Abstract. – OBJECTIVE: Endocrine Society classified patients with serum 25-hydroxyvitamin D [25(OH)D] levels below 20 ng/ml as deficiency, 20-30 ng/ml as insufficiency, and >30 ng/ml as replete. This study was planned to investigate the relationship between serum vitamin D level and homeobox 10 mRNA expression in women with polycystic ovary syndrome (PCOS).

PATIENTS AND METHODS: Thirty women with PCOS who failed the first IVF/ICSI attempt and were decided to have endometrial injury before second attempt were included in the study. Before the endometrial injury, the serum vitamin D levels of the women were measured, and they were divided into three equal groups as proposed by the Endocrine Society. Group 1 consisted of vitamin D deficient women (<20 ng/mL), Group 2 consisted of vitamin D insufficient women (20-30 ng/mL), and Group 3 consisted of vitamin D replete women (>30 ng/mL). Women in each group were injured with a Pipelle cannula during mid-luteal phase. Endometrial samples collected during injury were analyzed for HOXA10 mRNA expression by RT-PCR and correlated with serum vitamin D level.

RESULTS: When analyzing the results according to different vitamin D thresholds, as proposed by the Endocrine Society, HOXA10 mRNA expression was comparable between vitamin D deficient and vitamin D insufficient women. The HOXA10 mRNA expression of vitamin D replete women (Group 3) was found to be higher than both vitamin D deficient (Group 1) and vitamin D insufficient women (Group 2). HOXA10 mRNA expression of the women in Group 3 was 3.3-fold higher than Group 1 and 2.6-fold higher than Group 2. HOXA10 mRNA expression was correlated to the levels of vitamin D in the Group 3 (r=0.655, p=0.02). There was no significant correlation between serum vitamin D levels and endometrial HOXA10 mRNA expression of women in both Group 1 (r=0.343, p=0.06) and Group 2 (r=0.456, p=0.08).

CONCLUSIONS: Endometrium of women with PCOS with sufficient serum vitamin D levels express significantly higher HOXA10 mRNA than patients with low serum vitamin D levels.

Key Words: PCOS, Vitamin D, HOXA, Endometrium.

Introduction

Vitamin D is a steroid hormone and contributes to the realization of many physiological functions by acting on both calcium-dependent and calcium-independent pathways. Due to the widespread presence of vitamin D receptors (VDRs) in the whole body, it has led to the establishment of a link between vitamin D levels and many human diseases. Due to the intense expression of VDRs in the endometrium, ovary, granulosa cells and placenta1, it has come to the fore that there may be a close relationship between vitamin D levels and reproductive outcome, and studies have been carried out1-3 to explain this relationship. Although the results of studies1-3 comparing vitamin D levels and assisted reproduction results are mixed, most studies4,5 have shown that serum and follicular fluid vitamin D levels are highly correlated. Similarly, clinical pregnancy rates of individuals with high serum or follicular fluid vitamin D levels were found to be significantly higher than those with low vitamin D levels2,3. Each nmol/l increase in follicular liquid vitamin D level leads to an approximately 2.5% increase in clinical pregnancy rates3. Conversely, there are also studies4 reporting that oocytes obtained from patients with low FF vitamin D levels are more likely to produce high quality embryos and clinical pregnancy. On the other hand, Rudick et al5 emphasized that the relationship between vitamin D levels and pregnancy rates should be interpreted more carefully, since vitamin D measurement methods differ between studies1-3 and vitamin D-binding protein polymorphism is not taken into account.
Considering the above-mentioned conflicting evidence, we thought that vitamin D might exert its fertility-promoting effect through the endometrium. The fact that VDRs are common in the endometrium and regulate the expression of approximately one thousand genes was the basis of this thought. Homeobox genes are from the abdominal B gene family and are responsible for the release of growth factors and cytokines necessary for decidualization, implantation, and embryo survival. HOXA10, the level of which increases in the human and mouse peri-implantation period, provides successful embryo implantation. A study investigating the effects of vitamin D levels on HOXA10 gene has not been conducted yet. Therefore, this study was planned to investigate the possible relationship between serum vitamin D level and endometrial homeobox10 mRNA expression in women with polycystic ovary syndrome (PCOS). Since PCOS is an endocrine disorder characterized by both subfertility and defects in circulating vitamin D level, all participants were selected among the women who underwent ART with the diagnosis of PCOS. Thus, it was ensured that the participants were a homogeneous group.

**Patients and Methods**

Thirty women with PCOS who failed the first IVF/ICSI attempt and were decided to have endometrial injury before second attempt were included in the study. Before the endometrial injury, the serum vitamin D levels of the women were measured, and they were divided into three groups, as proposed by the Endocrine Society. Group 1 consisted of vitamin D deficient women (<20 ng/mL), Group 2 consisted of vitamin D insufficient women (20-30 ng/mL), and Group 3 consisted of vitamin D replete women (>30 ng/mL). Women in each group were injured with a Pipelle cannula during mid-luteal phase. Endometrial samples collected during injury were analyzed for HOXA10 mRNA expression by RT-PCR and correlated with serum vitamin D level. The study was performed according to the guidelines of the Helsinki Declaration on human experimentation and was approved by the Altinbas University Local Ethics Committee.

**Expression Analysis HOXA10 mRNA**

For expression analysis of targeted genes, total RNA was extracted from homogenized tissue material obtained from endometrium, using with GeneAll Hybrid–RTM RNA Kit (GeneAll, Korea). After isolation of total RNA, 1 µg of extracted RNA was used for cDNA synthesis, which performed with GeneAll 2X HyperScript TM One-Step cDNA Kit in accordance with the manufacturer’s instructions (GeneAll, Korea). The expression levels of HOXA10 mRNA were detected by using SYBR Green methodology (Eva Green PCR Master Mix, Biotium) in the Step-One Plus Real Time PCR System (Applied Biosystems, Foster City, CA, USA), with 25 µl of total reaction volume. The housekeeping GAPDH gene was used for normalization. The mRNA levels were calculated using the comparative ΔCt method (Ct of target gene - Ct of reference gene). Relative changes of mRNA expression levels of the analyzed HOXA10 gene were calculated using the 2^(-ΔΔCt) method (ΔC T treated - ΔC T untreated) and the data obtained from 2^(-ΔΔCt) method were used for statistical analysis.

**Statistical Analysis**

Data were analyzed with the use of the Statistical Package for Social Sciences software 21.0 for Windows package software (IBM Corp., Armonk, NY, USA). The normality distribution of data was tested with the Kolmogorov-Smirnov test. The continuous variables were analyzed by ANOVA test and Mann-Whitney U test. The categoric data were analyzed by means of the Pearson Chi-square test. Data are presented as the means ± SD. Spearman’s correlation analysis was used for detecting correlation between vitamin D and HOXA10 mRNA. A p-value of <.05 was considered statistically significant. Fold increases were considered to be positive for transcript overexpression when the corresponding mRNA level was ≥3-fold higher than that of initial transcript expression, negative if <2-fold.

**Results**

When analyzing the results according to different vitamin D thresholds HOXA10 mRNA expression was comparable between vitamin D deficient and vitamin D insufficient women. The HOXA10 mRNA expression of vitamin D deficient women (Group 3) was found to be higher than both vitamin D deficient women (Group 1) and vitamin D insufficient women (Group 2). HOXA10 mRNA expression of the women in Group 3 was 3.3-fold higher than Group 1 and 2.6-fold higher than Group 2. HOXA10 mRNA expression was correlated with the levels of vitamin D in the Group 3 (r = 0.655, p = 0.02). No significant correlation was observed between serum vitamin D and endo-
metrial HOXA10 mRNA expression in vitamin D deficient \((r=0.343, p=0.06)\) and insufficient women \((r=0.456, p=0.08)\). The mean value of HOXA10 mRNA by real time PCR in vitamin D deficient, insufficient, or replete women were 28, 27, and 23 arbitrary units (AU), respectively. The mean AU value of vitamin D replete women was significantly higher than both vitamin D deficient and insufficient women. The mean AU values of vitamin D deficient and sufficient women were comparable.

**Discussion**

Despite the abundance of opinions\(^5,9\) that Vitamin D provides its fertility promoting effect through the endometrium, sufficient studies have not been conducted\(^1-5\) to clarify these views. Our study is the first clinical study showing the relationship between vitamin D levels and receptivity genes. Most of the studies\(^3,10,11\) have suggested that the fertility outcomes of infertile individuals with sufficient vitamin D levels are better than those with vitamin D deficiency, and that this positive effect is provided by vitamin D on regulating folliculogenesis and stimulating oocyte developmental capacity. In good agreement with this, Xu et al\(^10\) reported that administration of vitamin D3 in primates both increased preantral follicle survival and regulated AMH levels, depending on the stage and administered dose. However, Drakopoulos et al\(^12\) reported that vitamin D levels did not have a significant effect on AMH and antral follicle count in the group of infertile patients who underwent ART. Although the results from clinical and experimental studies are consistent enough to imply a relationship among vitamin D, follicle maturation and embryo quality, data from clinical studies\(^10,12\) are strongly contradictory. For this reason, the ideas that vitamin D shows its positive effects on fertility through the endometrium have started to gain importance. The donor-oocyte recipient model was used to test whether vitamin D exerts its effect on the endometrium or oocytes. Since pregnancy rates were found to be low in egg donation recipients with a vitamin D level of <30 ng/ml, it was suggested that vitamin D exerts its effect through the endometrium\(^13\). However, in the cycles in which euploid blastocyst transfer was performed, no correlation was found between the patient’s vitamin D levels and pregnancy outcome\(^14,15\).

Both vitamin D and VDRs are synthesized in the endometrium independently of the phase of the cycle. Vigano et al\(^9\) showed that the 1 alpha OH-ase gene responsible for vitamin D synthesis is expressed in every period of the cycle. The same authors reported that VDRs are also expressed throughout the cycle and during decidua formation. These findings suggest that vitamin D plays an active role during decidualization required for successful embryo implantation. The net effects of vitamin D on the development of decidua, whose antiproliferative and immunomodulatory effects are clearly known, are not known\(^9\). Our study is the first to investigate the effects of vitamin D on HOXA10, the main gene responsible for endometrial receptivity. HOXA10 is a gene that increases expression in the implantation window and regulates decidualization\(^8\). In the absence of this gene, implantation does not occur\(^7\). When our patients were divided into three groups according to their vitamin D levels and HOXA10 mRNA expression was examined, we found that HOXA10 mRNA levels were significantly higher in Vitamin D replete women compared to vitamin D deficient and vitamin D insufficient women. High levels of HOXA10 in the presence of adequate vitamin D suggest that vitamin D contributes to the expression of receptivity genes. Detection of low HOXA10 levels in women with low vitamin D levels is strong evidence supporting a direct link between vitamin D and the expression of HOXA10.

**Conclusions**

Our study has some limitations due to the small number of cases and the evaluation of a single gene. However, despite all these limitations, this study is important as it shows the relationship between vitamin D levels and receptivity genes for the first time. The relationship between vitamin D and receptivity genes can be clarified with whole genome analyses of endometrium with the participation of more patients.

**Authors’ Contributions**

SSE designed the research study and wrote the manuscript. AE and SSE performed the research and analyzed the data. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

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Conflict of Interest
The authors declare that they have no conflict of interest.

Informed Consent
Verbal and written consent was obtained from patients before the study.

References