Impact of body mass index and vitamin D on serum AMH levels and antral follicle count in PCOS

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Abstract. – OBJECTIVE: To investigate the effects of body mass index (BMI) values and 25(OH) vitamin D levels determined by Endocrine Society on serum Anti Mullerian Hormone (AMH) and antral follicle count (AFC) in women with polycystic ovary syndrome (PCOS).

PATIENTS AND METHODS: Sixty infertile women with PCOS and 30 age-matched women with unexplained infertility (UEI), were included. Patients in PCOS and control groups were divided into three subgroups according to their BMI values as normal, overweight and obese. Each BMI group was divided into three subgroups according to vitamin D levels. While AMH and vitamin D levels were determined at first admission, AFC was measured on the third day of cycle.

RESULTS: BMI, AFC, and AMH levels of women with PCOS were significantly higher than the UEI group. AMH values of women with PCOS with normal BMI were found to be significantly higher than UEI controls with normal BMI. AMH values of overweight and obese PCOS patients and controls were similar. As BMI values of the PCOS group increased, vitamin D levels decreased significantly. Vitamin D levels of the patients in the PCOS group were found to be significantly lower than the control group. When evaluated according to BMI, the vitamin D levels of normal, overweight and obese women with PCOS were significantly lower than the UEI.

CONCLUSIONS: Rising BMI in PCOS leads to a significant decrease in vitamin D and AMH. Deficiency, insufficiency or normality of vitamin D do not affect the main markers of ovarian reserve.

Key Words: PCOS, AMH, AFC, 25(OH) vitamin D, BMI.

Introduction

Although the main structure from which polycystic ovary syndrome (PCOS) originates is the ovarian tissue, it may adversely affect the endocrine, metabolic and inflammatory pathways of the affected person in addition to its subfertility-producing effect. Indeed, most of the undesirable effects of PCOS on metabolic pathways and fertility occur due to the deterioration in follicle development and subsequent endocrine functions. The increase in the frequency of anovulation, hyperandrogenism, and insulin resistance, which are the main findings of PCOS, in the deficiency of vitamin D, a steroid hormone, has brought forward the idea that this hormone may have a role in the etiology of PCOS. The improvement of some symptoms of PCOS with vitamin D replacement strengthened the idea that this hormone may have a role in the etiology of PCOS. With the demonstration of the target receptor of vitamin D in the endometrium, fallopian tubes and granulosa cells, some studies comparing vitamin D levels with the main biomarkers of ovarian reserve in PCOS patients and subsequently meta-analyses have been published.

In fact, PCOS is not a clinical condition that negatively affects ovarian reserve. However, the reserve definition is used to determine the patient’s response to controlled ovarian stimulation. Antral follicle count (AFC) and anti-Mullerian hormone (AMH) levels are two main biomarkers used to determine ovarian reserve in PCOS patients. In PCOS, ovarian reserve markers often determine the stage of arrest in follicular development rath-
er than the primordial follicle pool. It is unclear whether and how 25-hydroxy vitamin D [25(OH) D] and adiposity affect ovarian reserve in PCOS. Although there are lean and normal-weight PCOS patients, obesity, a state of excess triglycerides accumulation, is one of the main metabolic abnormalities of PCOS. Subfertile women with PCOS have a higher risk of being obese than other subfertile women in the same age group without PCOS. Body mass index (BMI) is an easy and widely accepted method of assessing obesity. Reports on how BMI affects the dynamics of folliculogenesis in PCOS patients are unclear. AFC is the sum of the FSH sensitive follicles between 2 and 10 mm and can be easily measured on ultrasonography. Although BMI might negatively affect different stages of folliculogenesis, there is no clear relationship between BMI and AFC. AMH is a glycoprotein produced by granulosa cells that regulates the transition between early preantral and small antral follicles and is a reliable marker of ovarian reserve. In addition to studies reporting a positive or negative correlation between BMI and AMH, there are studies reporting no correlation between the two. Initial studies reported that vitamin D was associated with ovarian reserve, subsequent clinical studies failed to show a correlation between bioavailable vitamin D, AMH, AFC, and adiposity.

In the light of the above information, it is possible to see that there are not enough studies that clearly reveal the changes in AFC and AMH levels at different BMI and 25(OH)D levels. One of the main reasons for this difference between the study results is that the patient groups are heterogeneous and the participants are not sufficiently divided into subgroups. This study was, therefore, planned to investigate the effect of 25(OH) D and BMI on AFC and AMH levels by dividing PCOS patients into three groups according to their BMI values, then again into three subgroups according to the vitamin D levels proposed by the Endocrine Society. While the participants in the study group were selected from subfertile PCOS patients, the selection of patients in the control group from among unexplained infertility (UEI) cases determined by ultrasonography. In order to ensure the homogeneity of the groups, those ≥ 30 kg/m² were considered obese. Normo-, overweight or obese patients aged 20-35 years who applied for infertility evaluation and were diagnosed with UEI were included in the study. Patients were diagnosed as PCOS based on the revised Rotterdam criteria, which require two of the following three manifestations: (1) oligo- and/or anovulation, (2) clinical and/or biochemical hyperandrogenism, and (3) polycystic ovaries determined by ultrasonography. In order to be included in the control group, the individual must not have any of the Rotterdam criteria. Unexplained infertility is defined as the absence of conception despite 12 months of unprotected intercourse, not explained by anovulation. In addition to ovulatory values of serum lutecizing hormone (LH) surge or mid-luteal progesterone presence of normal semen parameters according to WHO criteria and presence of patent fallopian tubes during hysterosalpingogram or laparoscopy UEI was diagnosed. Unexplained infertile patients should not have clinical, laboratory and ultrasonographic findings of PCOS. All patients were recruited within a time interval of 4 months from the initiation of the study. The study was performed according to the guidelines of the Helsinki Declaration on human experimentation and was approved by the Local Ethics Committee. Participants were selected from among the infertile population who applied to BAU Medicalpark Hospital IVF-Center.

Patients and Methods

Sixty infertile women diagnosed with PCOS and 30 age-matched women with unexplained infertility (UEI), were included in the study. Patients were diagnosed as PCOS based on the revised Rotterdam criteria, which require two of the following three manifestations: (1) oligo- and/or anovulation, (2) clinical and/or biochemical hyperandrogenism, and (3) polycystic ovaries determined by ultrasonography. In order to be included in the control group, the individual must not have any of the Rotterdam criteria. Unexplained infertility is defined as the absence of conception despite 12 months of unprotected intercourse, not explained by anovulation. In addition to ovulatory values of serum lutecizing hormone (LH) surge or mid-luteal progesterone presence of normal semen parameters according to WHO criteria and presence of patent fallopian tubes during hysterosalpingogram or laparoscopy UEI was diagnosed. Unexplained infertile patients should not have clinical, laboratory and ultrasonographic findings of PCOS. All patients were recruited within a time interval of 4 months from the initiation of the study. The study was performed according to the guidelines of the Helsinki Declaration on human experimentation and was approved by the Local Ethics Committee. Participants were selected from among the infertile population who applied to BAU Medicalpark Hospital IVF-Center.

Patients in PCOS and control groups were divided into three subgroups according to their BMI values. Those with a BMI of 18.5-24.9 kg/m² were considered normal weight, those with a BMI of 25-29.9 kg/m² were considered overweight, and those ≥ 30 kg/m² were considered obese. Normo-weight, overweight and obese PCOS women were further divided into three subgroups according to their 25(OH) vitamin D levels as proposed by the Endocrine Society. According to this classification, values below 20 ng/mL indicated vitamin D deficiency, while values between 20-30 ng/mL indicated vitamin D insufficiency. Women with vitamin D levels >30 ng/mL were considered normal/replete.

The following inclusion criteria were applied: normo-weight, overweight or obese patients aged 20-35 years who applied for infertility evaluation between 2020-2021 and were diagnosed with PCOS. Normo-weight, overweight or obese patients aged 20-35 years who applied for infertility evaluation and were diagnosed with UEI. The patients were given detailed information about the importance of vitamin D in reproductive biology. Patients with possible causes affecting ovarian reserve and thus AFC and AMH levels were not
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included in the study. Absence of ovaries due to genetic diseases, poor responders, a history of previous ovarian surgery, ovarian endometrioma, functional hypothalamic amenorrhea, and a history of gonadotoxic drugs or radiotherapy were excluded from the study. Patients with metabolic problems such as diabetes, Cushing’s syndrome, congenital adrenal hyperplasia, androgen-secreting tumor, hyperprolactinemia were excluded from the study. In addition, women taking vitamin D or other hormonal or systemic medications and those who had a weight change of 10 kg or more in the last 3 months were also excluded. All participants underwent venous blood sampling for the determination of AMH and 25(OH) vitamin D levels at first admission irrespective of the day of the menstrual cycle. Both groups of participants had serum AMH and 25(OH)D sampled on the same day. AFC was measured on the third day of the menstrual cycle. AFC means the sum of antral follicles between 2-10 mm in both ovaries.

25(OH) Vitamin D Measurement

Using samples taken in lithium-heparin tubes total plasma 25(OH) vitamin D were measured with chemiluminescent enzyme immunoassay on the Cobas E 601 device (Roche Diagnostics GmbH, Mannheim, Germany) and results were given as ng/mL.

Anti-Mullerian Hormone Measurement

Using samples taken in lithium-heparin tubes serum AMH levels were measured with Gen II Beckman Coulter AMH ELISA kit in Cobas autoanalyzer (Roche Diagnostics GmbH, Mannheim, Germany) according to the manufacturer’s instructions. The analytical sensitivity of this assay was 0.03 ng/mL, and inter- and intra-assay coefficients of variations of this method were noted as 3.7 and 2.1%, respectively. The measuring range was noted as 0.02 ng/mL to 24 ng/mL, the limit of detection was smaller than 0.02 ng/mL.

Hormonal and Biochemical Analysis

Venous blood samples were obtained after an overnight fasting between the 2nd and 5th days of natural menstrual cycle for UEI patients or progesterin withdrawal bleeding for PCOS patients. Hemoglobin A1c (HbA1c), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG) were measured by photometric method with an ADVIA 2400 autoanalyzer (Siemens, Munich, Germany). Low-density lipoprotein cholesterol (LDL-C) was calculated with the Friedewald formula. Serum follicular stimulating hormone (FSH), LH, estradiol, and insulin were measured with chemiluminescent enzyme immunoassay on the Cobas E 601 device (Roche Diagnostics GmbH, Mannheim, Germany). Total testosterone, and dehydroepiandrosterone sulfate (DHEA-S) were measured via the radioimmunoassay method on the Cobas E 601 (Roche Diagnostics GmbH, Mannheim, Germany). Insulin resistance (IR) was evaluated by calculating homeostatic model assessment of insulin resistance [HOMA-IR = fasting blood glucose (mg/dL) x fasting insulin (mIU/L)/405]. The study was performed according to the guidelines of the Helsinki Declaration on human experimentation and was approved by the Local Ethics Committee. Informed consent was obtained from participants. The primary outcome of the study is to investigate the effects of 25(OH) vitamin D concentration and BMI values on serum AMH and AFC in PCOS patients.

Statistical Analysis

The Statistical Package for Social Sciences, version 21.0 (IBM Corp., Armonk, NY, USA) was used for statistical analysis. Vitamin D, AMH and other individual group parameters were assessed with one-sample Kolmogorov-Smirnov Z test and were found to be abnormally distributed. Hence, statistical comparisons between groups were performed by nonparametric Mann-Whitney U test. Spearman’s correlation analysis was used for detecting correlation between AMH, 25(OH) vitamin D and other parameters. Data are presented as mean±SD. For all comparisons, statistical significance was defined by \( p < 0.05 \).

Results

Demographic, hormonal and biochemical characteristics of both groups are shown in Table I. BMI, AFC, and serum AMH levels of PCOS patients were significantly higher than the control group. When the women in PCOS and control groups were divided into three subgroups as normal, overweight and obese, AMH values of PCOS patients with normal BMI were found to be significantly higher than UEI controls with normal BMI. AMH values of overweight and obese PCOS patients and controls were similar. As the BMI values of the patients in the PCOS group increased, 25(OH)D levels decreased significantly. As the BMI value of the patients in
the control group increased, serum 25(OH)D levels decreased, but this decrease did not reach statistical significance (Table II). Serum 25(OH)D levels of the patients in the PCOS group were found to be significantly lower than the control group. When evaluated according to BMI the 25(OH)D levels of normal, overweight and obese PCOS patients were significantly lower than the UEI controls. Among all patients in PCOS group, 56.7% (n = 34) were vitamin D deficient (<20 ng/mL) whereas 25% (n = 15) had vitamin D insufficiency (20-30 ng/mL) and the remaining 18.3% (n = 11) had replete vitamin D levels (>30 ng/mL).

Table I. Demographic, hormonal and biochemical characteristics of PCOS and UEI groups.

<table>
<thead>
<tr>
<th></th>
<th>PCOS (n = 60)</th>
<th>UEI (n = 30)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>24.6 ± 3.22</td>
<td>25.1 ± 2.09</td>
<td>0.07</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29.63 ± 4.56</td>
<td>26.12 ± 3.07</td>
<td>0.02</td>
</tr>
<tr>
<td>AMH (ng/mL)</td>
<td>4.28 ± 1.46</td>
<td>3.57 ± 0.80</td>
<td>0.03</td>
</tr>
<tr>
<td>AFC (number)</td>
<td>13.46 ± 3.66</td>
<td>9.34 ± 2.09</td>
<td>0.01</td>
</tr>
<tr>
<td>25(OH)D (ng/mL)</td>
<td>23.9 ± 3.44</td>
<td>28.4 ± 5.07</td>
<td>0.02</td>
</tr>
<tr>
<td>FSH (mIU/mL)</td>
<td>5.11 ± 0.30</td>
<td>4.90 ± 1.02</td>
<td>0.55</td>
</tr>
<tr>
<td>LH (mIU/mL)</td>
<td>8.13 ± 2.87</td>
<td>6.01 ± 2.04</td>
<td>0.03</td>
</tr>
<tr>
<td>Estradiol (pg/mL)</td>
<td>39.6 ± 5.33</td>
<td>33.5 ± 3.01</td>
<td>0.46</td>
</tr>
<tr>
<td>HbA1c</td>
<td>5.11 ± 1.12</td>
<td>5.03 ± 2.11</td>
<td>0.06</td>
</tr>
<tr>
<td>Insulin (uU/mL)</td>
<td>13.4 ± 3.01</td>
<td>6.91 ± 0.33</td>
<td>0.02</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.97 ± 0.44</td>
<td>1.44 ± 0.22</td>
<td>0.01</td>
</tr>
<tr>
<td>C-peptide (ng/mL)</td>
<td>2.63 ± 0.40</td>
<td>1.39 ± 0.20</td>
<td>0.03</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>38.3 ± 4.01</td>
<td>44.3 ± 5.11</td>
<td>0.04</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>131.4 ± 11.4</td>
<td>120.4 ± 8.55</td>
<td>0.49</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>110.3 ± 6.43</td>
<td>69.4 ± 2.05</td>
<td>0.68</td>
</tr>
<tr>
<td>Total Testosterone (ng/mL)</td>
<td>0.49 ± 0.03</td>
<td>0.32 ± 0.04</td>
<td>0.02</td>
</tr>
<tr>
<td>17-OH PG (ng/mL)</td>
<td>0.54 ± 0.22</td>
<td>0.47 ± 0.11</td>
<td>0.30</td>
</tr>
<tr>
<td>DHEA-S (UG/dL)</td>
<td>366.3 ± 14.5</td>
<td>320.4 ± 44.6</td>
<td>0.65</td>
</tr>
</tbody>
</table>

Table II. AMH and 25(OH) vitamin D levels classified according to the BMI values of the PCOS and UEI groups.

<table>
<thead>
<tr>
<th></th>
<th>AMH</th>
<th>25(OH) vitamin D</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PCOS</td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18.5-24.9 kg/m²</td>
<td>Normal</td>
<td>5.03 ± 2.09</td>
</tr>
<tr>
<td>25-29.9 kg/m²</td>
<td>Overweight</td>
<td>3.94 ± 0.49</td>
</tr>
<tr>
<td>≥ 30 kg/m²</td>
<td>Obese</td>
<td>3.87 ± 1.81</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>4.28 ± 1.46</td>
</tr>
</tbody>
</table>

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Table III. AMH and AFC of the PCOS group according to BMI and vitamin D status as proposed by Endocrine Society (ES).

<table>
<thead>
<tr>
<th>BMI</th>
<th>18.5-24.99 kg/m² Normal</th>
<th>25-29.9 kg/m² Overweight</th>
<th>≥30 kg/m² Obese</th>
</tr>
</thead>
<tbody>
<tr>
<td>25(OH)D*</td>
<td>&lt; 20 ng/mL</td>
<td>20-30 ng/mL</td>
<td>&gt;30 ng/mL</td>
</tr>
<tr>
<td>AMH</td>
<td>5.11 ± 2.08</td>
<td>5.02 ± 0.40</td>
<td>4.96 ± 2.01</td>
</tr>
<tr>
<td>AFC</td>
<td>12.6 ±2.60</td>
<td>12.9 ± 3.11</td>
<td>13.1±3056</td>
</tr>
</tbody>
</table>

*According to ES classification, values below 20 ng/mL indicated vitamin D deficiency, while values between 20-30 ng/mL indicated vitamin D insufficiency. Women with vitamin D levels > 30 ng/mL were considered normal/replete. BMI: Body Mass Index, 25(OH)D: 25-Hydroxy Vitamin D, AFC: Antral Follicle Count, AMH: Anti Mullerian Hormone.
AMH was positively correlated with AFC, LH, 17-hydroxyprogesterone (17-OH progesterone) and total testosterone. Age and AMH levels were negatively correlated in PCOS and control group. AMH levels were not correlated with HOMA-IR and insulin levels. No correlation was observed between 25(OH) vitamin D, AMH and AFC in the PCOS or control group. Both AFC and AMH levels did not vary according to serum 25(OH)D concentrations. While serum AMH and 25(OH)D levels were negatively correlated with BMI in the PCOS group BMI was not correlated with AMH in the control group. No significant correlation existed between serum AMH levels and dyslipidemia markers.

**Discussion**

The main finding of the current study is that the increase in BMI leads to a significant decrease in serum AMH and 25(OH) vitamin D levels in women with PCOS. AFC, on the other hand, was not affected by BMI values. While AMH levels of normal weight PCOS patients were found to be significantly higher than those of overweight and obese PCOS patients, AMH levels of overweight and obese PCOS patients were similar. AMH levels of all three BMI groups did not show a significant change compared to serum 25(OH) vitamin D values. AMH values were found to be similar in each value of vitamin D determined by the Endocrine Society. AMH and 25(OH) vitamin D levels of the patients in the control group did not change according to BMI. 25(OH) vitamin D levels of PCOS patients decreased in parallel with the increase in BMI. A negative correlation was found between BMI and 25(OH) vitamin D levels. Similarly, a negative correlation was found between increase in BMI and serum AMH levels. AMH and 25(OH)D values of the patients in the control group were not correlated with the increase in BMI. The mean AFC did not differ significantly between the vitamin D deficient, vitamin D insufficient and the vitamin D replete women with PCOS.

Data\(^2,5\) showing the relationship between serum 25(OH) vitamin D levels and AMH in PCOS patients are inconsistent. Consistent with literature, we found serum 25(OH) vitamin D levels to be significantly lower in PCOS patients compared to the control group. While some studies\(^1\) mentioned a positive correlation between vitamin D and AMH, some did not find any correlation\(^1,16\). A recent meta-analysis also reported that vitamin D supplementation had an effect on AMH levels according to the patient’s ovulatory status\(^\). Administration of vitamin D to anovulatory PCOS patients reduces AMH levels, while administration of vitamin D to ovulatory and non-PCOS patients increases AMH levels\(^\). Contrary to these data, Cappy et al\(^19\) reported that giving vitamin D had no effect on AMH values in cases with or without PCOS. More interestingly, there are also authors\(^20\) reporting that the administration of vitamin D to PCOS patients reduces AMH levels. It should be emphasized that the decrease in AMH levels after vitamin D in PCOS may be an indicator of the improvement of PCOS-related menstrual, metabolic and androgen increase-related problems rather than a negative effect on ovarian reserve\(^\).

When we classified the patients according to the 25(OH) vitamin D values, we found that normal or deficient vitamin D levels had no effect on ovarian reserve markers. Vitamin D deficiency (<20 ng/mL), insufficiency (20-30 ng/mL), or sufficiency (>30 ng/mL) did not significantly affect serum AMH and AFC levels. Neville et al\(^21\) reported that there was no correlation between vitamin D levels and AMH. Shapiro et al\(^22\) stated that AMH levels are not affected by vitamin D deficiency or normality. Drakopoulos et al\(^23\) showed that AMH and AFC levels of infertile women with deficient or normal vitamin D levels were not different. In a study\(^24\) comparing AMH and 25(OH)D levels in metabolic syndrome patients, they stated that there was no relationship between these two markers. The fact that there is no clear relationship between AMH and vitamin D levels does not necessarily mean that there is no relationship. Treatment of prostate cell cultures with vitamin D resulted in increased expression of AMH\(^25\). The presence of the vitamin D-response element in the promoter region of AMH is another important evidence of the close relationship between 25(OH) vitamin D and AMH\(^26\). The lack of a relationship between AMH and vitamin D in clinical studies may be due to the complexity of this relationship or the heterogeneity of patient groups. In support of this idea, in a recent meta-analysis\(^27\), it was reported that a correlation was found between vitamin D levels and AMH and AFC when the subgroup analysis of the studies was performed.

The most important finding of this study is that the increase in BMI values of PCOS patients leads to a decrease in both serum AMH...
and 25(OH) vitamin D levels. AMH values, which we found in the normal BMI value in our PCOS patients, decreased significantly with the increase in BMI. Consistent with our results, Georgopoulos et al\textsuperscript{28} and Freeman et al\textsuperscript{29} reported that BMI and AMH were negatively correlated. However, there are also studies\textsuperscript{10,15} reporting that obesity does not affect ovarian reserve markers. There are many data\textsuperscript{28,29} showing that follicle dynamics is impaired in obese cases. In obese patients with controlled ovarian hyperstimulation, serum estradiol levels are lower than in healthy controls, which is evidence of poor oocyte quality. Similarly, the fertilization capacity of the oocytes of obese cases is lower and the chance of clinical pregnancy is less. Low birth rates and high abortion rates in obese are other evidences that adiposity negatively affects oocyte quality\textsuperscript{30-32}.

We do not know by which mechanism an increase in BMI decreases AMH levels. However, AMH levels may be decreased in obese patients due to increased hemodilution\textsuperscript{33}. By affecting the release of adiposity leptin, it can reduce the release of AMH by impairing mural and cumulus granulosa cell functions \textit{via} the Janus kinase 2/signal transducer and activator of transcription 3 (JAK2/STAT3) pathway\textsuperscript{34}. The reduction of AMH secretion by leptin treatment is an important evidence of the relationship between adiposity and AMH\textsuperscript{34}. The relationship between vitamin D levels and BMI in obesity due to PCOS is not very clear. Baker et al\textsuperscript{35} reported no correlation between 25(OH)D and BMI in PCOS patients. On the other hand, Marques-Pamies et al\textsuperscript{36} showed that obesity and vitamin D levels were negatively correlated in patients who underwent surgery for morbid obesity. Since the accumulation of vitamin D in the adipose tissue due to its fat-soluble characteristic and its conversion to 25(OH)D are impaired in obese subjects, it is usual to have different results between studies\textsuperscript{37}.

Conclusions

Our study is stronger than previous studies in terms of some findings. First of all, not only we evaluated serum AMH levels in association with 25(OH) vitamin D, but we also assessed AFC and BMI. Secondly, although the patients in the control group were selected from the infertile population, it is important for homogeneity that they were selected from unexplained infertile patients whose metabolic parameters were not similar to PCOS. Third, we can say that the results are more reliable because AMH and 25(OH) vitamin D levels are studied on the same day and without waiting. Finally, we would like to state that the number of our cases is not sufficient to detect a nonlinear relation between vitamin D and AMH and AFC. Despite all these limitations, our results are important in showing that there is no direct relationship between vitamin D and ovarian reserve markers, AMH and AFC, in PCOS patients.

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

**Ethics Approval**
The study was performed according to the guidelines of the Helsinki Declaration on human experimentation and was approved by the Ethics Committee of Medeniyet University (2021-0747).

**Informed Consent**
Informed consent was obtained from participants at the time of enrollment.

**Authors’ Contribution**
Concept: Nur Dokuzeylül G"ung"or; Design: Kağan G"ung"or; Supervision: Nil"uf"er Celik, Asena Ayar Madenli; Resource: Kağan G"ung"or, Nur Dokuzeylül G"ung"or; Materials: Kağan G"ung"or, Nur Dokuzeylül G"ung"or, Murat Onal; Data collection and/or processing: Nur Dokuzeylül G"ung"or, Murat Onal; Literature search: Kağan G"ung"or; Writing: Nur Dokuzeylül G"ung"or, Kağan G"ung"or; Critical Reviews: Nil"uf"er Celik, Murat Onal.

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**Availability of Data and Materials**
The data supporting this article are available from the corresponding author on reasonable request.
References


