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Vitamin D status, polymorphisms, and clinical outcomes in couples undergoing assisted reproductive technologies: exploring the role of 25(OH)D levels

Rana Jreij[1], Rania Jounblat[2], Youmna Mrad[3], Elie Hajj Moussa[1], Maria Abdallah[1], Chadi Fakih[3], Fadia Najjar[1] and Aline Hamade[1*]

Abstract

Purpose Vitamin D status has been recognized as an important factor in human health, and its relevance in the context of couples utilizing assisted reproductive technologies is of particular interest.

Methods This study aimed to assess the levels of 25(OH)D by ELISA in the biological fluids (serum, semen, and follicular fluid) of 105 couples undergoing in vitro fertilization or intracytoplasmic sperm injection. Additionally, the association among 3 vitamin D receptor polymorphisms (Apa1, Fok1, and Taq1) and vitamin D status, as well as the clinical pregnancy rate after in vitro fertilization, were investigated.

Results The results revealed a high rate of hypovitaminosis in the women's group. Furthermore, a significant difference was observed in the fertilization rate ($P = 0.007$). Correlations were found between Apa1 polymorphism and the number of collected oocytes ($P = 0.014$), the number of embryos ($P = 0.001$), the number of 2 pronuclear ($P = 0.001$), and the frequency of clinical pregnancies ($P = 0.046$). Additionally, correlations were found between Taq1 SNP and the number of embryos ($P = 0.046$) and the serum vitamin D level ($P = 0.042$). The Fok1 polymorphism showed correlations with semen concentration ($P = 0.044$) and clinical outcome ($P = 0.032$).

Conclusion These results suggest the importance of systemic vitamin D for women prior to pregnancy. The findings emphasize the potential benefits of vitamin D supplementation in women before pregnancy and call for cautious interpretation of data regarding the impact of vitamin D on pregnancy.

Keywords Infertility, Serum, Follicular fluid, Semen, Vitamin D receptor, Polymorphism, IVF

*Correspondence:
Aline Hamade
aline.hamade@ul.edu.lb
Full list of author information is available at the end of the article

Introduction

Infertile couples make up about 15% to 20% of couples worldwide. Infertility is caused by multiple factors, such as environmental and nutritional factors, particularly vitamin D, which is a prohormone [1].

Vitamin D is involved in many physiological reproductive processes, including steroidogenesis, spermatogenesis, and acrosome reaction. It is correlated with sperm quality, ovarian reserve, polycystic ovarian syndrome, and endometriosis, among other conditions [2, 3]. It is also known that during pregnancy, vitamin D plays a vital role in embryogenesis, especially fetal skeletal development and calcium homeostasis [4].

The biologically active metabolite of vitamin D, 1,25-dihydroxyvitamin D, performs its principal actions by binding to the vitamin D receptor, a steroid intracellular hormone receptor that regulates gene transcription by interacting with the vitamin D receptor response elements of the target genes to produce various biological effects [5, 6].

The vitamin D receptor is located and expressed in male and female reproductive systems; thus, it plays an important role in male and female fertility even when assisted reproductive technologies are used.

The vitamin D receptor gene showed different polymorphisms [7, 8]. The most important single nucleotide polymorphisms (SNPs) are Apa1, Fok1, and Taq1. Studies have demonstrated that these SNPs may have an increased risk of developing some diseases such as bone problems, coronary artery disease, and cancer [9].

In a recent review Chen and Zhi discussed the potential functions of vitamin D in male and female reproductive systems and the associations between vitamin D and assisted reproductive technology (ART) outcomes [3]. They suggested the serious necessity for further studies because many controversial clinical findings on vitamin D levels and ART outcomes were revealed in this review. In fact, some studies showed that there is an association between Vitamin D in women and pregnancy outcomes [10, 11], and others did not show any association between the two [12].

It is known that vitamin D deficiency is highly prevalent around the world. Unfortunately, despite ample sunshine, vitamin D deficiency has long been a presupposition regarding vitamin D status in almost all countries of the Middle East, specifically Lebanon [13].

For all these reasons, this study aimed to investigate the effect of vitamin D levels in serum, follicular fluid, and semen on embryos quality and fertility. This study also aimed to determine the association between vitamin D receptor polymorphism and the outcome of the in vitro fertilization (IVF) in a population of Lebanese couples.

Materials and methods

Study subjects

One hundred and five couples suffering from infertility and having attempted IVF/Intracytoplasmic sperm injection (ICSI) as treatment in a fertility clinic in Beirut were selected to participate in this study. This study was conducted at the Lebanese University. Of the couples, 41% had never done previous IVF/ICSI, and 59% had attempted it at least once. Each patient was included once. Informed consent was obtained from all participants. The study protocol was approved by the Ethics Committee of Al Hayat Hospital (ethical authorization number: ETC-03/2019). Patients' DNA was extracted, serum was obtained, and semen from men and follicular fluid from women were also taken for Vitamin D measurement. In addition, each couple completed a questionnaire, including information on the couple (age, years of marriage, number of children, number of IVF attempts, etc.) and their medical history (health status, medication, etc.).

Semen analysis

Semen samples were obtained and analyzed. Sperm parameters analyzed included sperm concentration (million/ml) and sperm motility (%). Spermatozoa were classified as progressive motile (WHO Classes A and B), non-progressive motile (Class C), or immotile (Class D). These parameters were assessed after adding 10 µl of liquefied, well-mixed semen on a glass slide and counting the average of a few fields at a magnification of 200. The mean of spermatozoa per field was counted as millions/ml. This analysis was completed before the study directly after sample collection at the medical center in Beirut.

Ovarian stimulation and collection of oocytes and follicular fluid

The ovarian hyperstimulation protocols were selected by gynecologist Dr. Chadi Fakih. The "long standard" protocol was used with a daily GnRH agonist for the 100 female patients. The response to stimulation was monitored by serial hormonal blood tests and an ultrasound evaluation of follicular and endometrial growth. Ovulation was triggered with hCG when at least four follicles had reached 17 mm. The follicles were individually recovered by aspiration 35–36 h after induction of ovulation under neuroleptic anesthesia and guidance by vaginal ultrasound. Cumulus and coronal cells from individual collected oocytes were removed with hyaluronidase. Each mature oocyte was injected at standard magnification with a single sperm into a droplet of 30–40 µl buffer medium, with a viscous medium of polyvinylpyrrolidone to slow down the sperm. On Day 0 the injected oocytes were placed in a fertilization medium and on Day 1 in a cleavage medium at 37 °C (± 0.5 °C) in a dry atmosphere with CO_2 of 6% (± 0.5 °C). (The "one-step" culture medium had been applied). On Day 3, the embryos were analyzed according to (a) their fragmentation (0 = no fragmentation, f1: 1%–50%, F3: more than 50%); (b) the regularity of the blastomeres (0 = no different in size, B1: size different by a factor < 2, B2: size different by a factor 2–3, B3: different size by a factor > 3; (c) the ooplasmic appearance (homogeneous and clear, C1 abnormalities such as vacuole, inclusion, granulation, or incorrect staining, C2 abnormalities, C3 combined abnormalities); and (d) the number of blastomeres (> 6 on Day 3 or not). Follicular fluid was collected and then centrifuged at 14,000 × g for 12 min. All samples were stored at −20 °C until assayed.

Fertilization and maturation rates were calculated according to the following formulas:

Table 1 shows the primer sequences used for the VDR polymorphism analysis.

Genotyping

The genotyping of FokI (rs2228570)(C > T), ApaI (rs7975232)(A > C), and TaqI (rs731236) (T > C) polymorphisms was performed using the restriction fragment length polymorphism (RFLP) technique.

DNA samples were digested by Fok1, ApaI, and TaqI restriction enzymes from ThermoFisher Scientific. The mixtures were incubated at 37 °C for 5–15 min with FokI, at 37 °C for 1–16 h with ApaI, and at 65 °C for 1–16 h with TaqI to promote cleavage. The samples were then subjected to electrophoresis on 2% agarose gels to determine the lengths of the fragments and thus the genotyping results.

$$\text{Maturation rate} = (\text{number of oocytes fertilized} / \text{number of oocytes found}) \times 100$$

$$\text{Fertilization rate} = (\text{number of 2 pronuclear (PN)} / \text{number of oocytes fertilized}) \times 100$$

DNA amplification

The DNA sequence of interest, including the gene polymorphism fok1 (rs 2228570), was amplified by polymerase chain reaction (PCR). To perform the PCR reactions, Firepol 5X Master Mix (Solis BioDyne) was used, and the conditions were as follows: 94 °C for 5 min, 35 cycles at 94 °C for 30 s, 60 °C for 30 s, 72 °C for 1 min, and 72 °C for 5 min. The final volume was 20 µl, formed by 1 µl of patient DNA, 0.5 µl of each primer (forward and reverse) (Table 1), 4 µl of Master Mix, and 14 µl of nanopure water.

The DNA sequence, including the gene polymorphism Apa (rs7975232) and Taq (rs731236), was also amplified, with cycling parameters as follows: initial denaturation at 94 °C for 5 min, followed by 35 cycles of 94 °C for 30 s, 64 °C for 30 s, 72 °C for 30 s, and finally a 5 min extension at 72 °C and specific primers. The volume was 40 µl formed by 2 µl DNA, 1 µl of each primer (forward and reverse), 8 µl of Master Mix, and 28 µl of nanopure water.

Table 1 Primer sequences used to amplify the VDR gene

Primer	Forward	Reverse	DNA length
Fok1	AGCTGGCCCTGGCACTGA CTCTG	ATGGAA ACACCT TGCTTCTTC TCCCTC	265 bp
Apa1-TaqI	CAGAGCATGGACAGGGAGCAA	CACTTC GAGCAC AAGGGG CGTTAGC	495 bp

ELISA for 25(OH)D measurement

The enzyme immuno assay 25-hydroxyvitamin D EIA kit (Immuno Diagnostic System, USA) was used to measure the 25(OH) D level in all serum, follicular fluid, and semen in each couple. Serum concentration of 25(OH)D is considered the most reliable measure of overall vitamin D status and thus can be used to determine whether a patient is vitamin D sufficient.

The following ranges were used for the classification of 25(OH)D status in serum, deficient if VD < 20 ng/ml, insufficient if VD is between 20 and 30 ng/ml, and sufficient if VD is between 30 and 100 ng/ml.

Statistical analysis

Statistical tests were conducted to study significant correlations among the 3 SNPs; semen parameters (count and motility); VD levels in serum and semen in men; and the correlation among the polymorphism and follicular fluid characteristics, embryological outcome of the oocyte, VD level in serum, and FF in women, specifically IVF outcome.

Statistical analysis was performed using GraphPad Prism. 7 (GraphPad Software, Inc., San Diego, CA, USA). Chi-square analysis was used to compare genotypes and allele frequencies between groups. Differences among groups were evaluated using the nonparametric one-way analysis of variance (ANOVA). Correlation among different variables was evaluated using the Spearman and Pearson correlation test. *P*-values of less than 0.05 were considered significant.

Results

Sample description

In this study, 21.9% of couples were suffering from female infertility, 37.1% from male infertility, and 41.0% were idiopathic (Table 2).

The characteristics of the female patients are shown in Table 2. The mean age of female patients was 34.47 ± 6.569 (mean ± SD). The mean level of vitamin D was 14.10 ± 9.44 ng/ml in serum and 16.17 ± 8.6 ng/ml in follicular fluid.

The number of retrieved oocytes in the follicular fluid was 967, and 686 were identified as MII oocytes. The mean maturation rate was 73.193 ± 24.98%.

In addition, results shows that a high prevalence of female patients (79%) suffered from hypovitaminosis.

Male patients' characteristics are shown in Table 3. The mean age of male patients was 40.13 ± 7.971. The mean level of vitamin D3 in male patients' serum was 22.17 ± 7.48 ng/ml. In seminal fluid the mean level was 4.22 ± 1.42 ng/ml.

The mean concentration of semen was 37.10 ± 27.26 ($\times 10^6$/ml), with 31.10 ± 20.53% in progressive motility, 17.5 ± 9.13% in nonprogressive motility, and 49.08 ± 26.77% immotile (Table 3).

Correlation among women's serum, follicular fluid, vitamin D level, and IVF outcome

Of the 105 women undergoing IVF, 44 had a positive clinical pregnancy, and 61 had no pregnancy. Those who had a low vitamin D level in serum < 20 ng/ml were divided into 29 with a positive outcome and 54 with a negative outcome. Conversely, in the group with higher vitamin D levels (≥ 20 ng/ml), 15 had a positive outcome and 7 a negative one (Table 4). These results were significant with a *P*-value of 0.007 (Table 4). Thus, there was a strong correlation between pregnancy and serum vitamin D status in women.

However, no correlation was found between vitamin D levels in follicular fluid and IVF results ($P > 0.05$) (Table 4, Fig. 1). A significant difference was observed concerning the fertilization rate ($P = 0.042$). The women's age did not affect the vitamin D level in serum but did significantly affect the vitamin D level in follicular fluid ($P = 0.045$): the mean age was 35.20 ± 6.696 in the group with vitamin D < 20 ng/ml and 32.23 ± 5.722 in the group with vitamin D ≥ 20 ng/ml (Table 4).

A correlation between serum vitamin D levels in women and the pregnancy rate was observed (Table 4, Fig. 1).

Table 2 Characteristics of female patients

Previous IVF/ICSI attempts, n (%)	
0	43 (41%)
> = 1	62 (59%)
Vitamin D3 level in serum [ng/ml], mean ± (SD)	14.10 ± 9.44
Follicular fluid samples characteristics	
Number of oocyte retrieved, *n*	967
Number of identified MII oocytes, *n*	686
Maturation rate (%), mean (SD)	73.193 ± 24.98
Vitamin D3 level in follicular fluid [ng/ml], mean ± (SD)	16.17 ± 8.6
Embryological outcome of the oocytes	
Fertilization rate (%), mean ± (SD)	67.622 ± 31.42
2 Pronuclei, n	461
Number of embryo, n	430
Number of embryo transferred, n	267
IVF outcome	
Yes	44 (41.9%)
No	61 (58.1%)
Embryo cryopreservation, *n*	29

% percentage, *SD* standard deviation, *n* number

Table 3 Characteristics of male patients

Age [years], mean ± (SD)	40.13 ± 7.971
Vitamin D3 level in serum [ng/ml], mean ± (SD)	22.17 ± 7.48
Semen samples characteristics	
Viscosity; n (%)	
Normal	65 (63.7%)
Viscous	37 (36.3%)
Concentration ($\times 10^6$/ml), mean ± (SD)	37.10 ± 27.26
Progressive Motility (%), mean ± (SD)	
Progressive	31.10 ± 20.53
Non progressive	17.5 ± 9.13
Immotile	49.08 ± 26.77

% percentage, *SD* standard deviation, *n* number

Correlation between men's serum, semen, vitamin D level and IVF outcome

Table 5 shows that men's age did not affect the vitamin D level in serum. However, serum vitamin D level in men negatively affected semen quality: concentration and progressive motility were significantly higher in the men's group with a vitamin D level less than 20 ng/ml than the group with a vitamin D level of more than 20 ng/ml (Table 5).

But most importantly, men's vitamin D serum level had no significant effect on the IVF outcome ($P < 0.05$) (Table 5, Fig. 1).

VDR polymorphism in women and correlation with follicular fluid characteristics, embryological outcome of the oocyte, vitamin D level in serum and FF, and the IVF outcome

For female patients, the results indicated that the most common genotype for Taq1 polymorphism was Tt

Table 4 Characteristics of 105 women undergoing in vitro fertilization, by vitamin D serum and follicular fluid status

	Serum Vitamin D			*Follicular Fluid Vitamin D*		
	Vitamin D < 20 ng/ml	**Vitamin D ≥ 20 ng/ml**	***P* value**	**Vitamin D < 20 ng/ml**	**Vitamin D ≥ 20 ng/ml**	***P* value**
Patients' characteristics						
Number of patients; n (%)	83 (79%)	22 (21%)		79 (75.2%)	26 (24.8%)	
Age [years], mean (SD)	34.75 ± 6.875	33.41 ± 5.261	0.398	35.20 ± 6.696	32.23 ± 5.722	**0.045***
Vitamin D status [ng/ml], mean (SD)	10.01 ± 4.22	29.14 ± 7.98	**0.000***	13.14 ± 4.81	27.33 ± 10.26	**0.000***
Type of infertility, n (%)			0.540			0.450
Female	17	6		16	7	
Male	33	6		32	7	
Idiopathic	33	10		3	12	
Embryological outcome						
Number of oocytes retrieved, mean (SD)	9.45 ± 7.40	8.66 ± 5.92	0.651	9.05 ± 7.2	10.08 ± 6.89	0.531
Maturation rate (%), mean (SD)	72.387 ± 25.9	76.380 ± 20.7	0.516	71.978 ± 26.4	77.03519.7	0.380
Fertilization rate (%), mean (SD)	65.279 ± 31.6	76.881 ± 29.4	0.131	64.111 ± 32.6	78.716 ± 24.9	**0.042***
IVF outcome			**0.007***			0.175
Yes	29	15		30	14	
No	54	7		49	12	

SD Standard Deviation, *%* percentage, *SD* standard deviation, *n* number

Significant difference ($p < 0.05$, ANOVA, Spearman and Pearson) are indicated by a*

($n = 55$); the least common was tt ($n = 15$). For Apa1 polymorphism the most common genotype was Aa ($n = 47$), and the least common was aa ($n = 15$). And in the case of Fok1 polymorphism, the most common genotype was FF ($n = 62$), and the least common was ff ($n = 8$) (Table 6).

The statistical tests showed a correlation between Taq1 SNP and the number of embryos ($P = 0.046$), and the level of vitamin D in the serum ($P = 0.042$) (Fig. 2).

A correlation was also found between Apa1 polymorphism and the number of collected oocytes ($P = 0.014$), the number of embryos ($P = 0.001$), the number of 2 PN ($P = 0.001$), and the frequency of clinical pregnancies ($P = 0.046$). In the case of Fok1 SNP, no correlation was detected (Table 6).

For all 105 women, the percentage of AA genotype (48.8%) and Aa genotype (46.5%) with a positive IVF outcome was higher than the percentage of those with a negative IVF outcome, 34.42% and 44.26%, respectively. The percentage of the aa genotype was higher in the group with no pregnancy result (21.31% vs. 4.65%) with a P-value = 0.046 (Table 6).

The allelic frequency analysis Chi-square test reported that in the group with a pregnancy result, the "A" allele was more expressed (65.1% vs. 56.5%) although the "a" allele was more expressed in the group with negative results (43.4% vs. 34.9%) ($P = 0.029$) (Table 7).

VDR polymorphism in males and the correlation with semen parameters, vitamin D levels in serum and semen, and IVF outcome

The most common genotype in men was TT ($n = 45$) for Taq1 polymorphism, and the least common was tt ($n = 15$). In the case of Apa1 polymorphism, the most common genotype was Aa ($n = 50$), and the least common was aa ($n = 14$). For Fok1 polymorphism, the most common genotype was FF ($n = 63$), and the least common was ff ($n = 7$). There was no correlation between these SNPs and the IVF outcome. However, in the case of Fok1 polymorphism in men, a correlation was found only between Fok1 SNPs and semen concentration ($P = 0.044$), and the frequency of clinical pregnancies ($P = 0.032$) (Table 8).

In the men's group, the percentage of the Ff genotype (34.88%) and the ff genotype (13.95%) with a positive IVF outcome was higher than the percentage of those with a negative result (31.14% and 1.63%, respectively). The percentage of the FF genotype was higher in the group with a negative IVF outcome (67.21% vs. 51.16%) ($P = 0.032$) (Table 7).

Concerning allele distribution, the "F" allele was present with a higher percentage in the group with negative IVF results (82.78% vs. 68.6%) although the "f" allele was more expressed in the group with positive IVF outcomes (31.4% vs. 17.27%) with a P-value = 0.020 (Table 8). In addition, the results showed no correlation among Taq1,

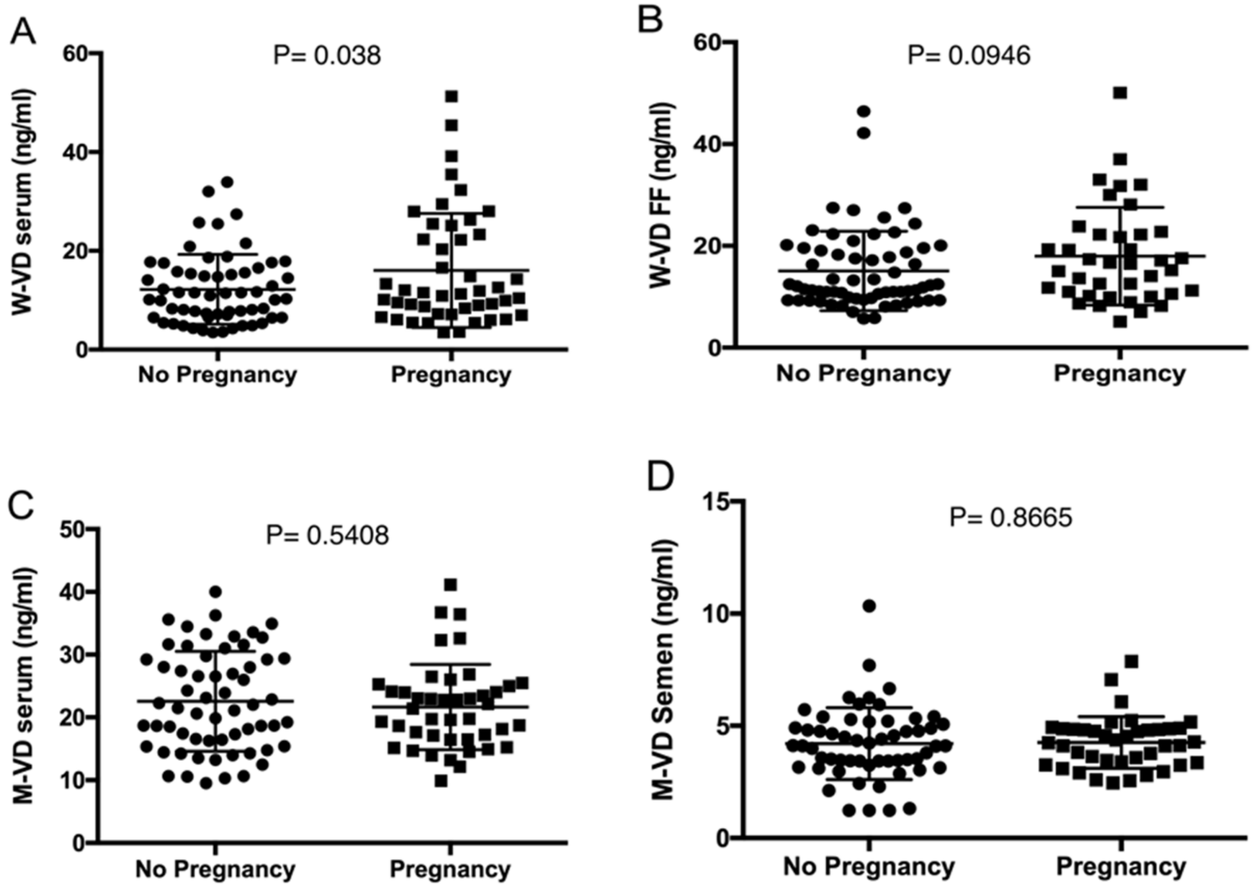

Fig. 1 Comparison of vitamin D levels in both groups (No pregnancy, Pregnancy). **A** Women Vitamin D serum level; **B** Female Vitamin D follicular fluid level; **C** Men Vitamin D serum level; **D** Men Vitamin D semen level. Correlation between vitamin D and pregnancy by Pearson Chi-square *P* value < 0.05 was significant

Apa1, and Fok1 polymorphism in males and vitamin D level in both serum and semen.

Discussion

This study showed a high prevalence of vitamin D hypovitaminosis among the enrolled women, with 79% affected. This prevalence is higher than that reported in in Karras et al. (2014), in which the vitamin D hypovitaminosis rate among pregnant women in the Mediterranean regions was approximately 50%–65% in most studies [14]. The results suggest that the women undergoing IVF had a higher incidence of hypovitaminosis than women who were not. These findings indicate that vitamin supplementation in childbearing women in Lebanon remains an important issue. The high prevalence of vitamin D deficiency in women may also contribute to other health complications during pregnancy, such as an increased risk of gestational diabetes and infants small for their gestational age [15].

Furthermore, significant correlations were observed between the serum vitamin D levels in women and the IVF outcome. Our results are consistent with the study conducted by Farzadi et al. (2015) [16] but contrast with those reported by Firouzabadi et al. (2014) [12].

Conversely, no correlation was found between the serum vitamin D level in men and IVF outcomes. Few studies have investigated the reproductive outcomes in men in relation to vitamin D status. It was reported that hypovitaminosis in men lead to significantly lower chances of pregnancy [17]. Moreover, only one study assessed vitamin D status in couples undergoing IVF. No correlation was found between 25(OH)D status in men and sperm quality or IVF outcomes [18].

A positive correlation was observed between serum vitamin D level and follicular fluid vitamin D levels, which is consistent with other studies [19, 20]. However, no correlation was found between serum vitamin D level and semen vitamin D level, and no previous research was found that studied this correlation.

Regarding follicular fluid vitamin D concentration and IVF outcome, our study found no correlation, which is similar to the findings of Firouzabadi et al. (2014) but

Table 5 Characteristics of 105 men undergoing in vitro fertilization, by vitamin D serum status

	Serum Vitamin D		
	Vitamin D < 20 ng/ml	**Vitamin D ≥ 20 ng/ml**	***P* value**
Patients' characteristics			
Number of patients	49 (47%)	55 (53%)	
Age [years], mean (SD)	38.45 ± 7.38	41.64 ± 8.23	0.041
Vitamin D serum status [ng/ml], mean (SD)	15.72 ± 2.94	27.92 ± 5.25	**0.000***
Vitamin D semen status [ng/ml], mean (SD)	4.09 ± 1.15	4.34 ± 1.63	0.388
Previous IVF/ICSI attempts, *n* (%)			0.171
0	24	19	
> 1	26	36	
Viscosity; n (%)			1.000
Normal	31	34	
Viscous	18	19	
Concentration ($\times 10^6$/ml), mean (SD)	42.82 ± 27.18	31.51 ± 26.44	**0.045***
Progressive Motility (%), mean (SD)			
Progressive	35.51 ± 20.74	27.18 ± 19.71	**0.038***
Non progressive	17.95 ± 9.23	17.09 ± 9.11	0.631
Immotile	43.67 ± 25.93	53.90 ± 26.81	0.051
Embryological outcome			
Fertilization rate (%), mean (SD)	64.01 ± 34.23	70.96 ± 28.491	0.262
IVF outcome			0.697
Yes	22	22	
No	28	33	

SD Standard Deviation,% percentage, *SD* standard deviation, *n* number

Significant difference ($p < 0.05$, ANOVA, Spearman and Pearson) are indicated by a*

contradicts the results reported by Ozkan et al. (2010), which demonstrated a higher clinical pregnancy rate with higher follicular fluid vitamin D levels [21]. Additionally, Ciepiela et al. (2018) reported a negative correlation between follicular fluid 25(OH)D levels and the oocytes' ability to undergo fertilization and subsequent preimplantation embryo development [20].

Furthermore, our study reported a significant difference in the fertilization rate between the two groups of women categorized by follicular fluid vitamin D levels (FF vitamin D: < 20 ng/ml and ≥ 20 ng/ml). A positive correlation was found between FF vitamin D levels and the fertilization rate but not with the number of oocytes retrieved. Similar results were reported by Farzadi et al. (2015).

Regarding the polymorphism, in the women's group, a positive correlation was found between Taq1 polymorphism and the serum vitamin D level, a result consistent with a study conducted in women with polycystic ovary syndrome (El-Shal et al. 2013) [22]. No correlation was observed with the follicular fluid vitamin D concentration or the number of collected oocytes. Similar results were reported by Reginatto et al. (2018) [23]. In the case of the Fok1 polymorphism, no significant associations were detected even with the IVF outcomes. However, the Apa1 SNP showed a significant difference in the number of collected oocytes, number of embryos, and 2PN detection. Moreover, a positive correlation between Apa1 SNP and the IVF outcome was observed. The "a" allele appears to increase the possibility of IVF failure. It is important to note that no previous work explored this particular correlation.

In the men's group, no correlation was found between Apa1 or Fok1 polymorphism and serum vitamin D level, which is consistent with some previous studies [24, 25] but contradicts a study that reported a relation between Apa1 SNP and serum vitamin D level [26]. Regarding the Taq1 polymorphism, no correlation was found with serum vitamin D levels, which differs from the results of another study in which a positive correlation was observed [25]. No previous literature has investigated the correlation between VDR polymorphism and semen vitamin D levels in men, and our study showed no correlation between the two.

One study showed a relation between Apa1 SNPs and sperm progressive motility (Hamade et al., 2014), which was not the case in this study not even with other SNPs. However, the Fok1 polymorphism was correlated with

Table 6 Correlation between VDR polymorphism in women and with follicular fluid characteristics, embryological outcome of the oocyte, vitamin D level in serum and FF, and the IVF outcome

	VDR-TaqI (rs731236; C/T)				VDR-ApaI (rs7975232; T/G)				VDR-FokI (rs2228570; C/T)			
	TT($n=34$)	Tt($n=55$)	tt($n=15$)	*p* value	AA($n=42$)	Aa($n=47$)	aa($n=15$)	*p* value	FF($n=62$)	Ff($n=34$)	ff ($n=8$)	*p* value
# of collected oocyte (Mean ± SD)	7.588 ± 6.055	9.66 ± 7.317	11.13 ± 7.971	0.211	11.56 ± 7.5	8.04 ± 6.64	6.33 ± 5.28	*0.014**	9.86 ± 7.09	8.38 ± 7.25	7.5 ± 6.09	
Maturation rate (%) Mean ± SD	72.09 ± 27.52	74.94 ± 24.26	68.93 ± 23.26	0.688	71.05 ± 23.17	75.211 ± 24.39	72.39 ± 32.72	0.737	72.56 ± 26.36	75.22 ± 22.89	68.46 ± 26.4	0.765
Fertilization rate (%) Mean ± SD	61.74 ± 35.66	71.04 ± 29.11	66.88 ± 29.66	0.405	67.76 ± 29.57	68.44 ± 31.95	62.91 ± 36.55	0.837	66.63 ± 31.45	69.88 ± 31.86	62.23 ± 33.14	0.796
# of embryos Mean ± SD	3.12 ± 2.447	4.71 ± 3.512	5 ± 3.185	0.046*	5.49 ± 3.874	3.61 ± 2.34	2.47 ± 1.959	*0.001**	4.38 ± 3.179	4.06 ± 3.311	3.50 ± 3.295	0.732
2 Pronuclei	3.42 ± 2.61	4.87 ± 4.019	5 ± 1.88	0.126	5.95 ± 4.02	3.53 ± 2.561	3 ± 2.418	*0.001**	4.76 ± 3.49	4.03 ± 3.32	3.5 ± 3.29	0.45
F-VD serum [ng/ml]	10.65 ± 5.63	15.78 ± 10.38	15.08 ± 11.12	*0.042**	14.84 ± 10.32	14.58 ± 9.45	9.71 ± 5.02	0.184	13.93 ± 10.34	13.47 ± 7.91	17.01 ± 8.57	0.131
F-VD FF [ng/ml]	13.96 ± 5.67	17.21 ± 9.28	17.11 ± 11.06	0.212	17.17 ± 9.65	16.21 ± 8.34	12.83 ± 5.49	0.267	15.46 ± 8.45	15.99 ± 8.03	22.00 ± 11.24	0.485
Frequency of clinical pregnancies				0.227				*0.046 **				0.822
No	24	29	8		21	27	13		36	21	4	
Yes	10	26	7		21	20	2		26	13	4	

SD Standard Deviation

Significant difference ($p < 0.05$, chi-square analysis) are indicated by a*

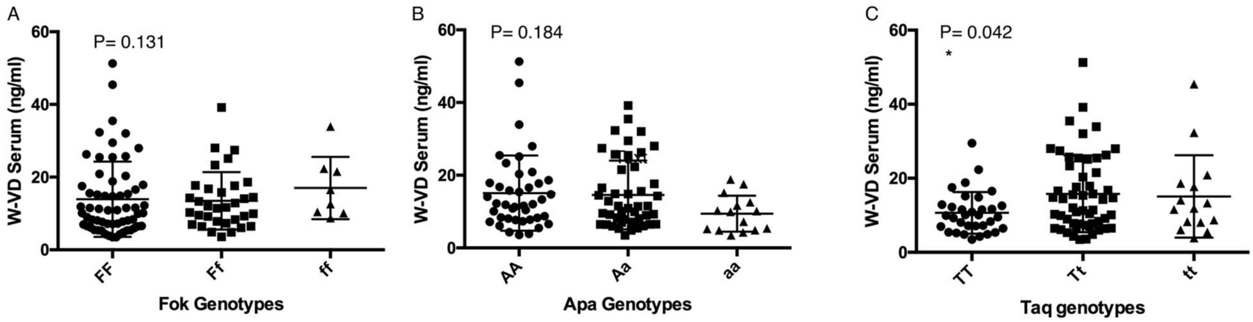

Fig. 2 Correlation between serum Vitamin D level and VDR polymorphism in women

Table 7 Correlation between the genotype and alleles frequency of Apa1 polymorphism in women and Fok1 in men and the IVF outcome

Polymorphisms		IVF outcome		P	OR	CI 95%
		Yes	No			
Women						
Apa I (rs7975232)	**Genotype**					
	AA	21 (48.8 %)	21 (34.42 %)	0.046*	0.54	0.279 - 0.911
	Aa	20 (46.5 %)	27 (44.26 %)			
	aa	2 (4.65 %)	13 (21.31 %)			
	Allele					
	A	62 (65.1 %)	69 (56.5 %)	0.029*		
	a	24 (34.9 %)	53 (43.4 %)			
Men						
FokI (rs10735810)	**Genotype**					
	FF	22 (51.16 %)	41 (67.21 %)	0.032*	2.201	1.025 - 4.235
	Ff	15 (34.88 %)	19 (31.14 %)			
	ff	6 (13.95 %)	1 (1.63 %)			
	Allele					
	F	59 (68.6%)	101 (82.78%)	0.020*		
	f	27 (31.4%)	21 (17.27%)			

OR odds ratio, *CI* confidence interval

Significant difference (p<0.05, chi-square analysis) are indicated by a*

sperm concentration ($P=0.044$). Furthermore, our study reported a correlation between Fok1 polymorphism in men and the frequency of clinical pregnancy ($P=0.032$). It was also found that the "f" allele was more prevalent in the group with positive IVF outcomes.

Conclusion

A high rate of vitamin D deficiency was observed in women using IVF. This deficiency correlated with pregnancy failure. Thus, a systemic supplementation of vitamin D along with sun exposure before pregnancy should be considered, which would also prevent other obstetric complications.

Overall, our study contributes to the growing body of evidence of the importance of vitamin D supplementation and genetic factors in the context of IVF. These findings emphasize the potential benefits of optimizing vitamin D status and considering genetic factors in fertility treatments to improve IVF success rates.

Study limitation

The study's findings are based on a sample of 105 infertile couples. To address this limitation, future research should consider expanding the sample size to include a larger and more diverse group of participants.

Table 8 Correlation between VDR polymorphism in men and semen parameters, vitamin D level in serum and semen, and IVF outcome

	VDR-TaqI (rs731236; C/T)				VDR-ApaI (rs7975232; T/G)				VDR-FokI (rs2228570; C/T)			
	TT ($n=45$)	**Tt ($n=43$)**	**tt ($n=15$)**	***p* value**	**AA ($n=40$)**	**Aa ($n=50$)**	**aa ($n=14$)**	***p* value**	**FF ($n=63$)**	**Ff ($n=34$)**	**ff ($n=7$)**	***p* value**
Viscosity (%)				**0.670**				**0.365**				**0.136**
Normal	*26*	*29*	*9*		*27*	*27*	*10*		*42*	*20*	*2*	
Viscous	*17*	*13*	*6*		*12*	*21*	*4*		*21*	*11*	*5*	
Concentration				**0.428**				**0.302**				**0.044***
Normal	*29*	*23*	*9*		*26*	*27*	*9*		*42*	*16*	*4*	
Oligozpermia	*15*	*19*	*5*		*12*	*22*	*5*		*20*	*17*	*2*	
Azoospermia	*0*	*1*	*1*		*2*	*0*	*0*		*1*	*0*	*1*	
Progressive Motility				**0.375**				**0.324**				**0.660**
Normal MP > 32%	25	19	6		22	20	8		32	14	4	
Abnormal MP < 32%	19	24	9		18	19	6		31	19	3	
Fertilization rate (%) Mean ± SD	**68.35 ± 3019**	**67.23 ± 33.09**	**62.62 ± 32.22**	**0.832**	**66.72 ± 34.50**	**65.91 ± 31.61**	**74.32 ± 21.18**	**0.673**	**62.32 ± 33.88**	**75.15 ± 28.11**	**74.31 ± 12.45**	**0.134**
M-VD serum [ng/ml]	**21.86 ± 7.79**	**22.55 ± 7.6**	**21.98 ± 6.73**	**0.661**	**22.05 ± 7.56**	**21.95 ± 7.05**	**23.31 ± 9.09**	**0.731**	**21.94 ± 7.46**	**23.24 ± 7.87**	**19.06 ± 5.11**	**0.287**
M-VD semen [ng/ml]	**4.21 ± 1.58**	**4.11 ± 1.10**	**4.53 ± 1.81**	**0.909**	**4.36 ± 1.5**	**4.11 ± 1.17**	**4.24 ± 2.03**	**0.829**	**4.25 ± 1.50**	**4.02 ± 1.12**	**5.02 ± 1.99**	**0.377**
Frequency of clinical pregnancies				**0.881**				**0.338**				***0.032****
No	27	26	8		21	33	7		41	19	1	
Yes	18	17	7		19	17	7		22	15	6	

SD Standard Deviation

Significant difference ($p < 0.05$, chi-square analysis) are indicated by a*

Authors' contributions
A.H, R.J and C.F: Conceptualization, Methodology. M.A: Writing—Original Draft. All authors: Writing—Review & Editing. All authors: Data Collection, Analysis. A.H and R.J: Supervision, Funding Acquisition. E.HM and A.H: Statistical Analysis.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Informed consent was obtained from all participants. The study protocol was approved by the Ethics Committee of Al Hayat Hospital (ethical authorization number: ETC-03/2019).

Consent for publication
I hereby provide consent for the publication of the manuscript detailed above.

Competing interests
The authors declare no competing interests.

Author details
[1]Laboratoire d'Innovation Thérapeutique (LIT), Departments of Life and Earth Sciences, Chemistry and Biochemistry, Lebanese University, Faculty of Sciences II, Fanar, Lebanon. [2]Laboratory of Cellular and Molecular Physiopathologies (CAMP), Department of Life and Earth Sciences, Faculty of Sciences, Lebanese University, Fanar, Lebanon. [3]Al Hadi Laboratory and Medical Center, Beirut, Lebanon.

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