Role of Vitamin D and Insulin Resistance in Polycystic Ovary Syndrome

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Abstract
Polycystic ovary syndrome (PCOS) is considered the commonest endocrinological disorder in childbearing age women with an incidence of 10%. Vitamin D has been shown in several studies to have a positive impact on female reproductive diseases, as well as PCOS. The diagnosis of PCOS, clinical symptoms, pathophysiology, hypovitaminosis D, insulin resistance, and the various methods for vitamin D and insulin assessment are all covered in this study. Androgen hypersecretion, oligo-ovulation, and/or ovarian morphologic features are used to diagnose this condition. Androgen excess, neuroendocrine causes, and insulin resistance are all part of the pathophysiology. Some female genital organs have been found to contain vitamin D receptors. Insulin regulates steroid biosynthesis in the ovaries and follicular maturation, so vitamin D is essential.

Key words
25 (OH) D, Polycystic Ovary Syndrome, Insulin resistance, Vitamin D

Introduction
With a global prevalence of 5–20 percent and 13% in fertile and 37.5% in secondary infertile patients in Egypt, polycystic ovary is the most common endocrinological irregularity in women of childbearing age [1]. This disorder is manifested by ovulatory dysfunction, excess androgen secretion in addition to polycystic ovarian morphology [2]. Also, several metabolic disorders are correlated to the syndrome such as resistance to insulin and obesity and these consequently elevate diabetes mellitus type 2 risk and heart diseases [3]. The definite etiology of the syndrome remains debatable and existing therapy had a moderate effect in controlling its symptoms and preventing its complications [2].

Menarche is normal or slightly delayed in PCO women, and abnormalities in the menstrual cycle occur in some cases of weight gain. PCOS women's menstrual abnormalities are characterized by oligo- or amenorrhea, as well as abnormal or missing ovulation, resulting in infertility [4]. Besides, the age of appearance of the syndrome is adolescence while diagnosis time is the thirties or forties because most of the manifestations do not become apparent until twenties or thirties, however some may occur at menarche [5].

Diagnosis of PCOS

1. Rotterdam criteria
Rotterdam criteria are considered the commonest criteria for diagnosing PCOS that are highlighted below:[6]
Presence of 2 out of 3 of the following characters:
- Oligovulation or anovulation.
- Signs of hyperandrogenemia.
- Ultrasonic criteria of PCO: 12 or more follicles with a diameter of 2 - 9 mm are existed in one ovary at a minimum and/or enlarged ovarian size > 10 ml as stated by Rotterdam criteria 6 as illustrated in figure (1).

2. National Institutes of Health Criteria (NIH) 7
That includes only 2 characters:
Clinical and/or biochemical hyperandrogenism.
Oligo/amenorrhea anovulation.

3. Androgen Excess Society (AES) 8
Hyperandrogenism with ovarian dysfunction or polycystic ovaries. They considered that androgen excess is a central event in polycystic ovary syndrome pathogenesis.

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Figure 1: Normal and polycystic ovary 7
Clinical Manifestations of PCOS

There is significant diversity in the clinical practice including variability in the same patient with time [10]. Symptoms of hyperandrogenemia involve the following:

1. Infertility.

The definition of infertility differs according to the female age, for example, if less than 35 years, it is the inability to conceive after 1 year of unprotected intercourse however after 35, it is the inability to conceive after only 6 months [11].

The clinical manifestations of infertility are correlated to Luteinizing hormone (LH) oversecretion (70%), the ratio of LH/FSH is elevated leading to an enhanced androgen biosynthesis in ovaries [12].

2. Hirsutism.

Hirsutism is recognized as the excessive development of terminal hair in women’s androgen-dependent areas. It is found in 50% - 80% of hyperandrogenic patients [10]. The hair growth rate differs corresponding to the diversity in 5α-reductase enzyme action that derives dihydrotestosterone (DHT) from testosterone. Hyperandrogenemia and insulin enhance 5-alpha reductase enzyme in hair follicles [13].

3. Acne.

It is a defect of the pilosebaceous unit, with face, neck, back, and chest lesions. It is established that patients undergo higher receptor sensitivity for androgens compared to the normal population [14]

4. Androgenetic Alopecia.

It is identified by hair fall in the central region of the scalp, with significant psychological and social consequences. Many factors shorten the anagen phase and miniaturize terminal follicles which involve 5-alpha-reductase elevation and the elevated concentration of androgen receptors [15].

Pathophysiology of The Syndrome

The pathophysiology of PCOS is explained in Figure (2) that includes:

A. Excessive adrenal and ovarian androgen biosynthesis.

In PCOS, luteinizing hormone (LH) hypersecretion causes enhancement in androgen biosynthesis by thecal cells. This is due to the enhanced pulse frequency of gonadotropin-releasing hormone (GnRH), resulting in LH levels elevation relative to follicle-stimulating hormone (FSH), ovarian impedance for FSH action, and hindrance of follicular development [16].

Despite the concept that androgens adversely influence follicles, androgens stimulate antral follicle development [17]. Upon follicle maturation, androgens hinder multiplication and stimulate apoptosis. This dual activity includes FSH action inhibition in larger antral follicles and stimulatory effect in smaller ones [18].

Theca, granulosa, and stromal androgen receptors (ARs) are responsible for androgen activity [19]. Typically, one follicle remains and becomes the dominant one [20]. Hyperestrogenism causes negative feedback on pituitary FSH secretion. The dominant follicle recompense for the decline in FSH stimulation through increased response to LH stimulation. Others undergo follicular atresia, probably due to FSH deficiency and hyperandrogenism.

B. Neuroendocrinological conditions.

PCOS is distinguished by higher LH pulse frequency and LH/FSH ratio. The primary characters of PCOS occur through the earlier puberty, associated with pulse generator reactivation of hypothalamic GnRH, gonadotropin release stimulation, and subsequent ovarian estrogen synthesis stimulation. The GnRH is secreted in discrete impulses that travel to gonadotrophs in the pituitary, causing impulsive secretion of LH and FSH [21]. Enhanced GnRH pulse frequency increases that of LH and attenuates FSH ones [22].

C. Insulin Resistance and hypersecretion.

Insulin enhances the cognate trophic hormones to stimulate ovarian and adrenal steroidogenesis. The compensatory hyperinsulinemia correlated to IR induces hyperandrogenemia in theca cells and declines the production of hepatic sex hormone-binding globulin (SHBG) which increases circulatory testosterone and enhances serum IGF-1 bioactivity through suppression of IGF-binding protein production. This leads to paradoxical activity according to tissue; resistance to insulin in the liver, skeletal muscle, and lipocytes, while insulin sensitive in steroidogenic tissues [23].

PCOS women have intrinsic insulin resistance (IR) unrelated to obesity or androgen concentrations [24]. Although lean PCOS women undergo IR; obesity aggravates IR [25]. PCOS pubescent girls with normal-weight undergo peripheral IR and fatty liver compared with control girls with normal-weight [26].

D. Obesity.

It is prevalent among pubescent girls and adult women with polycystic ovaries. In response to nutrients overconsumption, lipocytes undergo enlargement (hypertrophy) or the formation of novel lipocytes (hyperplasia). Hypertrophic lipocytes undergo hypoxia, propagation of free fatty acids, triglycerides, and lipogenesis [28]. Because of lipids overload in the adipose tissue, excess fats are stored in skeletal muscle, liver, and pancreas [29]. Fat partition in various storage sites negatively affects metabolic functions; fat deposition in the abdomen has pertained to a greater risk for cardiovascular disease [30].

Vitamin D

It is a lipid-soluble vitamin also identified as calciferol that is naturally produced in the body through the transformation of cholesterol to 7-dehydrocholesterol in the skin under sunlight or derived from dietary intake. It consisted of two forms: vitamin D2 (ergocalciferol); essentially human-made and present in fortified food. Vitamin D3 (cholecalciferol) has two supplies: production on the skin, animal-based foods [32].
**Vitamin D Absorption**

It is absorbed in the small intestine because of its lipid-solubility characters[33]. The efficacious vitamin D absorption relies on the fat availability within the gastrointestinal tract, which stimulates bile acids and lipase enzyme secretion [34]. Vitamin D is mainly included in chylomicrons to reach the systemic circulation. Chylomicrons are catabolized by lipoprotein lipase enzyme that presents in peripheral tissues, which are lipoprotein lipase-rich tissues, and that elucidates the cause of the vitamin D vanishing from plasma [35].

**Active Form Synthesis**

Vitamin D is inactive until it encounters the following subsequent reactions that are shown in Figure (3):

Firstly, it comes about within the liver to form 25-hydroxyvitamin D (25OHD) through 25-hydroxylase enzyme [36]. The other occurs in the kidney, intermediated by 1α-hydroxylase, which forms the calcitriol (1,25-dihydroxy vitamin D) from 25OHD [37]. Serum 25 (OH) D is the main indicator of vitamin D level because it is the principal circulating form[38]. Calcitriol renal production is managed by two hormones: up-regulated by parathyroid hormone (PTH) and down-regulated by fibroblast-like growth factor-23 (FGF23) [39]

Calcitriol is catabolized by the 24-hydroxylase enzyme that executes successive reactions resulting substantially in the conversion of calcitriol to calcitroic acid which is eliminated into feces [40]; a minute amount is excreted renally [41].
Vitamin D Physiological Actions

Management of Calcium and Phosphate Homeostasis

Calcitriol preserves plasma calcium levels by several mechanisms. Firstly, it enhances calcium and phosphate absorption through intestinal length without the need for PTH. Another mechanism that requires PTH, calcitriol is responsible for calcium mobilization from bones by stimulation of osteoclast production and activation [43]. Calcitriol promotes osteoclasts formation by induction of the protein secretion which is responsible for osteoclast synthesis and bone resorption [44]. Thirdly, calcitriol and PTH activate calcitriol reabsorption in the renal distal tubule when calcium is required [45]. Calcitonin hormone which is secreted in case of hypercalcemia prevents bone resorption and aids to restores normal calcium levels [45]. Phosphate deficiency enhances CYP27B1 to secrete more calcitriol, which promotes the small intestine for phosphate absorption.

Moreover, calcitriol induces osteocytes in the bone to secrete FGF23 that leads to phosphate renal excretion [46], also has feedback on vitamin D metabolism.

Regulation of Immune Function

Calcitriol derived from 25(OH)D in immune cells strengthens immunity by promoting the production of cathelicidin, a peptide with antibacterial activity [47]. Calcitriol typically suppresses the propagation of T helper cells and B cell immunoglobulin synthesis. However, in the case of inflammation, calcitriol enhances the proliferation and aggregation of regulatory T cells [48].

Fetal Growth

Sufficient vitamin D is also crucial for fetal growth. A study estimated the messenger ribonucleic acid (mRNA) and microRNA (miRNA) expression in 10 pregnant women with serum 25(OH)D level below 63.8nmol/l and 11 pregnant women whose 25(OH)D level were above 79.3 nmol/l. A variable expression level was detected in 305 genes and 11 miRNA between the two groups, those genes have a vital impact on organ development, carbohydrate, and lipid catabolism which affect pregnancy and fetal development [49].

Vitamin D and PCOS

Vitamin D has vital action in bone metabolism management and calcium homeostasis maintenance. Additionally, it has a role in cell differentiation, immune regulation, and neurogenesis [50]. Vitamin D is involved in infertility, due to vitamin D receptor (VDR), and 1 alpha-hydroxylase enzyme identification in several tissues [51]. VDRs were identified in some female reproductive organs such as the oocytes, uterus, and placenta and some studies reported that females with null vitamin D receptors were infertile and had folliculogenesis impairment [52].

More studies confirm the influence of hypovitaminosis D in metabolic disorders in PCOS women, including insulin resistance (IR) [53], obesity [54], and hypertension [55]. Results supported that vitamin D controls genes responsible for the metabolism of glucose and lipid [56].

Vitamin D has a positive impact on insulin by enhancing the expression of insulin receptors and thereby stimulating glucose transmission [57]. Insulin gene promoter contains vitamin D responsive component [58] and insulin gene transcription is stimulated by calcitriol [59]. Additionally, vitamin D manages calcium extracellularly and intracellularly which is essential in some tissue processes such as skeletal muscle and fat tissue which are insulin-responsive [57]. Moreover, insulin secretion is calcium dependant so, calcium influx dysfunction can negatively influence insulin secretion [60]. In a largest study with an RCT design among 104 obese, vitamin D deficient PCOS women did reveal a positive effect of weekly 50,000 IU vitamin D plus calcium 1000mg/day on insulin resistance [61].

In addition [61] a study demonstrated that vitamin D deficiency in PCOS women was correlated with metabolic risk factors, including insulin resistance and low HDL-C levels, independent of obesity measures [62]. Also, it has been reported that vitamin D might also have beneficial effects to the insulin responsiveness and androgen levels in PCOS [54]. Furthermore, another study reported that serum 25(OH)D is a significant predictor for insulin resistance in PCOS women [3].

Several studies reported that vitamin D shortage is prevalent in PCOS women and confirmed the association between lower vitamin D levels and resistance to insulin, hyperandrogenemia, and infertility [62]. Vitamin D regulates many genes that are vital for the metabolism of glucose and lipids, so its shortage may be the linkage between resistance to insulin and PCOS [3].

In a recent study, it is reported that vitamin D deficiency has been proposed as the possible missing link between insulin resistance and PCOS [63]. Low 25(OH)D levels are found to be significantly correlated with insulin resistance in women with PCOS [64]. Thus, genes involved in vitamin D metabolism have been suggested as candidate genes for the susceptibility to PCOS. A few polymorphisms in the VDR gene, such as Cdx2, Taq1, Bsm1, Apa1, and Fok1, were reported to play an influential role on insulin secretion and sensitivity in PCOS women [65]. The VDR Fok1 polymorphism was found to have a protective effect on the risk of type 2 diabetes mellitus, while the Bsm1 had a precipitating effect on the risk of type 2 diabetes. Besides, the Apa1 polymorphism was reported to confer a reduced risk of vitamin D deficiency [65].

Another study was enrolled in sixty patients of PCOS, they were divided into 3 subgroups based on vitamin D levels as a state of deficiency, insufficiency, and sufficiency. It demonstrated that the highest insulin resistance was noted in the vitamin D-deficient group, while the lowest resistance was noticed in the vitamin D sufficient group [66]. Other study reported that the serum vitamin D level in women with PCOS (n = 545) was lower than that of the control group (n = 145), being 25.7±32 ng/mL, respectively.

High prevalence of vitamin D deficiency has been found to be associated with metabolic syndrome which may have great impact on public health [67]. A study reported that the prevalence of vitamin D deficiency in women with PCOS is about 67-85%, with serum concentrations of 25(OH)D <20 ng/ml[68]. Also, the abnormalities that occurred in calcium and PTH levels which resulted from hypovitaminosis D may negatively affect follicular development and causes menstrual irregularities in women with the syndrome [2]. Besides, vitamin D regulates ovarian steroidogenesis and IGFBP-1 production [66].

However, vitamin D supplements led to beneficial effects on follicle maturation and menstrual cycle regulation [69]. A randomized clinical study enrolled in 67 PCOS patients with vitamin-D-deficiency and 54 non-PCOS but vitamin D deficient volunteers with matched age and body mass index. All subjects got oral cholecalciferol (50,000 IU weekly) for a period of 2 months and a daily 1500 IU for 1 month. The results illustrated that vitamin D supplements decrease levels of androgen and
intensify insulin sensitivity in vitamin-D-deficient subjects with the syndrome [70].

Other study reported that in PCOS patients with vitamin D deficiency, a 2-month treatment with 1500 mg calcium daily and 50,000 units of vitamin D on a weekly basis improved menstrual cycles in 7 out of 9 cases [71].

Another study evaluated the effects of vitamin D replacement in 15 women with PCOS. Treatment with a vitamin D3 analog (alphacalcidol) for 3 months showed an increase in the first phase of insulin secretion and improvement in lipid profiles.

Insulin

Insulin is an endocrine peptide hormone secreted by pancreatic β-cells in the islets of Langerhans and plays role in arranging nutrient availability response [72].

Insulin Resistance

Definition

It is identified by the state, in which insulin activity is insufficient to fulfill the peripheral tissue requirements, in spite of insulin hypersecretion. The concept can be illustrated by Himsworth observations [73], who noticed that concurrent glucose and insulin injection in diabetic patients caused one of the following outcomes. Some patients have stable or attenuated blood glucose which is insulin sensitive. Conversely, the trial significantly elevated blood glucose level that is an insulin impediment. As compensation for insulin resistance, fasting plasma insulin levels increase [74]. Target tissues and β dysfunction causes progression of fasting hyperglycemia and diabetes type 2 but this effect can be inverted by weight reduction and low-calorie regimens [75].

Classification of Resistance to Insulin

The resistance is classified into physiological, as in pregnancy and puberty, and pathological resistance. The pathological resistance is divided into primary resistance and secondary to certain dysfunction. The most popular type is metabolic syndrome which is identified by the collection of resistance to insulin, hyperglycemia, hypertension, dyslipidemia, hyperuricemia, and adiposity [76] that shown in table (1).

| Physiological | Aging | Puberty | Pregnancy | Obesity | Starvation | Diurnal variation |
|---------------|-------|---------|-----------|---------|------------|------------------|
| Secondary     | Stress (catecholamine†, surgery, illness) | Cushing’s | Acromegaly | Phaeochromocytoma | Glucagonoma |
| Hormone excess|       |         |           |         |            |                  |
| Growth hormone deficiency | Chronic renal failure, cirrhosis, haemochromatosis | T2D | PCOS |
| Organ failure  |       |         |           |         |            |                  |
| Primary       | ‘Metabolic syndrome’ | Insulin receptor defects (leprechaunism) | Syndromes (Prader-Willi, Laron dwarfism) |
| Genetic       | Congenital (generalized or partial lipodystrophy syndromes) | Acquired (HIV-1 protease inhibitor treatment) |

Table 1: Classification of resistance to insulin

Mechanism of Insulin Resistance

The resistance has multiple mechanisms. The deformity may include any step required for insulin signaling and can be categorized into prereceptor or post-receptor deformity. Prerreceptor resistance is scarce in humans and involves antibodies to insulin. Most of the resistance has pertained to post-receptor deformity. The main character of insulin resistance is a specific defect in PI3K-dependent signaling pathways [77]. Moreover, resistance is selective according to tissues that signaling may be efficacious in a certain tissue, while hindered in another [78].

Insulin and PCOS

Insulin is responsible for the modulation of ovarian steroidogenesis and follicular maturation. Insulin receptors are detected in granulosa and theca cells [79]. PCOS is identified by the diversity in insulin activity, implicated by the existence or the absence of resistance to insulin, in addition to the variations in resistance magnitude among affected women [80]. Incidence of resistance to insulin falls between 44% and 70% and the body recompenses for this impedance by oversecretion of insulin [81] which is involved in hyperandrogenemia, chronic anovulation through the following mechanisms [82] that illustrated in Figure (4):

1) Enhancement of ovarian and adrenal androgen biosynthesis.
2) Suppression of sex hormone-binding globulin (SHBG) that increases androgen bioavailability.
3) Through direct hypothalamic-pituitary’s effects that enhances luteinizing hormone prevalence).

Studies in pubescent [83] and adult PCOS women with hyperandrogenemia [84] have shown a positive correlation between insulin resistance or oversecretion with hyperandrogenemia and anovulation [85].

In a large prospective study, variable groups of patients with polycystic ovary and similar BMI were compared with each other and with BMI-matched controls. Patients with ovulatory dysfunction and hyperandrogenism had more resistance to insulin in comparison with controls [86].
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Estimation of vitamin D levels: Serum 25OHD

It is reported that circulating 25OHD levels are recently the optimal vitamin D indicator whatever the source (cutaneous synthesis, foods, or supplements) [88].  
Vitamin D Dietary Reference Intake (DRI) [89] and workshop of the Institute of Medicine (IOM) [90], proposed more studies to estimate the required vitamin D intake related to optimal concentrations of circulating 25(OH)D, according to age and race/ethnicity, taking into consideration the differences in UVB radiation exposures.  
Calcitriol is ineffective in vitamin D estimation, for several reasons. It has a short t1/2 (many hours), its synthesis is indirectly arranged by vitamin D consumption, its levels are modulated by another elements (such as PTH), and, even in the existence of severe hypovitaminosis D the calcitriol level may be normal or even elevated due to a renal enzyme up-regulation (1-alpha-hydroxylase).

Assays for Serum 25OHD

The assays currently available can be categorized into:

1. Binding: Antibody-based methods (Radioimmunoassay {RIA} and Chemiluminescence immunoassays {CLIA} and binding protein assay).
2. Chemical: (High performance liquid chromatography {HPLC}) and liquid chromatography-tandem mass spectrometry (LC-MS/MS)).

High-Performance Liquid Chromatography (HPLC)

Using HPLC followed by Ultraviolet quantitation is very stable and can be repeated several times. It provides isolated quantitation of 25- hydroxyvitamin D2 and D3. HPLC has some obstacles such as the large sample size, recommended preparation step, and interferences with other compounds measured in the ultraviolet spectrum so, high technical expertise is required [91].

Microplate Enzyme Immunoassay, Colorimetric

Principle

Sequential Competitive Method:

The required reagents involve an immobilized antibody, enzyme-antigen conjugate, and native antigen. After mixing an immobilized antibody, and a whole blood sample, a conjugation reaction come about between the native antigen for a restricted number of insoluble binding sites.  
After the washing step for removing any unreacted native antigen, the enzyme-conjugated antigen remained. The conjugate reacts with sites of the antibody unoccupied by the native antigen. Following another incubation, the bound fraction of antibody is isolated from unbound antigen by decantation or aspiration. The enzyme activity within the bound fraction of antibody is inversely proportional to the concentration of the native antigen. By using variable calibrators with a known concentration of antigen, a dose-response curve can be obtained from which an unknown antigen concentration can be attained.
Vitamin D Levels

25-hydroxy vitamin D level is classified into:
- < 12 ng/ml (< 30 nmol/l): Deficiency.
- 12-20 ng/ml (30 - 50 nmol/l): Insufficiency.
- 20 - 50 ng/ml (50 - 125 nmol/l): Sufficiency [89]

Assays of Insulin

Insulin assay can be generally divided into:
1. Immunoassays including ELISA, CLIA, RIA, and on-chip immunoassays.
2. Chromatography methods including HPLC-UV and LC-MS/MS.
3. Electrochemical biosensor.

Immunoassay

ELISA

Estimation of insulin levels quantitatively in human serum by a microplate enzyme immunoassay, colorimetric. ELISA has a good insulin selectivity with minimal protein intervention and so it is convenient for scientific research in which samples include physiological salts and albumin [92].

Principle

Immunoenzymometric Assay:

Enzyme-conjugated and immobilized antibodies (Ab) with higher affinity, specificity, and different epitope recognition, in excess, and native antigen are the primary reagents needed (Ag). The immobilization takes place on the surface of a microplate well in this operation. Exogenously inserted biotinylated monoclonal antibody interacts with streptavidin coated on the well. The reaction between the native antigen and the antibodies forms a soluble sandwich after combining biotinylated, enzyme-labeled antibodies with a serum containing the native antigen.

The complex is precipitated to the well at the same time, thanks to a high-affinity reaction between streptavidin and biotinylated antibody. By decantation or aspiration, the bound fraction of antibody is segregated from unbound antigen once equilibrium has been reached. The enzyme activity in the bound fraction is proportional to the native antigen concentration. Using well-known variable references.

Chromatography Methods

HPLC-UV

It is a greatly efficient detection technique based on column separation and detection under the maximum analyte UV absorption [93]. Liquid-liquid extraction (LLE) is a common process used for insulin extraction. Based upon the different chemical structures and affinities of insulin and its analogs with the chromatographic column, HPLC-UV is capable of obtaining complete separation and quantitation [94].

3. Electrochemical Biosensor

It is recognized as a device that depends on current estimation and/or voltage to detect the conjugation between the analyte and the recognition element in the biosensor [95]. This method has the following merit:

- It is uncomplicated, highly accurate, requires the least instrumentation, and evades the usage of sophisticated labeling strategies [96]. Conversely, the specificity for insulin is relatively low.

Insulin Levels

Serum insulin level is classified as:
- 0.7-9.0 μU/mL: Normal level in adults.
- > 9.0 μU/mL: insulin resistance [97]

Conclusion

Polycystic ovary is the most common endocrinological irregularity in women of childbearing age. This disorder is mainly diagnosed by Rotterdam criteria that include ovulatory dysfunction, hyperandrogenemia, and ovarian morphology. PCOS is accompanied by many complications such as resistance to insulin, obesity, and cardiovascular risk factors. Vitamin D receptors are recognized in many reproductive organs so, it is important to estimate vitamin D level in PCOS patients by any of the methods mentioned in the review. PCOS women have significantly lower serum 25(OH)D than control women, according to studies. Furthermore, lower vitamin D levels in PCOS women are linked to increased insulin resistance. To investigate the causality of this relation, more large randomized controlled trials are needed. Further randomized trials are required to clarify the role of supplementation with vitamin D in female fertility.

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