Magna Scientia Advanced Research and Reviews

eISSN: 2582-9394

Cross Ref DOI: 10.30574/msarr

Journal homepage: https://magnascientiapub.com/journals/msarr/

(RESEARCH ARTICLE)

Relationship between serum vitamin D levels and pregnancy rate of women with recurrent implantation failure undergoing IVF

Bita Saifi [1,*], Zahra Zandieh [2] and Mahsa Jalali [3]

[1] *Department of Basic Sciences, Faculty of Medicine, Mashhad Medical Sciences, Islamic Azad University, Mashhad, Iran.*
[2] *Department of Anatomy, School of Medicine, Iran University of Medical Sciences, Tehran, Iran.*
[3] *Armaghan Infertility and IVF Center, Armaghan Clinic, Mashhad, Iran.*

Magna Scientia Advanced Research and Reviews, 2023, 07(01), 049–056

Publication history: Received on 02 January 2023; revised on 12 February 2023; accepted on 14 February 2023

Article DOI: https://doi.org/10.30574/msarr.2023.7.1.0026

Abstract

Objective: Vitamin D deficiency has been shown to be associated with pregnancy complications due to the effect on implantation. However, the effect of vitamin D on the clinical outcomes of IVF in women with recurrent implantation failure is unclear. Present study aimed to investigate the effect of vitamin D status in recurrent implantation failure (RIF) patients undergoing in vitro fertilization (IVF) cycles.

Methods: A cross-sectional study was carried out on 52 women with RIF undergoing IVF treatments from 2018 to 2019. Blood samples were collected from participant after 2 weeks of embryo transfer and then serum 25-OH vitamin D levels were measured using enzyme-linked immunosorbent assay (ELISA) method. Clinical pregnancy and patients' characteristics were evaluated.

Results: The incidence of vitamin D deficiency, insufficiency and sufficiency among the studied population were 7.7%, 73.1% and 19.2%, respectively. Vitamin D levels were significantly associated with clinical pregnancy in patients with ≥30 years old, being overweight, duration of marriage and infertility duration ≥5 years and having history of abortion. By adjusting confounding factors in the multivariate analysis, we found a significant association between vitamin D levels and clinical pregnancy in RIF patients (p=0.007).

Conclusion: There is a significant correlation between vitamin D levels and clinical pregnancy rate in RIF woman undergoing IVF. However, more randomized, controlled trials are suggested.

Keywords: Vitamin D; *In vitro* fertilization; Pregnancy; Recurrent abortion

1. Introduction

Vitamin D indicates a vital function in the human physiology and its deficiency is associated with the increased risk of several cancers, cardiovascular, auto-immune and metabolic diseases, infections and obstetric complications [1]. Vitamin D3 (cholecalciferol), the most common form of vitamin D in the body is produced in the skin under the effect of sunlight [2]. Vitamin D levels are usually evaluated by measurement of serum concentration of active form 25-hydroxy vitamin D (25(OH)D) [2]. Recent investigations revealed that vitamin D effects the female reproduction health both in animals and humans [1]. Vitamin D involves in the induction of ovarian steroidogenesis and 1,25(OH) 2 D promotes production of progesterone, estradiol and estrone production [3]. This fat-soluble vitamin also functions in the endometrial development and initial implantation of embryo since expression of vitamin D receptors and metabolizing enzymes have been observed in the reproductive tissues [2, 4]. Vitamin D receptors have been found in the placenta,

* Corresponding author: Bita Saifi; Email: bita_saifi@yahoo.com

endometrium, ovaries, fallopian tubes, epithelial cells, decidua, hypothalamus and pituitary glands; hence vitamin D plays an important role in the oocyte development, anti-Mullerian (AMH) production and endometrial receptivity [5]. Some studies have shown that trophoblasts early in pregnancy respond to vitamin D. Vitamin D affects the inflammatory response locally, regulates genes involved in the implantation and induces decaudation for a successful pregnancy [6]. Pregnant women with vitamin D deficiency have pregnancy complications including preeclampsia, bacterial vaginosis, preterm labor and poor placentation [2, 5]. According to the statistics, almost 1 billion people have vitamin D deficiency and insufficiency globally, and 36 % of women in reproductive age have low vitamin D levels [3]. It has been shown that about 15 % of couples have infertility, and infertile women have vitamin D deficiency [3]. In vitro fertilization (IVF) is a common treatment for infertility [3].

Recurrent implantation failure (RIF) is characterized by failure in achieving clinical pregnancy following transferring at least six high quality embryos in ≥3 embryo transfer cycles [7]. The incidence of RIF in women undergoing IVF treatments is about 10%. Several factors may involve in the pathogenesis of RIF such as advanced maternal age, chromosomal factors, hormonal disorders, uterine abnormalities, infections, and maternal immunological factors [8].

Vitamin D, owing to its potential effect on the implantation, may have a significant function in the determination of assisted reproductive technology (ART) outcomes, particularly considering the high prevalence of vitamin D deficiency among women in reproductive age and those undergoing ART treatments [2, 4]. There are conflicting results regarding the association between vitamin D status and IVF outcomes [4]. Paffoni et al. showed that the adjusted odds ratio of 2.15 for clinical pregnancy rate in women with vitamin D > 20 ng/ml [1]. Furthermore, women with adequate vitamin D levels demonstrate higher rates of successful in vitro fertilization (IVF) [1]. Moreover, a previous study indicated that vitamin D regulates expression of genes involved in the endometrial receptivity [1]. Another study indicated that vitamin D status had no effect on the ongoing pregnancy rates [4]. Therefore, considering the effects of serum vitamin D levels on the fertility and contradictory results in this field and due to the genetic differences between the population of Iran and other populations, we aimed to investigate the association between serum vitamin D levels and clinical pregnancy rate in the women with RIF undergoing IVF.

2. Material and methods

2.1. Study design

This study was approved by the Ethics Committee of Mashhad Islamic Azad University (IR.IAU.MSHD.REC.1398, 024) and conducted according to Declaration of Helsinki 2002 principles. Information about each patient was recorded confidentially and suspiciously on the checklist with special code. After sinning informed consent participants enrolled to the study. In this cross-sectional study 52 women with RIF undergoing IVF treatments who referred to Armaghan Infertility between 2018 to 2019 were included. Demographic information including age, body mass index (BMI) were collected. Participants were women under 40 years old who referred to Armaghan Infertility due to male infertility. Excluding criteria were cervical or uterine abnormalities, chromosomal abnormalities, infectious diseases, congenital thrombophilia, history of polycystic ovary syndrome, autoimmune disease in men or women, endometriosis and women with a history of fetal biopsy. Participants underwent standard IVF protocol. Gonadotropin releasing hormone (GnRH) agonist or antagonist was used for pituitary suppression. Ovarian stimulation was performed using recombinant FSH (rFSH) alone or rFSH combined with recombinant luteinizing hormone (LH). When 2-3 follicles reached a diameter of 17–18 mm, oocyte maturation was induced with subcutaneous injection of human chorionic gonadotropin (hCG) (5000 or 10000) on day 2-3 of menstrual cycle. Oocytes were retrieved transvaginally 36 hours after trigger injection. Embryo transfer was performed on day 3 following oocyte retrieval. Two weeks after embryo transfer, βhCG test was carried out and then, 5 cc of heparinized blood was taken from participants. Serum levels of vitamin D was measured by ELISA method using 25 OH Vit D IDEAL TASHKHIS KIT (VIDAS@VITD, France, Marcy). Clinical pregnancy was defined as observation of at least one gestational sac on ultrasound at around 7 week's gestation with detectable heartbeat activity.

2.2. Statistical analysis

The results are shown as mean ± SD. Normality of the data was assessed using Shapiro-Wilk test and then Student's t test was used to analyze continuous variables. Logistic regression was used to examine the relationship between all variables. SPSS v 26 was used for data analysis and the significance level of the tests was considered <0.05.

3. Results

Patients' characteristics are demonstrated in Table 1. As shown in Table 1, the mean age of participants was 31.94±4.62 years old and almost had normal BMI (25.54±2.12 kg/m^2).

Table 1 Patients characteristics undergoing IVF treatment

Variable	N	%	MD±SD	Mix	Min
Age (years)			31.94±4.62	40	21
BMI (kg/m^2)			25.54±2.12	29.38	19.57
Duration of marriage (years)			6.44±3.25	16	1
Duration of infertility (years)			3.75±2.05	11	1
Number of abortions					
0	35	67.3			
1	17	32.7			
Number of previous IVF treatments					
0	27	51.9			
1	20	38.5			
2	3	5.8			
3	2	3.8			
Number of successful IVF					
0	47	90.4			
1	5	9.6			
Number of unsuccessful IVF					
0	29	55.8			
1	20	38.5			
2	2	3.8			
3	1	1.9			
Vitamin D levels (ng/mL)			20.37±8.12	40	6
IVF result (clinical pregnancy)					
-	28	53.8			
+	24	46.2			

Values are mean ± standard deviation or number of patients (%), BMI: body mass index, IVF: in vitro fertilization.

Furthermore, among participants, 24 individuals had successful IVF (46.2%). The mean serum concentration of vitamin D 15 days after IVF was 20.37±8.12 ng/ml which means 7.7% had vitamin D deficiency, 73.1% had insufficient levels and 19.2% had normal levels (Fig. 1).

Moreover, there was a significant association between clinical pregnancy and vitamin D levels (p=0.003), so that the mean vitamin D levels in patients with positive IVF result (23.88±7.86) was remarkably higher than those with negative result (17.34±7.18). Table 2 shows association between vitamin D levels and clinical pregnancy in different age and BMI groups. As depicted in Table 2, there were significant association between clinical pregnancy and vitamin D levels in patients ≥30 years old and those with overweight (p<0.0001 and p=0.03, respectively).

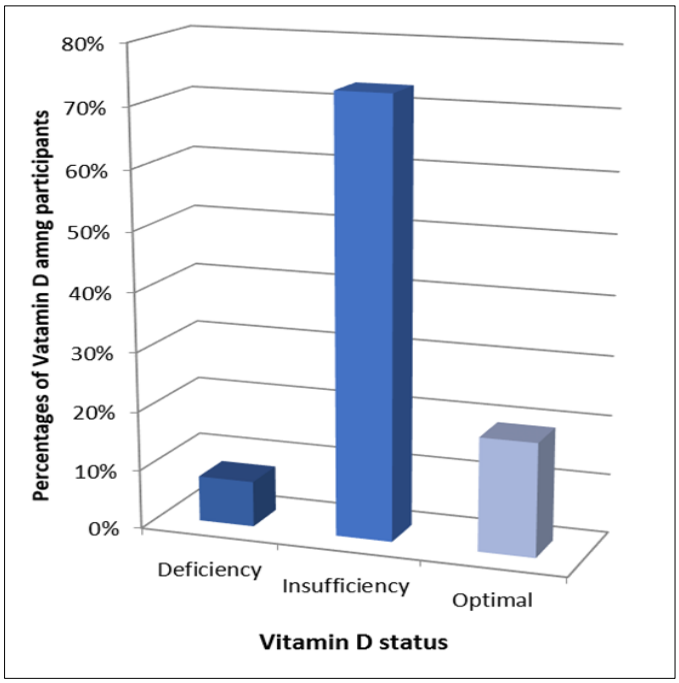

Figure 1 Vitamin D status among women with RIF undergoing IVF

Table 2 Association between vitamin D levels and IVF results (clinical pregnancy) in different groups of age and BMI

Variable	Mean±SD	Max	Min	P value
Age <30(years)				
IVF -	19.20±9.07	38	6	0.5
IVF+	16.83±7.44	27	9	
Age ≥30(years)				
IVF -	16.31±5.93	30	8	p<0.0001
IVF+	26.22±6.64	40	19	
Normal BMI (kg/m²)				
IVF -	18.54±8.04	38	6	0.05
IVF+	25.82±8.73	40	11	
Overweight (kg/m²)				
IVF -	16.44±6.58	30	8	0.03
IVF+	22.23±6.98	31	9	

Values are mean ± standard deviation, BMI: body mass index, IVF: in vitro fertilization

Moreover, association between vitamin D levels and IVF results in different groups of sociodemographic and clinical characteristics of the patients are summarized in Table 3. We found significant associations between vitamin D levels and clinical pregnancy in patients with duration of marriage ≥5 years, infertility duration ≥5 years, having history of abortion and IVF.

Table 3 Association between vitamin D levels and IVF results (clinical pregnancy) considering different sociodemographic and clinical characteristics of the participants

Variable	Mean±SD	Max	Min	P value
Duration of marriage <5 (years)				
IVF -	19.21±5.71	30	12	0.1
IVF+	24±7.75	12	11	
Duration of marriage ≥5 (years)				
IVF -	16.71±7.62	38	6	0.01
IVF+	23.80±8.20	40	9	
Infertility duration <5 (years)				
IVF -	18.36±7.53	38	8	0.1
IVF+	22.37±7.55	39	9	
Infertility duration ≥5 (years)				
IVF -	14.29±5.35	23	6	0.004
IVF+	29.60±6.88	40	21	
No history of abortion				
IVF -	19.31±8.05	38	6	0.1
IVF+	23.35±8.91	40	9	
History of abortion				
IVF -	13.80±3.26	19	10	P<0.0001
IVF+	25.14±4.78	30	19	
No history of IVF				
IVF -	16.93±5.81	29	9	0.05
IVF+	21.75±6.55	30	11	
History of IVF				
IVF -	17.81±8.72	38	6	0.02
IVF+	26±8.75	40	9	
Embryo transfer time				
3 days				
IVF -	17.14±6.95	38	6	0.004
IVF+	23.78±8.03	40	9	

Values are mean ± standard deviation, IVF: in vitro fertilization.

Logistic regression was used to investigate the effect of different variables on the IVF results. The results of logistic regression model show that by controlling the intervening variables, there is still a statistically significant relationship between serum vitamin D levels and clinical pregnancy (p=0.007, Table 4).

Table 4 Factor associated with clinical pregnancy in logistic regression analysis

Variable	B	SE	Wald χ^2	P value
Age (years)	-0.039	0.076	0.265	0.6
BMI (kg/m^2)	-0.079	0.170	0.177	0.6
Duration of marriage (years)	-0.217	0.161	1.805	0.1
Duration of infertility (years)	0.121	0.243	0.246	0.6
History of abortion	-0.0616	0.879	0.492	0.4
History of previous IVF	-0.0161	0.689	0.054	0.8
Vitamin D levels	0.133	0.050	7.224	0.007
Constant	-1.914	5.250	0.133	0.7

B, regression coefficient; SE, standard errors of regression coefficient; IVF, in vitro fertilization; BMI, body mass index

4. Discussion

Based on this cross-sectional study, the incidence of vitamin D deficiency among RIF women undergoing IVF treatments was 7.7% and vitamin D insufficiency is highly prevalent among these patients. Moreover, vitamin D levels was significantly associated with clinical pregnancy in the RIF patients.

Numerous studies have been performed to evaluate the correlation between vitamin D status and IVF outcomes, however the results are strongly contradictory [9-12]. Since 2018 till now, 5 meta-analysis studies have assessed the effect of vitamin D levels on the IVF outcomes [5, 9-12]. Chu et al. by reviewing 11 cohort studies indicated that women with sufficient vitamin D levels have greater live birth rate and chances of ART success compared to those with deficient or insufficient groups suggesting the impact of vitamin D in implantation [12]. However, they did not find a significant difference between sufficient and deficient groups for clinical pregnancy and chance of miscarriage which is against our result [12]. Furthermore, Zhao et al., Shen et al. and Cozzolino studies identified no association between vitamin D levels and IVF outcomes [5, 10, 11]. Lliuta et al. examined 3 meta-analysis studies including Zhao et al. and Chu et al. studies which was concocting of 15 cohort studies on 3711 women undergoing IVF treatments [9]. Women were divided into 3 groups of sufficient, deficient and insufficient based on the vitamin D levels. Then comparison was performed between the groups. The results showed that considering autologous and donor oocyte cycles, women in sufficient groups had higher ongoing pregnancy, biochemical pregnancy, live birth rates compared to the deficient group. Therefore, they concluded that better IVF outcomes is associated with the sufficient vitamin D levels [9]. In 2019, Chu et al. performed a cohort study on 500 women undergoing ART treatments. They find that live birth rate and clinical pregnancy and biochemical pregnancy rates are significantly associated with serum vitamin D levels. However, by adjusting confounding factors in multivariate analysis, results lost their statistical significance [2]. Indeed, most of the studies emphasize that high circulating vitamin D concentration is considerably associated with a better reproductive outcome [5, 9, 13-15]. However, when confounding factors were modified in multivariate analysis, no significant relationship can be observed between vitamin D deficiency and IVF outcomes [2, 3, 5, 9, 10]. In line with our finding Polyzos, Rudick, Paffoni and indicated a remarkable correlation between pregnancy rates and vitamin D deficiency [14, 16]. These discrepancies might be related to the differences in type of study, definitions of clinical pregnancy, analysis methods, number of embryos transferred, seasonal variations of vitamin D levels, age, ethnicity (due to vitamin D receptor gene polymorphisms), race and BMI of the studied patients, [3-5, 9, 11, 17]. In contrast to the previous study, present study was conducted on RIF women undergoing IVF. To best of our knowledge this is the first study evaluating effect of vitamin D levels on the clinical pregnancy in RIF patients undergoing IVF treatments. It has been shown that maternal vitamin D status may contributes to the development of RIF. Vitamin D modulates maternal immune responses to the embryo implantation in the RIF patients [18]. Vitamin D insufficiency or deficiency in the RIF women enhances natural killer (NK) cell cytotoxicity, number of interferon gamma (IFN-γ) or tumor necrosis factor (TNF) producing Th cells, and number of CD68 + macrophages on the endometrial cells which are risk factor for the development of RIF. Therefore, RIF women with vitamin D deficiency display impaired immunological tolerance [18]. One limitation of our study should be considered is that due to the time limitation, number of patients participated in this study was limited.

Abbreviations

AMH: anti-Mullerian; ART: assisted reproductive technology; BMI: body mass index; ELISA: enzyme-linked immunosorbent assay; GnRH: gonadotropin releasing hormone; hCG: human chorionic gonadotropin; LH: luteinizing hormone; IFN-γ : interferon gamma; IVF: in vitro fertilization; NK: natural killer; rFSH: recombinant FSH; RIF: recurrent implantation failure; TNF: tumor necrosis factor.

5. Conclusion

In summary, present study highlights vitamin D deficiency or insufficiency is associated with the lower clinical pregnancy outcomes in RIF's women undergoing IVF. However, high-quality, randomized," controlled trial studies with large sample size are needed to confirm suggested relationship.

Compliance with ethical standards

Acknowledgments

We appreciate patients for participating in this study.

Disclosure of conflict of interest

No conflict of interest.

Author contribution

N.A: acquisition of data and data analysis and approved the final version of manuscript, M.J: Performing experiments and approved the final version of manuscript, B.S: conception and design, supervision of the project, revising manuscript critically for important intellectual content and approved the final version of manuscript.

Statement of ethical approval

This study was approved by the Ethics Committee of Mashhad Islamic Azad University (IR.IAU.MSHD.REC.1398, 024) and conducted according to Declaration of Helsinki 2002 principles.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study."

References

[1] Paffoni A, Somigliana E, Sarais V, Ferrari S, Reschini M, Makieva S, Papaleo E, Viganò P. Effect of vitamin D supplementation on assisted reproduction technology (ART) outcomes and underlying biological mechanisms: protocol of a randomized clinical controlled trial. The "supplementation of vitamin D and reproductive outcome"(SUNDRO) study. BMC Pregnancy Childbirth. 2019;19(1):1-9.

[2] Chu J, Gallos I, Tobias A, Robinson L, Kirkman-Brown J, Dhillon-Smith R, Harb H, Eapen A, Rajkhowa M, Coomarasamy A. Vitamin D and assisted reproductive treatment outcome: a prospective cohort study. Reproductive health. 2019;16(1):1-10.

[3] Lv SS, Wang JY, Wang XQ, Wang Y, Xu YJAog. Serum vitamin D status and in vitro fertilization outcomes: a systematic review and meta-analysis. Archives of gynecology and obstetrics. Arch Gynecol Obstet. 2016;293(6):1339-45.

[4] Ha AN, Pham TD, Vuong LNJF. Association between Vitamin D Levels and Fertility Outcomes in Patients Undergoing IVF/ICSI. Fertility & Reproduction. 2020;2(03):85-92.

[5] Zhao J, Huang X, Xu B, Yan Y, Zhang Q, Li YJRB. Whether vitamin D was associated with clinical outcome after IVF/ICSI: a systematic review and meta-analysis. Reprod Biol Endocrinol. 2018;16(1):1-7.

[6] Ganguly A, Tamblyn JA, Finn-Sell S, Chan S-Y, Westwood M, Gupta J, Kilby MD, Gross SR, Hewison M. Vitamin D, the placenta and early pregnancy: effects on trophoblast function. J Endocrinol. 2018;236(2):R93-R103.

[7] Bashiri A, Halper KI, Orvieto RJRB, Endocrinology. Recurrent Implantation Failure-update overview on etiology, diagnosis, treatment and future directions. Reprod Biol Endocrinol. 2018;16(1):1-18.

[8] Sun Y, Zhang Y, Ma X, Jia W, Su YJFiE. Determining diagnostic criteria of unexplained recurrent implantation failure: A retrospective study of two vs three or more implantation failure. Front Endocrinol. 2021:851.

[9] Iliuta F, Pijoan JI, Lainz L, Exposito A, Matorras RJHF. Women's vitamin D levels and IVF results: a systematic review of the literature and meta-analysis, considering three categories of vitamin status (replete, insufficient and deficient). Hum Fertil. 2020:1-19.

[10] Cozzolino M, Busnelli A, Pellegrini L, Riviello E, Vitagliano AJF. How vitamin D level influences in vitro fertilization outcomes: results of a systematic review and meta-analysis. Fertil Steril. 20;114(5):1014-25.

[11] Shen C, Wang L, Wu X, Mao S, Fang CJC E. The relationship between vitamin D and IVF: a systematic review and meta-analysis. CLIN EXP OBSTET GYN. 2019;46(1):12-5.

[12] Chu J, Gallos I, Tobias A, Tan B, Eapen A, Coomarasamy AJHr. Vitamin D and assisted reproductive treatment outcome: a systematic review and meta-analysis. Hum Reprod.2018;33(1):65-80.

[13] Paffoni A, Ferrari S, Viganò P, Pagliardini L, Papaleo E, Candiani M, Tirelli A, Fedele L, Somigliana E. Vitamin D deficiency and infertility: insights from in vitro fertilization cycles. J Clin Endocrinol Metab. 2014;99(11):E2372-E6.

[14] Rudick BJ, Ingles SA, Chung K, Stanczyk FZ, Paulson RJ, Bendikson KAJF. Influence of vitamin D levels on in vitro fertilization outcomes in donor-recipient cycles. Fertil Steril. 2014;101(2):447-52.

[15] Environment and Reproductive Health Study Team Abadia Laura Gaskins Audrey J http://orcid. org/0000-0002-0904-7855 Chiu Yu-Han Williams Paige L Keller Myra Wright Diane L Souter Irene Hauser Russ Chavarro Jorge E jchavarr@ hsph. harvard. edu. Serum 25-hydroxyvitamin D concentrations and treatment outcomes of women undergoing assisted reproduction. AJCN. 2016;104(3):729-35.

[16] Polyzos NP, Anckaert E, Guzman L, Schiettecatte J, Van Landuyt L, Camus M, Smitz J, Tournaye H. Vitamin D deficiency and pregnancy rates in women undergoing single embryo, blastocyst stage, transfer (SET) for IVF/ICSI. Hum Reprod. 2014;29(9):2032-40.

[17] Laganà A, Vitale S, Ban Frangež H, Vrtačnik-Bokal E, D'anna RJERMPS. Vitamin D in human reproduction: the more, the better? An evidence-based critical appraisal. Eur Rev Med Pharmacol Sci. 2017;21(18):4243-51.

[18] Chen X, Diao L, Lian R, Qi L, Yu S, Liu S, Lin S, Xue Z, Zeng Y. Potential impact of maternal vitamin D status on peripheral blood and endometrium cellular immunity in women with recurrent implantation failure. Am J Reprod Immunol.2020;84(1):e13243.