson $r = 0.26$, $p = 0.04$).

No differences were observed for all sperm parameters between the groups below and above 14 alcohol units/week. To assess the effect of high alcohol consumption on semen quality we increased the “cut-off” from 14 to 35 alcohol units/week and regrouped the participants as follows: low to moderate alcohol consumption (MA) up to 35 alcohol units/week, and heavy alcohol consumption (HA) with more than 35 alcohol units/week. As presented in Table 2, this revealed a significant decrease in the percentage of spermatozoa with normal morphology in the HA group. Furthermore, the average values of this parameter for the HA group were below the reference range (2.5 (1.6–5.3), $p = 0.03$) compared with the MA group. For the MA group, spermatozoa with normal morphology were in the reference range.

The correlation analyses revealed that 25OHD levels positively correlated with sperm motility (total motility $r = 0.27$; $p = 0.04$ and progressive sperm motility $r = 0.28$; $p = 0.03$) and with the normal sperm morphology ($r = 0.29$; $p = 0.02$). Following these results, we applied a univariate regression analysis to test the effect of 25OHD on each of the spermogram parameters that were measured. Our results showed that 25OHD exhibited a significant effect on the motility of all sperm cells, including the development of progressive and non-progressive sperm cells ($\beta = 0.268$, $p = 0.040$), solely on the progressive motility ($\beta = 0.282$, $p = 0.030$), and on the lack of motility of the cells ($\beta = -0.266$, $p = 0.042$). Additionally, results disclosed that 25OHD levels could affect the percentage of normal sperm morphology ($\beta = 0.295$, $p = 0.023$).

A multiple regression analysis of vitamin D and all analyzed lifestyle indicators was performed to check possible multivariable effects on sperm quality. A multivariable effect on the percentage of normal sperm morphology was estab-

T a b l e 2

Comparison of spermogram parameters between the subgroups stratified according to the 25OHD levels, BMI, alcohol consumption and smoking

Spermogram parameters	Body Mass Index kg(cm ²)			25-Hydroxy Vitamin D, ng/mL		
	≤ 30 (n = 42) average 25.63 ± 2.61	> 30 (n = 18) average 33.50 ± 2.74	p	< 20 (n = 40) average 13.52 ± 4.72	≥ 20 (n = 20) average 26.08 ± 5.99	p
Sperm count, 10 ⁶ /mL	43.2 ± 30.4	29.4 ± 23.6	0.09	39.1 ± 29.2	38.7 ± 28.0	ns
Semen volume, mL	3.7 ± 1.7	3.1 ± 1.3	ns	3.7 ± 1.5	3.4 ± 1.8	ns
Sperm motility, (%)	53.1 ± 19.0	46.7 ± 16.1	0.07	48.0 ± 18.8	56.5 ± 16.3	0.09
Progressive sperm motility, (%)	38.4 ± 17.0	32.9 ± 13.0	0.07	34.1 ± 15.7	41.3 ± 15.9	0.09
Normal sperm morphology, (median, 25–75 percentile), %	4 (2–6.25)	2 (2–5.25)	0.05	3 (2–6)	4.5 (3–6)	0.01
Spermogram parameters	Smoking			Alcohol consumption		
	NS (n = 35) average 0	S (n = 25) average 17.16 ± 9.68	p	MA<35 (n = 44) average 13.11 ± 11.01	HA>35 (n = 16) average 52.60 ± 12.76	p
Sperm count, 10 ⁶ /mL	39.2 ± 32.4	41.4 ± 26.0	ns	42.2 ± 31.2	34.4 ± 24.9	ns
Semen volume, mL	3.8 ± 1.7	3.2 ± 1.5	ns	3.7 ± 1.7	3.3 ± 1.4	ns
Sperm motility, (%)	47.3 ± 19.3	56.5 ± 15.6	0.06	51.7 ± 17.5	49.8 ± 20.8	ns
Progressive sperm motility, (%)	33.2 ± 15.7	41.8 ± 15.3	0.04	36.3 ± 15.7	37.9 ± 17.2	ns
Normal sperm morphology, (median, 25–75 percentile), %	4 (2–6)	3 (2–6)	ns	4 (2–6.75)	2.5 (1.6–5.3)	0.03

Abbreviations: S (smokers); NS (non-smokers); A (alcohol consumption); MA (moderate alcohol consumption); HA (Heavy alcohol consumption). Data are presented as mean ± SD, *p* values < 0.05 were considered significant.

lished. The overall predictability of the model was $R^2 = 0.165$, $p = 0.045$, and no collinearity was reported between variables (VIF=1 for all variables). All indicators included in the model added to the significance of it with a 16.5%, yet the strongest predictor of the normal morphology was 25OHD ($\beta = 0.316$, $p = 0.020$, 95% [0.0508, 0.5824]).

Discussion. The marker for body vitamin D status is the concentration of serum circulating 25OHD levels [4]. In our study, significant differences were found in 25OHD levels between the groups stratified according to spermogram pa-

rameters. Normozoospermic CG had no vitamin D deficiency, with 25OHD mean levels somewhat higher than 20 ng/mL. In addition, a vitamin D deficiency was estimated for TG and OG. Furthermore, we analysed the spermogram parameter in the group with vitamin D insufficiency against participants with sufficient vitamin D serum levels (Table 2). A positive association was estimated between 25OHD mean levels and the percentage of morphologically normal sperm cells and sperm motility. These results support the hypothesis that sperm quality is closely related to vitamin D status. Our results are in accordance with other similar prospective studies in which the authors detected significantly higher 25OHD mean levels in a group of fertile men compared with unexplained infertile men (27.0 ng/mL vs. 23.6 ng/mL) in the Turkish population [5]. However, the infertile group was with vitamin D sufficiency unlike the observed deficiency in our study, this could be explained by the fact that the envisaged study was carried out in the period April - December covering the whole summer. In a study similar to ours, AZIZI et al. [6] compared vitamin D levels between a control group and a group with oligoasthenoteratozoospermia which showed vitamin D deficiency with mean levels of 25OHD quite close to the 25OHD values for our oligoasthenoteratozoospermic group, while the mean levels of 25OHD in the control groups differed substantially – 15.6 ng/mL vs. 20.39 ng/mL for our control group.

The hypothesis of how vitamin D may affect the sperm cells is based on the concept about the VDR receptor present inside spermatozoa. Local 25OHD inside sperm cells is converted into 1,25 dihydroxycholecalciferol, the active form of vitamin D by the cellular alpha-1-hydroxylase. The active form of vitamin D (1,25 dihydroxycholecalciferol) binds to the VDR inside sperm cells and activates a signal pathway releasing calcium from the intracellular calcium depoes in the neck of human spermatozoa. The manifestations of these non-genomic effects are related to inducing sperm motility, sperm capacitation, and the acrosome reaction [7].

BMI above 30 kg/m² has been suggested to be a key factor for infertility [8]. Excessive deposition of white adipose tissue, release of adipokines, and activated aromatase are the main cause of hormonal imbalance in men. Increased aromatase activity leads to the conversion of androgens into estrogens [9]. Another likely mechanism for reduced sperm quality in obesity is increased scrotal temperature. Elevated temperature in this area is associated with decreased sperm motility, and increased incidence of sperm DNA damage due to favourable conditions for oxidative stress in sperm [10]. According to BMI stratification, obese men had significantly lower normal sperm morphology compared with the non-obesity group ($p = 0.05$). Obesity group also showed worsened semen volume, sperm count, and sperm motility although not significantly (Table 2).

A possible reason for the impaired sperm morphology being more pronounced in obese men is obesity-induced systemic inflammation and oxidative stress [11]. Furthermore, increased levels of leptin – the peptide hormone that regulates ap-

petite and body weight – positively correlate with the accumulation of adipose tissue and induce the production of ROS [12]. The higher temperature has a negative effect on testicular Sertoli and Leydig cells and can accelerate the differentiation and spermatogenesis of spermatocytes. As a result of the reduced cycle of spermatogenesis damaged cells can be formed.

Tobacco pyrolysis produces approximately 4000 chemical compounds that are inhaled by smokers, including nicotine, carbon monoxide, cadmium, and other mutagenic compounds [13]. Some of these toxic compounds can inhibit the mitochondrial activity of spermatozoa, or disrupt their chromatin structure, thereby impairing the ability for reproduction [14]. Some act as oxidants, adding to the effects of obesity and alcohol consumption.

The results obtained in our study revealed a clear tendency for decreased semen volume in smokers compared with non-smokers confirmed by the significant negative correlation analysis. Other authors reported similar results [15]. Interestingly, a positive association between smoking and sperm motility was established and confirmed by the positive correlation analysis in our study (Table 2). In contrast, many other studies reported a negative association or no association between these two variables [16], while only a few others had found a positive association between smoking and sperm motility [15, 17]. It could be suggested that certain components in tobacco smoke may act on specific anatomic characteristics of the prostate-vesicular region, especially on seminal vesicles with a negative effect on the seminal vesicles volume, leading to low semen volume [18]. Regarding the increased sperm motility in smokers, some authors with similar results, attribute this effect to the nicotine [15, 17]. DAI et al. [17] observed that nicotine-induced overexpression of proteins involved in cytoskeleton regulation, such as profilin 1 (PFN1), can promote actin polymerization and improve sperm motility. It is important to note that the positive association between smoking and sperm motility should not be considered a beneficial effect of smoking. Although smokers' sperm exhibits increased motility, smokers most likely also have oxidation-induced sperm DNA fragmentation with a reduced fertilization capacity [19]. The inverse association between alcohol consumption and the percentage of normal morphology sperm cells was confirmed only for moderate-to-heavy alcohol consumers (volunteers above 14 alcohol units/week excluding those with below 14 alcohol units/week) through correlation analysis. In our study, the effects related to decreased sperm count and decreased percentage of normal sperm morphology seem to start manifesting only in heavy alcohol consumers but not for low to moderate alcohol consumers. This could be explained by the effect of alcohol on endocrine function, leading to increased serum prolactin (hyperprolactinemia), causing reduced sperm production.

The combined effect of all analysed lifestyle factors and vitamin D is only manifested in the percentage of normal sperm morphology. All four analysed factors can influence up to 16.5% the normal sperm morphology. Vitamin D is

the main factor affecting the spermatozoa morphology. Studies about the specific combined effect of analyzed factors are scarce. BOERI et al. [20] investigated the combined effect of smoking and alcohol consumption and concluded that both factors had a greater detrimental effect on semen parameters.

Conclusion. We conclude that different parameters of semen quality could be affected by vitamin D status and lifestyle related factors such as BMI, smoking, and alcohol consumption. Normal sperm morphology and motility are positively influenced by vitamin D status. Obesity may affect negatively normal sperm morphology, sperm count, and motility. Although stimulating sperm motility, the overall effects of smoking on sperm quality should be considered harmful, manifested by its negative influence on semen volume. Only relatively heavy alcohol consumption seems to affect negatively the percentage of normal sperm morphology and eventually the sperm count.

We believe that the results obtained in our study may significantly contribute to clarifying the combined effect of all analysed factors and may explain some idiopathic cases of infertility in apparently healthy young men. Vitamin D, BMI, smoking, and alcohol consumption altogether, contribute up to 16.5% to the affected normal sperm morphology. Men with the highest percentage of normal sperm morphology are those with higher vitamin D status and lower BMI, moderate alcohol consumption, and non-smokers.

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