The relation between vitamin D and the adolescents’ mid-luteal estradiol and progesterone

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Abstract. – OBJECTIVE: The aim of this study was to detect the effect of vitamin D (Vit. D) intake on the mid-luteal estradiol (E₂), and progesterone (P), and the relation between vit. D, and the adolescents’ mid-luteal E₂ and P.

PATIENTS AND METHODS: Eighty-five (85) adolescents were recruited for this cohort study after obtaining informed consent. After a detailed history and clinical examination, the body mass index (BMI) of the studied participants was calculated, followed by pelvic sonography to exclude any pelvic pathology. Participants’ blood samples were collected on days 21-22 of the menstrual cycle (mid-luteal) to measure the thyrotropin (TSH) (i.e., to exclude hypothyroidism), prolactin (i.e., to exclude hyperprolactinemia), glycosylated hemoglobin (HbA1C), (i.e., to exclude diabetes), E₂, P, and 25(OH)D. Participants received 50,000 IU of vit. D weekly for two months. Significant negative correlations between the 25(OH)D, and both the mid-luteal E₂ (r=-0.661; p<0.0001), and P (r=-0.521; p<0.00001) were detected in this study.

CONCLUSIONS: The mid-luteal E₂ and P statistically decreased after vit. D intake (50,000 IU of vit. D weekly for 2 months). Significant negative correlations between the 25(OH)D, and both the mid-luteal E₂ and P were detected in this study. The relation between vit. D and ovarian steroids, and the effect of vit. D intake on ovarian steroids need further larger studies.

Key Words: Vitamin D, Vit. D, Adolescents, Mid-Luteal, Estradiol, Progesterone.

Introduction

The Relation Between Vitamin D and the Adolescents’ Mid-Luteal Estradiol and Progesterone

The adequate vitamin D (Vit. D) concentrations have been associated with a range of reproductive outcomes¹².
Previous studies suggest a possible relation between vitamin D and polycystic ovary syndrome (PCOS).

Granulosa cells contain vitamin D receptors (VDR), and in-vitro stimulation of human ovarian cells with 1,25(OH)2D (1,25-dihydroxy vitamin D) affects the ovarian steroidogenesis.

Experimental studies reported an influence of vitamin D metabolites on the reproductive hormones production.

Mouse models showed an association between VDR defect and decreased aromatase enzyme (which converts the androgen to estrogen) activity and expression.

Moreover, the 1,25(OH)2D deficiency was associated with reduced corpus luteum function and progesterone (P) production.

In-vitro studies on the porcine granulosa cells shown no effect of 1,25(OH)2D on basal P production. At the same time, other studies showed a reduced progesterone synthesis after 1,25(OH)2D treatment.

The experimental effect of vitamin D on estrogen is clearer. Studies on human, porcine, and goat granulosa cells revealed a stimulatory effect of 1,25(OH)2D on both the estradiol (E2) and aromatase enzyme.

Moreover, the calcium metabolism hormones, including vitamin D levels are changed with endogenous estrogen changes (i.e., pregnancy and menopause), and with the use of oral contraceptive estrogen-containing tablets.

The relation between the 25-hydroxy vitamin D [25(OH)D] and steroid hormones in normal and PCOS women was inconsistently reported.

The role of vitamin D on ovarian steroids is not intensively studied. Therefore, the current study is designed to detect the effect of vitamin D intake on the mid-luteal E2 and P, and the relation between vitamin D, and the adolescents’ mid-luteal E2 and P.

**Patients and Methods**

Eighty-five (85) adolescents were recruited for this prospective cohort study, which was conducted in Aktobe-West Kazakhstan over two years (2021-2022), to detect the effect of vitamin D intake on the mid-luteal E2 and P, and the relation between vitamin D, and the adolescents’ mid-luteal E2 and P.

Participants were recruited for the current prospective cohort study, after the approval from the ethics committee of West Kazakhstan Medical University (No. 10, dated 04.10.2020) and after obtaining the informed consent from the adolescents themselves, and their guardians. The study followed ethics guidelines of the Helsinki Declaration.

After a detailed history, and clinical examination, the body mass index (BMI) of the studied participants was calculated, followed by a pelvic sonography to exclude any pelvic pathology (i.e., especially PCOS).

Inclusion criteria include adolescents (12-18 years old), with regular menstrual cycles, normal BMI 18.5-24.9 Kg/m2, and vitamin D deficiency.

Exclusion criteria include adolescents <12 years old or >18 years old, underweight (18.5 Kg/m2 BMI), overweight (25-29.9 Kg/m2) or obese (BMI ≥30 Kg/m2), with irregular menstrual cycles, known medical disorders (i.e., diabetes, or hypertension), known endocrine disorders (i.e., PCOS, thyroid, or hyperprolactinemia), received exogenous hormones or vitamin D supplement within the 6 months, and/or refused to participate.

Regular menstrual cycles were defined as menstrual flow on a regular basis every 21-35 days. The serum 25(OH)D is a reliable indicator for the vitamin D status. It reflects the cutaneous production of vitamin D and the vitamin D intake and it has a long half-life.

The normal serum vitamin D was defined as 25(OH)D >30 ng/mL, while <20 ng/mL 25(OH)D was defined as vitamin D deficiency.

The serum 25(OH)D was measured using the spectrophotometric method since it forms a pink chloroform that can be read at 500 nm wavelength when it binds with the antimony trichloride.

Diabetes was defined according to the American diabetic association (ADA) as a group of metabolic disorders characterized by hyperglycemia resulting from either deficient insulin secretion and/or insulin action.

Diabetes is diagnosed when the HbA1c ≥6.5% or fasting plasma sugar ≥126 mg/dL (7 mmol/L), or 2-hrs plasma glucose ≥200 mg/dL (11.1 mmol/L).

Hypertension is ≥140 mmHg systolic blood pressure, and/or ≥90 mmHg diastolic blood pressure (on two different days).

The diagnosis of PCOS was based on two criteria of the following ESHRE/ASRM criteria, 1) polycystic ovaries by ultrasound, 2) oligo-/anovulation, and 3) clinical evidence of hyperandrogenism (hirsutism and acne).

Participants’ blood samples were collected on days 21-22 of the menstrual cycle (mid-luteal)
to measure the thyrotropin (TSH) (i.e., to exclude hypothyroidism), prolactin (i.e., to exclude hyperprolactinemia), glycosylated hemoglobin (HbA1C), (i.e., to exclude diabetes), E₂, progesterone (P), and 25(OH)D.

The normal serum TSH ranges from 0.4-4.0 mIU/mL, normal serum prolactin <29 ng/mL, and normal HbA1C <6.5%.

The normal mid-luteal E₂ ranges from 60-200 pg/mL, and the normal mid-luteal P in adolescents with regular menstrual and ovulatory cycles is >7 ng/mL.

The participants received 50,000 IU of vit. D (Lamyra Pharmacare, Harrow, Middlesex, England) weekly for two months according to the hospital’s protocol, and on the 3rd month, the mid-luteal E₂, P, and 25(OH)D were measured.

The mid-luteal E₂, P, and 25(OH)D were compared before and after the vit. D intake to detect the effect of vit. D intake (50,000 IU weekly for 2 months) on the mid-luteal E₂ and P (primary outcome). The secondary outcome measured the relation between vit. D and the adolescents’ mid-luteal E₂ and P.

**Statistical Analysis**

The G Power 3.1.9.7 (Heinrich Heine Universität, Düsseldorf; Germany) with 0.05 probability, 0.95% power, 0.5 sample size, and Student’s t-test for statistical analysis was used for sample size calculation. Student’s t-test, and correlation analysis (Pearson’s correlation) were used for statistical analysis. p<0.05 was considered significant.

**Results**

Eighty-five (85) adolescents were recruited for this cohort study to detect the effect of vit. D intake (50,000 IU weekly for 2 months) on the mid-luteal E₂ and P (primary outcome), and the relation between vit. D and the adolescents’ mid-luteal E₂ and P (secondary outcome).

The normal serum vit. D was defined as 25(OH)D >30 ng/mL, while <20 ng/mL was defined as vit. D deficiency.

The mean age, weight, height, and BMI of the studied participants are presented in Table I. The mean 25(OH)D of the studied participants before vit. D intake was 13.4±3.01 ng/mL. The mid-luteal E₂ and P before vit. D intake were 109.3±15.7 pg/mL and 9.8±1.01 ng/mL, respectively (Table I).

The 25(OH)D of the studied participants statistically increased from 13.4±3.01 ng/mL to 58.5±2.07 ng/mL after vit. D intake (p=0.00036; 95% CI: -45.9, -45.1, -44.32). The mid-luteal E₂ and P statistically decreased from 109.3±15.7 pg/mL and 9.8±1.01 ng/mL, respectively to 40.7±10.52 pg/mL, and 5.2±0.73 ng/mL, respectively, after vit. D intake (p=0.00015; 95% CI: 64.5, 68.6, 72.7, and p=0.0016; 95% CI: 4.3, 4.6, 4.87, respectively), (Table II).

Significant negative correlations between the 25(OH)D, and both the mid-luteal E₂ (r =-0.661; p<0.00001), (Figure 1), and P (r=-0.521; p=0.00001), (Figure 2) were detected in this study.

**Table I.** Participants’ characteristics, 25(OH)D, mid-luteal estradiol, and progesterone.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Studied participants (n = 85 adolescents)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Years)</td>
<td>15.4 ± 1.3</td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>59.92 ± 4.3</td>
</tr>
<tr>
<td>Height (Cm)</td>
<td>159.0 ± 2.75</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>23.3 ± 1.4</td>
</tr>
<tr>
<td>25(OH)D (ng/mL)</td>
<td>13.4 ± 3.01</td>
</tr>
<tr>
<td>Estradiol (pg/mL)</td>
<td>109.3 ± 15.7</td>
</tr>
<tr>
<td>Progesterone (ng/mL)</td>
<td>9.8 ± 1.01</td>
</tr>
</tbody>
</table>

25(OH)D: 25-hydroxy vit. D. BMI: Body mass index; vit. D: Vitamin D. Data presented as mean ± SD (standard deviation).

**Table II.** Participants’ 25(OH)D, mid-luteal estradiol, and progesterone before and after vit. D intake.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Before vit. D intake (n = 85 adolescents)</th>
<th>After vit. D intake (n = 85 adolescents)</th>
<th>p-value (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25(OH)D (ng/mL)</td>
<td>13.4 ± 3.01</td>
<td>58.5 ± 2.07</td>
<td>0.00036* (-45.9, -45.1, -44.32)</td>
</tr>
<tr>
<td>Estradiol (pg/mL)</td>
<td>109.3 ± 15.7</td>
<td>40.7 ± 10.52</td>
<td>0.00015* (64.5, 68.6, 72.7)</td>
</tr>
<tr>
<td>Progesterone (ng/mL)</td>
<td>9.8 ± 1.01</td>
<td>5.2 ± 0.73</td>
<td>0.0016* (4.3, 4.6, 4.87)</td>
</tr>
</tbody>
</table>

*: Significant difference. 25(OH)D: 25-hydroxy vit. D; CI: Confidence Interval; vit. D: Vitamin D. Data presented as mean ± SD (standard deviation). Student’s t-test was used for statistical analysis.
The relation between vitamin D and the adolescents’ mid-luteal estradiol and progesterone

Figure 1. Correlation between 25(OH)D and estradiol.

Figure 2. Correlation between 25(OH)D and progesterone.
Discussion

The animals’ or the humans’ studies regarding the effect of vit. D and/or its metabolites on ovarian steroids are limited.

The relation between 25(OH)D and steroid hormones in normal women was inconsistently reported, and the role of vit. D on ovarian steroids is not intensively studied.

Therefore, eighty-five (85) adolescents were recruited for this cohort study. Participants received 50,000 IU of vit. D weekly for two months, and on the 3rd month, the mid-luteal E₂, P, and 25(OH)D were measured.

The mid-luteal E₂, P, and 25(OH)D were compared before and after the vit. D intake to detect the effect of vit. D intake (50,000 IU weekly for 2 months) on the mid-luteal E₂ and P.

The relations between vit. D and the adolescents’ mid-luteal E₂ and P were detected as secondary outcomes using the correlation analysis.

Although Hong et al. found the vit. D₃ increased E₂ production from the porcine cultured granulosa cells, Parikh et al. found that 1,25-(OH)₂D₃ stimulates the P production by 13%, and E₂ production by 9%, in human ovarian cells.

This study found that the mid-luteal E₂ and P statistically decreased from 109.3±15.7 pg/mL and 9.8±1.01 ng/mL, respectively, to 40.7±10.5 pg/mL, and 5.2±0.73 ng/mL, respectively, after vit. D intake (p=0.00015 and 0.0016, respectively).

Significant negative correlations between the 25(OH)D, and both the mid-luteal E₂ (p<0.00001, and <0.00001, respectively) were detected in this study.

Knight et al. reported significantly lower luteal P and reduced E₂ at higher 25(OH)D levels, with an inverse relationship between the 25(OH)D and both the E₂ and P.

The vit. D effect on the ovarian steroids is mediated through the CYP19 expression, which results in low aromatase enzyme with subsequent decreased E₂ production.

The identification of the vit. D receptors in the ovaries, uterus, and pituitary gland, supports the role of vit. D in ovarian steroidogenesis.

Moreover, Horii et al. found that high doses of 1,25(OH)₂D₃ reduce the corpus luteum function, and P production in female rats.

Vit. D has a beneficial role in insulin resistance (IR), and endometrial receptivity, but high levels and incorrect vit. D administration time, seem to have a detrimental effect on oocyte maturation. Therefore, Menichini et al. encourage a low dose of vit. D (400-800 IU/day), particularly in vit. D deficient PCOS women, with metabolic syndrome. Additionally, they suggested vit. D supplementation in selected women only during the luteal phase of the ovarian cycle (as luteal phase support).

Recently, Kiani et al. found that vit. D decreases the IR in PCOS. Similarly, inositol (carbo cyclic sugar isomer) lowers the IR, improves oocyte maturation, and restores normal ovulation in PCOS.

Benelli et al., found that myo-inositol (MI), and D-chiro-inositol (DCI) significantly increased the E₂ (from 47.0±18.2 to 107.4±92.86 pg/mL), (p<0.01) in obese PCOS women. They concluded that the MI plus DCI combination was effective with improved endocrine parameters in obese PCOS women.

The combination of MI and DCI improves the PCOS features. The alpha-lipoic acid (ALA) also has anti-inflammatory, antioxidant, and insulin-sensitizing activities. Few studies have suggested strengthening the MI effect in PCOS women by combining the ALA with MI. Lagana et al., concluded that in the absence of strong evidence, the ALA should not be recommended in the routine management of PCOS, even if combined with MI.

A placebo-controlled trial compared the effect of MI plus folic acid (treated group) to the folic acid (placebo) and found that the ovulation frequency was significantly higher (p<0.01), and the time of first ovulation was significantly shorter (p<0.05) in the treated group. The same study found that the effect of MI on follicular maturation was rapid, with increased circulating E₂ from the first week of MI treatment.

Gullo et al. found that cultured embryos in embryo culture media rich in MI showed more physiological cleavage rate, and MI depletion may be responsible for the poor oocyte quality, and increased risk of ovarian hyperstimulation syndrome for PCOS women.

Moreover, Dell’Edera et al. found that the combination of MI and DCI could prevent ma-
ternal gestational diabetes mellitus (GDM) andmacrosomia in women with >92 mg/dl fastingblood sugar during the first trimester of pregnancy.

Beck-Fruchter et al41, reported a significantassociation between the live birth and progesterone(14.65 vs. 11.62 ng/ml), (p=0.001) as well as E₂levels (355.12 vs. 287.67 pg/ml), (p=0.001).

Recently, the innovation of Artificial intelligence (AI) in the field of assisted reproductive technology (ART) could improve infertility treatment for better outcomes and higher success rates42. The introduction of AI in ART treatment requires certain precautions, to maintain the ethical frameworks, prioritizing human dignity, privacy, and data protection42. The AI should be under human control since it impacts the well-being of unborn children42.

Abdelazim et al16, reported increased risks of hypothyroidism, and hyperprolactinemia in PCOS women. They concluded that hypothyroidism and hyperprolactinemia are common endocrine disorders observed in PCOS women, and should be treated before starting the ART treatment for PCOS women.

The relation between hypothyroidism and hyperprolactinemia in PCOS women was explained by the high Thyrotropin-releasing hormone in hypothyroidism (TRH act as a dopamine antagonist)43. The hyperprolactinemia predisposes to anovulation, luteinized unruptured follicle syndrome (LUFS), luteal phase defect, and defective steroidogenesis with subsequent ovarian hyperandrogenism, increased PCOS severity, and infertility43.

The relation between vitamin D and ovarian steroids, and the effect of vitamin D intake on ovarian steroids need further larger studies.

The current cohort study was the first conducted in Aktobe-West Kazakhstan to detect the relation between vitamin D and the adolescents’ mid-luteal E₂ and P, and the effect of vitamin D intake (50,000 IU weekly for 2 months) on the mid-luteal E₂ and P.

This study found that the mid-luteal E₂ and P statistically decreased after vitamin D intake (50,000 IU of vitamin D weekly for 2 months). Significant negative correlations between the 25(OH)D, and both the mid-luteal E₂ and P were detected in this study. The relation between vitamin D and ovarian steroids, and the effect of vitamin D intake on ovarian steroids needs further larger studies.

Conclusions
The mid-luteal E₂ and P statistically decreased after vitamin D intake (50,000 IU of vitamin D weekly for 2 months). Significant negative correlations between the 25(OH)D, and both the mid-luteal E₂ and P were detected in this study. The relation between vitamin D and ovarian steroids, and the effect of vitamin D intake on ovarian steroids needs further larger studies.

Conflict of Interest
The Authors declare that they have no conflict of interests.

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Informed Consent
Informed consent was obtained by the participants themselves or their guardians.

Ethics Approval
The study was approved by the West Kazakhstan Medical University Ethics Committee (Approval No. 10, dated 04.10.2020).

Availability of Data and Materials
The data analyzed during this cohort study are submitted with the manuscript.

Authors’ Contribution
AD, AA, and AZ are responsible for the study concept, and design, literature review, data collection, final revision before publication. IAA, AK and ES are responsible for the literature review, Microsoft editing, and final revision before publication. RN, GG, SS, DA, and ZK are responsible for literature review, Microsoft editing, update of the references, and final revision before publication. IIS is the corresponding author responsible for literature review, Microsoft editing, and drafting, final revision before publication and submission for publication. All the authors have read and agreed to the published version of the manuscript.
References


