

## Effect of Vitamin D3 Supplement on the Semen Quality in Human Patients with Vitamin D Deficiency

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### ABSTRACT

Male fertility is a complicated process that can be hampered by a variety of lifestyle factors. This study aims to evaluate the effect of vitamin D3 deficiency on the semen quality and also to study the quality changes in the semen of patients with vitamin D3 deficiency, after supplementation with vitamin D3 for a month. This present study included 112 participants, who were classified into two groups according to the serum level of vitamin D3 (normal and deficiency groups). The semen was analyzed by the physical examination of both groups; also this study included follow-up patients with vitamin D3 deficiency, who were supplied with 5,000 IU of vitamin D3. The results showed a significant decrease in the motility of sperms in patients with vitamin D3 deficiency (27.16%±2.497%) compared to the standard group (36.71%±2.892%), and an increase in sperm motility in patients who consumed vitamin D3, after being followed up for a month (37.19%±2.836%). Moreover, the result showed a high and significant increase in the rate of deformities of sperms in patients with vitamin D3 deficiency (18.67%±1.074%) compared to the standard group (13.68%±0.8131%) and a decrease in the rate of deformities of sperms in patients who took vitamin D3 supplement for a month (13.54%±0.8116%). The study concluded that patients with a supplement intake of vitamin D3 had an effect on the semen quality, especially the motility and deformity of sperms in vitamin D3-insufficient men.

### 1. Introduction

Vitamin D3 is one of the fat-soluble vitamins that is naturally found in a few foods and also available as a dietary supplement (Ravisankar *et al.* 2015). Vitamin D is produced endogenously when ultraviolet (UV) rays from the sun strike the skin and trigger vitamin D3 synthesis (Rafeeq *et al.* 2020). Vitamin D3 is a nutrient that the body needs for building and maintaining healthy bones. This is because the body can only absorb calcium, the primary component of the bone, when vitamin D3 is present. Vitamin D3 also regulates many other cellular functions in the body. Its antioxidant, anti-inflammatory, and neuroprotective properties support body immunity, muscle function, and brain cell activity (Zhang *et al.* 2010). The amount of vitamin D3 in the body depends on many factors, including the time of day, season, and skin pigmentation. Vitamin D3

production might reduce or be completely absent during the winter season (Tsiaras and Weinstock 2011). Sunscreen, although important to prevent skin cancer, can also reduce vitamin D3 synthesis (Young *et al.* 2019). Bones might become thin, brittle, or deformed in hypovitamin D3 people. Vitamin D3 helps to prevent rickets and osteomalacia in children and adults. Vitamin D3, in addition to calcium, aids in the prevention of osteoporosis in the elderly (Chatterjee *et al.* 2014). Vitamin D3 can be obtained from food, especially fatty fish (Lips 2006). By converting 25-hydroxy vitamin D (25(OH)D) in the liver into 1,25-dihydroxy vitamin D (1, 25(OH)<sub>2</sub> D) in the kidney, the active metabolite of vitamin D can enter the cell and bind with the vitamin D-receptor, and then with a responsive gene, such as, a calcium-binding protein. The protein, such as osteocalcin or the calcium-binding protein, is generated after transcription and translation. Calcium absorption in the digestive system is mediated by the calcium-binding protein (Holt *et al.* 2002; Tsuprykov *et al.* 2021).

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Vitamin D3 deficiency occurs when people consume less than the recommended amount of vitamin D in their diet, have restricted sun exposure, have renal failure (cannot convert 25(OH)D to its active form), or a decrease in vitamin D absorption through the digestive system (Kennel *et al.* 2010). Vitamin D3 deficiency is more frequent in those with a milk allergy or lactose intolerance, as well as those who eat an ovo-vegetarian or vegan diet (Craig 2010). It has been estimated that one billion people worldwide have vitamin D3 deficiency or insufficiency. According to several studies (Dawson-Hughes *et al.* 2005; Holick 2006; Lips 2001), 40 to 100% of U.S. and European elderly men and women still living in the community (not in nursing homes) are deficient in vitamin D3.

Infertility is a significant health problem affecting around 15% of the couples worldwide. Most infertility is due to the male components of fertilization (Dissanayake *et al.* 2019). Fertility in men is a complicated process. To get a woman partner pregnant, the following must happen: Healthy sperms must be produced. This begins with the development and enlargement of the male reproductive organs throughout puberty. At least one of the testicles must function normally, and the body must produce testosterone and other hormones to trigger and maintain sperm production, and the sperm must be carried into the semen. Once produced in the testicles, delicate tubes must transport them until they mix with the semen and are ejaculated out of the penis (Petak *et al.* 2002). The vitamin D receptor (VDR), and the enzymes involved in D3 metabolism are located in germ cells, Leydig cells, Sertoli cells, spermatozoa, and the epithelial cells lining reproductive canal of male (Jensen 2014). The presence enzymes involved in vitamin D metabolism shows the reproductive organs in both animals and humans can control the local vitamin D response. "The presence of VDR in the male's testis shows that cholecalciferol may have both paracrine and autocrine effects, which possibly have a role in the regulation of testis function, thus impacting male infertility" (de Angelis *et al.* 2017). In both animal and human studies, the expression of vitamin D receptor (VDR) and enzymes involved in vitamin D metabolism in the reproductive system of male has been extensively studied.

The vitamin D receptor proteins were discovered in the seminal vesicles, prostate, epididymis, and germ cells, including spermatogonia, spermatocytes, and Sertoli cells. Although the VDR protein was detected in animal spermatozoa, it was inhibited in the epididymis tail. In the same vein, testicular

testosterone production enzymes were found to be downregulated in mice fed on a VDD diet (Cito *et al.* 2020). VDR demonstrated a heterogeneous pattern of localization in the human spermatozoa, consisting of the post-acrosome area, neck, and/or mid-piece, as determined by the reverse transcription polymerase chain reaction (RT-PCR) (Akhavizadegan and Karbakhsh 2017).

The special effects of vitamin D3 on fertility power of the male were found to be influenced by testis activities. Testicular action is made up of hormones production and spermatogenesis; both of which donate male reproductive potential. Hormone synthesis in the testis requires both germs and somatic cells, which are necessary for optimal spermatogenesis (Boisen *et al.* 2017). Experiments show that vitamin D3 has a positive influence on male fertility (Dabrowski *et al.* 2015), as it enhances the semen quality, mostly through non-genomic effects, and by modifying hormone synthesis through genomic and non-genomic mechanisms. Clinical trials in humans, on the other hand, are contentious. Vitamin D3 appears to play a role in the modulation of bioavailable testosterone rather than the total testosterone (Nandi *et al.* 2016). Furthermore, while observational studies reveal an increased incidence or risk of testosterone shortage in men with vitamin D3 deficiency, the majority of interventional trials report no effect of vitamin D3 treatment on the testosterone levels in the blood (Pilz *et al.* 2011). The effect of vitamin D3 on sperm quality has been reported to be the most constant. Vitamin D3 has been shown to have a positive association with sperm motility and to have direct effects on the spermatozoa, including non-genomic modulation of intracellular calcium homeostasis, activation of sperm motility, capacitation, and on the acrosome response molecular pathways (Ramlau-Hansen *et al.* 2011). This study aims to evaluate the effect of vitamin D3 on the sperm quality, and to study the quality of change in the semen of patients with vitamin D3 deficiency, after vitamin D3 supplementation for a month.

## 2. Materials and Methods

This cross-sectional study was carried out in Samawah, in Iraq, from April 2020 to June 2021, and included 112 participants who were classified in two main groups according to serum level of vitamin D3. The first group (n = 53) was with a level of vitamin D3 more or equal to 20 ng/ml (normal level), known as VDN, and the second group (n = 59) was with a vitamin

D3 level of less than 20 ng/ml (vitamin D deficiency), known as VDD. The semen from patients in both groups was evaluated by physical examination, using a semen analysis instrument. Patients in the second group were followed up and treated with vitamin D3 supplement (5,000 IU/day) (Bokharee *et al.* 2020) for a month and then an analysis of the semen was evaluated again to determine the improvement in the semen quality after treatment, and this group was referred to as VDS (n = 23).

### 2.1. Blood Collection

Blood was collected in the General Al-Hussein Hospital in Samawah, Iraq. The consent form was signed by all the human volunteers. Blood was collected immediately after semen collection and transferred into a plain tube. This was left to stand for 10 minutes to allow the blood to clot, then centrifuged at 1,600 rpm for 10 minutes (Abed *et al.* 2019). The serum was transferred into a new tube and kept in freezer until use.

### 2.2. Semen Evaluation and Processing

The semen samples were collected from all the males (n = 112), in the General Al-Hussein Hospital in Samawah city, Iraq, according to the medical protocol that was used for semen collection and analysis (Freshman 2002). Immediately after collection, each semen sample was assessed by an experienced operator, with regard to visual aspect, color, and density, and the ejaculated volume and pH were recorded. Physical and microscopic examinations of the sperms were performed and assessed using an automated sperm analyzer instrument.

### 2.3. Vitamin D test

25-OH Vitamin D was assayed using the MAGLUMI 25-OH Vitamin D assay kit, using a CL-900 instrument. In a Competitive Immunoluminometric Assay, this vitamin D test kit combined a “purified 25-OH Vitamin D antigen labeled as ABEI and a 25-OH Vitamin D monoclonal antibody labeled as FITC. The Sample, Calibrator, and Control were completely combined with the Displacing Reagent, FITC Label, and anti-FITC magnetic microbeads” and incubated at 37°C, to produce antibody–antigen complexes. The supernatant was decanted after sedimentation took place in “a magnetic field, followed by a single washing cycle. The sample antigen and the ABEI-labeled antigen competed with the FITC-labeled monoclonal antibody” after a second round of incubation and washing, forming antibody–antigen complexes. Following that, the first chemicals were

added, and the flash chemiluminescent process began. A photomultiplier detected the light signal as RLU in “less than three seconds, which was proportional to the quantity of 25-OH Vitamin D in the controls or samples” (Turpeinen *et al.* 2003).

### 2.4. Statistical Analysis

All results, curves, tables, and diagrams of this study were carried out using Microsoft Excel 2010 and the GraphPad prism statistical program (version 6).

### 3. Results

This cross-sectional study included 112 participants, classified into two groups according to Vitamin D3 value, where the first group was with a normal vitamin D3 (VDN) (n = 53) and second group with deficiency of vitamin D3 (VDD) (n = 59) with a mean level of serum vitamin D3 equal to 26.57±1.259 ng/ml and 8.956±0.2443 ng/ml, respectively, as in Figure 1 and Table 1.

The results in Figure 2 and Table 2 indicate that the average age of the groups was 36.09±1.053 years, 35.10±1.077 years, and 36.39±1.992 years in the group with normal vitamin D3 (VDN), the group with deficiency of vitamin D3 (VDD), and the group that treated with vitamin D3 supplement (VDS), respectively. The results show no significant difference in age among the participants in the three groups.

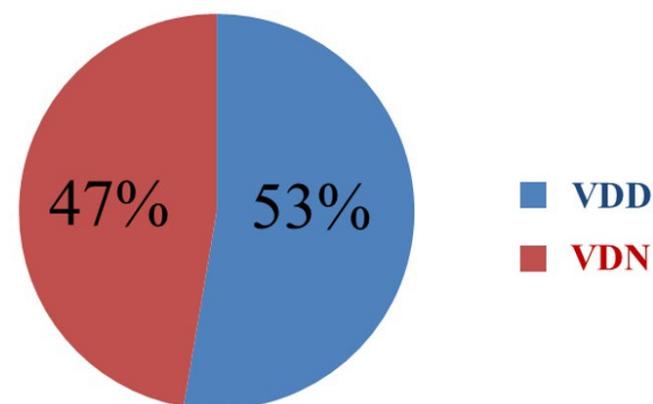


Figure 1. Percentage of samples in groups when VDN: vitamin D normal, VDD: vitamin D3 deficiency

Table 1. Mean value of serum vitamin D3 levels in the both groups

Groups	N	Mean±SE	p value	p value summary
VDN	53	26.57±1.2590	0.0001	S****
VDD	59	8.956±0.2443		

VDN: vitamin D normal, VDD: vitamin D3 deficiency

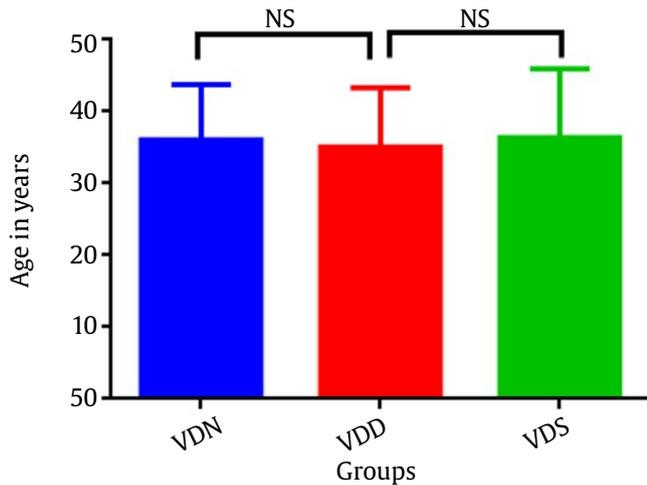


Figure 2. Age (years) in groups where VDN: group with vitamin D normal, VDD: groups with vitamin D3 deficiency, VDS: groups that treated with vitamin D3

Table 2. Age in years in groups

Groups	N	Mean±SE	p value	p value summary
VDN	53	36.09±1.053	-	-
VDD	59	35.10±1.077	0.5131	NS
VDS	23	36.39±1.992	0.5456	NS

VDN: Group with vitamin D normal, VDD: groups with vitamin D3 deficiency, VDS: groups that treated with vitamin D3

The results in Figure 3 and Table 3 indicate that the mean volume of semen samples from the participants is equal to 3.358±0.2223 ml, 3.797±0.2062 ml, and 3.739±0.2683 ml, in the VDN group, VDD group, and VDS group, respectively, and there is no significant difference between the VDD group and VDN group or between the VDS group and VDD group, with p values of 0.1509 and 0.8772, respectively.

The result in Figure 4 and Table 4 indicate the liquefaction time of the semen samples in the groups, where the mean values are 52.26±3.550 minutes, 50.17±2.916 minutes, and 54.35±4.569 minutes in the VDN group, VDD group, and VDS group, respectively. There is no significant difference (NS) between the groups in the liquefaction time of the semen samples.

The results in Figure 5 and Table 5 indicate the density of the semen samples in groups with a mean value equal to 22.06±0.7287 \*10<sup>6</sup>/ml, 17.65±1.399 \*10<sup>6</sup>/ml, and 18.49±1.636 \*10<sup>6</sup>/ml in the VDN group, VDD group, and VDS group, respectively, and the results show significant differences (S\*\*) between the VDN group and VDD group, with a p value of 0.0077, while there is no significant difference (NS) between the VDD group and VDS group, with a p-value of 0.7334.

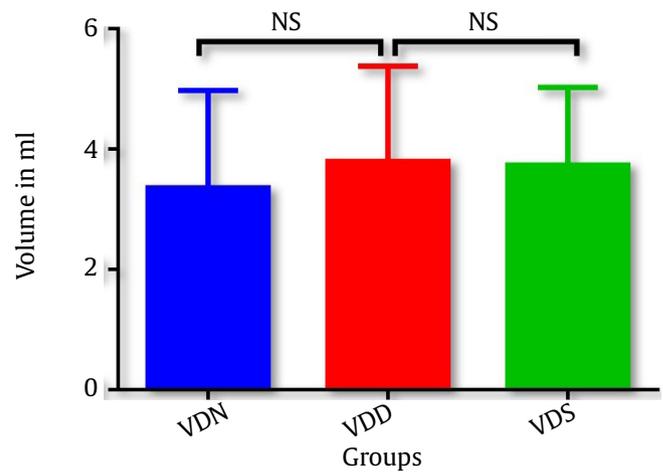


Figure 3. Volume of semen in groups where VDN: group with vitamin D normal, VDD: groups with vitamin D3 deficiency, VDS: groups that treated with vitamin D3

Table 3. Volume of semen in groups where p value ≤0.005

Groups	N	Mean±SE	p value	p value summary
VDN	53	3.358±0.2223	-	-
VDD	59	3.797±0.2062	0.1509	NS
VDS	23	3.739±0.2683	0.8772	NS

VDN: Group with vitamin D normal, VDD: groups with vitamin D3 deficiency, VDS: groups that treated with vitamin D3

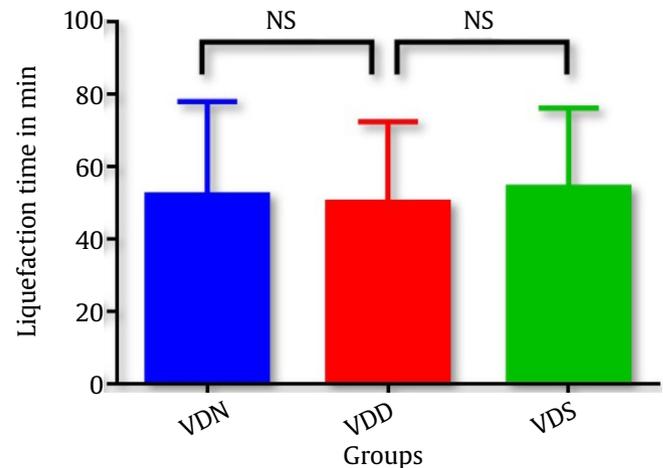


Figure 4. Liquefaction time of participants in groups where VDN: Group with vitamin D normal, VDD: groups with vitamin D3 deficiency, VDS: groups that treated with vitamin D3

Table 4. Liquefaction time of participants in groups, p value p value ≤0.005

Groups	N	Mean±SE	p value	p value summary
VDN	53	52.26±3.550	-	-
VDD	59	50.17±2.916	0.6468	NS
VDS	23	54.35±4.569	0.4475	NS

VDN: Group with vitamin D normal, VDD: groups with vitamin D3 deficiency, VDS: groups that treated with vitamin D3

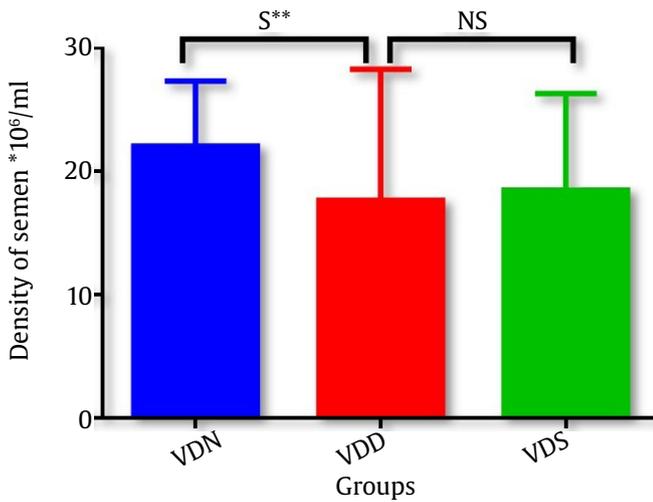


Figure 5. Density of semen samples in groups where VDN: group with vitamin D normal, VDD: groups with vitamin D3 deficiency, VDS: groups that treated with vitamin D3

Table 5. Density of semen samples in groups where p value  $\leq 0.005$

Groups	N	Mean $\pm$ SE	p value	p value summary
VDN	53	22.06 $\pm$ 0.7287		
VDD	59	17.65 $\pm$ 1.399	0.0077	S**
VDS	23	18.49 $\pm$ 1.636	0.7334	NS

VDN: Group with vitamin D normal, VDD: groups with vitamin D3 deficiency, VDS: groups that treated with vitamin D3

The results in Figure 6 and Table 6 indicate the motility percentages of sperm in the semen samples of participants with mean values of 36.71 $\pm$ 2.892%, 27.16 $\pm$ 2.497%, and 37.19 $\pm$ 2.836% in the VDN group, VDD group, and VDS group, respectively. The results show a significant difference (S\*) between the VDN and VDD groups with a p-value equal to 0.0135 and a significant difference (S\*) between the VDD and VDS groups with a p-value of 0.0247.

Finally, the results in Figure 7 and Table 7 indicate the rating of sperm deformity percentages in the semen samples of participants, when the mean values are 13.68 $\pm$ 0.8131%, 18.67 $\pm$ 1.074%, and 13.54 $\pm$ 0.8116% in the VDN, VDD, and VDS groups, respectively. The result shows a high significant difference (S\*\*\*) between the VDN and VDD groups with a p-value of 0.0004, and significant difference (S\*\*) between the VDD and VDS groups, with a p-value equal to 0.0055.

#### 4. Discussion

Vitamin D3, also known as cholecalciferol, can be found in fortified foods, animal foods (fatty fish,

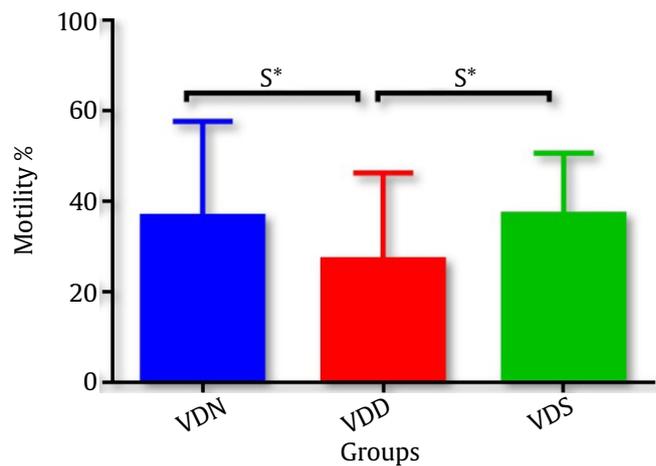


Figure 6. Motility percentages of sperm in semen samples of participants where VDN: group with vitamin D normal, VDD: groups with vitamin D3 deficiency, VDS: groups that treated with vitamin D3

Table 6. Motility percentages of sperm in semen samples of participants, p value  $\leq 0.005$

Groups	N	Mean $\pm$ SE	p value	p value summary
VDN	53	36.71 $\pm$ 2.892		
VDD	59	27.16 $\pm$ 2.497	0.0135	S*
VDS	23	37.19 $\pm$ 2.836	0.0247	S*

VDN: Group with vitamin D normal, VDD: groups with vitamin D3 deficiency, VDS: groups that treated with vitamin D3

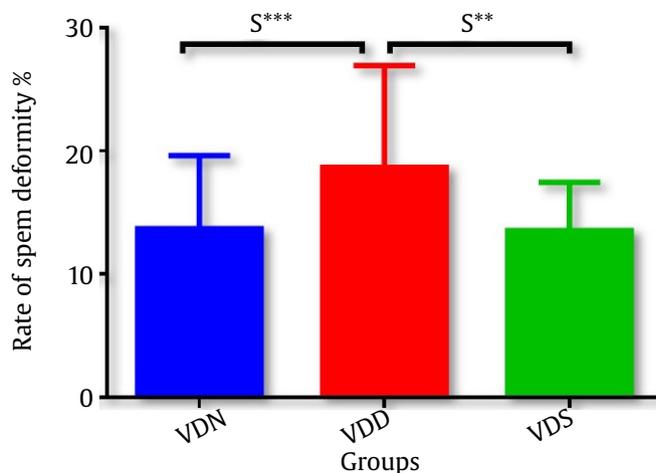


Figure 7. Rate of sperms deformities percentage in semen samples of participants where VDN: group with vitamin D normal, VDD: groups with vitamin D3 deficiency, VDS: groups that treated with vitamin D3

Table 7. Rate of sperms deformities percentage in semen samples of participants, p value  $\leq 0.005$

Groups	N	Mean $\pm$ SE	p value	p value summary
VDN	53	13.68 $\pm$ 0.8131		
VDD	59	18.67 $\pm$ 1.074	0.0004	S***
VDS	23	13.54 $\pm$ 0.8116	0.0055	S**

VDN: Group with vitamin D normal, VDD: groups with vitamin D3 deficiency, VDS: groups that treated with vitamin D3

cod liver oil, eggs, and liver), and supplements, as also they are produced internally when the skin is exposed to ultraviolet (UV) light from the sun (Tang *et al.* 2012). Many people believe vitamin D should be categorized as a hormone, and it has been dubbed the "neurosteroid" by some. Vitamin D deficiency has far-reaching health repercussions that go far beyond rickets or the consequences that occur with any other vitamin. Moreover, unlike other vitamins, it may be produced by your body when exposed to sunlight, and its active form, calcitriol, is similar to other hormones (estrogen, cortisol, and testosterone) (Cannell 2017; Kiraly *et al.* 2006).

Vitamin D synthesis in the skin decreases with age. In old people, the quantity of 7-dehydrocholesterol in the epidermis is lower than in young people, and they have a lower sensitivity to UV light, resulting in a 50% reduction in the synthesis of pre-vitamin D3 (Farage *et al.* 2008). In the result of this study there are no significant differences in the ages of participants between the groups of this study, as seen in Figure 2 and Table 2.

In humans, low vitamin D levels have been associated with poor sperm quality; males with low vitamin D levels produce much less sperm than men with normal vitamin D levels. According to one study, eating 2.7 grams of D-AA for three months increases the testosterone levels by 30–60% and sperm count by 60–100% in infertile males (Blomberg Jensen *et al.* 2016; Rehman *et al.* 2018). There are no significant differences, as shown in Figure 3 and Table 3, due to the effect of other factors as well, for example, men not being able to measure their actual semen volume. Some researchers suggest that adequate water and fluid intake may maximize the semen volume, but this increase will also be within normal limits.

Liquefaction time—normally it takes less than 20 minutes for semen to change from a thick gel into a liquid (Li and Winuthayanon 2017). An unusually long liquefaction time may indicate an infection, and this is in agreement with the result of this study, which shows no significant difference in the Liquefaction time of the semen in the samples between groups, as seen in Figure 4 and Table 4.

Vitamin D deficiency has also been linked to poor sperm quality in humans. The findings of this study corroborate with those of prior studies that have found a specific role for vitamin D in sperm motility

(Blomberg Jensen *et al.* 2018). When compared to males, who have had a normal vitamin D level, infertile men with vitamin D insufficiency have considerably reduced sperm production and motility as shown in Figure 6 and Table 6, which show significant difference in sperm motility between participants with normal vitamin D3 and the other groups of participants with vitamin D3 deficiency. In addition supplement 5,000 IU of vitamin D3 improved sperm motility in the third group. Vitamin D has been demonstrated to have a favorable relationship with sperm motility and to have a direct effect on the spermatozoa, including non-genomic control of intracellular calcium homeostasis and activation of molecular pathways involved in sperm motility, capacitation, and acrosome response (Maghsoumi-Norouzabad *et al.* 2021). In men from the general population, there is a constant positive relationship between 25-hydroxyvitamin D3 level and sperm total motility sperms and/or progressive motility of sperms (Elaghoury *et al.* 2020).

The link between vitamin D3 deficiency, morphology, and sperms count are debatable. Vitamin D3 has been shown to be either no effect or positively connected to sperm count (Salas-Huetos *et al.* 2017). Studies have shown a positive association between them, whereas, in this study, although with low samples, the result shows significant differences between the groups and role of vitamin D and deformity of sperms. In Figure 7 it can be seen that vitamin D supplementation improves the quality of sperm in vitamin D-deficient males and reduces the rate of sperm deformities (Azizi *et al.* 2018).

In conclusion, this study demonstrates that vitamin D3 supplementation improves the sperm quality in males with vitamin D insufficiency, especially the motility and sperm abnormalities.

### Conflicts of Interest

No conflicts of interest regarding the publication of this article.

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