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Evaluation of the effect of vitamin D3 supplementation on quantitative and qualitative parameters of spermograms and hormones in infertile men: A Randomized controlled trial

Leila Amini [a], Robabe Mohammadbeigi [b], Mohammadreza Vafa [c], Hamid Haghani [d], Amir Vahedian-Azimi [e], Leila Karimi [f], Shayesteh Jahanfar [g], Tannaz Jamialahmadi [h,i,j], Afsaneh Talebi [a,*], Amirhossein Sahebkar [k,l,m,n,**]

[a] Department of Midwifery, School of Nursing and Midwifery, Iran University of Medical Sciences, Tehran, Iran
[b] Clinical Research Development Unit (ShACkRDU), Iran University of Medical Sciences, Tehran, Iran
[c] School of Public Health, Iran University of Medical Sciences, Tehran, Iran
[d] Department of Biostatistics, School of Public Health, Iran University of Medical Sciences, Tehran, Iran
[e] Trauma Research Center, Nursing Faculty, Baqiyatallah University of Medical Sciences, Tehran, Iran
[f] Behavioral Sciences Research Center, Life Style Institute, Nursing Faculty, Baqiyatallah University of Medical Sciences, Tehran, Iran
[g] MPH Program, Central Michigan University, USA
[h] Biotechnology Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran
[i] Department of Food Science and Technology, Quchan Branch, Islamic Azad University, Quchan, Iran
[j] Department of Nutrition, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
[k] Halal Research Center of IRI, FDA, Tehran, Iran
[l] Neurogenic Inflammation Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
[m] Biotechnology Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad 9177948564, Iran
[n] Polish Mother's Memorial Hospital Research Institute (PMMHRI), 93338 Lodz, Poland

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ABSTRACT

Background: 25-Hydroxy Vitamin D3 is known to have an effect on reproductive system in both genders and may change the semen parameters in men.
Objective: Our study aimed to evaluate the effect of oral vitamin D3 supplementation on spermogram quantitative and qualitative parameters in infertile men.
Materials and Methods: This study was a triple-blind randomized controlled trial involving 62 infertile men with impaired spermatogonial tests. They were randomly divided into placebo and D3-supplemented groups. Spermograms and tests for LH (Luteinizing Hormone), FSH (Follicle Stimulating Hormone), TT (Total Testosterone), FT (Free Testosterone), SHBG (Sex Hormone Bonding Globulin), FAI (Free Androgen Index) and vitamin D3 levels were performed before and after the intervention.
Results: There were no significant differences between the two groups in parameters of the spermograms or serum levels of LH, FSH, TT, and FAI. In the intervention group, SHBG was significantly decreased after intervention ($p = 0.01$) and there was a significant increase in FT in the placebo group ($p = 0.03$).
Conclusion: The intake of vitamin D3 did not change the quality and quantity of spermograms and serum levels of LH, FSH, TT, and FAI but affected FT and SHBG. Further studies are still needed to clarify the biological role of vitamin D3 on fertility particularly on male fertility. This study lays a foundation for more extensive studies on male infertility.

* Corresponding author at: School of Nursing and Midwifery, Iran University of Medical Sciences, Tehran, Iran.
** Corresponding author at: Biotechnology Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran.
E-mail addresses: amini.l@iums.ac.ir (L. Amini), afsanehtalebi68@gmail.com (A. Talebi), sahebkara@mums.ac.ir, amir_saheb2000@yahoo.com (A. Sahebkar).

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1. Introduction

Male infertility is a growing problem in developed countries[1] and is responsible for 50 % of global infertility.[2] Infertility was solely attributed to the male partner in 23.7 % of Iranian couples, while 19.3 % of infertilities were related to the combination of male and female factors.[3]

Genetic disorders, varicocele, genital infection, systemic diseases, and environmental factors can cause male infertility. However, unknown factors also play a role in 30–40 % of the cases.[4,5]

Since most cases of infertility in men may be due to idiopathic sperm underproduction, determining the environmental, nutritional and preventable factors that emerge such inadequate production is of utmost importance.[6,7] Vitamin D3 deficiency is considered as an important public health problem.[8]

The presence of vitamin D3 receptors and the vitamin D3 metabolizing enzymes in the testicle, ejaculatory system, and adult sperm might indicate the effect of vitamin D3 on spermatogenesis and maturation of human sperm. Studies on animals and humans confirm that vitamin D3 is involved in many processes of the reproductive system in both genders.[9]

Vitamin D plays an important role in the metabolism of calcium and phosphorus ions. It is involved in the absorption of intestinal calcium and reabsorption of renal calcium; also it has an immediate impact on chondrocyte and osteoblast differentiation resulting in bone formation.[10]

It is hypothesized that vitamin D might have an effect on testosterone production *via* osteocalcin, -produced by osteoblasts- involved in bone metabolism. It has been postulated that vitamin D-induced osteocalcin expression might have an indirect relevant role in modulating testosterone production.[11]

For instance, Ramlau-Hansen et al. For instance, Ramlau-Hansen et al [12,13].

However, Hammoud et al. [14] confirmed a relationship between serum vitamin D3 and semen parameters. Therefore, more research is needed to determine the correlation between vitamin D and fertility in men. Other studies reported an association between high levels of vitamin D3 and high levels of testosterone in men.[14]

In this regard, the results reported by Hormozi et al. (2017) illustrated that vitamin D3 deficiency is not associated with low total and free testosterone levels. They found no association between vitamin D3 deficiency and levels of Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), Free Testosterone (FT), and Total Testosterone (TT). Also, a recent study found no relationship between Vitamin D3 and sperm parameters in infertile men.[15] The precise molecular mechanism which could link vitamin D to the production of testosterone is still unknown. Further studies are required to clarify the role of vitamin D on hormonal function and semen quality in infertile men. This requires a level of vitamin D which can be effective on the improvement in healthy reproductive parameters.[16] Considering the cost of treatment, negative psychosocial effects, lack of information regarding the effectiveness of vitamin D3, and recommendations for additional research (especially interventional studies), the present study aims to determine the effect of vitamin D3 supplementation on quantitative and qualitative parameters of spermograms in infertile men.

2. Materials and methods

2.1. Study design and participants

The trial was designed to investigate the effect of vitamin D supplementation on quantitative and qualitative parameters of spermograms and reproductive hormones in infertile men. This study was a randomized controlled triple-blind trial that included 62 infertile men under infertility treatment aged 20–45 years old. The study lasted a year. Infertile participants were referred to the fertility clinic for treatment. The standard deviation of sperm index in both control and treatment groups was considered 9.82 and 4.47, respectively",[17]. After placing these values in the formula, the number of samples in each group was estimated to be 25, considering 10 % sample loss, 35 people in each group were sampled. The inclusion criteria were physical and mental health (ascertained based on the records of the case); Body Mass Index of 18.5–30; no vitamin D3 supplement consumption during the past 3 months; no use of drugs affecting the levels of vitamin D3 for example glucocorticoids and anticonvulsants; no use of medications that affect spermatogenesis during the past 3 months for example Cimetidine, spironolactone; absence of azoospermia in the spermogram, suffering from idiopathic disruptive spermograms, no genital infection or history of taking medication for STDs (sexually transmitted disease) within the past 3 months for example Ciprofloxacin and Ofloxacin; absence of anatomical abnormalities of the reproductive system such as varicocele; no contact with pesticides, heavy metals and high levels of heat Based on their job; no smoking of either cigarette or hookahs during the past 3 months, no use of alcoholic drinks and illicit drugs; serum vitamin D3 levels $\leq$30 ng/l; Iranian nationality; and fertility of the spouse. Exclusion criteria were as follows: no more than one dose of vitamin D3 intake per day during the study, the incidence of complications diagnosed by a urologist and a nutritionist which prevented the continuation of vitamin D3 intake, and the use of other supplements or drugs during the study which were banned in the inclusion criteria.

2.2. Intervention

A total 62 patients were randomly assigned to receive either placebo or vitamin D3. Randomization was done in form of drawings: the placebo and vitamin D3 containers were identical and coded with numbers from 1 to 72 by a person who was not aware of the randomization process. All containers were placed in an opaque bag.[18] The participants then received the containers that were randomly taken out of the bag.

the intervention period in this study was 12 weeks since the spermatogenesis process lasted 75 days Vitamin D3 recipients received vitamin D3 50,000 IU tablets once a week for 8 weeks, and a maintenance dose of vitamin D3 in the remaining 4 weeks (50,000 IU vitamin D3 supplement monthly).[19] Placebo recipients received placebo tablets (oral paraffin).

2.3. Procedures

The eligible patients were informed on the study procedure and received written consent. Spermograms and serum levels of LH, FSH, TT, FT, Sex Hormone Bonding Globulin (SHBG), Free Androgen Index (FAI), and vitamin D3 level were evaluated prior to the intervention. The measurement of vitamin D3 was carried out using diagnostic kit 25)OH (D3 of (biorexfars company) with Elisa method, FSH and LH were measured by a kit from IBL-America company with Elisa method, and FT and SHBG were measured by a kit from Roch company with ECLIA method. To determine the hormonal and vitamin D3 parameters, a venous blood sample was taken between 8–9 A.M after 12 h of fasting. Finally, FAI was calculated for each subject as total testosterone level divided by the sex hormone-binding globulin (SHBG) level $\times$ 100. In order to conduct the spermogram test, individuals collected semen samples in sterile containers 3–7 days after sexual intercourse. The collected samples were placed in an incubator at 37 °C for 20 min and were subsequently tested.[20] The diagnosis was performed by a urologist as follows: all spermogram tests which had low sperm count, mobility impairment, or abnormal morphology were diagnosed as impaired spermograms. Normal values were considered Semen volume of 1/5 mL (1/4−1/7), Sperm concentration of 15 (10^6/mL) (12–16), Total Sperm Number of 39 million per ejaculate (33–46), Progressive Motility of (%) 32 (31–34), Sperm morphology of (%) 4 (3–4)[21] and Sperm Motile Index>160.[22]

The data collection instruments in this study included an individual and fertility information questionnaire, a semiquantitative food-

frequency questionnaire (FFQ), a sun exposure checklist, a spermogram record sheet, a drug side-effects record sheet, and a vitamin D3 supplementary checklist. The individual and fertility information questionnaire was prepared by the researchers and contained 16 questions as follows: 12 questions were related to demographic information, and the remaining 4 questions were focused on fertility data. The 24-hr food recall questionnaire was conducted to ensure that there were no significant differences between the two groups in terms of dietary intake. In addition, the sun exposure checklist included four questions to examine exposure to sunlight or lack thereof during the day and the use of sunscreen; it was supplied by the Ministry of Health and Medical Education as part of the National Food and Nutrition Program (Children and Adults). Moreover, the drug complaint checklist was to collect drug-related reports. Finally, the drug side-effects record checklist was provided by the Food and Drug Administration of the Ministry of Health. It is worth noting that in this study, no drug side-effects were reported and all questionnaires were approved by 8 faculty members and the validity of their content was confirmed.

The subjects, researchers, and statistics specialists were not informed of the contents of the containers (and consequently, were not aware which subjects belonged to which study group) until the end of the data analysis. Sampling continued until the sample size was completed. During the study, subjects were asked to retain their usual diet and lifestyle. Moreover, they were assured that they would receive all necessary treatments regarding any illness and would be examined by a specialist free of charge in case of any problems such as allergies or drug side-effects.

All participants were monitored to control the correct consumption of drugs and to check the development of any complications or allergies, they were followed up via telephone weekly. To improve and verify compliance, patients were asked to return the used bottles at the end of the study. At the end of the intervention serum levels of FSH, TT, FAI, SHBG, FT, and vitamin D3 were tested again. However, only vitamin D3 measurement was performed in the placebo group after the study.

2.4. Statistical analysis

Using the KS (Kolmogorov-Smirnov) test, the normal distribution of data was confirmed. Data were analyzed using SPSS (Statistical Package for Social Science) software version 16. Data analysis consisted of two parts: descriptive (frequency, mean, and standard deviation) and analytical (paired *t*-test, independent *t*-test, and Fisher's exact test). In this study, p-value of less than 0.05 was considered statistically significant.

2.5. Ethical consideration

This study was conducted as a randomized controlled trial and approved by the Ethics Committee of Iran University of Medical Sciences (Reference Number IR.IUMS.REC.1395.9311373019), and was registered at the Iranian Registry of Clinical Trials (Identification Number) IRCT2016111830947n1. All participants were informed about the aim of the study and signed an informed consent form before participating in the study.

3. Results

We randomly assigned 35 patients to the intervention and 37 patients to the control group; however, as described in Fig. 1, five patients in the intervention group and five patients in the control group were excluded.

A total of 62 infertile men participated in this study, the age and BMI range were 35–39 years and 25–29/9 kg/m^2. Other general characteristics of the participants are presented in Table 1. There was no significant difference in terms of length of the marriage, duration of infertility, infertility treatment history, employment status, and educational level

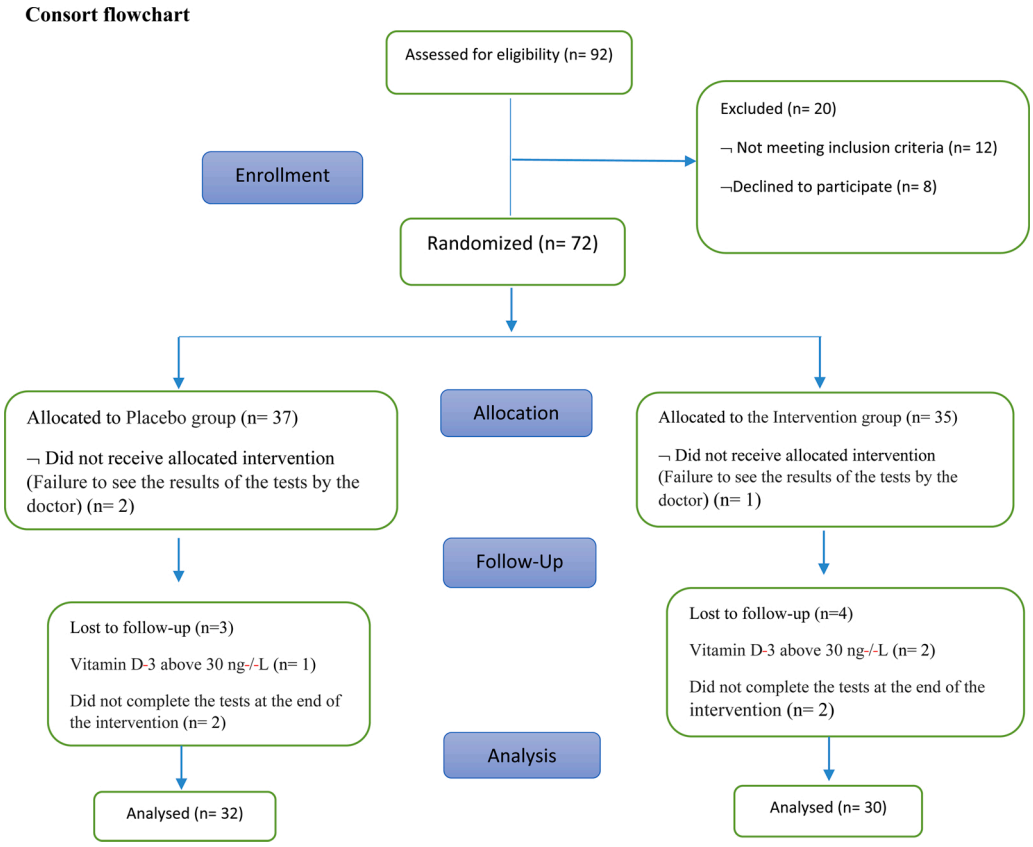

Fig. 1. The CONSORT Flow Diagram.

Table 1
demographic and fertility information of participants.

	Vitamin D3	Placebo	P-value
Age (years)	4.83±34.37	34.86 ± 4.65	0.68
Age of spouse (years)	4.38±29.65	29.86 ± 488	0.85
Body Mass Index	25.69 ± 1.94	25.47 ± 1.90	0.65
Length of marriage (years)	4.28 ± 2.22	5.30 ± 3.10	0.14
Duration of infertility (years)	1.68 ± 1.07	2.01 ± 1.26	0.27
Level of Education			0.53
Elementary	0%	6.2 %	
High school	43.3 %	34.4 %	
University	56.7 %	59.4 %	
Employment status			0.42
Self-employed	53.3 %	46.9 %	
Employee	33.3 %	46.9 %	
Manual Worker	13.4 %	6.2 %	
Economic situation			0.60
Weak	10 %	3.1 %	
Medium	73.3 %	78.1 %	
Good	16.7 %	18.8 %	
Infertility treatment history			0.46
Yes	16.7 %	9.4 %	
No	83.3 %	90.6 %	
Exposure time to sunlight			0.19
10−60 min	36.7 %	37.5 %	
60−120 min	46.6 %	59.4 %	
120< min	16.7 %	18.8 %	

between the experimental and placebo groups ($p > 0.05$) (Table 1). Fig. 1 shows the consort flowchart for the recruitment of the study population.

3.1. Indices of spermograms

As is noted in Table 2, the results of the paired t-tests showed that there was no significant difference between the placebo and vitamin D3 groups.

The mean and standard deviation for the quantitative and qualitative parameters of the sperm (before and 12 weeks after the intervention) were not significantly different in intervention and placebo groups.

3.2. Hormonal tests

In addition, as illustrated in Table 3, the mean and standard deviation for LH, FSH, TT, and FAI were not significantly different in Vitamin D3 receivers and the placebo group. However, the mean and standard deviation for SHBG ($P = 0.01$) in the intervention group demonstrated a statistically significant difference. Meanwhile, there was a significant difference in FT ($P = 0.03$) level between the intervention and placebo groups.

3.3. Seasonal variation

There was a significant statistical difference between vitamin D3 serum levels in the supplement group and placebo group prior to and after the study. It should be noted that sampling initiated during the winter and most samples were obtained during this season. Moreover, the intervention was conducted during the spring and summer, and it was applied to both groups.

4. Discussion

As demonstrated in the results, the use of vitamin D3 supplementation did not alter the quantitative and qualitative indices of the spermograms or serum levels of LH, FSH, TT, and FAI. There was a significant difference in terms of SHBG in the test group ($p = 0.01$) and FT in the placebo group ($p = 0.03$). In a similar study, Tartagni et al.[23], reported no significant difference between the two groups of men (Vitamin D deficient vs normal Vitamin D) in terms of sperm count, progressive sperm motility, morphology, and fertility. In this study, it was shown that the lack of variation in spermogram parameters can be due to several factors, including several plausible limitations associated with the semen analysis. Examples of such limitations include abnormalities in sperm (e.g. immature chromatin and fractured DNA) and other factors affecting semen components (e.g. sexual activity, and gonadal function).

As reported in the Tartagni's study, even though sperm parameters were not significantly different in the two groups in terms of concentration, mobility, and morphology, the ratio for fertility and live births were higher in a group with higher serum levels of vitamin D3; while the abortion ratio in the deficient group was increased. The results obtained in this study are consistent with the data about the role of vitamin D3 in the regulation of reproductive physiology in men. Tartagni et al. demonstrated that vitamin D3 acts as a protective genomic agent for the sperm DNA, albeit its precise role in the sperm nucleus is unclear.[23] Based on the results obtained in this study, it can be concluded that the effect of vitamin D on male fertility can be via affecting the sperm DNA, without directly affecting the sperm parameters.

According to the study by Blomberg Jensen et al.[24], a single dose of 300,000IU vitamin D followed by 1400 IU of vitamin D and 500 mg of calcium administered daily for 150 days had no effect on semen quality, serum FSH, and inhibin B levels in infertile men, compared to the placebo group. The rate of spontaneous pregnancies in treated couples was higher than in control couples. Vitamin D and calcium supplementation were not associated with semen quality on spontaneous pregnancies and live birth. It implies that the positive effect on live birth rate could either be an unforeseen finding or that the activated vitamin D is capable of improving gamete function in a yet unidentified manner.[24]

Karras et al.[25], provided evidence regarding a U-shaped relationship between vitamin D and the sexual gland function. First, in a study by RamlauHansen et al. in 2011 a decreasing pattern was shown in the volume of the amniotic fluid, the sperm count, and morphology for 307 men with high concentrations of vitamin D, even though this relationship was not significant. Second, in a study by Hammoud et al., in 2012 the U-shaped relationship between vitamin D concentration and semen quality was shown. It was reported that concentration, morphology, mobility, and total sperm count in men with vitamin D3 $\geq$ 20 ng/ml were higher than that of men whose vitamin D3 levels were $\leq$20 ng/mL. In this study, men with vitamin D3 $\geq$ 50 ng/ml had lower morphology, concentration, and percentage of moving sperm in comparison to vitamin D3 $\geq$ 20 ng. Therefore, both high and low levels of vitamin D3 can have negative effects on spermogram parameters; which may be due to the effect of vitamin D3 on the systemic and local levels of calcium and zinc.[14] The role of calcium in sperm maturation is well-defined. Vitamin D3 seems to play a role in transferring calcium in the epididymis.

In a cross-sectional study by Lerchbaum et al., in 2014 a U-shaped relationship between vitamin D and hypogonadism was reported as well since men with vitamin D serum levels of $>$ 102 *nm* and $\leq$ 43.9 *nm* had an increased risk of hypogonadism. Men with either low or high levels of vitamin D presented weaker glandular function compared to individuals with moderate levels of this vitamin.[25] This finding can also be effective in the results of the present study.

There was a significant statistical difference between vitamin D3 serum levels in the supplement group and placebo group, prior to and after the study. However, it should be noted that sampling initiated during the winter and most samples were obtained during this season. Moreover, the intervention was terminated during the spring and summer, and it was applied to both groups. Furthermore, the changes in vitamin D3 serum levels were significantly higher in the supplement group compared to the placebo group. Therefore, this statistical difference is justifiable. As reported and verified by Tartagni et al.[23], sampling in winter could affect vitamin D3 levelsand reduce its level.[23]

As opposed to the results of the present study, in a study by Deng XL et al. which was aimed to evaluate the efficacy and safety of vitamin D3

Table 2
the quantitative and qualitative indices of spermograms in vitamin D and placebo receivers before and after the intervention.

P-value	Placebo		P-value	Vitamin D3		
	After intervention	Before intervention		After intervention	Before intervention	
0.85	1.96 ± 1.1	1.95 ± 1.15	0.58	2.08 ± 1.04	2.13 ± 1.05	Semen volume(ml)
0.48	90.40 ± 13.37	88.90 ± 12.90	0.14	88.28 ± 13.64	85.44 ± 14.30	Sperm concentration(10^6/mL)*
0.25	101.29 ± 16.29	98.62 ± 16.28	0.13	100.42 ± 15.99	97.25 ± 16.62	Total Sperm Number(10^6/Ejaculate)*
0.07	64.71 ± 23.53	59.49 ± 22.71	0.23	60.48 ± 22.75	57.60 ± 22.64	Motile Sperm Concentration(10^6/mL)*
0.11	56.46 ± 20.31	51.94 ± 19.74	0.16	51.60 ± 20.89	48.60 ± 19.85	Total Motile Sperm Concentration(10^6/mL)*
0.47	14.84 ± 11.01	13.59 ± 9.11	0.39	14.00 ± 15.76	12.10 ± 8.77	Progressive Motility(%)
0.63	13.75 ± 6.77	13.25 ± 7.06	0.08	13.30 ± 11.16	10.40 ± 6.92	Sperm morphology(%)
0.29	40.43 ± 38.14	35.06 ± 25.20	0.08	36.90 ± 44.34	26.73 ± 23.98	Sperm Motile Index

Data presented as mean ± SD.
* Presented in a logarithmic scale.

in idiopathic oligoasthenosospermia, (2014), an increase in the count of sperms was observed following the medical treatment in the test group. This study also reported a significant statistical difference in the number of sperms with progressive motility between the control group and the test group.[17] Differences in the study population, such as variability in the selection of patients with spermogram abnormalities and diverse protocols of vitamin D supplementation, may influence the results of the study. It should be noted that few studies have reported the relationship between vitamin D3 and the concentration and the number of sperms. Most reports considered the relationship between vitamin D3 and sperm motility since the number of people with high levels of vitamin D3 is low. Therefore, to the best of our knowledge, there are no studies on the relationship between high levels of vitamin D3 and sperm parameters. A comparison between the mean value and the standard deviation of hormonal tests such as LH, FSH, SHBG, TT and FT in the men who participated in the supplement group demonstrated a statistically significant difference in the SHBG serum level before and after the intervention (P = 0/01). In terms of FT levels, the difference in the hormonal tests for the two placebo groups was statistically significant as well (P = 0/03).

Tak et al.[26] realized that 25-hydroxyvitamin D was related to TT ($p < 0.001$) and FT ($p = 0.008$). Vitamin D3 deficiency was associated with an increased risk of total and free testosterone deficiency and as a result, there was a positive correlation between 25-hydroxyvitamin D and testosterone. The reason for such difference could be not considering the seasonal changes in hormones and vitamin D3 intake.[26] Moreover, results from the hormonal tests in both the supplement and the placebo groups showed a statistically significant difference in the levels of FT, and SHBG (P = 0.03). Since insulin is a major SHBG regulator and low levels of SHBG is associated with insulin resistance and hyperinsulinemia, and as indicated in the current study, vitamin D3 affects the SHBG levels, more extensive studies can be beneficial in infertile men with diabetes.

As reported previously,[27] vitamin D3 level in Chinese male subjects is associated with levels of total testosterone, FT, SHBG, estradiol, and hypogonadism. In this study, this correlation was confirmed via obesity and insulin resistance.

To elaborate, the relationship between vitamin D3 and hypogonadism is clearly due to obesity and insulin resistance. Hence, in patients with controlled BMI, the relationship between vitamin D3 and hypogonadism will be reduced. On the contrary, the two groups in this study did not have any difference in BMI[27]. Lerchbaum et al., in 2019 investigated the effects of vitamin D3 on serum TT levels in men. One-hundred healthy men with serum TT levels < 10.4 nmol/l and 25-hydroxyvitamin D [25(OH)D] levels < 75 nmol/l participated in the trial. Subjects were randomized to receive 20,000 IU of vitamin D3/week (n = 50) or placebo (n = 50) for 12 weeks. They found no significant effect of vitamin D on androgen levels including TT, FT and FAI concentrations of middle-aged healthy men with low baseline serum TT levels.

In men with vitamin D serum levels of less than 50 nmol/l, a significant increase in the SHBG serum level was observed in the placebo group following the intervention, while the SHBG serum level remained unchanged in the vitamin D group.[28]

Table 3
The hormonal tests in vitamin D receiver's and placebo groups before and after the intervention.

P-value	Placebo		P-value	Vitamin D3		
	After intervention	Before intervention		After intervention	Before intervention	
0.29	4.37 ± 2.93	4.73 ± 2.63	0.95	4.71 ± 4.37	4.68 ± 2.93	FSH[a] (Iu/L)
0.63	3.58 ± 2.12	3.71 ± 1.50	0.69	4.10 ± 2.36	3.94 ± 3.73	LH[b] (Iu/L)
0.91	3.41 ± 1.25	3.38 ± 1.23	0.83	3.42 ± 1.04	3.46 ± 1.16	TT[c] (ng/dl)
0.03	11.91 ± 3.90	9.59 ± 3.76	0.94	10.36 ± 3.41	10.40 ± 3.79	FT[d] (pg/ml)
0.40	26.68 ± 13.53	27.40 ± 13.13	0.01	25.76 ± 9.99	28.73 ± 9.27	SHBG[e]($nmol/L$)
0.29	15.74 ± 9.74	14.03 ± 6.13	0.08	14.66 ± 6.05	13.07 ± 5.57	FAI[f]
0.01	19.43 ± 7.60	15.97 ± 6.86	0.00	35.27 ± 15.50	17.42 ± 6.98	VITD3 (ng/ml)

Data presented as mean ± SD.
● The mean and standard deviation of hormonal tests in supplement and placebo groups before and after the intervention.
● The results of the paired *t*-test showed that there was a statistically significant difference between the two groups of placebo and vitamin D3 in terms of hormonal markers.
[a] Follicle Stimulating Hormone.
[b] Luteinizing Hormone.
[c] Total Testosterone.
[d] Free Testosterone.
[e] Sex Hormone Bonding Globulin.
[f] Free Androgen Index.

In another study on men with normal TT serum levels, in the vitamin D group who received 20,000 IU of vitamin D3/week for 3 months, the SHBG serum levels were reduced, while no such change was seen in the placebo group. In terms of the obtained results, this study is similar to the present study. Also, the duration of treatment, was 12 weeks in both studies.[8]

Moreover, contradictory results in hormonal tests are due to confounding factors in the investigation of hormones, such as age, season, BMI, and different study methods. Failure to consider these factors may affect the outcome while adjusting these factors will emerge more precise results.

5. Conclusion

The results showed that using vitamin D3 supplementation did not change the quantitative and qualitative indices of the spermograms or hormonal markers, except for FT and SHBG.

Also, according to various studies, it is evident that there is still no general agreement on the optimal concentration of vitamin D3 for the efficiency of the reproductive system, and vitamin D3 deficiency is yet to be accepted as an indicator of poor reproductive health.

Therefore, more studies are needed to demonstrate the role of vitamin D3 in the treatment of male infertility.

5.1. Limitations

Given the process of spermatogenesis, and the fact that the expected time for the vitamins to have effect on semen is uncertain, it might be necessary to increase the duration for the vitamin D3 conservator dose supplementation. Among other potentially effective factors, one would measure sperm indices over 3–6 months after receiving the last dose. Therefore, the therapeutic dose of vitamin D3, duration of consumption, and the time interval between receiving the last dose of vitamin D3 and measurement of sperm indices can be effective. Due to the fact that the present article was an extract from the dissertation and the sufficient cost for checking the hormonal tests was limited, it was not possible to evaluate some other hormones (e.g. inhibin, TSH).

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CRediT authorship contribution statement

Leila Amini: Conceptualization, Methodology, Investigation, Resources, Writing - original draft, Supervision, Funding acquisition. **Robabe Mohammadbeigi:** Investigation, Writing - review & editing. **Mohammadreza Vafa:** Investigation, Writing - review & editing. **Hamid Haghani:** Methodology, Investigation, Writing - review & editing. **Amir Vahedian-Azimi:** Methodology, Formal analysis, Writing - review & editing, Supervision. **Leila Karimi:** Formal analysis, Investigation, Writing - review & editing. **Shayesteh Jahanfar:** Formal analysis, Writing - review & editing. **Tannaz Jamialahmadi:** Methodology, Writing - original draft. **Afsaneh Talebi:** Conceptualization, Resources, Writing - original draft, Supervision, Funding acquisition. **Amirhossein Sahebkar:** Methodology, Writing - original draft, Supervision.

Declaration of Competing Interest

None.

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