Vitamin D and aspects of female fertility

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ABSTRACT
The role of vitamin D in female reproduction has been intensively examined over the last few decades. A large body of evidence suggests that vitamin D might have beneficial effects on metabolic/hormonal parameters of PCOS and endometriosis, while it appears to be associated with IVF outcomes. However, due to the heterogeneity among observational and interventional studies, no cause-effect relationship has yet been established. The aim of this review is to analyze recent in vitro animal and human studies which examined the association of vitamin D with disease entities affecting female fertility potential. Recent research data strongly imply that vitamin D is implicated in female reproduction and might represent a beneficial and inexpensive therapeutic approach, in combination with first-line medical treatments, to female infertility.

Key words: Endometriosis, Female fertility, IVF, PCOS, Vitamin D

INTRODUCTION
Vitamin D is a secosteroid hormone mainly produced in the skin after sunlight exposure and is primarily known for its role in bone health and mineralization.1 In the last few years, the extraskeletal actions of vitamin D have emerged as a significant area of intensive scientific interest. The understanding that vitamin D receptor (VDR) and the enzymes required for the production of the active form of vitamin D are expressed in almost all human cells and tissues has linked vitamin D insufficiency/deficiency to many chronic diseases such as cancer, autoimmune and infectious diseases as well as cardiovascular diseases and diabetes mellitus type 2.1,2 Vitamin D deficiency, defined as serum 25-hydroxyvitamin D levels of <20 ng/ml, is estimated to affect about 50% of the population worldwide.1

Infertility is a hot topic in the field of public health, affecting about 48.5 million couples worldwide3 with significant psychological, medical and economic consequences. PCOS and endometriosis comprise the main causes of female infertility, with in vitro fertilization (IVF) offering a solution to this problem. Data accruing from studies undertaken either in animals
or humans point to a potential role of vitamin D in female fertility. In this context, epidemiological data have demonstrated a seasonality in human reproductive capacity, which could be partially explained by seasonal variation of vitamin D levels.

The aim of this review is to critically assess current literature data regarding the role of vitamin D in IVF and its association with PCOS and endometriosis.

METHODOLOGY

We searched Pubmed for English language publications up to February 2017 under the following terms: “Vitamin D and female infertility” and “Vitamin D and female reproduction” and “Vitamin D and PCOS” and “Vitamin D and endometriosis”, “Vitamin D and granulose cells” and “Vitamin D and IVF”. We also used the terms 25-hydroxyvitamin D or 25(OH)vitamin D or 1,25-dihydroxyvitaminD or 1,25(OH)2D3 instead of Vitamin D, and the term assisted reproduction technologies (ART) instead of IVF. Additionally, we included references on relevant topics from the reviewed articles in order to widen our search. Because a comprehensive background is a prerequisite for further discussions on vitamin D-induced effects, we provide a brief description of vitamin D metabolism and mechanism of action.

VITAMIN D METABOLISM AND MECHANISM OF ACTION

Vitamin D is a steroid hormone well known for its role in calcium homeostasis and bone mineralization. It is mainly produced in the skin after sunlight exposure. Diet and dietary supplements constitute alternative sources of vitamin D for humans. There are two distinct forms of vitamin D, that of D2 or ergocalciferol and D3 or cholecalciferol. Cholecalciferol is formed in the human skin from 7-dehydrocholesterol (7-DHC), a cholesterol precursor, in the presence of ultraviolet B radiation (UVB). UVB converts 7-DHC to previtamin D3, which is rapidly isomerized to vitamin D3. Ergocalciferol derives from several nutritional sources including green plants, mushrooms, fish fat and cod liver oil. Another source of vitamin D is commercially available vitamin D supplements.

Vitamin D, supplied either by UV irradiation of the skin or from the diet, is biologically inactive and requires two successive hydroxylations in the liver and in the kidneys by 25-hydroxylase (CYP27A1) and 1a-hydroxylase (CYP27B1) to produce its biologically active form, 1,25-dihydroxy-vitamin-D [1,25(OH)2D3] or calcitriol, respectively. It should be noted that the vitamin D status of the human body is best indicated by the circulating levels of 25(OH) VitD due to its longer half-life and higher serum concentration compared to 1,25(OH)2D3. Calcitriol acts through binding to specific nuclear receptor, VDR, which upon activation initiates multiple genomic effects. Calcitriol may also bind to a plasma membrane receptor mediating several non-genomic effects. VDR acts in concert with the retinoid X receptor (RXR), forming a heterodimer, as well as with other cofactors (repressors or activators) which regulate its function. The VDR-RXR heterodimer binds to vitamin D responsive elements (VDREs) located in the promoter region of target genes, thus regulating their transcription.

The main action of vitamin D is the absorption of calcium and phosphate from the gut, this required for bone mineralization. However, the wide distribution of VDR in almost all human tissues and the fact that 3% of the human genome is regulated by the vitamin D endocrine system point to a potential extra-skeletal role of vitamin D in various systems and organs, among them reproduction.

In vitro studies

In animal cells

VDR is expressed in the reproductive tissues (endometrium, ovaries and fallopian tubes) of cycling mice, especially during the estrous cycle, as well as in placenta, decidua and the ovaries of pregnant mice. Animal models have shown that vitamin D induces ovarian steroidogenesis. Vitamin D increased dehydroepiandrosterone sulfotranserase (SULT2A1) transcription, an enzyme that mediates sulfo-conjugation of endogenous hydroxysteroids. Moreover, calcitriol significantly decreased the expression of the anti-Mullerian hormone messenger RNA (AMH-mRNA) in hen granulosa cell cultures, whereas it increased the FSH receptor gene, indicating a positive role of vitamin D in follicular development and selection.
In human cells

VDR is expressed in human ovarian tissue and placenta. In terms of ovarian steroidogenesis, human ovarian cells stimulated progesterone, estradiol and estrone production in the presence of calcitriol. Furthermore, human granulosa cells cultured with vitamin D increased 3β-HSD messenger (mRNA) levels and progesterone release. In line with animal models, in vitro experiments in human models showed that vitamin D induces dehydroepiandrosterone sulfotranserase (SULT2A1). Vitamin D is also associated with markers of ovarian reserve, especially with AMH. Interestingly, a subsequent study demonstrated that the human AMH promoter contains a functional VDRE. Finally, human granulosa cells treatment with vitamin D resulted in a significant decrease in AMHR-II and FSH receptor mRNA. Given the inhibitory effects of AMH on the development of human granulosa cells, the decrease of AMH expression after vitamin D treatment may reflect a beneficial effect of vitamin D in the differentiation of these cells.

In vitro studies on endometriosis have yielded limited data. One of them showed that treatment of human endometriotic stromal cells (ESCs) with calcitriol significantly suppresses the interleukin(IL)-1β and tumor necrosis factor-alpha (TNF-α) induced inflammatory responses, mainly via reducing IL-8 mRNA expression and prostaglandin activity; viable ESCs numbers were also reduced. These results illustrate a potential immunomodulatory role of vitamin D in the inflammatory process of endometriosis.

With regard to IVF, an in vitro study investigated the immunomodulatory effect of vitamin D treatment on cytokine production [IL-6, IL-8, IL-10, transforming growth factor-β (TGF-β)] by endometrial cells of women with repeated implantation failure (RIF), the latter defined as three or more unsuccessful ART cycles after embryo transfer. Whole endometrial cells (WECs) and endometrial stromal cells (ESCs), obtained from RIF and normal fertile women, were treated with calcitriol. Endometrial cells from both the RIF group and the fertile group were capable of reducing cytokine production after calcitriol treatment, particularly IL-6 which facilitates the implantation process. On the other hand, WECs from both groups and ESCs only from the RIF group increased IL-8 and TGF-β production, respectively, which could be beneficial for RIF women. Moreover, calcitriol downregulated the increased amounts of interferon γ (IFN-γ) produced by unexplained recurrent spontaneous abortion (URSA) women, albeit increasing the secretion of TGF-β. Interestingly, in both studies whole and stromal endometrial cells were able to produce the active form of vitamin D, since they express 1α-hydroxylase. On the basis of these inconclusive data, the authors could not determine any clear effect of calcitriol on implantation, either positive or not, and highlighted the need for further research.

It has also been shown that in the fetoplacental unit, calcitriol affects human chorionic gonadotropin (hCG) production by human syncytiotrophoblasts and stimulates estradiol and progesterone synthesis by human placental cells. Meanwhile, the expression of human placental lactogen (hPL) was also reported to be regulated by calcitriol. In addition, human term placental trophoblasts were found to express calbindin-D28k (CaBP28k), which belongs to a large class of calcium binding proteins and might be associated with calcium transfer or cell development in human trophoblast. Moreover, a recent study showed a direct and beneficial effect of vitamin D on human extravillous trophoblast (EVT) invasion. Given that vitamin D deficiency is likely to increase the risk of pre-eclampsia and fetal growth restriction through inadequate EVT invasion, optimal vitamin D status could prevent these complications. Finally, human endometrial stromal cells cultured with calcitriol increased HOXA10 gene expression, which is crucial for the embryo implantation process.

Recent evidence from in vitro studies conducted in human and animal cells points to the functional role of the vitamin D endocrine system in the physiology of female reproduction. However, there are divergent results, possibly attributable to its two forms and several metabolites, the different concentrations of vitamin D used and to species-specific variations. Moreover, since various cell types are involved in the reproductive machinery and a local system mainly driven by 1α-hydroxylase activity is present in almost all of these cells responsible for the concentrations of the active metabolite at the tissue level, any relevant experimental data extracted from in vitro experiments must be interpreted with caution.
Animal studies

Data from animal studies also provide evidence of a clear role of vitamin D, either direct or indirect, in female reproductive functions. Diet-induced vitamin D deficient female rats demonstrated a reduction in overall fertility by 75% compared to vitamin D replete ones. Vitamin D deficient rat litters had small size and impaired neonatal growth. The impaired fertility rates were attributed to decreased impregnation and to increased number of pregnancy complications.\(^\text{30}\)

**VDR knockout mice (VDR\(^{-/-}\))**

VDR knockout mice manifested, apart from impaired bone formation and growth retardation, uterine hypoplasia and impaired folliculogenesis. Impressively, estrogen administration increased uterine weight of the VDR mutant mice, indicating a potential role of VDR in estrogen signaling.\(^\text{31}\)

Moreover, aromatase activity (the key enzyme in estrogen biosynthesis) and CYP19 gene (which encodes aromatase) expression were decreased in the ovaries of VDR null mutant mice (VDR\(^{-/-}\)). Biochemically, the VDR\(^{-/-}\) mice were hypocalceemic with elevated levels of FSH and LH indicative of hypergonadotropic hypogonadism. Calcium supplementation increased aromatase activity and CYP19 gene expression in the ovary but failed to correct the elevated gonadotropins. Despite the above endocrinological abnormalities some VDR\(^{-/-}\) mice with normocalcemia were fertile. The authors concluded that vitamin D has a potential role in estrogen biosynthesis through maintenance of normocalcemia and a direct effect on the expression of the aromatase gene.\(^\text{32}\) Interestingly, a study showed that VDR\(^{-/-}\) mice fed a high or medium calcium diet maintain 100% fertility.\(^\text{33}\)

**1-a hydroxylase knockout mice (1\(\alpha\)(OH)ase\(^{-/-}\))**

1-a hydroxylase knockout [1\(\alpha\)(OH)ase\(^{-/-}\)] female mice develop infertility with decreased estrogen and progesterone levels, elevated gonadotropins (FSH and LH), impaired follicular development, defective corpus luteum formation and uterine hypoplasia.\(^\text{34,35}\) Moreover, similarly to VDR\(^{-/-}\) mice, a high calcium diet given to 1\(\alpha\)(OH)ase\(^{-/-}\) mice improved their fertility.\(^\text{34}\) These results indicate that infertility is a secondary result of hypocalcaemia and not a direct effect of vitamin D due to the absence of VDR. However, an older study reported reduced reproductive capacity of vitamin D deficient female rats regardless of serum calcium concentration. Vitamin D or calcitriol supplementation restored fertility, suggesting a direct role of vitamin D on female infertility.\(^\text{36}\)

As concerns endometriosis and animal studies, vitamin D treatment of surgically induced endometriosis in rat models resulted in regression of the endometrial implants.\(^\text{37,38}\) Moreover, the VDR agonist elocalcitrol inhibited the development of endometriosis in a mouse model.\(^\text{39}\)

Interesting results emerged from a recent study on pregnant vitamin D deficient mice. According to their findings, maternal vitamin D deficiency is highly likely to contribute to the exposure of the developing fetus to higher glucocorticoid levels; this occurs through a reduction of placental 1\(\beta\)-HSD2 (1\(\beta\)-Hydroxysteroid dehydrogenase type 2) gene expression coding for the enzyme responsible for glucocorticoid inactivation while inducing the expression of the fetal head gene GILZ (glucocorticoid-induced leucine zipper) mainly regulated by glucocorticoids. Of note, a high exposure to glucocorticoids during this crucial period might be associated with adverse longterm health outcomes (mainly cardiometabolic and psychiatric disorders).\(^\text{40}\)

**Vitamin D and polycystic ovary syndrome (PCOS)**

Polycystic ovary syndrome (PCOS) is the most common endocrinopathy and the leading cause of infertility among women of reproductive age.\(^\text{41,42}\) It is a syndrome with clinical and biochemical heterogeneity which affects about 6-10% of women worldwide.

**(a) Vitamin D and insulin resistance**

Accumulating evidence suggests that vitamin D is associated with various metabolic and reproductive features of PCOS and thus may be involved in the pathogenesis of the syndrome. It is noteworthy that hyperinsulinemia and IR have a central role in the pathogenesis of PCOS, affecting the severity of clinical features independently of the presence of obesity. The following potential mechanisms linking vitamin D with IR have been proposed: (i) vitamin D improves insulin action by upregulating the expression of the insulin receptor and enhancing insulin responsiveness for glucose transport;\(^\text{43}\) (ii) 1,25(OH)\(_2\)D\(_3\) activates the
transcription of the VDRE of the human insulin gene which it has in its promoter;\textsuperscript{45} (iii) vitamin D regulates intracellular and extracellular calcium, which is crucial for insulin-mediated actions in insulin-responsive tissues;\textsuperscript{43} (iv) vitamin D exerts anti-inflammatory actions.\textsuperscript{2,9} However, most PCOS women are either overweight or obese. Obesity is associated with lower 25(OH)VitD levels, mainly due to the sequestration of the lipophilic vitamin in adipose tissue as well as due to lower sunlight exposure of obese subjects. These data raise the crucial question: Is vitamin D deficiency an additional risk factor aggravating IR in PCOS irrespectively of obesity? The following data attempt to address this issue.

\textit{(b) VDR gene polymorphisms and PCOS}

The role of VDR in the regulation of the human genome has motivated researchers to examine the contribution of the \textit{VDR} gene polymorphisms in metabolic and endocrine disturbances of PCOS. The results reflect an influence of \textit{VDR} gene variants in PCOS features; however, because they are as yet controversial, it is difficult to establish a clear association of \textit{VDR} polymorphisms with the development of PCOS.

\textit{VDR} \textit{ApaI}\textsuperscript{46,47} and \textit{BsmI} gene\textsuperscript{47} polymorphisms were associated with an increased risk of PCOS, after adjustment of results for age and body mass index (BMI). By contrast, other studies failed to find any association of these variants with PCOS susceptibility. In a recent case-control study, \textit{VDR} gene polymorphisms (\textit{TaqI, Apal, BsmI, FokI}) were not associated with the classic PCOS phenotype in Silesian women.\textsuperscript{48} An Indian case-control study also failed to show any significant association between \textit{VDR} gene variants and PCOS. However, \textit{Cdx2} and \textit{FokI} variants were associated with testosterone levels and infertility, respectively.\textsuperscript{49} The association between the \textit{VDR} gene rs757343 polymorphism and PCOS risk was examined in two studies, but both failed to observe any link.\textsuperscript{50,51}

One study reported an association of the \textit{VDR Apal} gene polymorphism with testosterone levels in PCOS women, whereas \textit{VDR Cdx2} variants were associated with insulin sensitivity.\textsuperscript{52} \textit{VDR BsmI} and \textit{VDR TaqI} gene polymorphisms were also associated with low SHBG levels and elevated LH levels, respectively.\textsuperscript{53}

\textit{(c) Observational studies}

Numerous observational studies investigated the association of 25(OH)VitD status with metabolic and endocrine parameters of PCOS (Table 1). In general, PCOS women had lower 25(OH)VitD levels compared to healthy controls. Among the PCOS population, obese women exhibited lower 25(OH)VitD levels than overweight or lean subjects.

\textit{i) 25(OH)VitD status and metabolic markers}

The potential effects of PCOS and obesity on PTH, vitamin D metabolites and metabolic aspects of the syndrome were investigated in a study of 291 PCOS women and 109 controls. Serum 25(OH)VitD levels were lower in controls compared to PCOS women, while increased body weight had a negative effect on vitamin D status. Moreover, 25(OH)VitD serum levels were inversely correlated with body mass index (BMI), PTH, insulin levels and the homeostasis model assessment of insulin resistance index (HOMA-IR), although these differences were BMI dependent.\textsuperscript{54} Additionally, another study reported that PCOS women had higher levels of 25(OH)VitD than controls, although after adjustment for age and BMI the significance was almost abolished.\textsuperscript{55} In line with the previous results, 25(OH)VitD levels were found to be higher in PCOS women than in controls. In the latter study, the quantitative insulin sensitivity check index (QUICKI) was used as a surrogate index of IR. Interestingly, PCOS subjects had lower QUICKI than controls at any concentration of 25(OH)VitD.\textsuperscript{56}

In contrast with the aforementioned findings, one study failed to confirm any difference in 25(OH)VitD levels between PCOS patients (\(n=37\)) and the control (\(n=70\)) group. However, the investigated population were adolescent females and only 13% of them were vitamin D sufficient (>30 ng/ml), which could have biased the results.\textsuperscript{57}

On the other hand, a recent study conducted in Australia showed that vitamin D levels were lower in overweight PCOS women than in overweight controls (31.6±11.3 versus 46.1±20.0 nmol/L), and this difference remained significant after adjustment for BMI and abdominal visceral fat. The hyperinsulinemic euglycemic clamp (HEC), the gold standard for the evaluation of insulin sensitivity, was used to evaluate
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Interestingly, low 25(OH)VitD levels in PCOS women was associated with higher levels of an anti-apoptotic protein, phosphoprotein enriched in diabetes gene product (PED/PEA-15). This inverse association could account for the dysregulated ovarian apoptosis seen in PCOS women.

A recent retrospective cohort study reported that PCOS infertile women with adequate 25(OH)VitD levels (>30 ng/ml) were more likely to achieve ovulation compared to those with 25(OH)VitD levels <20 ng/ml. Moreover, women achieving live births had higher 25(OH)VitD levels compared to those failing to carry out a live birth. Thus, an adequate 25(OH)VitD status could be a determining factor for a successful ovulation and pregnancy outcome for infertile PCOS women.

Finally, a prospective cohort study assessed reproductive parameters of PCOS and found that 25(OH)VitD deficiency (<25 nmol/L) was a significant predictive parameter for both follicle development and pregnancy in anovulatory infertile PCOS women who underwent clomiphene citrate (CC) stimulation. The heterogeneity of the studies could be explained by the variety of methodologies used for the assessment of VitD, the heterogeneity of study populations (small study samples, absence of control group) and the lack of adjustment for confounders, such as seasonality of 25(OH)VitD.

It could be concluded that the existing data converge towards a high prevalence of vitamin D deficiency among PCOS women and an inverse association with insulin sensitivity markers. However, the exact interrelationship between vitamin D status, obesity, IR and hyperandrogenism in PCOS still remains unclear and warrants further research.

(d) Interventional studies and PCOS

A notable number of interventional studies (Table 2) explored the therapeutic implications of vitamin D in the metabolic and reproductive aspects of PCOS. Meanwhile, recent meta-analyses of supplementation studies could not support a therapeutic effect of vitamin D treatment on metabolic disorders of the syndrome, apart from its positive effect on the reduction of serum triglycerides. In terms of hyperandrogenism markers, one meta-analysis con-
cluded that vitamin D treatment could improve follicle development and menstrual cyclicity, especially in combination with metformin,\(^8\) whereas the other found no beneficial effect.\(^8\)

Interestingly, recent studies indicate new potential pathways via which vitamin D could be implicated in the pathogenesis of PCOS. More specifically, advanced glycation end-products (AGEs) are involved in the pathological process of PCOS.\(^8\),\(^9\) The interaction of AGEs with their receptor (AGE-RAGE) induces pro-inflammatory gene activation resulting in cellular damage.\(^9\) These adverse effects are counteracted by an extracellular form of RAGE, the soluble receptor for AGEs (sRAGE), which binds to circulating AGEs, thereby inhibiting AGE-RAGE interaction. Based on the above data, 16 PCOS women and 35 controls were treated with vitamin D\(_3\) for 8 weeks. The improvement in vitamin D status of PCOS women was associated with a significant increase in sRAGE, indicating that vitamin D could exert anti-inflammatory actions by increasing sRAGE levels.\(^9\) Moreover, serum AMH levels in PCOS patients were reduced, thus vitamin D\(_3\) supplementation might improve ovary dysfunction and folliculogenesis in these women via normalization of AMH levels.\(^9\)

Apart from AGEs, TGF-β dysregulation may possibly be implicated in the pathophysiology of PCOS, given its role in angiogenesis, fibroblast activation and tissue fibrosis, which could explain morphological and vascular alterations of PCOS ovaries.\(^9\) PCOS women display an abnormal increase in TGF-β1 bioavailability, which is mainly attributed to the decreased levels of soluble endoglin (sENG), a circulating receptor that binds TGF-β1.\(^4\),\(^5\)

Recently, a study examined possible effects of vitamin D administration on TGF-β1 bioavailability in vitamin D deficient PCOS women. Vitamin D supplementation significantly increased serum sENG and decreased TGF-β1 bioavailability (TGF-β1/sENG). Moreover, vitamin D replacement decreased serum triglycerides, the Ferriman-Gallwey score and the menstrual interval. Further, the decrease in TGF-β1 bioavailability (Δ TGF-β1/sENG ratio) was associated with an improvement in lipid profile. These findings suggest that vitamin D induced decrease in TGF-β1 bioavailability in PCOS subjects might be a novel mechanism through which vitamin D exerts its beneficial effects on certain aspects of PCOS.\(^9\)

In conclusion, despite the abundance of existing literature data regarding supplementation studies, their results are inconsistent and no clear conclusion can be drawn about the effect of vitamin D administration on metabolic and reproductive parameters of PCOS. The aforementioned intervention studies are subject to several limitations, which partially explain the lack of apparent concordance. Firstly, some of them include a small sample size and/or lack randomization and allocation concealment, hence increasing the risk of selection bias. There is substantial heterogeneity with respect to 25(OH)VitD status of patients at baseline, the methodology used for 25(OH)VitD assessment, dosing regimen and intervention formulations, duration, use of concomitant therapies, all of which could contribute to the discrepancy of the observed results. Furthermore, these studies were conducted in different countries and at different seasons, factors which could also influence the results. In fact, lack of adjustment for confounders such as the seasonality of 25(OH)VitD or even the existence of residual confounders are significant shortcomings in the studies conducted. The use of a single baseline vitamin D measurement which may not reflect longterm vitamin D status could also affect the validity of the interventional studies.\(^6\) Moreover, the ultrasound criteria of diagnosis of PCOS and of the definition of ovulatory cycles varies among studies this also conducing to the low quality of many of them.

**Vitamin D and endometriosis**

Endometriosis is a common benign inflammatory disorder that affects 5 to 10% of women of reproductive age, the main clinical features including pelvic pain, dysmenorrhea, dyspareunia and infertility.\(^7\) The pathogenesis of endometriosis has not been well established, but it seems that an altered immune and inflammatory response enables the survival of endometrial implants.\(^7\)

The association between vitamin D and endometriosis is based on the following findings: 1) the human endometrium expresses VDR and 1α-hydroxylase, thus it could be a possible site of extrarenal synthesis and action of vitamin D;\(^8\) 2) vitamin D has immunomodulatory effects; macrophages, dendritic...
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<td>13 PCOS</td>
<td>USA</td>
<td>1500mg calcium carbonate daily and 50,000 IU Vitamin D₃ (ergocalciferol) weekly or biweekly</td>
<td>6 months</td>
<td>Restoration of menstrual cycles (7/13), improvement of acne (3/13) and pregnancy outcome (2/13)</td>
<td>Radioligand-binding assay</td>
<td>71</td>
</tr>
</tbody>
</table>
| Rashidi et al   | RCT          | 60 PCOS 3 groups (n=20) | Iran    | **Group 1**: 1000mg calcium and 400 IU Vitamin D per day  
**Group 2**: 1000mg calcium and 400 IU Vitamin D and 1500mg metformin per day  
**Group 3**: 1500mg metformin per day | 3 months treatment and 3 months follow up | Improvement of folliculogenesis and menstrual regularity in Group 2 | Not provided | 72        |
| Firouzabadi et al | RCT          | 100 PCOS 2 groups (n=50) | Iran    | **Group 1**: 1500mg metformin per day  
**Group 2**: 1500mg metformin/day plus 1000mg calcium/day plus 100,000 IU Vitamin D₃/month | 6 months | Improvement of menstrual abnormalities, follicle development and infertility in Group 2 (non-statistically significant) | RIA | 73        |
| Asadi et al     | RCT          | 110 PCOS 2 groups (n=55) | Iran    | **Group 1**: 300,000 IU cholecalciferol once  
**Group 2**: Placebo | 2 months | Endometrial thickness (thicker) in Group 1  
No significant difference in pregnancy outcome between the two groups | Not provided | 74        |
| Wehr et al      | Single Arm   | 46 PCOS      | Austria | 20,000 IU cholecalciferol per week | 24 weeks | Decrease of fasting and stimulated glucose, C-peptide levels, TG, estradiol levels  
Improvement of menstrual frequency (50%)  
Increase of total cholesterol and LDL | Enzyme immunoassay | 75        |
| Selimoglu et al | Single Arm   | 11 PCOS      | Turkey  | 300,000 IU Vitamin D₃ orally, single dose | 3 weeks | Decrease in HOMA-IR  
No significant change in DHEAS, total and free testosterone, androstendione | RIA | 76        |
| Pal et al       | Single Arm   | 12 PCOS      | USA     | Vitamin D₃ 2000 IU daily and Vitamin D₃ 50,000 IU monthly (modified to 50,000 IU weekly) and calcium 530mg/day | 3 months | Reduction in total testosterone and androstendione levels  
Reduction in BP  
No change in IR parameters | RIA | 77        |
| Razavi et al    | RCT          | 60 PCOS 2 groups (n=30) | Iran    | **Group 1**: Vitamin D 200 IU, Vitamin K 90 μg, Calcium 500mg twice a day  
**Group 2**: Placebo | 8 weeks | Reduction in serum free testosterone, DHEAS in Group 1 | ELISA | 78        |
| Kotsa et al     | Single Arm   | 15 PCOS      | Greece  | 1 μg alphacalcidol/day | 3 months | Increase in first phase insulin secretion  
Increase in HDL and decrease in TG | RIA | 79        |

AMH: anti-Mullerian hormone; APO-A1: apolipoprotein A1; BP: blood pressure; DHEAS: dehydroepiandrosterone sulfate; FG: Ferriman-Gallwey score; HDL: high-density lipoprotein; HOMA-IR: homeostasis model assessment–insulin resistance; IR: insulin resistance; LDL: low-density lipoprotein; PCOS: polycystic ovary syndrome; PTH: parathyroid hormone; QUICKI: quantitative insulin-sensitivity check index; RCT: randomized control trial; RIA: radioimmunoassay; sENG: soluble endoglin; sRAGE: soluble form of receptor for advanced glycation end-products; TG: triglycerides; TGF-β1: transforming growth factor beta 1; VLDL: very low-density lipoprotein.
TABLE 2. Vitamin D and PCOS – INTERVENTIONAL STUDIES.

<table>
<thead>
<tr>
<th>Author</th>
<th>Study Design</th>
<th>Participants</th>
<th>Country</th>
<th>Intervention</th>
<th>Duration</th>
<th>Main Results</th>
<th>25(OH)D Measurement</th>
<th>Reference</th>
</tr>
</thead>
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<tr>
<td>Ardabili et al</td>
<td>RCT</td>
<td>50 PCOS 2 groups (n₁=24, n₂=26)</td>
<td>Iran</td>
<td>Group 1: 50.000 IU Vitamin D₃/20 days Group 2: Placebo orally</td>
<td>2 months</td>
<td>Reduction in TG, total cholesterol, VLDL, PTH in Group 1 No change in HOMA-IR, QUICKI, insulin levels No change in HDL-C, LDL-C, Apo-A1</td>
<td>Chemoluminescence Immunoassay</td>
<td>82</td>
</tr>
<tr>
<td>Raja-Khan et al</td>
<td>RCT</td>
<td>28 PCOS (n₁=13, n₂=15)</td>
<td>USA</td>
<td>Group 1: 12.000 Vitamin D₃/day Group 2: Placebo orally</td>
<td>12 weeks</td>
<td>No change in HOMA-IR, QUICKI, insulin levels</td>
<td>RIA</td>
<td>81</td>
</tr>
<tr>
<td>Asemi et al</td>
<td>RCT</td>
<td>104 PCOS 4 groups (n=26)</td>
<td>Iran</td>
<td>Group 1: 1000 mg/day calcium plus Vitamin D placebo Group 2: 50.00 IU/week Vitamin D plus calcium placebo Group 3: 1000 mg calcium/d plus 50.000 IU/week Vitamin D Group 4: calcium placebo plus Vitamin D placebo</td>
<td>8 weeks</td>
<td>Decrease in insulin levels, HOMA-IR, TG, VLDL and increase in QUICKI in Group 3</td>
<td>ELISA</td>
<td>83</td>
</tr>
<tr>
<td>Garg et al</td>
<td>RCT</td>
<td>32 PCOS 2 groups (n₁=15, n₂=17)</td>
<td>India</td>
<td>Group 1: Metformin (500 mg × 2 for weeks and 500 mg × 3 for 6 weeks) plus Vitamin D₃ (120.000 IU once monthly) Group 2: Metformin (500 mg × 2 for weeks and 500 mg × 3 for 6 weeks) plus placebo</td>
<td>6 months</td>
<td>No significant difference in HOMA-IR and insulin secretion</td>
<td>Chemiluminescence Immunoassay</td>
<td>84</td>
</tr>
<tr>
<td>Irani et al</td>
<td>RCT</td>
<td>16 PCOS 35 Controls</td>
<td>USA</td>
<td>50.000 IU of Vitamin D₃ orally once weekly</td>
<td>8 weeks</td>
<td>Increase in serum sRAGE levels and decrease in serum AMH levels in PCOS</td>
<td>Immunoassay</td>
<td>92</td>
</tr>
<tr>
<td>Irani et al</td>
<td>RCT</td>
<td>68 PCOS 2 groups (n₁=45, n₂=23)</td>
<td>USA</td>
<td>Group 1: 50.000 IU Vitamin D₃ orally once weekly Group 2: Placebo</td>
<td>8 weeks</td>
<td>Increase in serum sENG and decrease in TGF-β1 bioavailability (TGF-β1/sENG ratio) in Group 1 Decrease in FG score, TG, menstrual interval in Group 1</td>
<td>Immunoassay</td>
<td>93</td>
</tr>
</tbody>
</table>

AMH: anti-Mullerian hormone; APO-A1: apolipoprotein A1; BP: blood pressure; DHEAS: dehydroepiandrosterone sulfate; FG: Ferriman-Gallwey score; HDL: high-density lipoprotein; HOMA-IR: homeostasis model assessment–insulin resistance; IR: insulin resistance; LDL: low-density lipoprotein; PCOS: polycystic ovary syndrome; PTH: parathyroid hormone; QUICKI: quantitative insulin-sensitivity check index; RCT: randomized control trial; RIA: radioimmunoassay; sENG: soluble endoglin; sRAGE: soluble form of receptor for advanced glycation end-products; TG: triglycerides; TGF-β1: transforming growth factor beta 1; VLDL: very low-density lipoprotein.
cells and lymphocytes express the VDR, while the active metabolite of vitamin D, 1,25(OH)₂D₃, acting through the VDR was found to induce the destruction of microbial agents and to inhibit antigen presentation and maturation of dendritic cells. Moreover, vitamin D exerts antiproliferative effects on lymphocytes, especially on Th1 cells, promoting a shift from Th1 to Th2 phenotype.₁,²,⁹

Data linking vitamin D and endometriosis emerge mainly from observational studies and show conflicting results. Several studies failed to note any difference between serum 25(OH)VitD levels in patients with endometriosis and healthy subjects.⁹⁹,¹⁰⁰ However, VDR and 1α-hydroxylase expression was higher in the endometrium and ovaries of women with endometriosis compared to healthy subjects, although this difference was statistically significant only for 1α-hydroxylase, implying a higher local production of the active metabolite calcitriol and/or an increased action of vitamin D.¹⁰⁰

On the other hand, recently published data show an inverse relationship between vitamin D level and endometriosis, as women with greater 25(OH)VitD level had a 24% lower risk of developing endometriosis than women with lower levels.¹⁰¹ What is more, in this large prospective cohort study the researchers found that higher consumption of dairy foods was associated with a lower risk of endometriosis. Similarly, a Japanese study reported lower 25(OH)VitD levels in women with severe endometriosis compared to controls and women with mild endometriosis.²⁰ Recently, an observational study found a high rate of hypovitaminosis D [25(OH)VitD serum level <30 ng/ml] in a cohort of 49 women with a single ovarian endometrioma.¹⁰²

In contrast, significantly higher levels of 25(OH) VitD were observed in the serum of women with endometriosis compared to healthy individuals (24.9±14.8 ng/ml vs 20.4±11.8 ng/ml).¹⁰³

Additionally, VDBP in serum, peritoneal fluid (PF) and urine was examined as a possible biomarker of endometriosis. A study using proteomic technologies for VDBP analysis demonstrated its presence in the PF of women with endometriosis, whereas one vitamin D binding protein (DBP) isoform (DBPE) was expressed to a lower degree in the PF of women with untreated endometriosis compared to the control group.¹⁰⁴

However, another study found no difference between serum and peritoneal fluid levels of VDBP of women with endometriosis compared to the control group.¹⁰⁵ VDBP levels were also evaluated in urine samples of women with endometriosis and found to be significantly higher in women with endometriosis, albeit the sensitivity (58%) and specificity (76%) of this method limits its diagnostic value.¹⁰⁶ Moreover, it has been shown that VDBP expression is significantly higher in ectopic endometrial tissue in comparison with the normal endometrium, suggesting a plausible local role of VDBP in the progression of the disease.¹⁰⁷

Finally, in a cross-sectional study, VDBP was increased in all samples of women suffering from endometriosis compared to the control group.¹⁰⁸ Interestingly, a subsequent analysis of the samples identified a specific allele of VDBP (GC*2) to be about 3-fold higher in all endometriosis groups than in the control group. This specific VDBP polymorphism (high expression of GC*2) could be responsible for an insufficient activation of macrophages leading to an altered immune response, which enables the development of endometriosis.¹⁰⁸ The role of VDR gene polymorphisms (Apal, TaqI, FokI, BmsI) in the pathogenesis of endometriosis and infertility associated with the disease has been studied but no association has been identified.¹⁰⁹

Taken together, these data may indicate a plausible implication of vitamin D in the pathogenesis of endometriosis through exerting an autocrine/paracrine role in the endometrial microenvironment; however, further research is needed to determine whether vitamin D supplementation could have a role as an adjuvant therapy in the treatment of endometriosis or is merely a confounding factor.

Vitamin D and in vitro fertilization (IVF)

The implication of Vitamin D in the outcome of ART (clinical pregnancy and live birth) has been examined in numerous studies. Nevertheless, the data are inconsistent and a clear role of vitamin D in the success of IVF outcome remains elusive.

In the first experimental attempt to link vitamin D status with IVF outcome, vitamin D sufficient women undergoing IVF had higher pregnancy and implan-
vation rates compared with the vitamin D deficient women. The beneficial impact of high vitamin D status on IVF outcome could be attributed to the effects of vitamin D on the endometrium, since vitamin D status was not significantly associated with ovarian response parameters.

In line with the previous study, a retrospective-cohort study found a positive relationship between vitamin D status and IVF success only in non-Hispanic whites compared to Asians, indicating that the role of vitamin D in IVF should be evaluated in relation to ethnic origin. Furthermore, it was also hypothesized that the positive effects of vitamin D on ART outcome may be mediated through endometrial pathways.

In order to test this hypothesis, a subsequent study investigated the influences of vitamin D status on ART outcomes in donor-recipient cycles. Egg-donation recipients with non-replete vitamin D status [25(OH) VitD <30 ng/ml] had reduced clinical pregnancy and live birth rates. Moreover, no correlation between recipient vitamin D status and ovarian stimulation parameters, fertilization rates, embryo quality or the number of embryos transferred was noted, suggesting that vitamin D appears to exert its effects on fertility via the endometrium. However, donor characteristics were not included in the latter study, which could confound these results.

The majority of the following studies demonstrated a positive relationship between higher vitamin D status and IVF outcome. Recent data imply that vitamin D deficiency (<20 ng/ml) compromises pregnancy achievement in women undergoing Day 5 (blastocyst stage) single embryo transfer (SET). The lower clinical pregnancy rates were attributed to a harmful effect of vitamin D deficiency on endometrial receptivity.

Conversely, a prospective observational study found that elevated follicular fluid (FF) 25(OH)VitD levels in combination with decreased FF glucose levels were associated with poorer embryo quality and negative IVF outcome, indicating a potential negative role of vitamin D at the oocyte level. However, the above observation is in contrast to the detrimental effects of vitamin D deficiency on the endometrium reported in previous studies. Additionally, two Iranian studies failed to demonstrate any significant association between serum or FF vitamin D levels and implantation or pregnancy rates. However, the high prevalence of vitamin D deficiency in the examined population limits the strength of their research.

Furthermore, vitamin D status was not associated with clinical pregnancy rates of women undergoing euploid embryo transfer, frozen-thawed embryo transfer and clinical pregnancy rates among oocyte recipients. An interventional trial examined potential effects of vitamin D insufficiency treatment on fertility outcomes regarding frozen-thawed embryo transfer cycles but did not show any association. Finally, a recent meta-analysis failed to document any correlation between vitamin D deficiency and pregnancy rates in women undergoing IVF. The heterogeneity among the aforementioned studies and their contradictory results highlight the need for further research so as to clarify the exact association of vitamin D status and IVF success.

**Vitamin D status and female fertility**

Endocrine Task Force guidelines define vitamin D deficiency as a 25(OH)VitD level of <20 ng/ml, vitamin D insufficiency as a 25(OH)VitD level of 21-29 ng/ml and vitamin D sufficiency as a 25(OH)VitD level of ≥30 ng/ml. The Institute of Medicine Committee defines vitamin D deficiency at a level below 20 ng/ml. These definitions however are related only to skeletal health and an optimal level of vitamin D levels for its non-skeletal actions has not been established. Of interest, according to the Endocrine Society Task Force, vitamin D3 or vitamin D2 are the suggested treatment options for vitamin D deficiency. On the other hand, literature data indicate that vitamin D3 is more effective in increasing 25(OH)VitD levels than vitamin D2. Clearly, the optimal level of vitamin D in female fertility and the type of supplements required for the treatment of vitamin D deficiency are two significant issues which merit further research and need to be addressed.

**CONCLUSION**

There are a large number of in vitro, animal as well as human observational studies which strongly point towards an association between vitamin D and female fertility. Research data indicate that vitamin D might be implicated in the pathogenesis and prevention of endometriosis, while vitamin D status has
been linked to IVF outcome. Furthermore, vitamin D supplementation in PCOS women ameliorated some of the metabolic and, mainly, the reproductive disorders. Although promising, these data are not sufficient to establish a cause-effect relationship between vitamin D status and fertility issues. Moreover, there is still no general consensus as to the minimum level of vitamin D optimal for female reproductive health and fertility, while the screening of vitamin D status in women undergoing IVF is still under debate. Vitamin D administration is a well-tolerated and inexpensive treatment. However, whether vitamin D supplementation could be a novel adjunct agent in the treatment of metabolic/hormonal aspects of PCOS and endometriosis is a question still awaiting an answer. Large-scale, high quality dose-response RCTs with longer follow-up are needed 1) to determine the exact role of vitamin D in female IVF outcome, evaluated by indices such as the number of oocytes retrieved, the number and the quality of the embryo formed, the clinical pregnancy rates as well as the live birth rates and 2) to identify threshold effects of vitamin D supplementation on hormonal, metabolic and reproductive outcomes in PCOS.

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