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Effects of Vitamin D Supplementation on Semen Quality and Reproductive Hormones in Patients with Asthenozoospermia: A Randomized Double-Blind Placebo-Controlled Clinical Trial

Alireza Gheflati; MSc[1], Seyed Ali Mohamad Mirjalili; MD[2], Mojtaba Kaviani; PhD[3], Amin Salehi-Abargouei; PhD[1], Elham Hosseini-Marnani; MSc[1] & Azadeh Nadjarzadeh; PhD[*1]

[1] *Department of Nutrition, School of Public Health, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
[2] *Department of Urology, School of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
[3] *School of Pure and Applied Science, School of Nutrition and Dietetics, Acadia University, Wolfville, NS, Canada.*

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Corresponding author:
azadnajarzadeh@ssu.ac.ir
Department of Nutrition, School of Public Health, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

Postal code: 8915173160
Tel: +98 35 38209114

ABSTRACT

Background: Animal models and observational studies have suggested a favorable role of vitamin D in male reproduction. However, randomized clinical trials investigating the effect of vitamin D supplementation on male fertility are limited. Therefore, this study aimed to examine the effect of vitamin D supplementation on semen quality, reproductive hormones, and anthropometric measurements in vitamin D deficient males with Asthenozoospermia. **Methods:** Forty-four males with infertility were randomly assigned to the vitamin D group (DG, supplemented with 9 pearls of vitamin D containing 50000 IU vitamin D_3 once a week for 12 weeks) and placebo group (PG, received 9 pearls of placebo once a week for 12 weeks). Semen quality markers (sperm count, morphology, sperm motility, semen volume), total testosterone, sexual hormone binding globulin (SHBG), free androgen index (FAI), and anthropometric measurements (weight, body mass index (BMI), waist circumference (WC), hip circumference (HC), and waist to hip ratio (WHR) were measured at the baseline and the end of the study. **Results:** Serum 25-OH-D levels were significantly higher in the DG compared with the PG. In a multivariate adjusted model, WC significantly decreased in the DG in comparison to the CG (-0.90 ± 0.67 cm VS 0.49 ± 0.38 cm). A marginally-significant increase was observed after vitamin D supplementation for SHBG compared to the baseline value in DG (11.69 ± 5.79, P = 0.05). Compared to the baseline value, sperm immotile was decreased after vitamin D supplementation in the DG (-12.35 ± 5.13, P = 0.02). However, no statistical significant differences were observed in the semen quality markers (sperm count, morphology, motility, and volume), total testosterone, free androgen index, and other anthropometric values. **Conclusions:** Vitamin D supplementation did not improve semen quality markers, reproductive hormones, and other anthropometric measurements in vitamin D–deficient infertile men compared to the control group.

Keywords: *Vitamin D; Male infertility; Semen quality; Reproductive hormones*

Introduction

Infertility is defined as the inability to conceive after 12 months of regular intercourse without contraception (Monga *et al.*, 2004). In the past two decades, the rate of infertility has remained

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unchanged, so that 49 million couples were affected by this health issue by 2010 (Skakkebaek *et al.*, 2015). According to the World Health Organization (WHO), infertility is defined as a sperm concentration of less than 15 million per milliliter (oligozoospermia), less than 40% of sperm with motility (asthenozoospermia), and less than 4% with normal morphologic features (teratozoospermia) (World Health Organization, 2010a). Several treatment procedures have been recommended to treat affected couples including assisted reproductive techniques (ARTs) (Reproduction *et al.*, 2016), empirical drugs (clomiphene citrate and Tamoxifen) (Chua *et al.*, 2013), and other miscellaneous therapies such as androgens (Dohle *et al.*, 2003), alpha-blockers (Yamamoto *et al.*, 1986) and magnesium supplementation (Závaczki *et al.*, 2003) . However, none of these options were effective based on the European Association of Urology statement in treating semen quality disorders (Jungwirth *et al.*, 2015). However, a large body of research supports the beneficial effects of lifestyle modifications on the sperm quality (Gopalan and Naidu, 1972, Jensen, 2014, Tüttelmann *et al.*, 2012).

Recently, special attention has been paid to the effect of vitamin D supplementation on reproductive outcomes in both genders (Anagnostis *et al.*, 2013). One of the earliest findings was that vitamin D maintains calcium and phosphorus hemostasis (Holick, 2007). Gradually, evidences suggested that vitamin D deficiency increased the risk of cancers (Lappe *et al.*, 2007), autoimmune diseases (Szodoray *et al.*, 2008), diabetes (Pittas *et al.*, 2007), and cardiovascular diseases (Pilz *et al.*, 2011b). Some evidences indicated other effects of vitamin D (Bikle, 2009, Lerchbaum and Obermayer-Pietsch, 2012a) in modulating reproductive process in addition to sex steroid hormones (Blomberg Jensen *et al.*, 2010, Kinuta *et al.*, 2000). The vitamin D receptor (VDR) is expressed in most organs (Jensen, 2014). Vitamin D is not biologically active; it has to be activated by hepatic 25-hydroxylation that converts cholecalciferol into metabolite 25-hydroxyvitamin D (25OHD). Renal 1a-hydroxylase converts 25OHD into the active metabolite 1,25-(OH)2D3 that binds and activates VDR (Bouillon *et al.*, 2008). Previous studies revealed that detrimental mutations in VDR are associated with a wide range of diseases such as osteoarthritis (Colombini *et al.*, 2013), prostate cancer (Ingles *et al.*, 1997), and diabetes (Ogunkolade *et al.*, 2002). However, these mutations are highlighted in infertility and reproductive disorders (Boisen *et al.*, 2017).

Some specific mechanisms have been proposed through which vitamin D influences male fertility remain unclear. Vitamin D receptors exist in human testis (Habib *et al.*, 1990) and also sperm cells (Corbett *et al.*, 2006). The metabolizing enzymes in the human testis, ejaculatory tract, and sperm cells indicate the role of vitamin D in spermatogenesis (Blomberg Jensen *et al.*, 2010). It was shown that internal sperm Ca^{2+} quantities provide sufficient Ca^{2+} for the induction of sperm motility (Kong *et al.*, 2007). Aquila et al. revealed that 1,25-(OH)2D3 increased intracellular sperm Ca^{2+} (Aquila *et al.*, 2009). Moreover, 1,25-(OH)2D3 can influence the sperm extra testicular motility by its potential action on acrosin activity (Aquila *et al.*, 2009).

Previous animal studies have shown lower fertility rates among vitamin D–deficient males in comparison with vitamin D–sufficient ones (Kwiecinski *et al.*, 1989, Uhland *et al.*, 1992). A study on mutant mice without VDR showed decreased sperm count and motility along with histological testis abnormality (Kinuta *et al.*, 2000). In another attempt, Jensen et al. reported that vitamin D supplementation had no effect on sperm quality (Blomberg Jensen *et al.*, 2017a); while Alzoubi et al. revealed sperm motility improvement in idiopathic male infertility patients with low vitamin D following oral vitamin D administration (vitamin D, 5000 IU, daily for two months) (Alzoubi *et al.*, 2017). Clinical trials over vitamin D supplementation on infertility are rare. Therefore, we aimed to investigate the effect of vitamin D supplementation in infertile male patients with vitamin D deficiency on sperm quality parameters, especially sperm motility. Moreover, we assessed its

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potential effects on sexual hormone binding globulin (SHBG) and testosterone.

Material and Methods

Study participants: Outpatients attending Yazd Research & Clinical Center for Infertility affiliated with Yazd University of Medical Sciences underwent medical screening before participating in the study. According to the sample size formula suggested for randomized clinical trials, our sample size was estimated as 22 patients in each group considering $\alpha = 0.05$, test power of 90%, sperm motility as a key variable, and 10% attrition. Eligible participants were defined by having 1) 18-45 years of age, 2) sperm motility disorder based on WHO criteria; passing at least one year from the time the couple decided to have a baby, 3) low levels of 25-hydroxy-D (25-OH-D) < 30 ng/ml.

In total, 44 eligible individuals were recruited as new cases of asthenozoospermia patients diagnosed by urologist. The exclusion criteria were as follows: 1) intake of vitamin D and calcium supplements in the last 3 months, 2) participants who had varicocele, cryptorchidism, microorchidism, and individuals with azoospermia and a history of vasectomy, 3) intake of less than 90% of the vitamin D pearls.

Ethical considerations: This study was conducted according to the guidelines provided by the Declaration of Helsinki. Furthermore, Ethics Committee in Shahid Sadoughi University of Medical Sciences approved the study protocol. Oral assent and informed written consent were obtained from all patients after providing them with detailed explanations about the study methods, purposes, advantages, and risks at baseline. This trial was registered at www.irct.ir with registration code: IRCT20120913010826N29.

Study design: This randomized, parallel, double-blind, and placebo-controlled clinical trial was conducted in the Infertility Research Center, Yazd, Iran from October 2017 to July 2018. Participants were randomly divided to 2 groups (in 1:1 ratio) to take either vitamin D supplementation (DG) or the placebo (PG) for 12 weeks. DG received 9 pearls containing 50.000 IU vitamin D3 every 8 consecutive weeks and only one pearl in the third month for maintaining the dose. Patients in the PG received 9 placebo pearls, containing paraffin oil to take once a week and only one placebo in the third month, which was similar to the intervention group.

Appearance of the placebo pearls (color, shape, size, and packaging) was completely similar to the vitamin D pearls. All supplements were manufactured by Zahravi Pharmaceutical, Co., Tabriz, Iran. Supplements were coded as A or B and were placed in a similar sealed envelope by a third person. Patients were randomly assigned into the study groups using a computer-generated random sequence. The randomization sequence was concealed until the last participant completed the final visit. A trained clinician who was blinded to the treatment allocation recruited and assessed all participants. The follow up sessions with the patients were performed once a week by a phone call to ensure that all capsules were consumed. Compliance was assessed by counting the remained pearls. Individuals who consumed less than 90% of the capsules were excluded from the analysis. Moreover, as participants were provided with a tracking chart; they were supposed to fill it out and return it at the next visit. Participants were asked not to change their daily physical activity throughout the study and their dietary intakes were assessed using a 3-day food records completed during the study. Nutritionist IV software (First Databank), modified for Iranian foods, was used to calculate energy and nutrient intake of participants based on a 3-day food record.

*Measurements***:** All measurements were performed at the study baseline and 12 weeks after the intervention. The patients' body weight was measured in minimal clothing and without shoes using a digital scale (model BF511; Omron, Japan) to the nearest 0.1 kg. Height measurement was carried out in standing position, without shoes, and by a non-stretched tape measure (Seca, Germany) with 0.1 cm accuracy. Body mass index (BMI) was calculated by weight (kg) divided by height squared (m) and expressed as kg/m^2. Waist

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circumference (WC) was obtained as the lowest amount between iliac crest and lateral costal margin. Hip circumference (HC) was recorded over the largest part of buttocks. Other anthropometric indices were assessed by body composition analyzer (model BF511; Omron, Japan). Physical activity of participants was determined by multiplying metabolic equivalent (MET) derived from a valid self-reported physical activity questionnaire by hour and day (MET/h/d). A questionnaire that was categorized into nine groups ranging from sleep/rest (MET 0.9) to vigorous physical activity was used for assessing the participants' physical activity (MET 6) (Aadahl and JØrgensen, 2003). Participants were asked not to change their usual physical activity level throughout the study.

Fasting blood samples were collected from 8 am and 10 am. Consequently, 10 ml blood sample of peripheral venous was obtained after a 12-h overnight fasting at the beginning and end of the study. Blood samples were centrifuged at 3500 rpm for 10 min and aliquots of serum were stored in -70 °C refrigerator. Serum 25-OH-D levels were measured by a commercial ELISA kit (IDS, Boldon, UK) with inter- and intra-assay CVs of 4.4 to 6.6%. Sexual hormone binding globulin (SHBG) and serum total testosterone were assessed using commercial kits (diameter, Milano, Italy). Inter- and intra-assay CVs were 3.4 to 5.0% and 3.9 to 5.7%, respectively. In addition, free androgen index (FAI) was calculated as the ratio of total testosterone to SHBG.

Semen samples were taken by masturbation in a room near the laboratory and kept at 37 °C. Patients were asked not to ejaculate for at least 48 h before sampling. Semen analysis was conducted considering the WHO guidelines (World Health Organization, 2010b). Sperm parameters like sperm count (10^6/ml), motility, viability, and normal morphology were assessed on behalf of 200 spermatozoa for each sample. Sperm count and motility were evaluated by means of Makler chamber using a light microscopy (Olympus Co., Tokyo, Japan). The viability and morphology were assessed by Eosin and Papanicolaou staining tests, respectively.

Data analysis: All statistical analyses were performed using SPSS for Windows (SPSS Version 20; SPSS Inc., Delaware). Kolmogorov-Smirnov test was run to assess the normal distributions of variables. Log transformation was performed for non-normally distributed variables. Independent t-test was applied to assess the difference in means of general characteristics, dietary intake, mean of biomarkers at the baseline and at the end of study and changes in biomarkers between DG and PG. Paired t-test was performed to compare the baseline and endpoints values within each group. Crude and a multivariate-adjusted model (adjusted for baseline weight, baseline values, and smoking) were conducted to compare mean changes between the intervention and control groups. Analysis of covariance and independent student t-test were performed in crude and in multivariate-adjusted models analysis, respectively.

Results

Of 44 recruited patients, 4 persons withdrew from the study due to low compliance or personal reasons (**Figure 1**). In total, 40 individuals were randomly allocated into two groups; DG and PG (twenty in each group).

Table 1 summarizes the baseline characteristics of patients. The mean of age was 32.3 ± 5.8 years in the DG and was 33 ± 5.48 years in the PG (P = 0.69). As revealed in **Table 1**, no statistically significant difference was found in weight (P = 0.63), BMI (P = 0.15), WC (P = 0.43), and HC (P = 0.60).

As demonstrated in **Table-2**, no statistically significant differences were observed between the DG and PG in energy intake, carbohydrate, fat, protein, saturated fat, cholesterol, sodium, potassium, calcium, iron, zinc, Selenium, Vitamin B6, Vitamin B12, Vitamin C, Vitamin E, and fiber intake as well as physical activity level in the baseline. Furthermore, none of these parameters showed significant differences before and after the intervention with regard to the DG and PG. In

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addition, no significant change was found in the mean change of all measurements.

Table 3 demonstrates the change of anthropometric indices in both groups. As it is represented, anthropometric indices were not significantly different between the two groups at the beginning of the study. According to the results, within group analysis showed no significant differences in mean weight, BMI, WC, HC, and WHR in both groups, although, the comparison between two groups showed a marginally significant decreased in WC in the DG ($P = 0.08$). Additionally, the results of adjustments represent a significant decrease of WC in the DG ($P = 0.03$).

Table 4 displays the effect of intervention on semen quality and reproductive hormones in both groups after 12 weeks of the intervention. Between-group analysis showed significant differences for serum 25-OH- D levels between the two groups ($P < 0.001$). Among sperm quality markers, only sperm motility (immotile form) decreased significantly in the DG ($P = 0.02$). However, sperm count, sperm motility (progressive), (non-progressive), sperm morphology, and sperm volume did not change significantly in the DG. Compared with the baseline values, SHBG was marginally increased in the DG ($P = 0.05$). The intervention did not significantly change the other markers. The multivariate adjusted model between two groups revealed no significant differences between the two groups (**Table 4**).

Table 1. Comparison mean (±SE) of baseline characteristics of participants in both groups.

Variables	Vitamin D group (n = 20)	Placebo group (n = 20)	P-value[a]
Age (y)	32.30 ± 1.30	33.00 ± 1.22	0.69
Height (cm)	174.7 ± 1.56	171.15 ± 1.89	0.15
Weight (kg)	75.14 ± 3.42	77.71 ± 4.12	0.63
Body mass index (kg/m^2)	24.47 ± 0.86	26.34 ± 0.97	0.15
Waist circumference (cm)	92.33 ± 2.73	95.38 ± 2.69	0.43
Hip circumference (cm)	99.04 ± 1.67	100.44 ± 2.06	0.60

[a]: *Student t-test*

Table 2. Comparison mean (±SE) of nutrients and calorie intake within and between groups.

Variables	Before	After	P-value[a]	Change
Energy (kcal)				
Vitamin D group	2150.59 ± 111.39	2132.68 ± 121.94	0.60	-17.90 ± 34.03
Placebo group	2284.65 ± 123.08	2328.68 ± 118.53	0.05	44.03 ± 21.21
P-value[b]	0.42	0.25		0.13
Carbohydrate (g)				
Vitamin D group	309.36 ± 15.08	303.79 ± 18.59	0.56	-5.56 ± 9.54
Placebo group	337.52 ± 17.37	338.44 ± 16.05	0.85	0.92 ± 4.93
P-value	0.22	0.16		0.54
Protein (g)				
Vitamin D group	79.14 ±5 .41	81.55 ± 5.55	0.53	2.40 ± 3.83
Placebo group	92.55 ± 7.93	92.84 ± 7.74	0.93	0.29 ± 3.29
P-value	0.17	0.24		0.67
Fat (g)				
Vitamin D group	68.21 ± 4.67	67.16 ± 5.69	0.79	-1.04 ± 3.86
Placebo group	65.51 ± 5.39	70.17 ± 4.74	0.11	4.65 ± 2.84
P-value	0.70	0.68		0.24
Saturated fatty acids (g)				
Vitamin D group	19.67±1.89	19.43 ± 2.17	0.85	-0.23 ± 1.29
Placebo group	20.32±2.38	21.95 ± 2.05	0.36	1.63 ± 1.7
P-value	0.83	0.40		0.39

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Table 2. Comparison mean (±SE) of nutrients and calorie intake within and between groups.

Variables	Before	After	P-value[a]	Change
Cholesterol (mg)				
Vitamin D group	310.20 ± 48.08	428.20 ± 59.41	0.06	118.0 ± 60.6
Placebo group	403.58 ± 70.93	441.70 ± 78.30	0.35	38.11 ± 40.34
P-value	0.28	0.89		0.28
Fiber (g				
Vitamin D group	15.22 ± 1.36	15.27 ± 1.37	0.94	0.04 ± 0.69
Placebo group	15.98 ± 1.27	17.01 ± 1.69	0.48	1.03 ± 1.43
P-value)	0.68	0.42		0.54
Sodium (mg)				
Vitamin D group	1081.47 ± 198.04	988.23 ± 203.07	0.36	-93.24 ± 100.46
Placebo group	1271.04 ± 176.48	1262.97 ± 145.02	0.92	-8.06 ± 85.12
P-value	0.47	0.27		0.52
Potassium (mg)				
Vitamin D group	1975.02 ± 138.66	2058.50 ± 160.36	0.52	83.47 ± 128.43
Placebo group	2096.17 ± 178.41	1952.99 ± 194.99	0.22	-143.18 ± 113.50
P-value	0.59	0.67		0.19
Calcium (mg)				
Vitamin D group	662.20 ± 105.85	663.72 ± 104.05	0.98	1.52 ± 63.31
Placebo group	669.53 ± 57.55	650.24 ± 58.99	0.32	-19.28 ± 18.95
P-value	0.95	0.91		0.75
Iron (mg)				
Vitamin D group	17.46 ± 1.22	18.02 ± 1.05	0.46	0.56 ± 0.74
Placebo group	19.41 ± 1.14	18.43 ± 1.16	0.36	-0.97 ± 1.05
P-value	0.25	0.79		0.24
Zinc (mg)				
Vitamin D group	7.82 ± 0.78	8.67 ± 0.91	0.18	0.85 ± 0.62
Placebo group	7.23 ± 0.56	7.66 ± 0.56	0.49	0.43 ± 0.61
P-value	0.54	0.35		0.63
Selenium (mg)				
Vitamin D group	0.08 ± 0.006	0.08 ± 0.009	0.97	0.0003 ± 0.009
Placebo group	0.08 ± 0.009	0.10 ± 0.01	0.12	0.018 ± 0.01
P-value	0.81	0.21		0.21
Vitamin B6 (mg)				
Vitamin D group	1.41 ± 0.23	1.40 ± 0.21	0.90	-0.01 ± 0.08
Placebo group	1.40 ± 0.17	1.28 ± 0.17	0.27	-0.12 ± 0.48
P-value	0.98	0.67		0.42
Vitamin B12 (µg)				
Vitamin D group	3.45 ± 0.65	4.08 ± 0.87	0.55	0.63 ± 1.06
Placebo group	4.53 ± 1.28	3.54 ± 1.11	0.23	-0.99 ± 0.80
P-value	0.45	0.70		0.23
Vitamin C (mg)				
Vitamin D group	79.47 ± 13.24	87.20 ± 14.28	0.58	7.72 ± 13.86
Placebo group	105.11 ± 15.08	88.94 ± 16.22	0.36	-16.16 ± 17.42
P-value	0.20	0.93		0.29
Vitamin E (mg)				
Vitamin D group	2.19 ± 0.19	2.16 ± 0.23	0.91	-0.02 ± 0.25
Placebo group	3.09 ± 0.68	4.37 ± 1.10	0.17	1.28 ± 0.91
P-value	0.22	0.06		0.18

[a]: paired t-test; [b]:Student t-test.

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Table 3. Comparison mean (±SE) of anthropometric indices in both groups at baseline and after 12 weeks.

Variables	Before	After	P-value[a]	Change
Weight (kg)				
Vitamin D group	75.14 ± 3.42	75.47 ± 3.74	0.57	0.33 ± 0.59
Placebo group	77.71 ± 4.12	77.57 ± 4.08	0.45	-0.14 ± 0.18
P-value[b]	0.63	0.70		0.44
Body mass index (kg/m^2)				
Vitamin D group	24.47 ± 0.86	24.28 ± 0.95	0.36	-0.18 ± 0.19
Placebo group	26.34 ± 0.95	26.27 ± 0.94	0.30	-0.07 ± 0.06
P-value	0.15	0.14		0.59
Waist circumference (cm)				
Vitamin D group	92.33 ± 2.73	91.42 ± 2.92	0.19	-0.90 ± 0.67
Placebo group	95.38 ± 2.69	95.87 ± 2.75	0.21	0.49 ± 0.38
P-value	0.43	0.27		0.08
Hip circumference (cm)				
Vitamin D group	99.04 ± 1.67	98.34 ± 1.86	0.24	-0.70 ± 0.58
Placebo group	100.62 ± 1.88	100.44 ± 2.06	0.66	0.18 ± 0.40
P-value	0.60	0.39		0.22
Waist to hip circumference				
Vitamin D group	0.92 ± 0.01	0.92 ± 0.01	0.54	-0.003 ± 0.004
Placebo group	0.94 ± 0.01	0.94 ± 0.01	0.58	0.002 ±0.004
P-value	0.33	0.23		0.41

[a]: Paired t-test; [b]: Student t-test.

Table 4. Comparison mean (±SE) of semen quality and reproductive hormones in both groups at the baseline and after 12 weeks.

Variables	Before	After	P-value[a]	Change
Vitamin D (nmol/l)				
Vitamin D group	16.26 ± 1.71	49.92 ± 3.11	<0.001	33.66 ± 4.03
Placebo group	17.79 ± 1.36	31.17 ± 2.25	<0.001	13.38 ± 1.67
P-value[b]	0.49	<0.001		<0.001
SHBG (mmol/l)				
Vitamin D group	36.12 ± 3.41	47.82 ± 6.85	0.05	11.69 ± 5.79
Placebo group	28.78 ± 3.36	32.73 ± 3.56	0.11	3.95 ± 2.39
P-value	0.13	0.06		<0.001
Testosterone (nmol/l)				
Vitamin D group	4.20 ± 0.39	4.61 ± 0.46	0.26	0.41 ± 0.36
Placebo group	3.09 ± 0.24	3.40 ± 0.29	0.14	0.31 ± 0.20
P-value	0.01	0.03		0.81
Free androgen index				
Vitamin D group	0.12 ± 0.01	0.16 ± 0.03	0.97	0.03 ± 0.15
Placebo group	0.19 ± 0.05	0.26 ± 0.10	0.98	0.07 ± 0.07
P-value	0.25	0.33		0.97
Sperm count (million/ml)				
Vitamin D group	45.00 ± 9.72	53.9 ± 10.19	0.11	8.90 ± 5.36
Placebo group	54.94 ± 11.34	46.13 ± 9.61	0.24	-8.81 ± 7.32
P-value	0.51	0.58		0.05
Sperm progressive motility (%)				
Vitamin D group	21.50 ± 1.84	25.10 ± 3.10	0.17	3.60 ± 2.57
Placebo group	22.9 ± 1.59	24.75 ± 2.22	0.52	24.75 ± 2.22
P-value	0.56	0.92		0.65

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Table 4. Comparison mean (±SE) of semen quality and reproductive hormones in both groups at the baseline and after 12 weeks.

Variables	Before	After	P-value[a]	Change
Sperm non-progressive motility (%)				
Vitamin D group	10.60 ± 0.84	9.40 ± 0.99	0.31	-1.20 ± 1.16
Placebo group	10.70 ± 0.91	9.90 ± 0.56	0.46	-0.80 ± 1.08
P-value)	0.93	0.66		0.80
Sperm immotile (%)				
Vitamin D group	67.85 ± 1.82	55.50 ± 4.98	0.02	-12.35 ± 5.13
Placebo group	66.40 ± 1.79	65.35 ± 2.49	0.74	-1.05 ± 3.11
P-value	0.57	0.08		0.02
Sperm morph (normal %)				
Vitamin D group	2.65 ± 0.33	2.80 ± 0.34	0.19	0.15 ± 0.20
Placebo group	2.35 ± 0.26	2.05 ± 0.24	0.95	-0.30 ± 0.25
P-value	0.48	0.08		0.38
Semen volume (ml)				
Vitamin D group	3.39 ± 0.33	3.28 ± 0.42	0.68	-0.11 ± 0.28
Placebo group	3.48 ± 0.33	3.52 ± 0.38	0.92	0.04 ± 0.46
P-value	0.86	0.67		0.77

[a]: Paired t-test; [b]: Student t-test

Discussion

Our findings demonstrated that consuming 50,000 IU vitamin D for 12 weeks decreased WC significantly. However, no significant change was observed on semen quality markers (sperm count, morphology, sperm motility, semen volume), total testosterone, SHBG, FAI, and other anthropometric measurements (weight, BMI, HC, and WHR). To the best of our knowledge, this study is one of the first randomized clinical trials in this area.

After completion of the intervention, serum 25-OH-D levels of the DG and PG were 49.9 nmol/l and 31.17 nmol/l, respectively. The mean serum 25-OH-D concentrations of the DG was significantly higher than the PG. Seasonal variation in the endogenous vitamin D synthesis might explain the increased vitamin D levels in the PG partly, because most men were recruited during winter and spring (Alzoubi *et al.*, 2017).

A large body of research has revealed the association between some nutrients intake such as folic acid, polyunsaturated fatty acids, and plant based diets; however, rare studies are available over the effect of Vitamin D supplementation and infertility outcomes. Therefore, further investigations are needed to clarify this matter appropriately (Panth *et al.*, 2018). Nevertheless, we aimed to investigate the effect of vitamin D supplementation on infertility outcomes and assessed nutrient intakes and physical activity as confounding effects. In fact, we wanted to minimize the confounding effects on the final results and assessing nutrient intake was not our primary aim.

Although many recent investigators (Lerchbaum and Obermayer-Pietsch, 2012b) studied the role of vitamin D on semen quality and spermatogenesis, it is not fully understood. For example, Blomberg Jensen et al. investigated the association of semen quality and vitamin D status in 300 men. The results of their study showed a positive correlation between serum 25-OH-D concentrations and sperm motility and progressive motility. Furthermore, Vitamin D deficient men (<10 ng/ml) had a lower proportion of progressive motile in comparison to men with sufficient vitamin D status (> 30 ng/ml) (Blomberg Jensen *et al.*, 2011). Some evidences from observational studies suggest an association between vitamin D and semen quality, but the data from RCTs are very rare. In a randomized clinical trial on 117 Jordanian males, idiopathic infertility

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men with low vitamin D levels were prescribed with oral vitamin D, 50,000 IU daily for two months. The results demonstrated that vitamin D supplementation had beneficial effects on sperm motility in vitamin D deficient idiopathic male infertility patients (Alzoubi *et al.*, 2017). In a randomized placebo-controlled clinical trial, 121 male patients with chronic prostatitis or chronic pelvic pain syndrome were prescribed a synthetic form of vitamin D_3 for 3 months. The findings of this study showed a significant decrease in IL 8, resulting in an improvement in semen quality and motility (Tiwari, 2009).

Similar to our findings, the results of an RCT on 235 men showed that vitamin D supplementation did not improve semen quality markers in vitamin D insufficient infertile males. The participants in this study received cholecalciferol 300,000 IU initially. Later, the treatment group received 1400 IU cholecalciferol plus 500 mg of calcium once a day for 150 days, while the control group received the placebo (Blomberg Jensen *et al.*, 2017b).

Semen quality parameters are not the best predictor markers of male fertility (Guzick *et al.*, 2001). However, they are still practical in clinical use because better alternatives are lacked (Zinaman *et al.*, 2000). For instance, total sperm count and sperm volume are susceptible to inter- and intra-individual changes, which may support results of the current study (Carlsen *et al.*, 2005).

Previous observational studies indicated some controversial associations between vitamin D status and serum androgen levels. For instance, Wher et al, investigated 2299 German men and reported that vitamin D was directly associated with FAI and inversely associated with SHBG (Wehr *et al.*, 2010). However, the results of another cross-sectional study on 307 healthy men demonstrated that vitamin D had a negative and positive relationship with FAI and SHBG, respectively (Ramlau-Hansen *et al.*, 2011). The number of clinical trials over the effect of Vitamin D consumption on FAI and reproductive hormones on males with infertility is limited. In contrast with our findings, in a 1-year clinical trial on 54 healthy men with deficient vitamin D levels, consuming either 83 µg vitamin D increased testosterone levels significantly (Pilz *et al.*, 2011a). However, in the current study, we did not observe any significant changes in the testosterone levels, which could be induced by the short intervention period in our study (12-weeks).

This study has some limitations. We could not perform our study throughout a year to control vitamin D variations in different seasons. Therefore, we failed to control the variation of endogenous vitamin D in different seasons. Moreover, the small sample size and short interventional period are among other limitations.

The main strengths of our study are as the following. Our research is the first study to investigate the effect of vitamin D supplementation on semen quality markers and reproductive hormones in Asian countries. Second, the generalizability of our results is acceptable since the Infertility Research Center Yazd is a referral research center in Iran and many patients refer to this venter for therapeutic purposes from all over the country. Third, we had a low attrition rate (4 people) and good compliance. In conclusion, our study demonstrated that vitamin D supplementation had no effect on sperm quality markers or reproductive hormones in vitamin D deficient males with Asthenozoospermia. However, Vitamin D treatment decreased WC significantly compared with those men in the placebo group. Although a large body of research from animal studies and human studies (exclusively cross-sectional studies) suggest that vitamin D is involved in many reproductive system functions in both genders, the supporting randomized clinical trials in this regard are rare. Thus, new well-designed clinical trials with superior methodological designs are needed to provide new insights to the role of vitamin D on the treatment of male infertility.

Conflict of interest

Authors declared no conflict of interests.

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Authors' contributions

Gheflati AR, Nadjarzadeh A, Mirjalili SAM and Salehi-Abargouei A designed, performed and conducted the design theory of the study. Hosseini-Marnani E supported in statistical analyses and Gheflati AR wrote this article. Nadjarzadeh A and Kaviani M revised and approved the final version of the article. All authors have approved the final article.

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