https://doi.org/10.37939/jrmc.v27i2.2046

25 0H Vitamin D Levels in Pregnant Females: The Hidden Time Bomb

Fatima-Tuz-Zahra[1], Mehnaz[2], Noreen Atzaz[3], Shabana[4], Asma Iqbal[5], Sami Saeed[6]

Abstract

Objective: To assess and correlate changes in vitamin D levels in three trimesters of pregnancy.
Material and methods: This longitudinal study was conducted in collaboration with the Gynecology & Obstetric and Pathology department of Fauji foundation hospital, Rawalpindi. Eighty pregnant females were included in the study and tested for vitamin D at three points of time i.e. first trimester gestational age: (7-13 weeks), second trimester (20-26weeks), and third trimester (33 weeks till term). Pregnant females at their first visit and healthy nonpregnant females (controls) were analyzed for vitamin D, vitamin B12, folate, ferritin, and Hemoglobin (Hb) levels and compared using the Mann- Whitney U test for vitamin D and independent sample t-test for other parameters. Vitamin D and Hemoglobin (Hb) testing was done during the first, second, and third trimesters among pregnant females and compared between three groups using the Kruskal Wallis test and paired t-test respectively.
Results: Mean age of the pregnant females was 35 years. Mean vitamin D levels showed a significant decline from 30.7 ng/ml vs 19.5 ng/ml vs 18.6 ng/ml for the first, second, and third trimesters respectively (p-value<0.05). The frequency of vitamin D deficiency was 15.1% for pregnant females at their first visit which progressed to 20% and 25% in the second and third trimesters respectively.
Conclusion: Increased vitamin D deficiency and insufficiency rates exist in pregnant females, which progresses to further decline in the ensuing trimesters.
Keywords: Vitamin D, Pregnancy, Vitamin B12, Hemoglobin (Hb), Folate

[1] Assistant Professor of Pathology, Rawalpindi Medical University; [2] Assistant Professor of Pathology, Fauji Foundation Hospital; [3] Post graduate trainee, Fauji Foundation Hospital; [4] Lecturer Foundation University, Islamabad; [5] Assistant Professor of Gynaecology, Fauji Foundation Hospital; [6] Professor of Pathology, Fauji Foundation Hospital.
Correspondence: Dr Fatima Tuz Zahra, Assistant Professor of Pathology, Rawalpindi Medical University. **Email:** fatizuhra2@gmail.com
Cite this Article: Zuhra, F. T., Mehnaz, Atzaz, N., Shabana, Iqbal, A., & Saeed, S. (2023). 25 0H Vitamin D Levels in Pregnant Females: The Hidden Time Bomb . *Journal of Rawalpindi Medical College*, *27*(2). https://doi.org/10.37939/jrmc.v27i2.2046.
Received October 17, 2022; accepted May 03, 2023; published online June 24, 2023

1. Introduction

Maternal and child health care programs advocate that only healthy mothers produce healthy babies. The provision of a healthy in-utero environment cannot be fulfilled by nutritionally deficient mothers. Amongst the macro and micronutrients required in pregnancy: iron, vitamin D and B_{12} are of prime importance. (1)
Vitamin D (cholecalciferol) is essentially a hormone. Vitamin D facilitates intestinal calcium absorption and thus maintenance of adequate calcium levels required for bone mineralization, skeletal development, and growth.(2) It also plays a key role in other metabolic processes including glucose metabolism, regulation of immune responses, and growth. Calcidiol form which is the major circulating form is measured because of its longer half-life and ease of analysis. Vitamin D levels are affected by myriad factors varying from dietary intake, skin pigmentation, and the actice of skin covering to acute infections and inflammation. (3),(4) Vitamin D deficiency triggers the release of Parathyroid hormone (PTH). This causes the mobilization of calcium from bones and in turn bone mineral density is reduced. Long-term hypovitaminosis D causes osteomalacia, osteoporosis, and an increased tendency of fractures.
Optimal levels of vitamin D are still an ongoing debate, according to endocrine society guidelines the cutoff < 20 ng/ml (50 nmol/L) is used for labelling deficiency, for insufficiency levels are between 20-29 ng/ml. (5) Similarly unanimous consensus on optimal serum level during pregnancy has not been developed. (6) Vitamin D status of non-pregnant females has been thoroughly investigated among different age groups (7). However, Changes in vitamin D status in pregnant females are still under investigation (8).
Studies on both Asian and Western pregnant women have reported an increased frequency of vitamin D deficiency and insufficiency. According to Bhowmik et al. Prevalence of hypovitaminosis D was 48.7% in first trimester females taking deficiency threshold as <50ng/ml. As per F Ahmad et al., the prevalence of vitamin D deficiency was 17.3% and insufficiency was 47% when taking <30ng/ml as the threshold (9).
Pakistan experiences sunshine all year round however Pakistani women are particularly prone to develop vitamin D deficiency because of dark skin and the religious practice of covering the body when going out of the home (10). Furthermore, the diet of young females is lacking in vitamin D-rich food due to non-affordability in the lower class and junk food preference in the higher class (11). One of the most vulnerable groups is pregnant women. The deficiency

begins slowly in the early reproductive years it is aggravated in pregnancy which is a period of enhanced vitamin D requirement and then the climax is unveiled in the menopausal years with accelerated osteoporosis and increased tendency of fractures[12]. Vitamin D deficiency is associated with reduced bone mineral density post-delivery which further progresses to bone complications later in life [13]. Thus, hypovitaminosis D can be regarded as a slow ticking time bomb working silently to a devastating end. According to Raia et al vitamin D deficiency was associated with intrauterine growth retardation and increased risk of stillbirth Similarly significant neonatal adverse outcomes include rickets, increased incidence of respiratory infections and even behavioural issues. [14], [15] Considering the huge implications of vitamin D deficiency in pregnant females, this dynamic process can be stopped with early intervention in the form of vitamin D supplements in pregnancy. A safe dose of 1200-1500 IU supplementation can be done during pregnancy according to endocrine society guidelines [16].

Fauji foundation hospital caters to low-middle-class families of ex-soldiers as its entitled patients. The average daily intake of vitamin D in these women is suboptimal. They do not have access to vitamin D-fortified foods and most of them are fully clad according to the Islamic cultural norms with minimal sun exposure. Moreover, to cover the increased nutritional requirements of pregnancy, only ferritin, folate and calcium-based supplements not containing vitamin D were prescribed. Keeping this background in mind we undertook this study to evaluate if these supplements are meeting the nutritional requirements of pregnant females and how are they affecting vitamin D levels.

We sought to correlate changes in vitamin D levels with pregnancy trimesters and determine the frequency of vitamin D deficiency and insufficiency in pregnant females at different stages of pregnancy.

2. Materials & Methods

This was a longitudinal study between May 2022 till August 2022, carried out after approval from the institutional review board at Fauji Foundation Hospital Rawalpindi. Pregnant females visiting the Gynaecology and obstetric OPD were included after written informed consent. A sample size of eighty was calculated using Rao soft calculator keeping the confidence interval at 95. Both primi and multigravida were included. These females were tested at three points based on their gestational age. Pregnant females had their first blood sample taken between 7-13 weeks on their booking visit. The second sample was taken at 20-26 weeks when they reported for the oral glucose tolerance test or anomaly scan. The third sample was taken at 33-39 weeks of gestational age. Exclusion criteria included non-pregnant females and pregnant females with systemic or autoimmune ailments. Baselines demographic data including age, parity, educational status, dietary intake, and use of multivitamins was recorded on a structured proforma. A 7 ml venous blood sample was drawn from the antecubital vein for pathology lab tests. From the syringe 2 ml was transferred to the EDTA tube for Blood complete picture analysis while the rest 5ml was taken in the clot activator tube. After centrifugation at 1500 rpm for 10 min, serum was separated for special chemistry immunoassay tests: serum vitamin D, B_{12}, folate, and ferritin. The serum was stored in aliquots and kept at -80 C till batch analysis. Blood CP sample was taken in an EDTA tube and run on a fully-automated haematology analyzer Sysmex XN-1000. Vitamin D, B_{12}, folate and ferritin were assayed on a special chemistry chemiluminescence-based immunoassay analyser Advia Centaur after ensuring quality control. A cutoff of less than <20ngml was chosen for vitamin D deficiency and insufficiency was considered as levels between 20-29 ng/ml. Adequate or optimal levels were greater than or equal to 30ng/ml [5].

All the demographic data and lab test results were entered into SPSS and analyzed using SPSS version 25. Mean SD were determined for quantitative variables whereas frequencies and percentages were calculated for qualitative variables. Since vitamin D was found to have a non-normal distribution as determined by the Kolmogrov-Smirnov test, non-parametric statistics were applied to it. Means of test results were compared using the Mann-Whitney U test for vitamin D and an independent t-test for other parameters between first-trimester pregnant females and controls. Kruskal Wallis test was used for the comparison of means of vitamin D results between the three trimesters of pregnant females.

3. Results

A total of 102 pregnant females were included in the study however 22 were lost to follow-up and did not report for the second or third-trimester vitamin D sample. Eighty pregnant females participated in the study. The age range was between 22 and 41 years.

The mean age of pregnant females was 35 years. Mean Hb, platelets, vitamin D, B12, folate, ferritin and fasting glucose were 10.50 ±1.29 g, 279 ± 83.3mm^3, 23.1±7.7 ng/ml, 225.01±98.8 ng/ml, 8.4± 6.1 ng/ml, 20.2±41.8 ng/ml and 4.4±0.5 mmol respectively (Table 1). In the non-pregnant control group, significantly, higher levels were observed for values of Hb, vitamin D and B12, ferritin and folate (p-value<0.05) (Table-1).

Table-1 Demographic and baseline laboratory parameters of the study population.

Parameters	**Patients n=80**
Hbg	10.58±1.29
Platelets mm3	279.8±85.4
Vitamin D ng/ml	23.1±7.57
Vitamin B12 ng/ml	225.9±73.08
Folate ng/ml	8.08±6.12
Ferritin ng/ml	20.2±12.67
Fasting Glucose mmol	4.45±0.50

In the first-trimester vitamin D level observed at 7-13 weeks of gestation was 30.7 ng/ml. The levels ranged between 15.9- 49.4 ng/ml respectively. The frequency of vitamin D deficiency was 15.2 per cent; insufficiency was 40.7 per cent and 44.1 per cent had sufficient vitamin D levels. For the second trimester (20-26 weeks of gestation) the vitamin D level was 20.2± ng/ml. The levels ranged between 8-32 ng/ml respectively. The frequency of vitamin D deficiency was 20 per cent. In the last trimester, the minimum value of 16 ng/ml whereas a maximum of 28 ng/ml was observed. The Vitamin D level was 18.6±2.4 and the frequency of vitamin D deficiency increased to 25 per cent (Figure1).

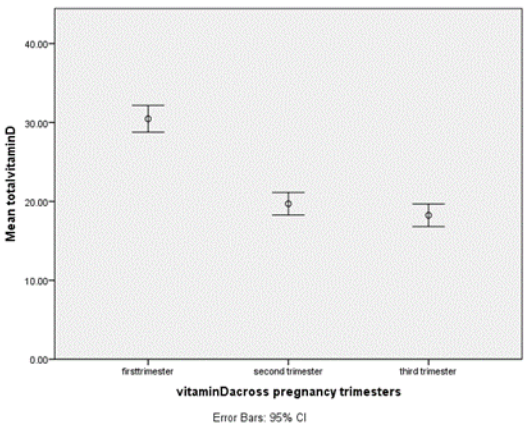

Figure-1 Distribution of vitamin D among three groups of pregnant females

Vitamin D levels among the first vs. second trimesters (30.7±5vs 19.7±5) and second vs. third trimesters (30.7±5 vs 18.2±3) were compared with each other by applying the Wilcoxon Signed-Rank test. Vitamin D was found to be significantly lower in the second and third trimesters compared to the first p-value <0.001(Table 2). Haemoglobin and hematocrit were compared in a similar fashion using paired t-tests and showed a significant decline in second and third trimesters p-value <0.001(Table 2). According to Kruskal Walli's test results, a significant difference was observed between the means of vitamin D for first, second and third-trimester pregnant females. 30.7 ng/ml vs 19.5 ng/ml vs 18.6 ng/ml p-value<0.05. (Table 3). The Wilcoxon-Signed Rank test was used to compare vitamin D levels between two trimesters. Paired t-test was applied to compare haemoglobin levels between two trimesters of pregnant females. The Kruskal-Wallis test was used for the comparison of vitamin D across three trimesters. One-way ANOVA was applied to compare haemoglobin between three trimesters of pregnant females.

5. Discussion

The present study shows more than half of the subjects(pregnant females) in the first trimester were either vitamin D deficient or insufficient and this deficiency increased with the growing weeks of pregnancy. These results are in line with the results from previous studies on the Pakistani population which show an even higher frequency of deficiency in first-trimester females [17]. Mustafa et al. found 44.8% of first-trimester females deficient while taking 20ng/mL as the cutoff [18].

Pakistan, India, and Bangladesh are regarded as hotspots of vitamin D deficiency with >20 per cent deficiency status among pregnant women. [19] International Studies in Asia and beyond have reported similar data regarding vitamin D deficiency [20].

Table-2 Comparison of Vitamin D and Hemoglobin among different groups of pregnant females.

Parameters	First trimester levels	Second trimester levels	Second vs first Levels p-value	Third trimester levels	Third vs first Levels p-value
25(OH)D ng/ml	30.7±5.7	19.7±5.8	<0.001	18.2 ±3.1	<0.001
Haemoglobin g/dl	10.5±2.1	10.1±3.1	<0.01	9.9±2.6	<0.01

P value <0.05 was considered significant

In this study we aimed to find changes in 25(OH)D levels across the three Pregnancy trimesters. This study is one of the first studies in Pakistan that evaluated vitamin D at different gestational weeks in pregnant women. Our results showed a progressive decrease in levels across the three trimesters from 30.7 to 19.6 and 18.2 ng/ml was found comparable to a Japanese study by Takaota et al that showed the decline in vitamin D levels across three pregnancy trimesters [21]. The levels decreased from 13.8 ng/mL to 11.4 ng/ml and 11.5 ng/ml. One of the possible mechanisms of this decline could be the volume expansion occurring in pregnancy. Plasma volume expands during pregnancy with the maximum occurring in the second trimester. This is responsible in part for anaemia and the recording of low Hb in the second and last trimester The plasma volume expands by 1.13 L till term. [22]. In the study by Takaota after the second trimester mean vitamin D improved from 11.4 to 11.5 ng/ml how ever in our study the levels consistently declined from 19.6 to 18.2 ng/ml after the effect of volume expansion decreased. Therefore these results are attributed to low vitamin D reserves in our pregnant females compounded by insufficient diet and supplementation in this critical period.

Table-3 Vitamin D and Hemoglobin among different trimesters of pregnant females

Parameters	First trimester n=80	Second trimester n=80	Third trimester n=80	P value
	Mean±SD	Mean±SD	Mean±SD	
Vitamin D ngml	30.7±15.6	19.5±5.8	18.24±3.14	0.01*
Haemoglobin g	10.58±2.1	10.1±3.1	9.9±2.6	0.01*

P value <0.05 was considered significant

Another mechanism is the enhanced requirement in the latter phases of pregnancy for the growing foetus which requires it for skeletal mineralisation. Pregnancy causes physiological changes in calcium and vitamin D metabolism. Only the calcidiol 25 OH-D crosses the placenta whereas the other forms 1,25(OH)D do not. Placenta has its key share in calcidiol metabolism and thus may contribute to reduced 25 OH-D levels. [23]

As per Tsuprykov et.al, there s a reported opposite correlation of 25(OH) D levels with 1,25(OH) D concentration. Total 1,25(OH) D levels rise to double at 12 weeks of pregnancy and then continue to increase due to the increase in binding proteins however the free form or physiologically active form stays relatively unchanged on the contrary 25(OH) D levels are reduced due to increased demand in pregnancy [24].

Our results are in contrast to a Swedish study which shows an increase in vitamin D levels with the progression of pregnancy from 55 nmol/L to 64 nmol/L [25]. In a study by Savrad et al, the levels of vitamin D increased across the trimesters of pregnancy from 67.5 nmol/L to 84 nmol/L[26]. These studies could not differentiate whether the increase was from supplementation or a normal physiological increase since all these women were on vitamin D supplements.

One minor reason for hypovitaminosis D could be related to Asian complexion. Dark-skinned Asian populations are more prone to vitamin D deficiency as compared to their fair-toned counterparts. The first standardized set of vitamin D studies carried out in Europe found deficiency to be greater in dark-skinned minority groups than in the local population. [(3)]

Vitamin D supplementation early on in pregnancy can lead to healthy outcomes averting long-term morbidity in the form of bone diseases. The current recommendation for intake of vitamin D during pregnancy is from (400-600) IU per day for the attainment of levels at 25-50 nmol/L(10-20ng/ml). The efficacy of this dosage needs to be validated in the Pakistani population. Screening for vitamin D should be mandatory. The screening of vitamin D should be added to baseline tests conducted at the booking visit since most of the pregnant females after bearing the burden of multiple pregnancies and belonging to low socioeconomic status are either vitamin D deficient or insufficient.

The strength of the study was its longitudinal design with patient follow-up from the booking visit till the last trimester. Further studies are needed to assess the effect of hypovitaminosis D on a pregnant woman by measuring PTH(parathyroid hormone) and bone profile and also the inclusion of postpartum samples.

5. Conclusion

A high prevalence of vitamin D deficiency and insufficiency is observed among pregnant females visiting obstetric OPD at our tertiary care centre. The frequency of Vitamin D deficiency increased in pregnant females with ensuing pregnancy trimesters.

CONFLICTS OF INTEREST- None

Financial support: None to report.

Potential competing interests: None to report

Contributions:

F.T.Z, S.S - Conception of study

N.A, S, A.I, S.S - Experimentation/Study conduction

F.T.Z - Analysis/Interpretation/Discussion

M, A.I, S.S - Manuscript Writing

S.S - Critical Review

F.T.Z- Facilitation and Material analysis

References

[1] Bhowmik B, Siddique T, Majumder A, Mdala I, Hossain IA, Hassan Z, et al. Maternal BMI and nutritional status in early pregnancy and its impact on neonatal outcomes at birth in Bangladesh. BMC Pregnancy Childbirth. 2019;19(1):413. doi 10.1186/s12884-019-2571-5. PMID:

[2] Ashley B, Simner C, Manousopoulou A, Jenkinson C, Hey F, Frost JM, et al. Placental uptake and metabolism of 25(OH)vitamin D determine its activity within the fetoplacental unit. Elife. 2022;11:10–2. doi: 10.7554/eLife.71094. PMID: 35256050; PMCID: PMC8903835.

[3] Cabaset S, Krieger JP, Richard A, Elgizouli M, Nieters A, Rohrmann S, et al. Vitamin D status and its determinants in healthy pregnant women living in Switzerland in the first trimester of pregnancy. BMC Pregnancy Childbirth. 2019 8;19(1):10. doi: 0.1186/s12884-018-2150-1.

[4] Burke NL, Harville EW, Wickliffe JK, Shankar A, Lichtveld MY, McCaskill ML. Determinants of vitamin D status among Black and White low-income pregnant and non-pregnant reproductive-aged women from Southeast Louisiana. BMC Pregnancy Childbirth. 2019 2;19(1). DOI: 10.1186/s12884-019-2246-2

[5] Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Guidelines for preventing and treating vitamin D deficiency and insufficiency revisited. J Clin Endocrinol Metab. 2012;97(4):1153–8. doi: 10.1210/jc.2011-2601

[6] Chen F, Wang Y, Guo Y, Wang J, Yang A, Lv Q, et al. Specific higher levels of serum uric acid might have a protective effect on bone mineral density within a Chinese population over 60 years old: A cross-sectional study from northeast China. Clin Interv Aging. 2019;14:1065–73. doi: 10.2147/CIA.S186500.

[7] Akhtar S. Vitamin D Status in South Asian Populations – Risks and Opportunities. Crit Rev Food Sci Nutr. 2016;56(11):1925–40. doi: 10.1080/10408398.2013.807419.

[8] Yang C, Jing W, Ge S, Sun W. Vitamin D status and vitamin D deficiency risk factors among pregnancy of Shanghai in China. BMC Pregnancy Childbirth. 2021;21(1):431. doi: 10.1186/s12884-021-03889-0.

[9] Ahmed F, Khosravi-Boroujeni H, Khan MR, Roy AK, Raqib R. Prevalence and predictors of vitamin d deficiency and insufficiency among pregnant rural women in bangladesh. Nutrients. 2021;13(2):449. doi: 10.3390/nu13020449

[10] Junaid K, Rehman A, Jolliffe DA, Wood K, Martineau AR. High prevalence of vitamin D deficiency among women of child-bearing age in Lahore Pakistan, associating with lack of sun exposure and illiteracy. BMC Womens Health. 2015;15(1):83. doi: 10.1186/s12905-015-0242-x

[11] Riaz H, Finlayson AE, Bashir S, Hussain S, Mahmood S, Malik F, et al. Prevalence of Vitamin D deficiency in Pakistan and implications for the future. Expert Rev Clin Pharmacol 20161;9(2):329–38.doi.org/10.1586/17512433.2016.1122519

[12] Mansur JL, Oliveri B, Giacoia E, Fusaro D, Costanzo PR. Vitamin D: Before, during and after Pregnancy: Effect on Neonates and Children.Nutrients 2022 1;14(9):1900. doi: 10.3390/nu14091900.; .

[13] Yoshikata H, Tsugawa N, Watanabe Y, Tsuburai T, Chaki O, Hirahara F, et al. 25-Hydroxyvitamin D profiles and maternal bone mass during pregnancy and lactation in Japanese women. J

Bone Miner Metab. 2020;38(1):99–108. doi: 10.1007/s00774-019-01032

[14] Pilz S, Zittermann A, Obeid R, Hahn A, Pludowski P, Trummer C, et al. The Role of Vitamin D in Fertility and during Pregnancy and Lactation: A Review of Clinical Data. Int J Environ Res Public Heal.2018;15(10):2241. doi: 10.3390/ijerph15102241.

[15] Raia-Barjat T, Sarkis C, Rancon F, Thibaudin L, Gris JC, Alfaidy N, et al. Vitamin D deficiency during late pregnancy mediates placenta-associated complications. Sci Rep [Internet]. 2021;11(1): 20708. doi.org/10.1038/s41598-021-00250-5

[16] Mithal A, Kalra S. Vitamin D supplementation in pregnancy. Indian J Endocrinol Metab. 2014;18(5):593-6. doi: 10.4103/2230-8210.139204

[17] Nasir JA, Imran M, Arif S, Zaidi A. Pattern of Vitamin D among Pakistani Pregnant Women. J Coll Physicians Surg Pakistan. 2018;28(3):233–7. doi: 10.29271/jcpsp.2018.03.233.

[18] Mustafa G, Asadi MA, Iqbal I, Bashir N. Low vitamin D status in nursing Pakistani mothers in an environment of ample sunshine: A cross-sectional study. BMC Pregnancy Childbirth. 2018;18(1):426. doi: 10.1186/s12884-018-2062-0.

[19] Kiely ME, Wagner CL, Roth DE. Vitamin D in pregnancy: Where we are and where we should go. J Steroid Biochem Mol Biol. 2020;201:105669. doi: 10.1016/j.jsbmb.2020.105669

[20] Kanth N. Status of Vitamin D deficiency in pregnant women and their infants in rural areas of Karachi, Sindh. J Soc Obstet Gynaecol Pakistan. 2020;10(3):176–80.

[21] Takaoka N, Nishida K, Sairenchi T, Umesawa M, Noguchi R, Someya K, et al. Changes in vitamin D status considering hemodilution factors in Japanese pregnant women according to trimester: A longitudinal survey. PLoS One. 2020;15(10):e0239954 doi.org/10.1371/journal.pone.0239954

[22] De Haas S, Ghossein-Doha C, Van Kuijk SMJ, Van Drongelen J, Spaanderman MEA. Physiological adaptation of maternal plasma volume during pregnancy: a systematic review and meta-analysis. Ultrasound Obs Gynecol. 2017;49(2):177–87. doi: 10.1002/uog.17360.

[23] Karras SN, Wagner CL, Castracane VD. Understanding vitamin D metabolism in pregnancy: From physiology to pathophysiology and clinical outcomes. Metabolism. 2018 ;86:112–23. doi: 10.1016/j.metabol.2017.10.001.

[24] Tsuprykov O, Elitok S, Buse C, Chu C, Krämer BK, Hocher B. Opposite correlation of 25-hydroxy-vitamin D- and 1,25-dihydroxy-vitamin D-metabolites with gestational age, bone- and lipid-biomarkers in pregnant women. Sci Rep. 2021;11(1):1923. doi.org/10.1038/s41598-021-81452-9

[25] Lundqvist A, Sandström H, Stenlund H, Johansson I, Hultdin J. Vitamin D status during pregnancy: A longitudinal study in Swedish women from early pregnancy to seven months postpartum. PLoS One. 2016;11(3):e0150385. doi: 0.1371/journal.pone.0150385

[26] Savard C, Bielecki A, Plante AS, Lemieux S, Gagnon C, Weiler HA, et al. Longitudinal Assessment of Vitamin D Status across Trimesters of Pregnancy. J Nutr. 2021 ;151(7):1937–46. doi: 10.1093/jn/nxab060.