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Original Article

Preconception Vitamin D Level and In Vitro Fertilization: Pregnancy Outcome

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ABSTRACT

Objective: Vitamin D deficiency impairs female fertility and the success of in vitro fertilization (IVF). The recommended serum 25-hydroxyvitamin D (25(OH)D) level in IVF-conceived pregnancies is still debated. We aimed to explore the relationship of the preconception serum 25(OH)D level with pregnancy outcome following IVF treatment. We also explored the utility of the currently recommended serum 25(OH)D cutoff of ≥50 nmol/L for women undergoing IVF therapy.
Methods: Retrospective cohort of women who had undergone IVF therapy. Of the women who started IVF therapy (n = 354), 218 completed the study. They were divided into 2 groups: (1) women who achieved a successful pregnancy (pregnant group, n = 160) and (2) those who did not achieve a successful pregnancy (nonpregnant group, n = 58). Preconception serum samples were analyzed for reproductive hormones, fasting glucose, insulin, and 25(OH)D levels.
Results: Overall, the median (interquartile range) age, body mass index, and hemoglobin A1c level were 32 (6) years, 25.7 (7.4) kg/m^2, and 5.2% (0.6%), respectively. The 25(OH)D level was significantly higher at preconception in the pregnant group (56.4 [21.4] vs 47.9 [29.16] for nonpregnant, $P = .001$). The preconception 25(OH)D level was a significant predictor of IVF outcome (B = 0.04; 95% CI, 1.01-1.06; $P = .001$), with greater IVF success associated with a serum 25(OH)D level of ≥50 nmol/L (odds ratio, 0.46; $P = .01$).
Conclusion: Preconception 25(OH)D sufficiency (≥50 nmol/L) is associated with successful pregnancy outcome following IVF therapy.

Introduction

Vitamin D has effects on bone health, immunoregulation, and control of inflammation and plays a role in the regulation of female reproduction, with its receptors expressed in cells throughout the female reproductive tract, including the ovaries, placenta, and endometrium.[1-3] Vitamin D is implicated in the regulation of ovarian follicular growth and menstrual cyclicity through effects on steroidogenesis (including the production of estrogen and progesterone).[4,5] Indeed, the serum vitamin D level is correlated with the antimüllerian hormone level and, as such, can be used as an indicator of ovarian reserve.[4,6] Furthermore, vitamin D deficiency is associated with polycystic ovary syndrome and endometriosis, both of which are known risk factors for infertility.[7] The preconception serum vitamin D level is also correlated with the outcomes of assisted reproductive technologies possibly through effects on ovarian maturation, embryo quality, and implantation.[8,9]

In the general population, a serum vitamin D level of 30 to 50 nmol/L (12-20 ng/mL) is considered insufficient, whereas a level of ≤30 nmol/L (≤12 ng/mL) indicates vitamin D deficiency.[10] The U.S. Institute of Medicine recommends a vitamin D level of ≥50 nmol/L (≥20 ng/mL) in nearly all healthy individuals, whereas the

Abbreviations: BMI, body mass index; hCG, human chorionic gonadotropin; IVF, in vitro fertilization; 25(OH)D, 25-hydroxyvitamin D.

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Endocrine Society recommends a higher serum vitamin D level of ≥78 nmol/L (≥30 ng/mL) for overall health.[2] Although there is some consensus regarding the definition of vitamin D sufficiency within the general population, there remains contention regarding the optimal serum vitamin D level for successful pregnancy outcomes in women undergoing in vitro fertilization (IVF) therapy. To address this, we explored the relationship between the preconception serum vitamin D level and outcome of IVF treatment (pregnancy vs nonpregnancy). We also explored the validity of the currently recommended maternal preconception serum vitamin D level of ≥50 nmol/L for IVF-conceived pregnancies.

Highlights

- Vitamin D deficiency impairs female fertility and in vitro fertilization (IVF) success. The U.S. Institute of Medicine recommends a vitamin D level of ≥50 nmol/L (≥20 ng/mL) in nearly all healthy individuals, whereas the Endocrine Society suggests ≥78 nmol/L (≥30 ng/mL) for overall health
- The recommended vitamin D level is still debatable in IVF-conceived pregnancies
- Preconception maternal vitamin D sufficiency, including the recommended maternal cutoff of ≥50 nmol/L, is associated with a successful pregnancy outcome following IVF
- Initiating daily supplementation of vitamin D at preconception is important for all pregnant women, especially for those at risk (obese, limited sun exposure, vegetarian, and dark skin), to meet the optimal level

Clinical Relevance

The assessment and treatment of preconception maternal vitamin D deficiency (<50 nmol/L) are important before undergoing any infertility treatment as a means to improve in vitro fertilization and pregnancy outcomes.

Materials and Methods

Subjects and Procedure

A retrospective cohort study was conducted at IVF clinics in the United Arab Emirates. All women aged 18 to 39 years with a body mass index (BMI) of 18.5 to 38 kg/m^2 and who were undergoing their first IVF cycle were included. We recorded anthropometric data (body weight and height) and details from standard medical history questionnaires. The exclusion criterion was any chronic medical condition (eg, hepatic, renal, or cardiovascular disease). The study included the following2 groups of women who had undergone IVF treatment: (1) women with a successful pregnancy (pregnant group) and (2) those who did not achieve a successful pregnancy (nonpregnant group). The study complied with the code of ethics within the Declaration of Helsinki, and ethical approval was obtained from local authorities. Informed consent was sought from all participants prior to their inclusion in the study.

IVF Intervention

All women underwent controlled ovarian stimulation. IVF/intracytoplasmic sperm injection treatment was administered using a gonadotropin-releasing hormone antagonist protocol. Briefly, daily recombinant follicle-stimulating hormone was administered for 12 days, and a daily gonadotropin-releasing hormone antagonist injection (0.25 mg) was commenced from day 5 of stimulation. The ovarian follicle size and number were monitored with ultrasound scanning and serum measurement of blood test of female reproductive hormones (follicle-stimulating hormone, luteinizing hormone, estrogen, and progesterone). Recombinant human chorionic gonadotropin (hCG) (250 μg) was administered by injection for final maturation of follicles. Egg retrieval was scheduled for 36 hours later, and embryos were then transferred on day 5. Progesterone (oral tablet, 10 mg 3 times/d; injection, 50 mg/d; and vaginal pessary, 400 mg twice/d) and estrogen (oral tablet, 2 mg 3 times/d) therapies were administered after embryo transfer.

The first pregnancy test was conducted 10 days after embryo transfer with serum β-hCG. The first ultrasound scan was performed 4 weeks following a positive B-hCG result to detect a fetal heartbeat. A clinically confirmed pregnancy was based on a positive serum β-hCG test result in addition to a gestational sac observed on ultrasound scan. A negative β-hCG test result and/or no visible gestational sac represented an unsuccessful pregnancy outcome.[11] Unsuccessful pregnancy outcomes included “biochemical” pregnancies (confirmed by a positive β-hCG result but no visible sac detected on ultrasound) and ectopic pregnancies (confirmed by abnormal implantation of the embryo in an extrauterine location).[11] For the purposes of our study, we excluded biochemical and ectopic pregnancies.

Outcome Measures

Baseline fasting blood test results were obtained, including plasma glucose, serum insulin, female reproductive hormones, and 25-hydroxyvitamin D (25(OH)D). The plasma glucose level was measured by an enzymatic reference method with hexokinase-glucose-6-phosphate dehydrogenase. The reproductive hormone, insulin, and 25(OH)D levels were measured with an electrochemiluminescence immunoassay using Cobas E immunoassay analyzers (Roche Diagnostics). The homeostatic model assessment of insulin resistance was calculated as follows: (fasting plasma insulin level × fasting plasma glucose level)/405.[12]

Statistical Analysis

Data analysis was performed using the Statistical Package for Social Sciences software version 21.0 for Windows. Data were nonnormally distributed (identified using the Shapiro-Wilk test) and, therefore, presented as median and interquartile range. The baseline anthropometric and biochemical data from the 2 groups (pregnant vs nonpregnant) were compared using the nonparametric Mann–Whitney *U* test for 2 independent samples. Binary logistic regression predicted any association between the preconception serum 25(OH)D levels and IVF outcome (with the pregnant/nonpregnant outcome as a dependent variable) and adjusted for BMI and age variables. The association between a serum 25(OH)D cutoff of 50 nmol/L and IVF outcome was tested with the χ^2 test.

Results

Baseline Data

Of a total of 693 women who were screened, 339 either did not start IVF treatment (high baseline reproductive hormones as per physician) or did not have an embryo transfer (because of poor

Table 1
Comparison of Anthropometric, Metabolic, and Endocrine Parameters at Baseline and 12 Weeks of In Vitro Fertilization Treatment for Pregnant and Nonpregnant Women

Variables	Baseline		P value[a]
	Pregnant (n=160)	Nonpregnant (n=58)	
Age (y)	32.0 (7.0)	32.0 (7.0)	.5
Weight (kg)	65.5 (20.0)	64.2 (16.07)	.94
Body mass index (kg/m^2)	24.9 (7.38)	26.3 (5.82)	.06
Female hormones			
FSH (IU/L)	6.46 (2.53)	7.07 (2.67)	.1
LH (IU/L)	5.99 (3.21)	5.57 (2.8)	.27
Estrogen (pg/mL)	42.04 (24.0)	44.03 (22.04)	.81
Progesterone (ng/mL)	0.23 (0.23)	0.25 (0.22)	.46
Metabolic and endocrine			
Fasting glucose (mg/dL)	86.3 (8.15)	87.5 (10.65)	.7
Fasting insulin (μIU/mL)	9.09 (6.75)	9.94 (7.29)	.24
HbA1c (%)	5.28 (0.56)	5.13 (0.5)	.18
HOMA-IR	1.95 (1.5)	2.05 (1.6)	.33
TSH (μIU/mL)	1.71 (1.29)	1.95 (1.46)	.34
Vitamin D (25(OH)D) (nmol/L)	56.4 (21.41)	47.9 (29.16)	.001

Abbreviations: FSH = follicle-stimulating hormone; HbA1c = hemoglobin A1c; HOMA-IR = homeostatic model assessment of insulin resistance; LH = luteinizing hormone; TSH = thyroid-stimulating hormone; 25(OH)D = 25-hydroxyvitamin D.
Data are presented as median and interquartile range (Q3-Q1).
[a] $P < .05$ vs pregnancy by the independent test.

embryo quality, genetic abnormalities, and/or ovarian hyperstimulation). The remaining 354 women were recruited into the study. Of these, 218 women completed the study, and there were 136 dropouts because some participants had already left the country before the 12-week tests and others withdrew consent because of failed pregnancy. From those who completed the study, participants were divided into the following 2 groups: (1) women with a successful pregnancy (n = 160, pregnant group) and (2) those who did not achieve a successful pregnancy (n = 58, nonpregnant group). Biochemical and ectopic pregnancies (n = 10) were excluded. The pregnant group comprised singleton (n = 104, 65%) and multiple (n = 56, 35%) pregnancies. Overall, the ethnic groups of the women included Arabian Gulf nationals (53%), Far East (20%), Middle Eastern (15%), Europeans (8%), and African (4%).

The baseline data (reported as median and [interquartile range]) included age of 32 (6) years, BMI of 25.7 (7.4) kg/m^2, hemoglobin A1c level of 5.2% (0.6%), and thyroid-stimulating hormone level of 1.82 (1.4) μIU/mL. The preconception serum 25(OH)D level was significantly lower in the nonpregnant (46.9 [29.16] nmol/L) than in the pregnant group (56.4 [21.4] nmol/L; $P = .001$; Table 1). For all other baseline preconception data (including anthropometric, hormonal, and biochemical parameters), these were equivalent between the pregnant and nonpregnant groups.

IVF Success Rate and Serum 25(OH)D Level

Overall, 40% of women (n = 88) had a preconception serum 25(OH)D level of <50 nmol/L. Table 2 shows that 63% of those who had 25(OH)D levels of ≥50 nmol/L had a successful pregnancy compared with 37% who did not have a successful pregnancy (odds ratio, 0.46; 95% CI, 0.25-0.86; $P = .014$). On the other hand, all other variables did not show statistically significant differences according to the 25(OH)D levels with the exception of the BMI ($P = .039$).

There was a significant association between the maternal serum 25(OH)D level and success of IVF outcome (pregnancy vs nonpregnancy). Furthermore, the preconception serum 25(OH)D level was a significant determinant of pregnancy outcome following IVF treatment (B = 0.03; 95% CI, 1.008-1.042; $P = .003$), even after adjusting for BMI and age (B = 0.03; 95% CI, 1.008-1.042; $P = .003$) (Table 3).

A preconception serum 25(OH)D level of <50 nmol/L was associated with a nonpregnancy IVF outcome ($P = .01$). Conversely, a preconception serum 25(OH)D level of ≥50 nmol/L was associated with a successful clinically confirmed pregnancy outcome (odds ratio, 0.46; 95% CI, 0.25-0.86; $P = .01$).

Discussion

We demonstrated that the maternal preconception serum 25(OH)D level predicts the pregnancy outcome of IVF treatment. Furthermore, a preconception serum level of 25(OH)D of ≥50 nmol/L is associated with a greater likelihood of a successful pregnancy outcome following IVF treatment. Based on the current Institute of Medicine guidelines, vitamin D deficiency (<30 nmol/L) and insufficiency (30-50 nmol/L) affected 19% and 69%, respectively, of women included in our study overall.

Overall, the median age of subjects was below the high-risk age group of 35 years. Furthermore, the preconception hemoglobin A1c and homeostatic model assessment of insulin resistance levels were within the normal range. However, several women included in the study were overweight at preconception (BMI, 24.9-29.9 kg/m^2), an important risk factor for adverse pregnancy outcome following IVF treatment.[13] In contrast to previous studies that reported inverse correlations between the serum 25(OH)D level and age and obesity, we did not find such associations.[2] In addition, the 25(OH)D level independently predicted the IVF outcome, regardless of the age and BMI of women.

The high prevalence of 25(OH)D insufficiency/deficiency in the participants in our study likely stems from a relatively low exposure to sunlight within the Gulf because women usually wear long robes and head coverings for religious reasons.[2,14-16] In addition, women usually undertake a blood test before the initiation of IVF treatment to rule out any medical issues (eg, diabetes mellitus and vitamin D deficiency). If vitamin D is deficient, supplements are prescribed. Despite the presence of vitamin D deficiency, this does not usually result in any delay in IVF treatment resulting from the administration of vitamin D supplements. Indeed, the usual practice is that vitamin D deficiency is only treated (with the prescription of an oral multisupplement containing 400 IU of vitamin D) once a pregnancy is confirmed clinically.

In our retrospective study, the preconception maternal serum 25(OH)D level (within a recommended cutoff of ≥50 nmol/L) predicted the pregnancy outcome of IVF. The preconception serum 25(OH)D level of ≥50 nmol/L increases the chance of a successful pregnancy by 0.46 times. Our data are consistent with those from another study.[4] Maternal serum 25(OH)D likely regulates hCG expression and secretion and stimulates estrogen and progesterone production within trophoblasts that in turn facilitates optimal implantation and maintenance of a healthy pregnancy.[5] However, some controversy exists within the literature regarding the precise role of maternal 25(OH)D in the optimization of pregnancy, with other studies having reported that the fertilization rate and markers of embryo quality were not associated with the maternal serum 25(OH)D level.[5] We did not explore the effects of the maternal serum 25(OH)D level on IVF-related oocyte data, fertilization rate, or embryo quality. Given the likely importance of maternal serum 25(OH)D for optimal IVF pregnancy outcome based on our data, it seems reasonable to ensure proper treatment of maternal vitamin D deficiency/insufficiency (with oral vitamin D replacement therapies) prior to IVF treatment, even if this results in a delay to the initiation of IVF therapy by a few weeks.[17,18] A daily supplementation of 10 μg (400 IU) of vitamin D is recommended for

Table 2
Comparison of Different Variables at the Baseline According to Vitamin D Levels Using the Mann–Whitney *U* Test

Parameters	Vitamin D level		Odds ratio	95% CI		*P* value
	<50 nmol/L (*n*=145)	≥50 nmol/L (*n*=73)		Lower	Upper	
Successful pregnancy[a]						
No	31 (21.4%)	27 (37%)	0.46	0.25	0.61	.014
Yes	114 (78.6%)	46 (63%)				
Age (y)	32.0 (6.5)	32.0 (5.5)				.602
Weight (kg)	63.4 (15.4)	67.1 (21.5)				.205
BMI (kg/m^2)	25.2 (6.15)	26.7 (8.65)				.039
HbA1c (%)	5.2 (0.6)	5.26 (0.48)				.455
Fasting glucose (mg/dL)	86.3 (7.7)	87.0 (11.45)				.547
Fasting insulin (μIU/mL)	8.72 (6.9)	9.79 (6.98)				.223
HOMA-IR	1.9 (1.55)	2.2 (1.65)				.233
FSH (IU/L)	6.7 (2.615)	6.59 (2.395)				.989
LH (IU/L)	5.99 (3.15)	5.71 (2.78)				.460
Estrogen (pg/mL)	41.3 (22.05)	46.54 (26.43)				.637
Progesterone (ng/mL)	0.23 (0.2185)	0.25 (0.235)				.601
Vitamin D (25(OH)D) (nmol/L)	56.4 (12.34)	35.67 (19.28)				<.001

Abbreviations: FSH = follicle-stimulating hormone; HbA1c = hemoglobin A1c; HOMA-IR = homeostatic model assessment of insulin resistance; LH = luteinizing hormone; TSH = thyroid-stimulating hormone; 25(OH)D = 25-hydroxyvitamin D.
Data are presented as median and interquartile range (Q3-Q1); $P < .05$ versus a 25(OH)D level of ≥50 nmol/L by the independent test.
[a] χ^2 test.

Table 3
Logistic Regression Analysis Using In Vitro Fertilization Outcome (Pregnant/Nonpregnant Dependent Variable) and Preconception Vitamin D, BMI, and Age as Independent Variables ($n = 218$)

Independent variables	Unadjusted					Adjusted				
	B	*P* value	OR	95% CI		B	*P* value	OR	95% CI	
				Lower	Upper				Lower	Upper
Vitamin D (25(OH)D)	0.03	.003	1.03	1.008	1.042	0.02	.004	1.02	1.008	1.041
BMI	…	…	…	…	…	−0.05	.175	0.96	0.895	1.020
Age	…	…	…	…	…	−0.03	.418	0.97	0.906	1.042

Abbreviations: BMI = body mass index; OR = odds ratio; 25(OH)D = 25-hydroxyvitamin D.

all pregnant women, which can be initiated at preconception, and up to 20 μg (800 IU) for those women at risk of vitamin D deficiency/insufficiency (obese, limited sun exposure, vegetarian, and dark skin).[19] Women with vitamin D deficiency are prescribed higher doses of vitamin D supplements (vitamin D injection of 50 0000 IU weekly or 5000 IU 4 times a week depending on the level), and this is decided by the treating endocrinologist.

Our study has some limitations. Some markers of IVF outcome were not assessed, including infertility diagnosis, parity and ovarian reserve, the number and quality of eggs retrieved, and embryo quality. To better elucidate the role of the maternal serum 25(OH)D level on IVF pregnancy outcome, its level in serum and follicular fluid should be measured in future studies, and the number and quality of the oocyte pool should be evaluated.[20] In addition, the 25(OH)D level of the male partner was also not measure, which may influence embryo quality. Recent studies suggest that immune and/or inflammatory abnormalities may contribute toward embryo implantation failure and pregnancy loss. Maternal serum 25(OH)D plays a key role in regulating anti-inflammatory cytokines (eg, interleukin 4 and interleukin 10)[8,20,21] and has important immunomodulating activities, which should be explored in the context of IVF-related embryo implantation and pregnancy success in future studies. Importantly, our data only reveal the association, and not the causality, of the maternal serum 25(OH)D levels with IVF-related pregnancy outcomes. Therefore, it is not possible to conclude that the maternal serum 25(OH)D levels influence IVF-related pregnancy outcomes with confidence. An alternative explanation is that other factors, such as mental health or a specific medical condition, may have influenced both sunlight exposure and IVF-related pregnancy outcomes. Furthermore, we did not assess other possible factors that are associated with both 25(OH)D deficiency/insufficiency (eg, poor diet or certain bone diseases) and suboptimal IVF-related pregnancy outcomes. The vitamin D binding protein level was not measured and could have been an interesting marker to test and determine its correlation with IVF success. Future studies should explore a broader ethnicity and the effect of season variability on the maternal serum 25(OH)D levels and IVF-related pregnancy outcomes.[2,8]

In conclusion, preconception maternal 25(OH)D sufficiency (serum 25(OH)D level of ≥50 nmol/L) is associated with successful pregnancy outcomes following IVF treatment. It will be important to explore the effects of vitamin D supplementation and correction of vitamin D deficiency/insufficiency prior to IVF treatment on pregnancy outcomes.

Disclosure

The authors have no multiplicity of interest to disclose.

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Author Contributions

A.C., T.M.B. and H.A.H. conceived and planned the project; A.C. and H.A.H. were in charge of the analysis of results; A.C. wrote the manuscript. All authors provided critical feedback and helped in the preparation of this manuscript.

Data Availability

Data that support the findings of this study are available on request from the corresponding author. The data are not publicly available because of privacy or ethical restrictions.

References

1. Boyle V. Vitamin D and pregnancy. *Obstet Gynaecol Reprod Med*. 2014;24(10):315–316.
2. Ferrari D, Lombardi G, Banfi G. Concerning the vitamin D reference range: pre-analytical and analytical variability of vitamin D measurement. *Biochem Med*. 2017;27(3):453–466.
3. Keane KN, Cruzat VF, Calton EK, et al. Molecular actions of vitamin D in reproductive cell biology. *Reproduction*. 2017;153(1):R29–R42.
4. Zhao J, Huang X, Xu B, Yan Y, Zhang Q, Li Y. Whether vitamin D was associated with clinical outcome after IVF/ICSI: a systematic review and meta-analysis. *Reprod Biol Endocrinol*. 2018;16(1):13.
5. Rudick B, Ingles S, Chung K, Stanczyk F, Paulson R, Bendikson K. Characterizing the influence of vitamin D levels on IVF outcomes. *Hum Reprod*. 2018;27(11):3321–3327.
6. Butts SF, Seifer DB, Koelper N, et al. Vitamin D deficiency is associated with poor ovarian stimulation outcome in PCOS but not unexplained infertility. *J Clin Endocrinol Metab*. 2019;104(2):369–378.
7. Pilz S, Zittermann A, Obeid R, et al. The role of vitamin D in fertility and during pregnancy and lactation: a review of clinical data. *Int J Environ Res Public Health*. 2018;15(10):2241.
8. Pacis MM, Fortin CN, Zarek SM, Mumford SL, Segars JH. Vitamin D and assisted reproduction: should vitamin D be routinely screened and repleted prior to ART? A systematic review. *J Assist Reprod Genet*. 2015;32(3):323–335.
9. Chu J, Gallos I, Tobias A, Tan B, Eapen A, Coomarasamy A. Vitamin D and assisted reproductive treatment outcome: a systematic review and meta-analysis. *Hum Reprod*. 2018;33(1):65–80.
10. Baldini D, Malvasi A, Kosmas I, Baldini GM, Silvestris E. Increased bioavailability of Vitamin D improved pregnancy outcomes in in vitro fertilization cycles, only in patients over 36 years: a cross-sectional study. *Eur Rev Med Pharmacol Sci*. 2021;25:4964–4972.
11. Annan JJ, Gudi A, Bhide P, Shah A, Homburg R. Biochemical pregnancy during assisted conception: a little bit pregnant. *J Clin Med Res*. 2013;5(4):269–274.
12. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*. 1985;28(7):412–419.
13. Stubert J, Reister F, Hartmann S, Janni W. The risks associated with obesity in pregnancy. *Dtsch Arztebl Int*. 2018;115(16):276–283.
14. Sampat N, Al-Balushi B, Al-Subhi L, Al-Adawi S, Essa MM, Qoronfleh MW. Vitamin D: public health status regional gulf region. *Int J Nutr Pharmacol Neurol Dis*. 2019;9(4):117–135.
15. Singh P, Kumar M, Al Khodor S. Vitamin D deficiency in the Gulf Cooperation Council: exploring the triad of genetic predisposition, the gut microbiome and the immune system. *Front Immunol*. 2019;10:1042.
16. Al Saleh Y, Beshyah SA, Hussein W, et al. Diagnosis and management of vitamin D deficiency in the Gulf Cooperative Council (GCC) countries: an expert consensus summary statement from the GCC vitamin. *Arch Osteoporos*. 2020;15(1):35.
17. Shahgheibi S, Farhadifar F, Pouya B. The effect of vitamin D supplementation on gestational diabetes in high-risk women: results from a randomized placebo-controlled trial. *J Res Med Sci*. 2016;21:2.
18. Dastorani M, Aghadavod E, Mirhosseini N, et al. The effects of vitamin D supplementation on metabolic profiles and gene expression of insulin and lipid metabolism in infertile polycystic ovary syndrome candidates for in vitro fertilization. *Reprod Biol Endocrinol*. 2018;16(1):94.
19. Royal College of Obstetricians and Gynaecologists. Vitamin D in pregnancy. *Sci Impact Pap*. 2014;43:1–11.
20. Grzechocinska B, Dabrowski FA, Cyganek A, Wielgos M. The role of vitamin D in impaired fertility treatment. *Neuro Endocrinol Lett*. 2013;34(8):756–762.
21. Conti P, Kempuraj D. Impact of vitamin D on mast cell activity, immunity and inflammation. *J Food Nutr Res*. 2016;4(1):31–39.