Serum and follicular fluid vitamin D and follicular response among infertile women undergoing ICSI

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Abstract

Background Some studies have shown the relation between vitamin D levels and the success rate of fertility in couples treated with Assisted Reproductive Technology (ART). The aim of this study is to determine Association of vitamin D level in serum and follicular fluid with follicular response in infertile women in an ART program.

Method: In this cross sectional study 81 infertile women were placed under treatment for induction ovulation according to a long protocol. Follicular fluids were extracted from follicles over 14 mm and blood samples collected on the same day. Follicular fluid and blood samples were centrifuged and stored at -80 C. Then level of 25-OH-vit D was measured by immunoassay method.

Result: The mean age of the subjects was 32.91 ± 4.83. The average BMI of patients was 27.63 ± 3.97. There was no significant correlation between the follicular concentrations of vitamin D and the number of oocytes as well as serum concentration of vitamin D and the number of oocytes (Respectively, P = 0.95 and P = 057). There was also no relation between the numbers of ovarian follicles and follicular concentrations of vitamin D as well as the number of ovarian follicles and serum concentration of vitamin D (P=0.07).

Correlation analysis revealed a high relationship between serum and follicular fluid vitamin D (P<0.001).

Conclusion: Different concentrations of vitamin D in serum or follicles have no significant correlation with the number of ovarian follicles and mature oocytes.

Key words: Vitamin D, infertility, Intracytoplasmic sperm injection, Oocyte, follicular fluid

Introduction

The World Health Organization estimates that approximately 10-25% of couples have infertility problems. Infertility has affected about 80 million people worldwide [1, 2]. Vitamin D is a steroid hormone and induces its effects on the nucleus by connecting its receptors to this part of the cell. This hormone is effective on almost all body cells and has a positive relation with the body's health from cancer to heart disease [3, 4]. Vitamin D can play an important role in growth and differentiation of various tissues, reducing the risk of chronic diseases such as cancer, autoimmune diseases, infectious diseases and cardiovascular disease [5-11]. Studies have shown that Vitamin D receptors exist in most tissues and cells in the body which in itself shows the role of vitamin D [5]. Among the various physiological functions of vitamin D, some seem an important role in reproductive physiology [12] in a way that Vitamin D receptors are also found in reproductive tissues such as ovary and uterus [13, 14].

More information confirming the association between vitamin D and reproduction arise from studies carried out on receptors. Vitamin D receptors can be observed in reproductive organs like the uterus and ovaries [15]. Recent studies have shown that Vitamin D plays an important role in female fertility by promoting the synthesis of two important hormones in reproduction which are estrogen and progesterone. The correct balance of these two hormones is essential for reproductive health and menstrual cycles [16]. Vitamin D may also be associated with endometriosis and the possibility of pregnancy after IVF operation [17]. Lack of vitamin D shows up to 85% companionship, in women with polycystic ovary syndrome. The deficiency of this vitamin is also associated with PCOS symptoms such as menstrual irregularities and infertility [17]. Patients with PCOS have hypervitaminosis D and average levels of vitamin D are lower in these patients compared to non-PCOS people [18]. It has been shown that vitamin D supplementation can improve insulin resistance as well as the sequence of menstrual cycles [19, 20] which in itself can increase the chances of pregnancy. Adequate levels of vitamin D are also important for fetal development during pregnancy [21]. Vitamin D deficiency is especially common in the Middle East. Factors such as avoiding exposure to the sun, using sunscreen, type of dressing of women and the high number of skin pigments in Asian people can be among the main reasons for this deficiency [22]. The prevalence of vitamin D deficiency in Australia is between 70 to 80 percent among children and pregnant women while this percentage falls to 23 in young adults [23]. According to studies of the Endocrinology Research Center of Tehran University of Medical Sciences, the prevalence of this problem is in oscillation from 40 to 80 percent in different parts of Iran [24]. Some of the studies have shown the relation between the level of vitamin D and the success rate of fertility in couples treated with ART, in a way that women with vitamin D deficiency have shown a lower fertility rate compared to women who had higher vitamin D levels [25, 26]. It has been shown in some studies that women with higher levels of vitamin D in serum and follicular fluid will have significantly higher clinical pregnancy after IVF and ET and high levels of vitamin D are significantly associated with better parameters of controlled ovarian stimulation [12]. But other studies have mentioned the lack of significant effect of vitamin D in the follicular fluid and blood in predicting outcomes related to reproductive techniques [27]. Thus, the objective of our study is to evaluate the companionship of vitamin D level in serum and follicular fluid with follicular response in infertile women undergoing ART treatment.

Materials and Methods

This is a cross-sectional study. Sampling has been carried out using census method and includes all women with infertility undergoing IVF or ICSI who referred to infertility center of Fatemeh Zahra of Babolin 2015 during the period of 8 months (October to March). Inclusion criteria included age between 18 and 40, normal Hormone, thyroid and prolactin tests and exclusion criteria included Cancellation of treatment cycles, performing ART for genetic identifications, underlying medical condition such as heart, liver and kidney diseases and the use of drugs that interfere with the metabolism of vitamin D.

Written consent is initially signed by infertile women after explaining the method for women eligible for study inclusion and demographic data are collected by questionnaire. Then, infertile women will be placed under treatment for ovulation induction according to the protocol of infertility center of Fatemeh Zahra (Long Protocol) which is in from of prescribed Buserelin acetate (Cinnagen, Iran) with the initial dose of 0.5mg / d in mid luteal phase which is reduced to 0.25mg / d after occurrence of menses and completion of pituitary suppression and will continue until the day of oocyte retrieval. Gonal F (Cinnagen, Iran) starts from the second day of menses based on age and the number of antral follicles and their dose is regulated by monitoring Estradiol and TVS. HCG (Daroupakhsh, Iran) is administered at a dose of IU10000 when the size of follicle reaches to 16-18 mm and TVS guide of ovaries are removed 34 to 36 hours later. Follicular fluid is taken from follicles over 14 mm and heparinized blood sample of these women is taken on the same day to measure the level of 25-hydroxy vitamin D. Blood samples and follicular fluid sample are placed at -80 ° centigrade after centrifugation and sent to the laboratory on a weekly basis. Vitamin D measurement is done by immunoassay method using the bioactiva kit. It should be noted that Serum vitamin D levels less than 20ng / dl are considered as deficient levels of vitamin D and levels between 20-30ng / dl are considered as inadequate levels and levels higher than 30ng / dl are considered as adequate levels of vitamin D according to the Endocrine Society [27]. Analysis of the data was done using the SPSS19 application and descriptive statistical indexes including frequency and average and chi-square test was used to compare qualitative variables between the two groups.

Results

82 patients entered the study from whom 1 subject was excluded (due to corruption of test and unwillingness of the patient for re-testing). Finally, 81 infertile patients were evaluated. The average weight and BMI and type of infertility and cause of infertility and average FSH LH hormone did not have significant difference in groups with inadequate levels of vitamin D and vitamin D deficiency and adequate levels of vitamin D (Table 1).

Table 1: Demographic and hormonal characteristics of sampled patients

	Patient Groups (serum vitamin D, ng/ml)			
	Vit D < 20	20 ≤ D < 30.0	D ≥ 30.0	P value
Age (year)	33±4.99	31.63±4.82	35.14±2.61	0.27
BMI (kg/m^2) [a]	27.61±3.70	27.78±5.27	27.48±1.15	.98
Infertility				.32
Primary	41(70.7%)	14(87.5%)	4(57.1%)	
Secondary	17(29.3%)	2(12.5%)	3(42.9%)	
Infertility cause				
Unexplained	19(32.8)	4(25)	0	
Tubal factor	9(15.5)	0	1(14.3)	
Ovulatory disorder	4(6.9)	1(6.3)	2(28.6)	.18
Male factor	21(36.2)	7(43.8)	2(28.6)	
Uterine malformation	1(1.7)	1(6.3)	0	
Mix	21(36.2)	7(43.8)	2(28.6)	
Hormonal assessment				
FSH (mlu/ml)	6.88±3.12	6.88±2.87	8.88±6.48	.34
LH(mlu/ml)	5.44±3.41	6.04±3.50	6.17±8.65	.81

Values are reported as mean±SD or n (%)

40 patients had number of follicles less than 5 (49.4%), 32 patients had between 5 to 10 (39.5%) and 9 patients had more than 10 (11.1%). The average number of oocytes was 6.62 ± 4.98. The average serum levels of vitamin D was 15.12 ± 13.14 and the average vitamin D follicular fluid was 23.32 ± 13.54.

Pearson correlation test showed a significant positive relation between serum and follicular levels of vitamin D (rp=0.67, p<0.001) (Figure 1 - next page).

Correlation obtained between the number of oocytes and levels of vitamin D in the serum was not statistically significant (rp=-0.028, p=0.8) (Figure 2). Pearson correlation between the number of oocytes and levels of vitamin D in follicular fluid did not show a significant relation (rp=0.029, p=0.79) (Figure 3). Kendall and Spearman correlations between the number of follicles and serum vitamin D levels were not statistically significant (rc=0.078, p=0.36)(rs=-0.098, p=0.38) (Figure 4). Kendall and Spearman correlations between the number of follicles and follicular fluid vitamin D levels were not statistically significant (rc=0.014, p=0.88)(rs=-0.014, p=0.88) (Figure 5).

Figure 1: Correlation of serum and follicular fluid vitamin D

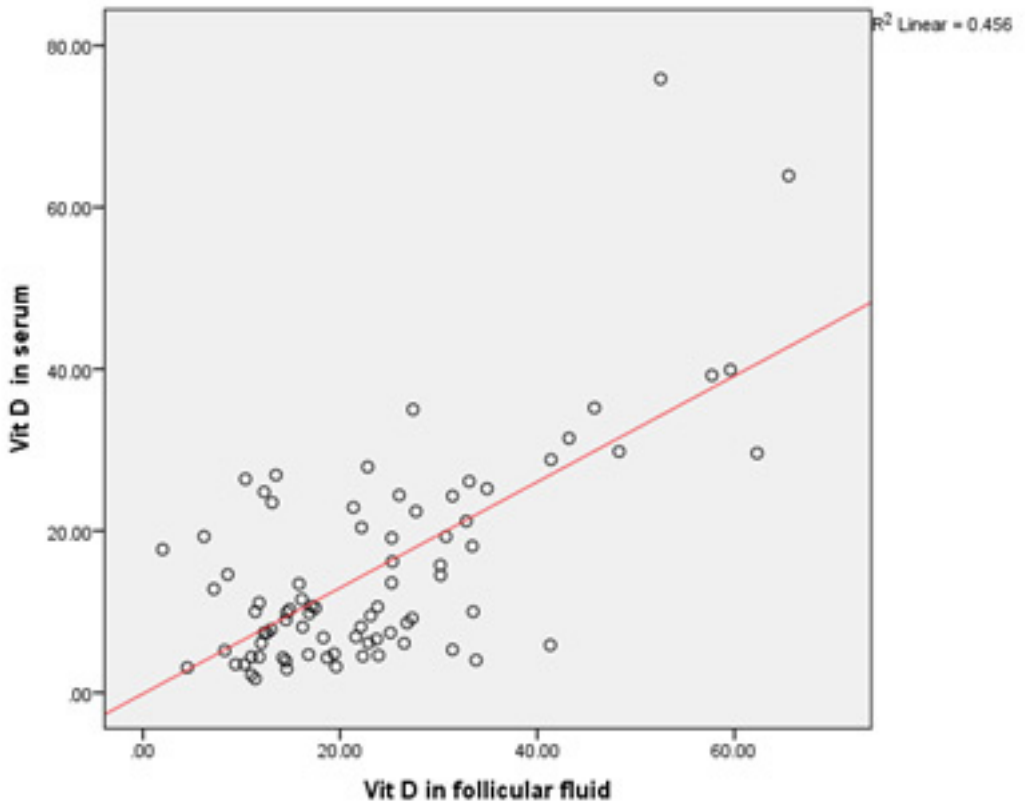


Figure 2: Correlation of oocytes numbers and serum vitamin D

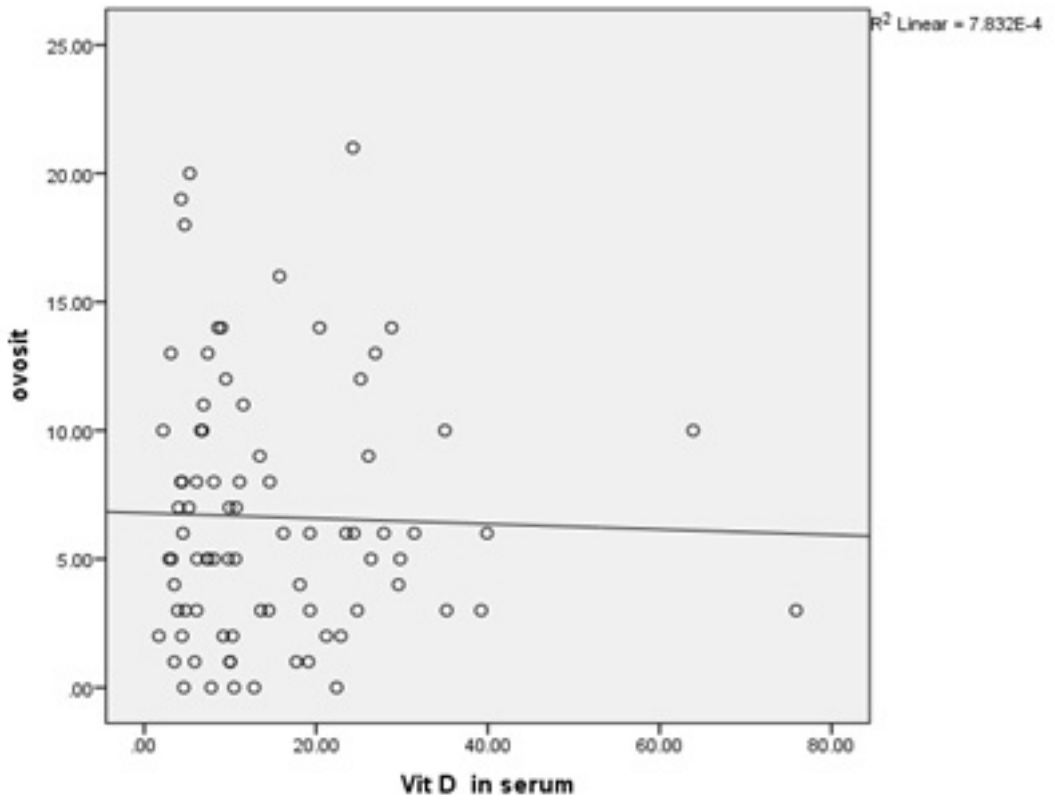


Figure 3: Correlation of oocytes numbers and follicular fluid vitamin D

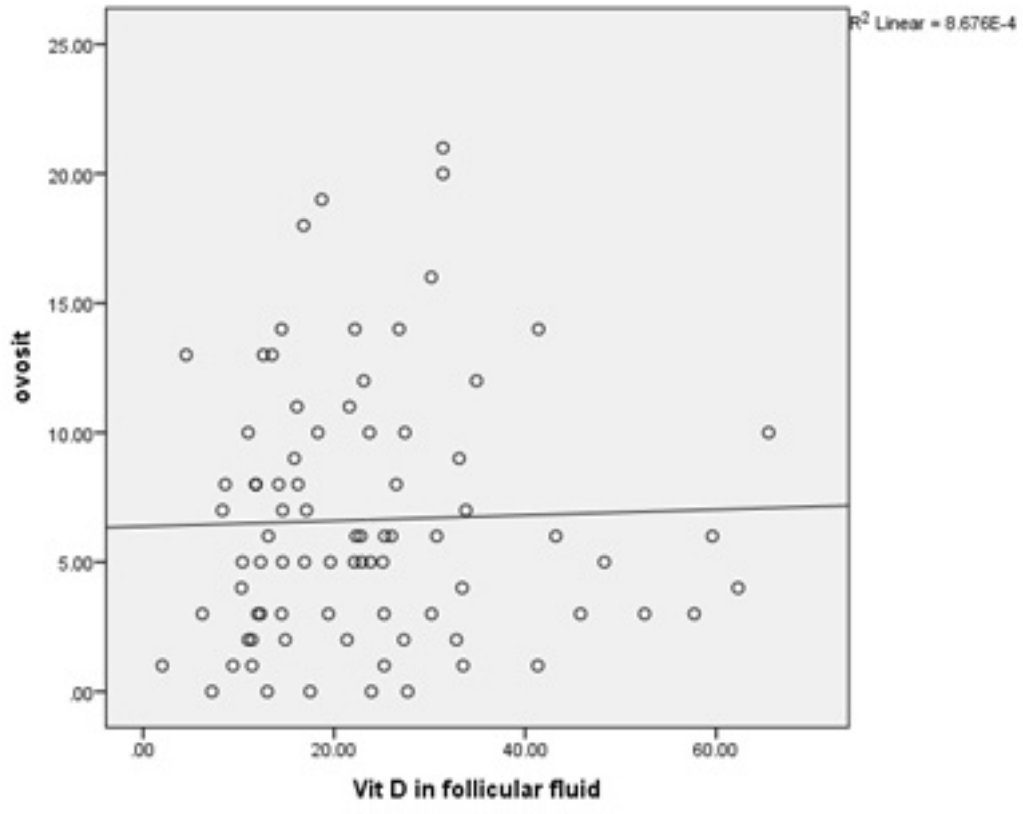


Figure 4: Correlation of follicle number and serum vitamin D

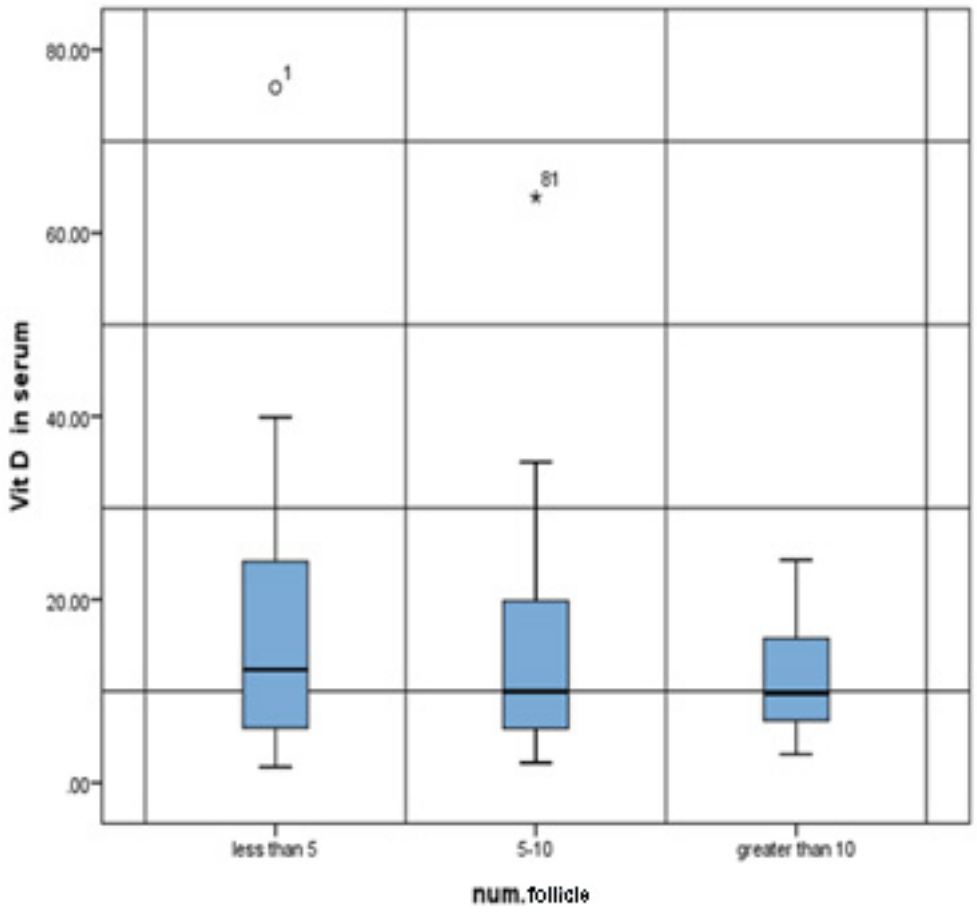
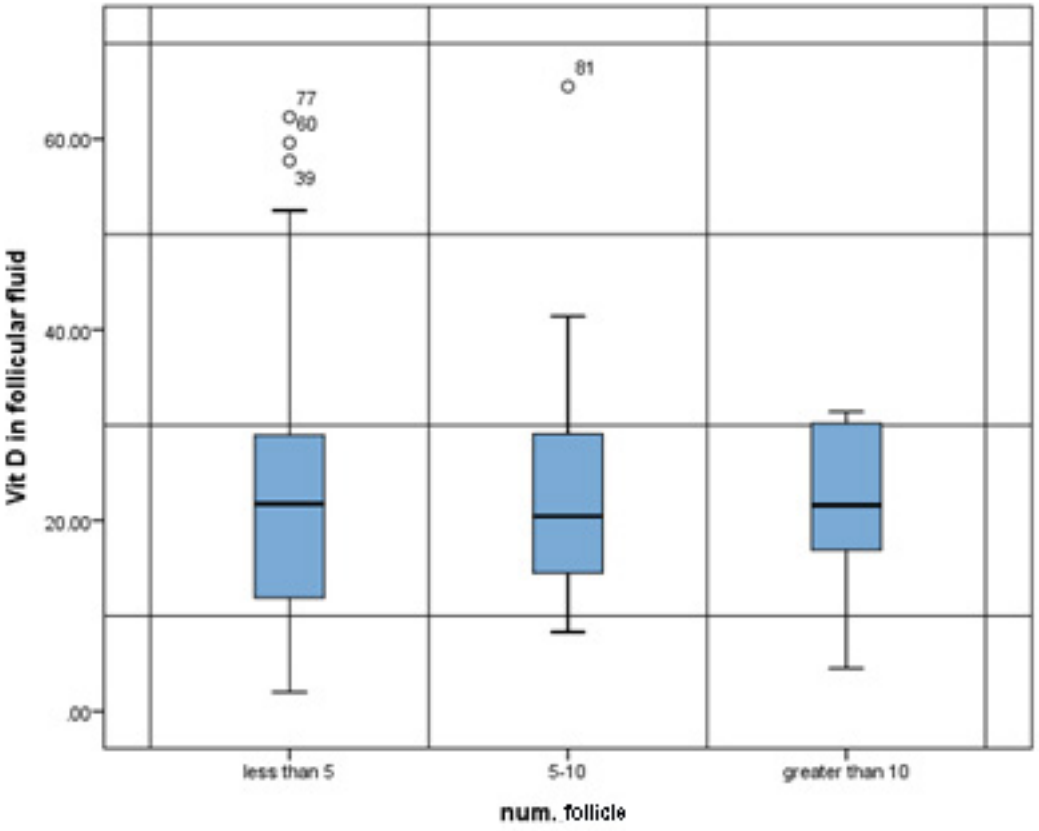


Figure 5: Correlation of follicle number and follicular fluid vitamin D



Discussion

The present study has been carried out in order to determine the possible role of serum and follicular fluid vitamin D levels on follicular response in 81 infertile women referred to infertility center of Fatemeh Zahra in Babol during 8 months (from August to March of2015). The results of our study showed a 72 percent prevalence of vitamin D deficiency in infertile women and only 8% of patients had adequate levels of vitamin D. It was determined in a study carried out by Garabedian and colleagues on 173 infertile women that 55% of them had vitamin D deficiency [15]. Rudick and colleagues examined the effects of vitamin D on the clinical outcome of IVF in another study, out of 188 infertile women, 109 patients (58%) suffered from a lack of vitamin D (with levels less than 30ng / ml) [28]. Vitamin D status is different among various communities which can be due to different factors affecting the level of vitamin D. Seasons, geographic location, dressing, using sunscreen and skin pigmentation in people are among these factors [29].

Some of the hormones and metabolites are effective on the number and maturation of oocytes which are supplied by follicular fluid. Follicular fluid is also an important environment for oocyte development. Increased or decreased compositions of this fluid affect the number and quality of oocytes and embryo. A part of the composition of follicular fluid originates from serum and metabolic changes in serum may affect the biochemical compounds of follicular fluid. Thus, growth and maturation of follicle are affected by metabolite concentrations of blood [30, 31]. Our study also showed a significant positive correlation between serum vitamin D levels and follicular fluid vitamin D levels which was similar to the results obtained from the study of Firouzabadi and Anifandis [32, 33].

The results of our study showed that different concentrations of vitamin D in serum and follicular fluid do not have significant relation with the number of ovarian follicles and oocytes. A few studies have examined the serum and follicular fluid vitamin D levels at the same time and most of the studies have only evaluated the serum level of vitamin D or follicular fluid level of vitamin. In the study of Farzadi and his colleagues, no significant relation was observed between the follicular fluid vitamin D levels and number and quality of oocytes but follicular fluid levels of vitamin D had a significant positive correlation with the level of implantation and IVF results [34]. In the study of Polyzos and colleagues, no significant relation was observed between vitamin D levels and fertility and there was also no relation between serum vitamin D deficient and ovarian reserve and ovarian response to ovulation induction [25]. Anifandis and colleagues also did not find any relation between the follicular fluid levels of vitamin D and the number of oocytes in their study which examined the effect of vitamin D level and glucose level follicular fluid [33]. Only in a study which compared the serum vitamin D level with follicular fluid similar to our study, Ozkan and his colleagues found out by evaluating the infertile women undergoing IVF that higher levels of 25-hydroxyvitamin D in serum and follicular fluid are associated with higher rates of clinical pregnancy after IVF and high levels of

of vitamin D in serum and follicular fluid are significantly associated with better parameters of controlled ovarian stimulation [35]. Rudick and colleagues confirmed that vitamin D status is associated with the success of IVF in non-Hispanic white women in populations with high ethnic diversity but no beneficial effects of adequate levels were observed among the Asian population and in fact, vitamin D is inversely related to IVF success [28]. This matter probably could explain the results of our study.

Conclusion

The present study showed that there is a significant correlation between serum vitamin D levels and follicular vitamin D levels but there is no relation between serum and follicular vitamin D levels and the number of oocytes and ovarian follicles in infertile women.

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