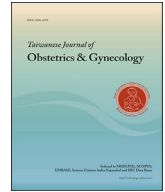




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## Original Article

## Follicular fluid 25-hydroxyvitamin D levels determine fertility outcome in patients with polycystic ovary syndrome

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## ABSTRACT

**Objective:** To determine the possible relationship between follicular fluid 25-hydroxyvitamin D [25(OH)D] levels and fertility outcome of women who underwent IVF/ICSI with the diagnosis of lean polycystic ovary syndrome.

**Materials and methods:** Thirty patients who were diagnosed with PCOS according to the Rotterdam criteria and decided on IVF/ICSI were included in the study. Thirty patients who were scheduled for IVF/ICSI for reasons other than PCOS and matched in terms of age and BMI were taken as the control group (non-PCOS). According to BMI values, patients in both PCOS and non-PCOS groups were lean. Women in both groups were aged 21–35 years with a normal BMI (18.5–24.9 kg/m<sup>2</sup>) and first IVF/ICSI attempt. Both groups of patients were followed up using the antagonist protocol. Vit D levels were measured in serum and follicular fluid (FF) samples taken on the day of oocyte collection. The correlation between FF vit D levels, the number of total oocytes, MII oocytes and 2 PN zygotes, HOMA-IR, hormonal and demographic parameters, clinical pregnancy rate (CPR), live birth rate (LBR), and miscarriage rate were evaluated.

**Results:** At the time of oocyte retrieval women with PCOS had similar serum Vitamin D compared to non-PCOS women (21.8 (12.6–24.8) ng/ml vs 22.3 (11.5–25.1) ng/ml,  $p < 0.54$ ). In FF, assessed on the day of oocyte retrieval, the concentration of Vitamin D was similar in women with PCOS when compared to non-PCOS women (11.2 (9.2–14.4) ng/ml vs 13.3 (11.1–17.4) ng/ml,  $p < 0.06$ ). For both groups, Vitamin D levels were lower in FF compared to serum vit D. A positive correlation was found between serum and FF Vitamin D concentrations in the full cohort. A positive and significant correlation was found between FF-vit D levels and the number of total oocyte ( $r = 0.344$ ,  $p < 0.04$ ) and MII oocyte ( $r = 0.404$ ,  $p < 0.02$ ) in the PCOS group. The number of total oocyte, MII oocyte and 2 PN zygotes of the PCOS group were significantly higher than the non-PCOS group. Positive pregnancy test rate, clinical pregnancy and live birth rates were similar in both groups. The miscarriage rates in the non-PCOS group were significantly higher than in the PCOS group. A positive and significant correlation was also found between FF vit D levels and positive pregnancy test ( $r = 0.566$ ,  $p < 0.03$ ) and CPR ( $r = 0.605$ ,  $p < 0.02$ ) in PCOS group. There was no correlation between FF-vit D levels and live birth and miscarriage rates in neither the PCOS nor the non-PCOS group.

**Conclusions:** Both serum and FF 25-hydroxyvitamin D level of women with PCOS at the time of oocyte retrieval are similar to non-PCOS controls. While FF 25-hydroxyvitamin D levels correlate with total and MII oocyte counts, positive pregnancy test and CPR, it does not correlate with miscarriage and live birth rates.

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## Introduction

Vitamin D is a fat-soluble micronutrient, which is synthesized in the skin through sunlight or ingested in the diet. It acts through the vit D receptors (VDR) in the target cell. VDR is widely found in both male and female germ cells [1]. Since Vit D acts through VDR, it is

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also accepted as a steroid hormone by some authors. In a limited number of studies conducted in the last decade, it has been reported that vitamin D deficiency leads to decreased fertility [2,3]. Pre-ART vit D replacement provides an approximately three-fold increase in intrafollicular vit D levels [4]. In line with this clinical pregnancy and live birth rates were found to be significantly higher in patients who underwent vit D replacement in the pre-ART period compared to those who did not receive vit D replacement [5]. Antral follicle counts were also found to be high in patients with high serum Vit D levels [6]. However, there are studies reporting that elevated serum vit D only increases the fertilization rates and does not affect clinical pregnancy and live birth rates [3]. The main reason for the different results between the studies may be that the patient groups are not homogeneous and the IVF outcome is determined only according to serum vit D levels.

Since serum vit D levels are affected by many parameters, studies have designed to determine IVF outcome according to follicular fluid vit D levels [1,3,7]. It has been reported that intrafollicular vit D levels and reproductive parameters were correlated rather than serum vit D levels [8,9]. Ozkan et al. [9] showed that women with higher FF vitamin D level are significantly more likely to achieve clinical pregnancy [9]. Despite studies comparing serum and FF vit D levels and ART outcome in the infertile population consisting of heterogeneous patients, there is no study comparing FF vit D levels and IVF/ICSI outcome in polycystic ovary syndrome (PCOS) [1,3,7]. PCOS is an endocrine disease that is one of the most common causes of subfertility in women of reproductive age, characterized by defects in follicle development and ovulatory function. A close relationship between changes in serum vit D levels and androgen synthesis and insulin resistance has been reported [10,11]. Decreased serum vit D levels have been reported in PCOS patients compared to non-PCOS controls [12]. Similarly, it has been reported that vit D supplementation in PCOS patients increases dominant follicle formation [13]. Although a recent study found significantly lower vit D levels in the follicular fluid of women with PCOS compared to non-PCOS controls [7] they did not compare FF vit D with ART outcome. For this reason, this study was planned to determine the relationship between serum and FF-vit D levels and hormonal and demographic parameters of patients who underwent IVF/ICSI due to PCOS. In addition, the relationship between FF-vit D levels and embryological parameters, clinical pregnancy, live birth and miscarriage rates were also evaluated.

## Material and methods

Thirty patients who were diagnosed with PCOS and decided on IVF/ICSI were included in the study. The diagnosis of PCOS was made according to the revised Rotterdam criteria which require two of the following three manifestations [1]: oligo and/or anovulation [2], clinical and/or biochemical hyperandrogenism, and [3] polycystic ovaries determined by ultrasonography. Thirty patients who were scheduled for IVF/ICSI for reasons other than PCOS and matched in terms of age and BMI were taken as the control group. According to BMI values, patients in PCOS group were lean. The patients in the control group were selected among the patients diagnosed with male factor infertility or unexplained infertility. Patients with androgenic skin manifestations or ovulatory dysfunction were not included in the control group. Women in PCOS and control groups were aged 21–35 years with a normal BMI (18.5–24.9 kg/m<sup>2</sup>) and first IVF/ICSI attempt. Women with BMI values compatible with obesity or overweight were not included in the study groups. Each group underwent hormonal and radiological examination. Semen analysis and hysterosalpingography was performed in both groups. Demographics characteristics, age, and body mass index of participants in both groups were recorded. For

assessing basal hormone levels women with PCOS were subjected to progesterone induced withdrawal bleeding to determine their follicular phases. Serum samples were taken on the third day of the cycle of the patients in PCOS and control groups for complete hormonal assays including luteinizing hormone (LH), follicle stimulating hormone (FSH), testosterone, and insulin. The fasting serum insulin, LH, FSH, testosterone, estrogen and progesterone levels were measured in autoanalyzer by using electrochemiluminescence immunoassay. Homeostatic model assessment [HOMA-IR] formula was used for calculating insulin resistance. The study was performed according to the guidelines of the Helsinki Declaration on human experimentation and was approved by the Local Ethics Committee.

Both groups of patients were followed up using the antagonist protocol. Recombinant follicle stimulating hormone (Gonal-F, Merck Pharmaceutical Group Inc, Turkey) was initiated as the initial dose on the third day of the menstrual cycle. Initial rFSH doses of PCOS and control groups were different. Transvaginal ultrasonography was used for monitoring the folliculogenesis. GnRH antagonist (Cetrotide 250 µg, Merck Serono, Turkey) was added daily when the leading follicle reached a diameter of 14 mm. When the mean diameter of two or three leading follicles reached 17 mm or more, triptoreline acetate (Gonapeptyl 0.1 mg/ml, Ferring, Turkey) was injected to trigger ovulation. In the control group single dose of recombinant hCG was used to induce ovulation. The oocyte pick-up was carried out either 35 or 36 h after ovulation trigger. Vit D levels were measured in serum and follicular fluid samples taken on the day of oocyte retrieval. Embryo transfers were performed under ultrasound guidance using a soft tip catheter. A single top quality embryo on day 5 was transferred to each patient. Luteal phase support with progesterone was continued until the day of the pregnancy test.

The primary outcome measures of our study are;

- (i) To compare serum and FF-vit D levels both within and between groups.
- (ii) To correlate the relationship between FF-vit D levels and total oocytes, MII oocytes, 2 PN zygotes, HOMA-IR, hormonal and demographic parameters.
- (ii) To determine the relationship between FF-vit D level and positive pregnancy test, clinical pregnancy rate (CPR), live birth rate (LBR), and miscarriage rate. For this reason, the dominant follicle vit D level was correlated with the outcome of the oocyte after ICSI.

Clinical pregnancy rate defined as evidence of a gestational sac, confirmed by ultrasound examination at the 4th week of transfer. Live birth rate defined as delivery of a live fetus after 24 completed weeks of gestational age. Serum beta-hCG levels were measured in all patients on the 12th day of embryo transfer. The loss of fetus before 20 weeks of gestation was defined as miscarriage.

## Follicular fluid sampling

For assessment of 25-hydroxyvitamin D and sex steroids in FF, the dominant follicle was sampled and collected FF was stored at 20C until analysis. The following criteria were followed when selecting the dominant follicle; (i) follicle diameter >17–18 mm, (ii) follicle containing M II oocytes, (iii) being the first follicle to be reached and aspirated if possible, and (iv) minimal blood contamination. One dominant follicle from both ovaries was selected and aspirated. Flushing was not performed unless it was necessary. A separate test tube was used for the dominant follicle in each ovary. If MII oocyte was detected in the tube, FF was evaluated. If MI or GV oocyte was detected in the first aspirated dominant follicle, the

dominant follicle in the other ovary was evaluated. If the follicles in both ovaries carried MII oocytes, the follicle with the least number of erythrocytes was included in the study. The cumulus cells in the FF sample were removed using 10% hyaluronidase solution. FF with matched MII oocytes was centrifuged at  $5000\times g$  for 5 min, and the supernatants were aliquoted and stored at  $-20^{\circ}\text{C}$ . Patients with poor response to rFSH, those with hydrosalpinx and those dominant follicle without MII oocyte were not included in the study. Patients who received vit D supplementation in the last six months were excluded from the study. Following thawing of FF and serum samples, 25-hydroxyvitamin D was measured using the chemiluminescent immunoassay. Total 25-hydroxyvitamin D was measured in serum and FF during oocyte retrieval. The measurement of 25 (OH) D levels was performed using an enzyme chemiluminescence immunoassay (ECLIA, Elecsys Vitamin D total II, Roche Cobas E602, Japan). The intra- and inter-assay coefficients of variations were 10 and 15%, respectively.

### Statistical analysis

The data was analyzed with the use of the Statistical Package for Social Sciences software 21.0 for Windows package software (SPSS). Normality of data was examined by the use of the Shapiro–Wilk test. Pearson's Chi-square and Fischer's exact test were used to compare categorical variables. Continuous variables were analysed using the Mann–Whitney U-test depending on the normality of the distribution. Data are presented as mean  $\pm$  SD. Categorical data are described either by number of cases or percentages. A *p* value  $< 0.05$  was considered statistically significant in all tests.

### Results

Age, BMI and number of IVF/ICSI trials were similar in both groups. Serum LH, total testosterone, insulin and HOMA-IR were significantly higher in the PCOS group compared to the control group. FF-estradiol and progesterone levels of women with PCOS and control group were similar. The duration of rFSH use was significantly higher in the PCOS group than in the non-PCOS controls. The increase in the duration of use was due to keeping the initial rFSH dose low in the PCOS group. The number of total oocyte, MII oocyte and 2 PN zygotes of the PCOS group were significantly higher than the non-PCOS group. Positive pregnancy test, clinical pregnancy and live birth rates similar in both groups. The miscarriage rates in the control group were significantly higher than in the PCOS group (Table 1).

One dominant follicle was aspirated from each ovary of 30 patients in the PCOS group. The total number of aspirated dominant follicles was recorded as 60, and 50 of them contained MII oocytes, and FF-vit D levels were measured in 30 of them. The median diameter of the aspirated dominant follicles was  $18.6 \pm 2.1$  mm. Both in the PCOS and non-PCOS control group, the concentration of serum and FF- vitamin D was similar (Table 2). At the time of oocyte retrieval women with PCOS had similar serum Vitamin D compared to non-PCOS control women (21.8 (12.6–24.8) ng/ml vs 22.3 (11.5–25.1) ng/ml,  $p < 0.54$ ). In FF, assessed on the day of oocyte retrieval, the concentration of Vitamin D was similar in women with PCOS when compared to non-PCOS women (11.2 (9.2–14.4) ng/ml vs 13.3 (11.1–17.4) ng/ml,  $p < 0.06$ ). For both groups, Vitamin D levels were lower in FF compared to serum vit D ( $P < 0.01$  and  $p < 0.03$ ).

Correlation analysis revealed a positive correlation between serum and FF Vitamin D concentrations in the full cohort. A strong and significant positive correlation was found between FF-vit D levels and serum Vit D levels in the PCOS group ( $r = 0.877$ ,  $p < 0.001$ ). A positive correlation was found between FF-vit D levels

and the number of total oocyte ( $r = 0.344$ ,  $p < 0.04$ ) and MII oocyte ( $r = 0.404$ ,  $p < 0.02$ ) in the PCOS group. No correlation was found between the number of 2 PN zygotes and FF-vit D. There was no significant correlation between FF-vit D levels and age, BMI, total testosterone, FSH, LH and HOMA-IR values. A positive and significant correlation was found between serum and FF-vit D levels in the non-PCOS control group ( $r = 0.677$ ,  $p < 0.001$ ). There was no significant correlation between FF-vit D levels and total oocytes, MII oocytes and other hormonal and demographic parameters in non-PCOS controls. There was no significant correlation between the duration of rFSH use and vitamin D levels and other demographic, laboratory and embryological parameters. A positive and significant correlation was found between FF vit D levels and positive pregnancy test and CPR in PCOS. There was no significant correlation between FF vit D and positive pregnancy test rates and CPR in the non-PCOS group. Likewise, there was no correlation between FF-vit D level, live birth and miscarriage rates in neither the PCOS group nor the patients in the non-PCOS group (Table 3).

### Discussion

Follicular fluid of PCOS patients hosts impaired expression of AMH, inhibin B, activin A, tumor necrosis factor and many other molecules [14]. The FF vitamin D dynamics of PCOS patients have started to attract the attention of researchers in the last decade. Then, the vitamin D ligand protein is intensely expressed in FF of PCOS cases [15]. Recent study investigating proteomic profiling of follicular fluid from women with PCOS revealed upregulated vitamin D binding protein [16]. Overexpression of FF vitamin D binding protein in women with PCOS has been a proof that vitamin D is used in follicle development [15]. Kong et al. [17] reported that granulosa cell proliferation and apoptosis in the follicles of PCOS patients are regulated by microRNA-9 and via the VDR. Despite many studies were designed for investigating vit D levels in the serum of PCOS patients, only a few studies have studied FF vit D levels and reported lower values than non-PCOS controls [7,18]. However, any study comparing FF vitamin D levels with ART outcome has not been made.

Our study is the first to compare follicular fluid vit D levels and ART outcome in patients undergoing IVF/ICSI due to PCOS. We found similar serum and follicular fluid vit D levels in both women with PCOS and non-PCOS. Although there was a positive and significant correlation between serum and FF vit D levels in both groups, FF vit D levels were lower than serum. In previous studies, they reported that serum and FF vit D levels were highly correlated [9,19]. The presence of decreased vit D in follicular fluid compared to serum suggests that the passage of circulating vit D into follicular fluid is restricted by some unknown mechanisms. Reporting of a 2.8-fold increase in FF vit D levels in patients undergoing vit d replacement suggests the existence of a saturable transport system between the serum and the follicle [4]. On the other hand, the excessive use of 25 (OH)D during folliculogenesis, especially in granulosa cells, may explain the decreased FF vit D levels compared to serum [4]. Since granulosa cells contain a large amount of VDR and provide hormone and growth factor support to the developing oocyte, intrafollicular vit D levels may be lower than serum [20]. Defective follicular development in vitamin D receptor knockout mice is another evidence of vit D requirement during folliculogenesis [21]. Moreover, it has been reported that adding Vit D3 to granulosa cell culture induces estradiol and progesterone synthesis by increasing VDR, steroidogenic acute regulator,  $3\beta$ -hydroxysteroid dehydrogenase mRNA expression and cyclic adenosine monophosphate release [22]. Since developing follicles express VDR in proportion to follicle size [22] we may have found low FF vit D levels because we only evaluated the dominant follicles at the

**Table 1**  
Demographic, hormonal and reproductive characteristics of PCOS and control groups.

	PCOS (n = 30)	Non-PCOS (n = 30)	p
Age (y)	26.9 ± 0.11	28.1 ± 1.04	0.55
BMI (kg/m <sup>2</sup> )	24.8 ± 1.50	24.1 ± 0.33	0.06
Infertility duration (y)	3.22 ± 1.22	3.65 ± 0.33	0.08
Endometrial thickness on the day of ET (mm)	9.11 ± 1.05	8.90 ± 2.13	0.40
Testosterone (ng/mL)	0.90 ± 0.04	0.53 ± 0.32	0.01
LH (mIU/ml)	10.9 ± 2.03	5.11 ± 3.2	0.02
FSH (mIU/ml)	5.11 ± 2.06	5.05 ± 2.08	0.33
Insulin (mU/L)	13.2 ± 3.06	7.09 ± 3.87	0.02
HOMA-IR	3.90 ± 2.04	1.44 ± 0.31	0.01
Total day of rFSH administration (day)	14.3 ± 3.22	10.2 ± 2.65	0.02
Total rFSH dose (IU)	2230.6 ± 410.4	2345.6 ± 480.4	0.01
E2 on hCG day (pg/mL)	2345.5 ± 669.2	2250.6 ± 579.1	0.02
Progesterone on hCG day (ng/mL)	1.13 ± 0.40	0.98 ± 0.20	0.53
Total oocyte	18.2 ± 1.03	11.6 ± 1.32	0.01
MII oocyte	12.5 ± 1.07	7.11 ± 3.06	0.02
2 PN zygotes	9.11 ± 2.09	5.22 ± 2.01	0.01
Good quality embryo	8.20 ± 1.04	3.01 ± 0.33	0.03
ET	Single ET	Single ET	NA
Cryopreserved embryo	7.03 ± 1.01	2.03 ± 0.55	0.01
Positive pregnancy test	12 (40%)	13 (43.3)	0.63
Clinical pregnancy (%)	10 (33.3%)	11 (36.6%)	0.07
Miscarriage (%)	1/10 (10%)	2/11 (18.1%)	0.04
Live birth (%)	9 (30%)	9 (30%)	0.33

Data presented as means ± SD. BMI; body mass index, FSH; follicle-stimulating hormone, LH; luteinizing hormone, PCOS; polycystic ovary syndrome, MII; mature oocyte, \*p < 0.05.

**Table 2**  
Comparison of serum and FF vitamin D levels in PCOS and non-PCOS groups.

	Serum vit D (ng/mL)	Follicular fluid vit D (ng/mL)	p-value
PCOS	21.8 (12.6–24.8)	11.2 (9.2–14.4)	0.01
Non-PCOS	22.3 (11.5–25.1)	13.3 (11.1–17.4)	0.03
p-value	0.54	0.06	

**Table 3**  
Correlation analysis of FF vitamin D and reproductive parameters.

	PCOS (n = 30)		Non-PCOS (n = 30)	
	Follicular Fluid Vit D			
	r	p	R	P
Serum vit D	r = 0.877	<0.001	r = 0.677	<0.001
Total oocyte	r = 0.344	<0.04	r = 0.277	0.60
MII oocyte	r = 0.404	<0.02	r = 0.255	0.08
2 PN zygotes	r = 0.122	0.40	r = 0.320	0.32
HOMA-IR	r = 0.410	0.56	r = 0.322	0.40
BMI	r = 0.201	0.10	r = 0.409	0.22
Positive pregnancy test	r = 0.566	<0.03	r = 0.544	0.09
CPR	r = 0.605	<0.02	r = 0.677	0.37
LBR	r = 0.443	0.36	r = 0.331	0.07
Miscarriage	r = 0.209	0.44	r = 0.109	0.60
Days of rFSH use	r = 0.340	0.60	r = 0.401	0.32

highest level of the developmental period. FF vit D levels might have been different if we had evaluated the entire cohort of follicles. When all these findings and our data are evaluated together, due to the intensity of metabolic events occurring in the follicle during controlled ovarian stimulation, decreased FF vit D levels compared to the circulation may be encountered due to the use of excessive amounts of vit D.

When we compared PCOS patients and non-PCOS control patients, we found both serum and FF vit D levels to be similar. The mean serum vit D level of the PCOS group was 21.8 ng/ml, while the mean serum vit D level of the control group consisting of patients without PCOS was 22.3 ng/ml. The Endocrine Society classified patients with serum 25(OH)D levels below 20 ng/ml as deficiency,

patients with 21–29 ng/ml as insufficiency, and patients with more than 30 ng/ml as full tanks. Since the serum vit D values of PCOS and control group patients included in our study were between 21 and 29 ng/ml, they were considered as insufficiency group [23]. Therefore, being diagnosed with PCOS did not lead to a decrease in serum and follicular fluid vit D levels. Unlike our results, Masjedi et al. [7] found that vit D levels were significantly lower in follicle fluid samples taken on the day of oocyte collection of patients who were scheduled for IVF/ICSI due to PCOS, compared to controls. However, the authors did not specify serum vit D levels. In addition, our and their vit D measurement methods are different. Moreover, age, BMI and infertility duration of PCOS patients included in our study were different from Masjedi et al. [7].

Studies comparing Vit D levels with ART outcome have shown conflicting results. In addition to studies reporting that Vit D deficiency reduces both implantation and clinical pregnancy rates [2,24], there are also studies reporting that Vit D has no effect on fertility outcome [25,26]. It has been reported that both implantation and clinical pregnancy rates are positively affected in women replete in vitamin D [9]. In the present study, no significant difference was found between the PCOS and the non-PCOS groups in positive pregnancy test, CPR, and LBR. However, miscarriage rates were found to be significantly higher in the control group. In contrast to the control group we found that the likelihood of obtaining a positive pregnancy test after embryo transfer was higher in women with high FF vit D levels. There was a significant correlation between FF vit D levels and positive pregnancy test rates in PCOS. In good agreement with this, Farzadi et al. [27] showed that FF concentrations of vitamin D at the time of oocyte pick-up were significantly higher in women who became pregnant compared to those who were not. Unlike our study, the participants in Farzadi's study were not PCOS patients. Another study showed that follicular fluid vitamin D levels were associated with clinical pregnancy rates [9]. Authors also reported that for every nmol/l increase in FF vitamin D level, a 2.4% increase was found in clinical pregnancy rates [9]. Likewise, a recent meta-analysis reported that women with vit D insufficiency or deficiency have a lower chance of having a positive pregnancy test than women with sufficient vit D.

The closer the vitamin D levels are to normal, the higher the rate of obtaining a positive pregnancy test [5].

We found a positive and significant correlation between clinical pregnancy rates and FF vit D levels in the PCOS group. The positive picture we found in positive pregnancy test rates continued in clinical pregnancy rates as well. It has been reported that clinical pregnancy rates in women replete in vitamin D are higher than in patients with vitamin D deficiency [5]. Vitamin D levels of patients in both the PCOS and control groups in our study are within the limits of insufficiency. Although serum and FF vitamin D levels of the patients in the control group were similar to those of the PCOS group, we did not find a significant correlation between clinical pregnancy rates and FF vitamin D levels in control patients. In line with our results, Liu et al. [3]. Reported that patients with high serum vitamin D levels had high fertilization rates, but clinical pregnancy, implantation and live birth rates were not proportional to the amount of vit D. Likewise, no significant correlation was found between LBR, miscarriage rates and FF vit D levels in both PCOS and control groups. We may not have detected a correlation between LBR and FF vitamin D levels, since it has been reported that the rates of live births are higher in women replete in vitamin D [5] and our patients in both groups did not meet this requirement. We did not detect any correlation between serum and FF vitamin D levels and miscarriage in our patients in both groups. Consistent with our results, in a meta-analysis evaluating the results of six studies, it was reported that there was no relationship between vitamin D levels and miscarriage [5].

We do not know by which mechanism Vit D increases achieving positive pregnancy test and clinical pregnancy rates. It has been reported that Vit D increases implantation by regulating main receptivity gene, homeobox10, and immune tolerance to the fetus [25,28]. Liu et al. [3] classified infertile patients scheduled for ART according to their serum vit D levels and reported that the group with the highest serum 25 (OH) D levels had the highest fertilization rates. Vit D may also increase implantation and clinical pregnancy by increasing blastocyst ploidy. It has been reported that higher vitamin D level in the FF could be related with higher oocyte quality [27]. Consistent with this, Arnaz et al. [29] reported that individuals with Vit D levels >20 ng/ml during ART produced more euploid embryos than those with vit D deficiency (<20 ng/ml). Conversely, Ciepiela et al. [1] reported that oocytes obtained from top quality embryos had low FF vitamin D levels. Anifandis et al. [26] showed that women with a sufficient FF vitamin D had a lower quality of embryos. In addition, clinical pregnancy rates in patients with high FF vitamin D levels were reported to be lower than in patients with low vitamin D levels [26]. We did not evaluate the ploidy status of embryos in our study. However, we found a positive and significant correlation between FF vit D levels and the number of total and MII oocytes. In oocyte donation cycles, Rudick et al. [30] found the live birth rates of recipients with deficient serum vit D levels to be significantly lower than those of vit D replete recipients (31% vs 59%). In the light of this result, the author suggested that vit D exerts its fertility-enhancing effect through the endometrium. However, the study design and the data obtained are not sufficient to make a definite conclusion. Regardless of the underlying mechanism, there is a positive and significant relationship between FF vit D levels and clinical pregnancy rates in patients undergoing IVF/ICSI due to PCOS. Taken together, this positive relationship between FF vit D and positive pregnancy test and clinical pregnancy rates may be due to Vit D-mediated increase in endometrial receptivity [5,31] or increase in euploid blastocyst count. The last sentence I stated should be confirmed by further studies so that it is not speculation. Our study has some limitations, especially the small number of cases. The second handicap is that we did not measure active vitamin D levels.

## Conclusions

The main findings of our study can be listed as follows; (i) We found similar serum and follicular fluid vit D levels in both PCOS and non-PCOS controls. (ii) Although there was a positive and significant correlation between serum and FF vit D levels FF vit D levels were lower than serum. (iii) While FF vit D levels correlate with total and MII oocyte counts, and CPR, it does not correlate with miscarriage and live birth rates. Despite the some limitations this study is important in terms of shedding light on future scientific studies as it is the first study to compare FF vitamin D levels and ART outcome in women with PCOS.

## Declaration of competing interest

There is no conflict interest.

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