

VITAMIN D AND SEMEN QUALITY IN URBAN, YOUNG, HEALTHY MEN (ANDROLS)

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ABSTRACT

Background and Objective

Our aim was to evaluate whether the blood concentration of 25(OH)D₃ is associated with semen quality and sperm morphology parameters in young men.

Material and Methods

Healthy, urban volunteers aged 20–35 were recruited from universities, clubs and societies in the macroregion of Lower Silesia (Poland). We evaluated medical history, lifestyle factors and environmental threats, collected semen samples, and evaluated vitamin D levels. We acquired data for 177 subjects.

Results

The mean concentration of 25(OH)D₃ was 13.7 ± 8.9 ng/mL. Only a minority of the included subjects (18%) had a serum 25(OH)D₃ concentration above the lower limit (20 ng/mL). In total, 39% had severe vitamin D deficiency (<10 ng/ml). None of the studied semen parameters was correlated with the serum concentration of 25(OH)D₃; we also found no correlations after adjusting for alcohol consumption, cigarette smoking, carrying a mobile phone in pant pockets, body mass index, caffeine consumption and physical activity.

Conclusion

Our data indicate that the serum concentration of 25(OH)D₃ was not correlated with semen quality in a healthy, young urban population with prevalent vitamin D insufficiency.

Vitamin D is involved in regulating a range of body functions. Sufficient vitamin D production has been associated with a variety of clinicopathological parameters, including a lower risk of diabetes, a lower incidence of autoimmune disease and improved bone health. A growing amount of evidence suggests that vitamin D may also play a role in human reproduction.

Exploration of the role that vitamin D might play in reproductive physiology is justified by the burden known to be caused by vitamin D insufficiency¹ and previous reports describing its effect on the deterioration of semen quality and fertility rates.² Accordingly, we found it tempting to consider the relationship between vitamin D and andrological evaluations.³

Some authors have suggested that vitamin D activity may be associated with semen quality.^{4,5} Both animal and human studies^{6,7} have found links between vitamin D status and certain features of spermatozoa. For example, the enzymes responsible for vitamin D metabolism have been implicated as markers of semen quality.⁸

A reliable parameter that is often used to describe vitamin D status is 25(OH)D₃,⁹ which is commonly assessed during medical checkups. Although a correlation between the blood concentration of 25(OH)D₃ and semen parameters has been reported,⁶ this relationship has not been confirmed in other settings and populations.^{4,7,10} It has been also suggested that most data are related to men with sufficient vitamin D levels.¹⁰ In our region, vitamin D insufficiency and deficiency are relatively common.¹¹ We therefore sought to determine whether the blood concentration of 25(OH)D₃ is correlated with semen quality in a sample of young, healthy, urban men.

METHODS

The present investigation is part of a project entitled Andrological Status of Young Men in Lower Silesia (AndroLS). The study was approved by the local Bioethics Committee and all included procedures were conducted in compliance with the tenets of the Declaration of Helsinki regarding human subjects and the European Communities Council Directive of 24 November 1986 (86/609/EEC). Informed consent was obtained from all participants included in the study. The detailed characteristics of the participants,

their flow through the study protocol, and the laboratory methodology were described in our previous publications.^{12–14}

In the included men, the mean age was 24.6 ± 3.6 years old, and the mean body mass index was 24.0 ± 2.8 . Among the participants, 14% smoked cigarettes, and 92% drank alcohol. One out of every three enrollees exercised regularly, and 100% of them had accomplished at least 12 years of education.

Semen samples (one per subject) were collected from the participants between December and February. The samples were acquired in an andrology laboratory-associated room and immediately analyzed by a single experienced medical analyst according to the instructions in the World Health Organization (WHO) 2010 diagnostician laboratory manual and using a Sperm Class Analyzer (SCA; CASA System MICROPTIC S.L., Barcelona, Spain). The analysis comprised measurements of the following: semen volume; semen consistency; sperm count and concentration; and spermatozoa motility, vitality, and morphology (% of normal; % of abnormal sperm with an amorphous head, a round head, a tapered head, a microcephalic head, a macrocephalic head, cytoplasmic droplets, a vacuolated head, an abnormal middle-piece, an abnormal tail and double-headed sperm). We also evaluated time of liquefaction, pH and leukocytes.

Blood samples were obtained in the morning after the subject had fasted for at least nine hours. The serum was then separated and stored at -70°C . The concentration of 25(OH)D₃ was measured using electrochemiluminescence (ECLIA) in an Elecsys system (Roche, Switzerland). The intra- and inter-assay coefficients of variation were 5.6% and 8.0%, respectively. The limit of detection was 4 ng/mL (10 nmol/L). In four of our cases, the serum concentration of 25(OH)D₃ was missing. Full data were available for 173 men.

STATISTICAL ANALYSIS

Variables with a non-normal distribution were log-transformed. The variable “semen consistency” was categorized into two ranks, intact or increased, because there was a low number of abnormal observations.

The relationships between semen quality parameters and the concentration of vitamin D were evaluated using Pearson's two-sided correlation coefficient, which was also used for type 0-1 variables, and is equivalent to the Student's t-test for independent samples. Then, partial correlations were performed to correct for body mass index (BMI), WHR (waist-hip ratio), alcohol intake (yes-no), cigarette smoking (yes-no), carrying a cellular phone in a pants pocket (yes-no), working with a laptop on one's knees (yes-no), wearing tight clothing (yes-no), using a sauna (yes-no), caffeine consumption (mg/day), and physical activity (MET total) between the evaluated semen variables and the concentration of vitamin D. A nominal alpha of 0.05 was considered statistically significant. All calculations were performed using R Core Team (2017) software (R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>).

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RESULTS

The present report is derived from a cross-sectional study of an initial population of 5000 young, healthy men from Lower Silesia (Poland). In our previous publications, we discussed in detail the overall quality of semen in the sample¹³ and its associations with physical activity¹² and addiction.¹⁴

The mean (\pm SD) concentration of 25(OH)D₃ in the volunteers was 13.7 ± 8.9 ng/mL, the mean semen volume 3.1 ± 1.5 mL, the mean sperm concentration $60 \pm 44 \times 10^6$ /mL, the mean total sperm count $170 \pm 137 \times 10^6$ /ejaculate, and the mean percent of normal forms $14.7 \pm 6.5\%$.

We found a minority of the studied men (17%) to be vitamin D sufficient (>20 ng/mL). Only 4% of them had concentrations above 30 ng/mL, which is suggested to be the lower limit of the optimal level.

A level in the range between 10 and 20 ng/mL was found for 40% of the participants. Nearly 40% of the included men had a 25(OH)D₃ concentration that was below the threshold for deficiency (<10 ng/mL) (Figure 1; Table 1).

FIG. 1 Concentrations of 25(OH)D₃ in the studied men.

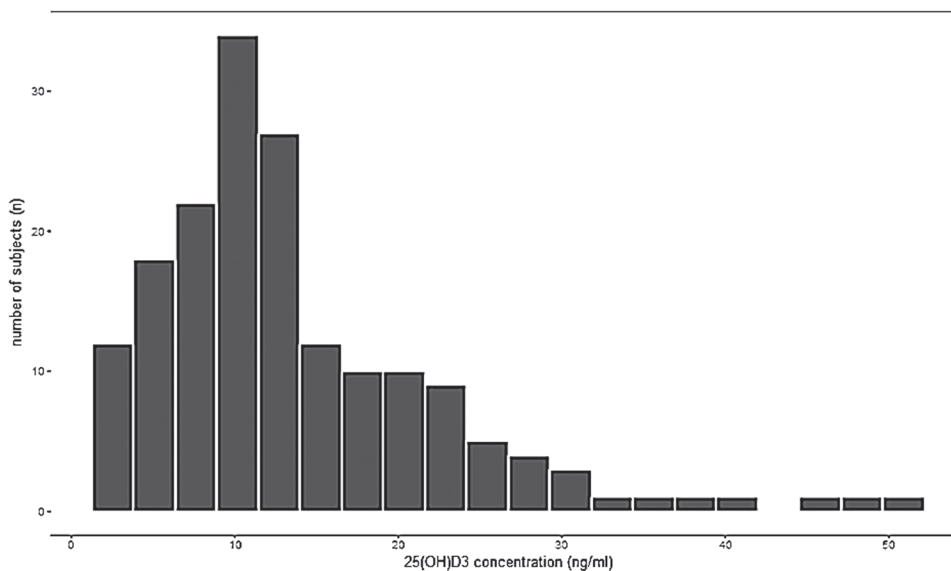


TABLE 1 Concentrations of 25(OH)D₃ Observed in the Studied Men according to Vitamin D Insufficiency/Deficiency Criteria

25(OH)D ₃ concentration [ng/mL]	Frequency <i>n</i> (%)	% Valid
<10	69 (39)	39.9
10–20	73 (41,2)	82.1
>20	31 (17,5)	100.0

Our statistical analysis of the data revealed no significant correlations between vitamin D levels and semen variables in the investigated subjects (Table 2). Furthermore, we observed no correlations after adjusting for alcohol intake, smoking cigarettes, being exposed to the electromagnetic field of a mobile phone (i.e., carrying the device in a pants pocket), body mass index, overall caffeine consumption and the level of everyday physical activity (Table 2).

We found no correlation between vitamin D status and time of liquefaction, pH, leukocytes or detailed characteristics of spermatozoa morphology (data not presented).

DISCUSSION

The outcomes of our study suggest that the blood concentration of 25(OH)D₃ is not related to semen quality in young, healthy men. We would like to stress that this observation was made in a sample in which vitamin D insufficiency/deficiency was prevalent. Such a situation is typical for our part of Europe.¹¹ We did our best to exclude potential bias resulting from confounding factors.

Vitamin D has established roles in calcium/phosphate homeostasis, bone and cardiovascular health, immunity and carcinogenesis.¹⁵ An increasing volume of evidence indicates that vitamin D also affects male reproduction.^{3,16}

Receptors for vitamin D (VDR) are abundantly present in the reproductive system, including human spermatozoa.¹⁷ Germ cells and the cells lining the reproductive tract are directly influenced by active forms of vitamin D. They also express enzymes that metabolize 25(OH)D₃ and can therefore regulate the intracellular activation of VDR.¹⁸ Additionally, some

of the effects evoked by sun exposure or treatment with cholecalciferol/calcitriol are non-genomic.¹⁹

It has previously been noted that in couples undergoing assisted reproduction, pregnancy rates are correlated with the vitamin D status of the male partner.²⁰ For example, the importance of interactions between metabolites of vitamin D and spermatozoa within the female reproductive tract have been reported.²¹ Interestingly, the authors of a recent interventional study in rats suggested that vitamin D deficiency was harmful to the reproductive system and could be reversed by reverting the calcium/phosphorus balance.²² Hence, the concentration of calcium in the seminal fluid may play a special role in reproduction.²³

A large portion of the population is not adequately exposed to sunlight and/or does not have a high enough intake of products containing cholecalciferol. Vitamin D insufficiency and deficiency are associated with a range of suspected clinical conditions, although these links are questioned by some researchers.²⁴

In line with the definition of the Institute of Medicine²⁵ and local guidelines,²⁶ we set a 25(OH)D₃ concentration <20 ng/mL (50 nmol/L) as an indicator of vitamin D deficiency. In our sample, nearly 40% of the men had vitamin D levels in the severely deficiency range (below 10 ng/mL). These data are especially intriguing because the participants were young and more physically active than older individuals and the general population. It should be noted that the collection of material (blood and semen) was carried out between December and February.

It is easier to obtain semen samples for evaluation from men seeking medical advice (typically in infertility clinics) than from healthy subjects. A few studies performed in subfertile/infertile men have identified

TABLE 2 Pearson's Correlation Coefficients (With 95% Confidence Intervals) between Serum 25(OH)D₃ Concentrations and Semen Parameters, As Follows: Without Corrections (R) and with Adjustments for Cigarette Smoking, Alcohol Consumption, Carrying a Telephone in a Pants Pockets, BMI, WHR, Caffeine Consumption and Physical Activity (R')

	r	p	r'	p
Semen volume [mL]	-0.02 (-0.17, 0.13)	0.787	0.02 (-0.18, 0.22)	0.833
Semen consistency	-0.03 (-0.18, 0.12)	0.708	-0.04 (-0.23, 0.16)	0.705
Sperm concentration [$\times 10^6$ /ml]	-0.06 (-0.21, 0.10)	0.480	-0.13 (-0.32, 0.07)	0.211
Total sperm count [$\times 10^6$ /ejaculate]	-0.06 (-0.21, 0.09)	0.411	-0.14 (-0.33, 0.06)	0.176
Progressive motility sperm [%]	-0.03 (-0.18, 0.12)	0.652	-0.12 (-0.31, 0.08)	0.224
Non-progressive motility [%]	-0.01 (-0.16, 0.14)	0.912	-0.03 (-0.22, 0.17)	0.901
Immotile sperm [%]	0.08 (-0.07, 0.23)	0.299	0.07 (-0.13, 0.26)	0.510
Vital sperm [%]	0.00 (-0.15, 0.15)	0.975	-0.07 (-0.26, 0.13)	0.490

BMI = body mass index; WHR = waist to hip ratio.

a link between vitamin D and semen quality.^{4,23} In a Danish cohort of 1248 infertile subjects, the percent of motile spermatozoa was significantly higher in men with 25(OH)D₃ > 30 ng/mL than in those with 25(OH)D₃ < 10 ng/mL (23). Similarly, in a sample of Chinese men of mixed fertility status ($n=559$), the concentration of 25(OH)D₃ was indicative of the motility and morphology of their spermatozoa.⁷ Another interesting report from China suggested that there is a positive association between the concentration of 25(OH)D₃ and conception rates in men with idiopathic oligoasthenozoospermia.²⁷ In agreement with the above findings, an *in vitro* study showed that active metabolites of vitamin D may alter sperm quality by increasing their motility.⁶

The amount of data related to the quality of sperm and 25(OH)D₃ concentrations in healthy men is more modest than that for subfertile/infertile subjects. In a study of 300 men selected from the general population, a correlation was identified between a low concentration of 25(OH)D₃ (< 10 ng/mL) and a lower percent of motile, progressively motile and morphologically normal spermatozoa.⁶ However, the above-mentioned population was different from ours in that in their study, only 44% of the participants had vitamin D

insufficiency (25[OH]D₃ < 20 ng/mL), whereas in our study, this number was higher than 80%. The proportion of men with severe vitamin D deficiency was also considerably higher in our sample.

It is difficult to draw comparisons between our data and those obtained in other studies. For example, a Danish investigation of 307 young men included a relatively small proportion of men with vitamin D deficiency or insufficiency (6% and 17%, respectively).¹⁰ Although other authors have reported relationships between vitamin D status and semen parameters, these associations often disappear after adjustments are made for confounding factors. Again, in the previously mentioned study of a Chinese population, the relationships between vitamin D and semen quality parameters in fertile subjects ($n = 195$) were marginally significant/non-significant.⁷

Our findings are similar to those reported in a study performed in Iran. The authors of a recently published study evaluated the concentration of 25(OH)D₃ in 278 men who visited an infertility clinic. They noted that there was no correlation between sperm parameters and vitamin D levels in normospermic men.⁴ However, the percentages of subjects with vitamin deficiency and insufficiency in their study (9% and

44%, respectively) were considerably lower than the percentages observed in our sample (40% and 80%, respectively). Nonetheless, the apparently different sun exposure of these populations should be considered.

It is worth mentioning that 25(OH)D₃ levels are not likely to be associated with testosterone concentrations⁷ and that supplementation with vitamin D does not increase sex steroid levels.²⁸

Our results are not clearly in alignment with the hypothesis that vitamin D supplementation should be especially beneficial in men with severe vitamin D deficiency.¹⁸ However, the design of our project makes verification of this concept impossible.

A strength of the present study is that we enrolled men who were not seeking medical advice. It is difficult to encourage this population to take part in this type of investigation because of their lack of interest in addition to religious and cultural reasons.

The participants in this study were derived from a relatively homogenous population. There are no significant minority populations in Lower Silesia. We therefore hoped to avoid any potential impact of genetics on serum vitamin D levels. This region of Lower Silesia is one of the most industrialized regions in Poland, and in terms of environmental pollution, it can be compared to other developed parts of Europe.

The semen samples collected for this study were analyzed in a laboratory by a single, experienced medical analyst according to the WHO recommendations. The electrochemiluminescence assay used in this study is a reliable method for evaluating 25(OH)D₃ concentrations, achieving results that are comparable to high-performance liquid chromatography.²⁹

The blood samples obtained from the participants were taken during a period in which skin exposure to UVB radiation was negligible. They received no doses of vitamin D that would exceed a supplementary dosage.

The high percentage of subjects included in this study who had vitamin D inadequacy mirrored the corresponding percentage in the population. For our analysis, it was important that many of the included subjects were truly vitamin D-deficient.

A potential bias of our study is that we used a “one-time semen analysis” as a surrogate marker for fertility/infertility. This limitation reduces applicability

of our results in not recommending checking vitamin D levels in men seeking diagnosis and treatment for male infertility. The included subjects reported a higher level of physical activity than it is reported for the general population; all of the included men had more than twelve years of education; we were unable to acquire double semen samples. We did not evaluate dihydroxycholecalciferol levels in our subjects. This parameter has not been studied in a large number of studies, although it may provide value in studies exploring the suspected effects in 1,25(OH)₂D₃ in the reproductive tract.⁸

CONCLUSIONS

In a sample of healthy, young, urban men with unchecked fecundity and prevalent vitamin D insufficiency, we found that semen quality was not related to serum concentrations of 25(OH)D₃.

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