Evaluation of Vitamin D and Anti-Müllerian Hormone Levels in Iraqi Infertile Women

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Abstract
Although the mechanism underlying the relationship between vitamin D insufficiency and reproduction is unknown, research suggests that it may have a direct deleterious impact on ovarian function. This is primarily because vitamin D insufficiency can affect gonadal function. The anti-müllerian hormone (AMH) is one of the most important biomarkers produced by granulosa cells and plays a key role in folliculogenesis. This study wanted to look at and compare vitamin D and AMH levels in infertile and fertile women, as well as the relationship between them in both groups. A hundred infertile and fertile women participated in the study. Anti-müllerian, prolactin, follicle-stimulating, and luteinizing hormones, as well as 25-hydroxyvitamin D, were estimated. Vitamin D deficiency was found in 72% of infertile women (n = 36), compared to 48% (n = 24) of the fertile group. There was no significant link between 25-hydroxyvitamin D and AMH in both groups. Infertile women exhibited a significantly lower serum AMH and higher body mass index. This study's findings suggested that the correlation between vitamin D and ovarian reserve markers was unlikely to present. However, the infertile group has a more significant vitamin D deficiency and insufficiency rate.

Keywords: anti-müllerian hormone, reproduction, vitamin D

Introduction
Infertility is a complex illness with substantial medical and emotional consequences. It is a common condition characterized by the inability to get pregnant after a year or more of unprotected sex.1 Vitamin D insufficiency is a serious health issue affecting people of all ages worldwide because of its critical role in bone health.2 Vitamin D levels have been linked to various illnesses in numerous studies, and it has an enormous consequence on gene expression in reproductive organs, implying that it has a role in fertility. The mechanism underlying this relationship is unknown. However, the discovery of the Vitamin D receptors and the enzyme 1-hydroxylase in organs such as the uterus, ovary, and placenta may explain how vitamin D shortage affects gonadal function. As a result, vitamin D is becoming a new factor in reproductive health.3

The most frequent approach for determining vitamin D levels is to check the blood concentration of 25-hydroxyvitamin D. It is the most accurate measure of vitamin D levels, which has a half-life of three weeks and hence is a correct indicator of vitamin D status.4 According to several studies summarized in Grzechocinska, et al.,’s study,5 vitamin D interacts with the anti-müllerian hormone (AMH) gene, directly altering its synthesis, and hence ovarian activity is sustained longer in women with higher vitamin D levels. According to some study, AMH production may be positively regulated by vitamin D,6 while others found no link.7

The AMH is a granulosa cell-produced dimeric glycoprotein thought to be one of the most important ovarian reserve markers.8 Its main role in the ovary is to prevent early stages of ovarian follicle growth, preserve ovarian reserve, and regulate the influence of follicle-stimulating hormone (FSH) on follicles.9 Thus, this study aimed to look at and compare vitamin D and AMH levels in infertile and fertile women and the relationship between serum AMH and vitamin D levels in both groups

Method
This study was conducted in Mosul City, Iraq, at various centers. The Medical Research Ethics Committee of the College of Medicine at the University of Mosul, Iraq, approved this study. Before collecting data, all partici-
pants were informed about the study’s goals and received their agreement. This was a case-control study with 100 women ranging from age 18 to 40 years old. They were split into two categories: control, which consisted of 50 seemingly healthy fertile women, and case, which consisted of 50 infertile women with unexplained infertility. Records of smoking, usage of steroids, hormonal drugs, or oral contraceptive pills, vitamin D insufficiency, endometriosis, autoimmune disease, polycystic ovarian syndrome, thyroid disorders, tubal cause, or male cause were all ruled out.

After an overnight fast, a sample of five mL of venous blood was drawn from each woman on the second day of her menstrual cycle to assess the biochemical markers 25-hydroxyvitamin D, AMH, FSH, luteinizing hormones (LH), and prolactin hormone. The mini VIDAS assay, a bioMérieux automated quantitative enzyme-linked fluorescent assay (ELFA) technology, was used to measure all parameters.10,11 The body mass index (BMI) = weight (kg)/height$^2$ (m$^2$), and according to the World Health Organization (WHO), a BMI of less than 18.5 kg/m$^2$ is considered underweight, 18.5-24.9 kg/m$^2$ is considered normal weight, (25-29.9 kg/m$^2$) is overweight, and a BMI of 30 kg/m$^2$ or above is considered obesity.12

The Institute of Medicine and the Endocrine Society Clinical Practice Guidelines stated that 25-hydroxyvitamin D values below 20 ng/mL indicate a deficiency, between 20 and 30 ng/mL suggest insufficiency, and over 30 ng/mL indicate normal.4 The data in this study were analyzed using the SPSS Program, and the mean and standard deviation were used to express the findings. The differences between the groups were investigated using the Student’s t-test, and Pearson’s correlation test was used to identify the link between vitamin D and AMH.

### Results

A case-control study has been handled on 100 women, 50 fertile and 50 infertile, both groups having ages ranging from 18 to 40 years. The controls had a BMI between 19.4-26.6 kg/m$^2$, while the patients had a BMI between 18.9-35 kg/m$^2$. Table 1 displays descriptive statistics for each of the groups studied using the Student’s t-test. In some cases, the BMI was considerably greater (p-value = 0.019). There was no discernible age difference between the two groups.

Table 2 shows that the case group has an elevated percentage of vitamin D deficiency than the control. Table 3 demonstrates that 62% of the case group were overweight and obese (40% overweight and 22% obese), while only 26% of the controls were overweight. Table 4 demonstrates that the amount of serum AMH in patients is significantly lower than in controls (p-value = 0.0001). Vitamin D had no significant link with AMH in both groups, according to Table 5 using Pearson’s correlation test.

### Discussion

The importance of vitamin D in fertility becomes more widely recognized. Several studies have pointed to a link between efficient infertility therapies and adequate vitamin D levels.13 Ovarian function and AMH production may be affected by vitamin D, according to study.14 In young women, serum levels of AMH are thought to rise quickly after a high-dose vitamin D supplement.15

The BMI in the case group of this study (mean±SD = 25.38±5.09) was significantly higher than that of the controls (mean±SD = 23.6±2.29) with a p-value = 0.019, and 40% of infertile women were overweight, with 22% obese, compared to 26% of the fertile group were overweight. This followed Dağ and Dilbaz’s study, which

### Table 1. Characteristics of Respondents Based on Age and Body Mass Index

<table>
<thead>
<tr>
<th>Variable</th>
<th>Case (n = 50)</th>
<th>Control (n = 50)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18-40</td>
<td>31.20</td>
<td>32.58</td>
<td>0.259</td>
</tr>
<tr>
<td>18.5-24.9 kg/m$^2$</td>
<td>25.38</td>
<td>23.6</td>
<td></td>
</tr>
<tr>
<td>18.9-35 kg/m$^2$</td>
<td>20</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>≥30 kg/m$^2$</td>
<td>9</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Note: SD = Standard Deviation

### Table 2. The Percentage of Vitamin D Levels in Both Groups

<table>
<thead>
<tr>
<th>Vitamin D</th>
<th>Case (n = 50)</th>
<th>Control (n = 50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deficiency (&lt;20 ng/mL)</td>
<td>36 72%</td>
<td>24 48%</td>
</tr>
<tr>
<td>Insufficiency (20-30 ng/mL)</td>
<td>10 20%</td>
<td>9 18%</td>
</tr>
<tr>
<td>Normal (&gt;30 ng/mL)</td>
<td>4 8%</td>
<td>17 34%</td>
</tr>
</tbody>
</table>

### Table 3. The Percentage of Body Mass Index in Both Groups

<table>
<thead>
<tr>
<th>Body Mass Index</th>
<th>Case (n = 50)</th>
<th>Control (n = 50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18.5-24.9 kg/m$^2$</td>
<td>19 38%</td>
<td>37 74%</td>
</tr>
<tr>
<td>25-29.9 kg/m$^2$</td>
<td>20 40%</td>
<td>13 26%</td>
</tr>
<tr>
<td>≥30 kg/m$^2$</td>
<td>11 22%</td>
<td>0 0</td>
</tr>
</tbody>
</table>

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found that being overweight and obese can impair fertility. This could be owing to a negative relationship between BMI and the amount of estradiol generated, resulting in a reduction in estradiol concentration as BMI rises.

In this study, 72% of women in the case group were vitamin D deficient, 20% had insufficient vitamin D, and 8% were of normal vitamin D levels, which were similar to the findings of Lata, et al., finding that 64.28% of infertile women had vitamin D insufficiency. Dressler, et al., discovered that 98.2% and 81.3% of infertile women in two different clinics had vitamin D insufficiency or deficiency.

In this study’s investigation, it was found that vitamin D levels in the case and control groups were not significantly different. Even though the case group’s levels were lower, implying that vitamin D may play a function in fertility. According to the Rudick, et al., study, Caucasian women with vitamin D insufficiency have a lower pregnancy rate (37%) than women with adequate vitamin D (78%).

The case group had a considerably lower level of AMH than the controls (p-value = 0.0001). This was in line with the findings of Lata, et al.,’s study, which discovered lower AMH in cases (p-value = 0.005), that may be a factor in infertility in this group because AMH is a reflection of ovarian reserve and responsiveness, which influence reproduction in women, excluding other sources of infertility. The FSH, LH, and prolactin hormone levels did not differ significantly among the groups studied, and they were all within normal ranges.

Despite the fact that there is no concurrence on whether vitamin D affects AMH generation, it has been suggested that it directly affects ovarian function. Vitamin D has a positive correlation with AMH; according to Merhi, et al., the AMH stimulant was also found to be influenced by vitamin D in another investigation of human granulosa cells. These studies clashed with this study, which established no significant linkage between AMH and vitamin D. This was in line with the results of Lata, et al., which established no connection between vitamin D levels and AMH. This study’s findings were also consistent with Drakopoulos, et al.,’s study, also a retrospective analysis of 340 polycystic ovary syndrome and ovulatory women revealed that AMH levels were not linked with serum vitamin D concentrations.

**Conclusion**

Finally, this study’s results revealed no significant correlation between AMH and vitamin D levels in both fertile and infertile women. Even though the infertile group has an elevating percentage of vitamin D deficiency. Therefore, to do a routine of vitamin D testing and therapy for deficient individuals to avoid depletion of ovarian reserve appears to be ineffective. Nonetheless, vitamin D may have a role in human reproduction, and ongoing prospective and translational research initiatives are desperately needed to investigate the possible impact of vitamin D insufficiency on reproductive outcomes.

**Abbreviations**


**Ethics Approval and Consent to Participate**

All process of this study was recognized by the ethical committee of University of Mosul (Reg. MREC/2018-8).

**Competing Interest**

The authors declare that there are no significant competing financial, professional, or personal interests that might have affected the performance or presentation of the work described in this manuscript.

**Availability of Data and Materials**

All raw or complementary data are available upon request.
Authors’ Contribution
SKH, ZMAH, and BHA did the experimental process, analysis, and manuscript writing.

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