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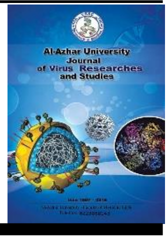

# Effect of Vitamin D Supplementation on Ovulation Induction Among Women with Poly Cystic Ovary Syndrome

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## Abstract

Polycystic ovary syndrome represents the most common cause of anovulatory infertility. Deficiency of vitamin D is common, and it's linked to ovarian insufficiency and infertility. The purpose of our study was to determine the effect of supplementation of vitamin D in inducing ovulation in PCOS patients. This prospective controlled clinical study was done in Obstetrics & Gynecology Department in Al-Zahra University Hospital. 50 PCO patients were analyzed in each group. Vitamin D group: included 50 PCOS patients who used 50 mg clomiphene citrate twice daily to induce ovulation starting from the 3rd day of menstrual period and continue for 5 days added to 10,000 IU of oral vitamin D twice a week and calcium 1250 mg twice daily for 1 month before induction of ovulation and continued for 3 induction cycles. Control Group: included 50 PCOS patients who used 50 mg clomiphene citrate twice daily to induce ovulation starting from the third day of menstrual cycle and for 5 days added to a placebo tablet which given twice a week and calcium 1250 mg twice daily for period of 1 month prior to ovulation induction and continued for 3 induction cycles. Vitamin D supplementation showed significant benefits according to ovulation after adding to clomiphene citrate during ovulation induction among PCOS patients.

**Keywords:** Polycystic ovary syndrome, Insulin resistance, Vitamin D, Vitamin D deficiency, Clomiphene citrate, Body mass index, Metabolic syndrome.

## 1. Introduction

Polycystic ovary syndrome (PCOS) is a prevalent illness that affects 4–18% of women of reproductive age. [1-4]. Anovulatory infertility is a common result of polycystic ovary syndrome whose clinical symptoms include anovulation or oligo-ovulation, infertility, menstrual disturbance, polycystic ovaries and hyperandrogenism [5].

WHO recognizes infertile couples as those who failed in conceiving despite repeated unprotected coitus for one year [6].

PCOS remains an enigmatic condition, despite years of research. The pathophysiology is complex and is thought to be a result of interaction between genetics, epigenetics, ovarian dysfunction, endocrine, neuroendocrine and metabolic alteration [3, 7, 8].

PCOS is an androgen excess condition with reproductive and metabolic dysfunctions including infertility, insulin resistance (IR), hyperinsulinemia, and dyslipidemia [9].

Sonographic criteria of polycystic ovaries from the 2003 Rotterdam conference include ≥ 12 small cysts (2 – 9 mm in diameter) or an raised ovarian volume (more than 10 mL) or both [10].

Treatment of PCOS depends on a woman's goals and the severity of the endocrinal dysfunction. It includes lifestyle modification [11], clomiphene citrate [12, 13] combination oral contraceptive pills [14], metformin [15], vitamin D [16, 17], laparoscopic ovarian surgery [18], IVF [18] and acupuncture [19].

About 80–90% of the body's total vitamin D is formed by the skin after sunlight exposure, while a small amount comes from diet or supplements [17, 20, 21].

Vitamin D is considered sufficient if it is > 30 ng/ml, and insufficient if it is 20–29 ng/ml ,while considered deficient if it is < 20 ng/ml [16, 22] .

Vitamin D deficiency (VDD) is very prevalent worldwide [16] and causes primary ovarian insufficiency [23] and infertility [24, 25]. VDD produces PCOS through insulin resistance (IR) which raises the risk of diabetes and cardiac disease. [22, 26, 27]. The purpose of our study was to detect effect of supplementation of vitamin D in inducing ovulation in PCOS patients.

## 2. Patients and Methods

This prospective controlled clinical study was done in Obstetrics & Gynecology Department in Al-Zahra University Hospital. Patient were one hundred patients diagnosed with PCO seeking for fertility were invited to participate in this study.

### 2.1 Justification of sample size:

The sample size for this study was calculated according to Arkin, 1984 using the following equation [30]:

$$N= \frac{(Z_{\alpha})^2 * (SD)^2}{(d)^2}$$

The following factors were used to determine the sample size:

-The confidence level was set at 95%.

-The study's power was at 80%.

Total sample size = 49.64 ≈ 50 samples in each group.

### 2.2 Inclusion criteria:

100 patients aged 20-35 years with PCO diagnosed by the presence of two of the three diagnostic characteristics listed below:

a) Oligo- or anovulation

b) Picture of hyperandrogenism [hirsutism, acne or androgenic alopecia].

c) Ultrasonic signs of polycystic ovaries [≥12 follicles in each ovary with diameter of 2 – 9 mm and raised ovarian volume > 10 mL in cycle days 3 – 5].

### 2.3 Exclusion criteria:

1- The Age < 20 or > 35 years.
2- Any endocrinological disorder.
3- Any chronic medical disorder.
4- Women with other concomitant cause of infertility or pelvic pathology. Vitamin D or calcium supplementation, oral contraceptive pills or other hormonal treatment within last three cycles.

5- Mental illness causing patients to be unable to comprehend the study's purpose.

### 2.4 Design & Randomization:

Computer generated random numbers concealed in 100 sealed envelopes. Patients were randomly classified into two groups 50 patients each.

### 2.5 Vitamin D (intervention) Group:

Included 50 PCOS patients who used 50 mg clomiphene citrate twice daily to induce ovulation starting from the 3rd day of menstrual period and continue for 5 days added to 10,000 IU of oral vitamin D twice a week [29] and calcium 1250 mg twice daily for 1 month before induction of ovulation and continued for 3 induction cycles [30].

### 2.6 Control Group:

Included 50 PCOS patients who used 50 mg clomiphene citrate twice daily to induce ovulation starting from the third day of menstrual cycle and for 5 days added to a placebo tablet which given twice a week and calcium 1250 mg twice daily for period of 1 month prior to ovulation induction and continued for 3 induction cycles[30] **.**

A complete clinical evaluation was performed on all patients, which included the history, general, abdominal, and pelvic examinations, pelvic ultrasound, and a hormonal profile. At day 3 of the spontaneous menstrual cycle in regularly menstruating women or after withdrawal cycle in amenorrheic women, blood samples were withdrawn for follicle stimulating hormone (FSH), luteinizing hormone (LH), thyroid-stimulating hormone (TSH), prolactin, estradiol (E2) and total testosterone. On day 21 of the cycle, serum progesterone was tested **.**

We didn't detect vitamin D levels in the start because it was too costly to do so. In anovulatory women using clomiphene citrate for ovulation induction, many therapies were employed as adjuvant therapy. We assumed that using Vitamin D for empirical purposes was safe, widespread, and simple [30].

### 2.7 Technique of transvaginal ultrasound:

At the first, the cervix was scanned followed by the uterus and then the adnexa and cul-de sac. Because the field of the view is so small, it may be necessary to gently slide the transducer further up or down the vagina to visualize the iliac blood vessels and then follow them to the ovary. The ovary is much easier to visualize when it contains follicles, as they are echo free. Then the scan was analyzed for monitoring follicular development.

Serial transvaginal ultrasound was performed using vaginal transducer of the LOGIQ V5 ultrasound machine (GE MEDICAL SYSTEMS, CHINA CO.) to detect the number and size of follicles from day 10 of the cycle and day after day until the main follicle achieved a diameter of 18–20 mm, then a single dosage of 10 thousand IU of human chorionic gonadotropin was delivered intramuscularly to induce ovulation. Endometrial thickness was also measured in millimeters in the longitudinal section from the anterior subendometrial zone to the opposite side, this included two endometrial layers. During the treatment cycles, all patients were encouraged to have timed intercourse.[30]

The primary outcome was the ovulation rate. It was observed for 3 cycles of induction after starting clomiphene citrate if pregnancy didn ’ t happen. The secondary outcomes included clinical, biochemical pregnancy and side effects. A progesterone level of ≥ 19 nmol/l was used to determine ovulation. [30]. The presence of biochemical pregnancy was determined by a positive hCG level in the blood, while ultrasound identification of the gestational sac confirmed clinical pregnancy. Treatment was maintained for up to three induction cycles or until pregnancy was achieved. Withdrawal bleeding was done by norethisterone therapy in patients who failed to ovulate and remained amenorrheic for more than 6 weeks.

## 3. Results

50 patients were analyzed in each group (Figure 1). There was no statistically significant difference in the demographic data of the patients, or the baseline hormone levels between the two groups at the time of randomization (Table 1 and 2). Vitamin D and control groups didn’t show statistically significant difference according to demographic data including BMI, oligomenorrhea, amenorrhea, hirsutism and acne after 3 induction cycles (Table 3). Also, vitamin D and control groups didn’t show statistically significant difference in 3rd day of the period according to ovarian volume on both sides after three cycles of induction and number of antral follicles of left ovary after 1st and 3rd cycles of induction (Table 4). Added to that, vitamin D and control groups didn’t show statistically significant difference in in day of ovulation according to endometrial thickness and progesterone level after induction of ovulation (Table 5). After the induction cycles, 62 percent of women who got Vitamin D achieved successful ovulation, compared to 36 percent in the placebo group with P.value 0.007 (Table 6). The vitamin D group had a higher rate of clinical and chemical pregnancy than the control group. However, there was no significant difference between studied groups according to chemical pregnancy, clinical pregnancy, oligomenorrhea, amenorrhea and acne as P-values were >0.05 (Table 6). Also, there was no significant difference between studied groups regarding the side effects including GIT upsets, blurring of vision, blurring of vision, headache, constipation, breast tenderness and hot flushes as P-values were > 0.05 (Table 7).

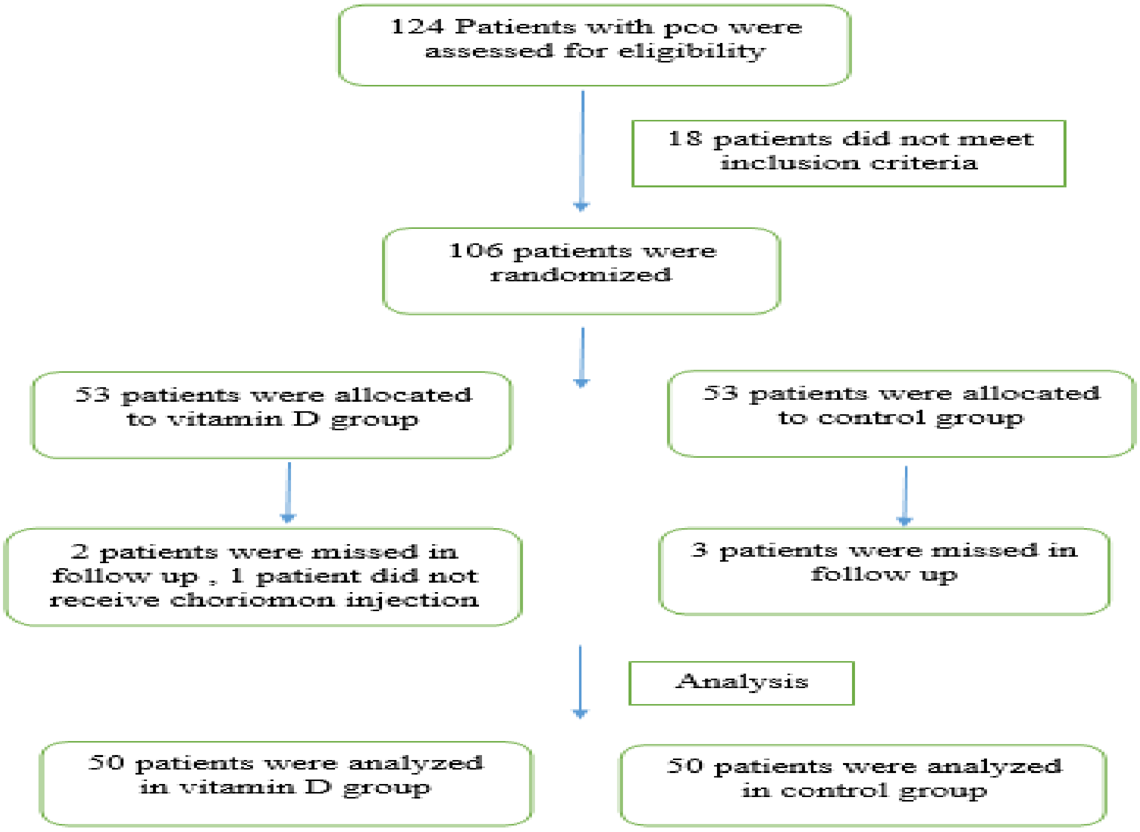


**Figure (1):** Patient flowchart of studied groups.

**Table (1):** Demographic data of the studied groups in 1st visit (before intervention).

| Variable | Vitamin D group | Control group | P.value |
|---|---|---|---|
| Age (years) | 26.38 ± 3.81 | 27.70 ± 3.73 | 0.083 |
| BMI (Kg/m$^2$) | 26.54 ± 3.75 | 27.31 ± 2.71 | 0.243 |
| Duration of infertility (months) | 26.62 ± 15.93 | 30.10 ± 16.24 | 0.282 |
| 1ry Infertility | 35 (70%) | 30 (60%) | 0.295 |
| 2ry Infertility | 15 (30%) | 20 (40%) | 0.295 |
| Oligomenorrhea | 35 (70%) | 36 (72%) | 0.826 |
| Amenorrhea | 15 (30%) | 14 (28%) | 0.826 |
| Hirsutism | 29 (58%) | 35 (70%) | 0.211 |
| Acne | 19 (38%) | 25 (50%) | 0.227 |

**Table (2):** Hormonal profiles of the studied groups in 1st visit (before intervention).

| Variable | Vitamin D group | Control group | P.value |
|---|---|---|---|
| FSH (mIU/ml) | 5.1 ± 0.97 | 5.2 ± 1.17 | 0.660 |
| LH (mIU/ml) | 11.39 ± 2.04 | 11.35 ± 2.49 | 0.935 |
| S.Estradiol (E2) (pg/ml) | 30.28 ± 8.43 | 31.27 ± 10.21 | 0.596 |
| S.Testosterone (ng/dl) | 6.72 ± 2.38 | 6.92 ± 2.19 | 0.676 |
| S.Prolactin (ng/ml) | 15.57 ± 5.35 | 15.68 ± 4.8 | 0.914 |
| TSH (µIU/ml) | 1.93 ± 0.82 | 2.29 ± 1.04 | 0.058 |
| S.Progesterone (nmol/L) | 4.70 ± 0.69 | 4.86 ± 0.85 | 0.329 |

**Table (3):** Comparison between studied groups according to demographic data after induction of ovulation

| Variable | | Vitamin D group | Control group | P.value |
|---|---|---|---|---|
| BMI (Kg/m$^2$) | After 1st cycle of induction | 26.43 ± 3.92 | 26.99 ± 2.84 | 0.409 |
| | After 2nd cycle of induction | 26.63 ± 3.93 | 26.93 ± 2.87 | 0.670 |
| | After 3rd cycle of induction | 26.84 ± 3.96 | 27.27 ± 2.77 | 0.556 |
| Oligomenorrhea | After 1st cycle of induction | 34 (68%) | 36 (72%) | 0.663 |
| | After 2nd cycle of induction | 32 (64%) | 35 (70%) | 0.523 |
| | After 3rd cycle of induction | 28 (56%) | 33 (66%) | 0.305 |
| Amenorrhea | After 1st cycle of induction | 15 (30%) | 14 (28%) | 0.826 |
| | After 2nd cycle of induction | 14 (28%) | 14 (28%) | 1.0 |
| | After 3rd cycle of induction | 13 (26%) | 14 (28%) | 0.822 |
| Hirsutism | After 1st cycle of induction | 28 (56%) | 34 (68%) | 0.216 |
| | After 2nd cycle of induction | 25 (50%) | 34 (68%) | 0.067 |
| | After 3rd cycle of induction | 24 (48%) | 33 (66%) | 0.069 |
| Acne | After 1st cycle of induction | 19 (38%) | 24 (48%) | 0.313 |
| | After 2nd cycle of induction | 18 (36%) | 22 (44%) | 0.190 |
| | After 3rd cycle of induction | 16 (32%) | 22 (44%) | 0.216 |

**Table (4):** Comparison between studied groups according to ultrasound in 3rd day of the period after induction of ovulation.

| Variable | | | Vitamin D group | Control group | P.value |
|---|---|---|---|---|---|
| Right ovary | Ovarian volume | After 1st cycle of induction | 11.28 ± 0.88 | 11.62 ± 0.75 | 0.040* |
| | | After 2nd cycle of induction | 11.35 ± 0.86 | 11.69 ± 0.75 | 0.042* |
| | | After 3rd cycle of induction | 11.38 ± 0.86 | 11.85 ± 0.70 | 0.007* |
| | N. of antral follicles | After 1st cycle of induction | 13.78 ± 2.14 | 14.58 ± 2.37 | 0.080 |
| | | After 2nd cycle of induction | 13.79 ± 2.31 | 14.35 ± 2.14 | 0.221 |
| | | After 3rd cycle of induction | 13.95 ± 1.99 | 14.12 ± 1.89 | 0.699 |
| Left ovary | Ovarian volume | After 1st cycle of induction | 11.21 ± 0.78 | 11.58 ± 0.75 | 0.017* |
| | | After 2nd cycle of induction | 11.29 ± 0.77 | 11.62 ± 0.74 | 0.033* |
| | | After 3rd cycle of induction | 11.29 ± 0.77 | 11.73 ± 0.72 | 0.008* |
| | N. of antral follicles | After 1st cycle of induction | 13.56 ± 2.03 | 14.82 ± 2.41 | 0.006* |
| | | After 2nd cycle of induction | 13.77 ± 2.38 | 14.24 ± 1.85 | 0.273 |
| | | After 3rd cycle of induction | 13.45 ± 1.44 | 14.23 ± 1.76 | 0.026* |

**Table (5):** Comparison between studied groups according to ultrasound in day of ovulation after induction of ovulation.

| Variable | | | Vitamin D group | Control group | P.value |
|---|---|---|---|---|---|
| Right ovary | Ovarian volume | After 1st cycle of induction | 11.91 ± 1.33 | 11.96 ± 0.76 | 0.813 |
| | | After 2nd cycle of induction | 12.24 ± 1.19 | 12.52 ± 1.16 | 0.245 |
| | | After 3rd cycle of induction | 12.56 ± 1.40 | 12.37 ± 1.12 | 0.481 |
| | N. of antral follicles | After 1st cycle of induction | 13.18 ± 2.54 | 14.08 ± 2.75 | 0.092 |
| | | After 2nd cycle of induction | 12.83 ± 2.90 | 13.41 ± 2.82 | 0.324 |
| | | After 3rd cycle of induction | 12.71 ± 2.44 | 13.30 ± 2.44 | 0.259 |
| | N. of mature follicles | After 1st cycle of induction | 7 (14%) | 5 (10%) | 0.538 |
| | | After 2nd cycle of induction | 12 (24%) | 12 (24%) | 1.0 |
| | | After 3rd cycle of induction | 15 (30%) | 7 (14%) | 0.053 |
| | size of mature follicles | After 1st cycle of induction | 18.99 ± 0.70 | 18.98 ± 0.61 | 0.980 |
| | | After 2nd cycle of induction | 19.03 ± 1.14 | 19.18 ± 0.81 | 0.738 |
| | | After 3rd cycle of induction | 19.37 ± 1.43 | 18.92 ± 0.82 | 0.474 |
| Left ovary | Ovarian volume | After 1st cycle of induction | 11.68 ± 1.11 | 11.85 ± 0.89 | 0.405 |
| | | After 2nd cycle of induction | 12.20 ± 1.26 | 11.98 ± 0.86 | 0.325 |
| | | After 3rd cycle of induction | 12.44 ± 1.45 | 12.27 ± 0.78 | 0.506 |
| | N. of antral follicles | After 1st cycle of induction | 12.88 ± 2.27 | 14.38 ± 2.88 | 0.005* |
| | | After 2nd cycle of induction | 12.70 ± 2.44 | 13.69 ± 2.37 | 0.046* |
| | | After 3rd cycle of induction | 12.20 ± 1.80 | 13.28 ± 2.29 | 0.016 |
| | N. of mature follicles | After 1st cycle of induction | 5 (10%) | 3 (6%) | 0.461 |
| | | After 2nd cycle of induction | 14 (28%) | 5 (10%) | 0.022* |
| | | After 3rd cycle of induction | 13 (26%) | 8 (16%) | 0.220 |
| | size of mature follicles | After 1st cycle of induction | 20.18 ± 0.83 | 19.00 ± 0.44 | 0.066 |
| | | After 2nd cycle of induction | 19.36 ± 0.92 | 19.26 ± 1.02 | 0.835 |
| | | After 3rd cycle of induction | 19.08 ± 0.95 | 19.26 ± 0.99 | 0.692 |
| Endometrial thickness | | After 1st cycle of induction | 11.39 ± 1.80 | 10.29 ± 0.83 | 0.001* |
| | | After 2nd cycle of induction | 10.44 ± 1.57 | 9.51 ± 0.92 | 0.001* |
| | | After 3rd cycle of induction | 9.61 ± 1.39 | 9.06 ± 0.71 | 0.023 |
| Progesterone level (nmol/L) | | After 1st cycle of induction | 10.53 ± 4.78 | 9.75 ± 4.28 | 0.399 |
| | | After 2nd cycle of induction | 15.64 ± 5.21 | 13.47 ± 4.66 | 0.037* |
| | | After 3rd cycle of induction | 21.40 ± 8.60 | 18.08 ± 6.83 | 0.049* |

**Table (6):** Comparison between studied groups according to ultrasound in day of ovulation after induction of ovulation.

| Variable | Vitamin D group | Control group | P.value |
|---|---|---|---|
| Ovulation of mature follicle | 31 (62 %) | 18 (36 %) | 0.009* |
| Chemical pregnancy | 20 (40 %) | 15 (30 %) | 0.295 |
| Clinical pregnancy | 16 (32 %) | 13 (26 %) | 0.509 |
| Oligomenorrhea | 28 (56 %) | 33 (66 %) | 0.305 |
| Amenorrhea | 11 (22 %) | 10 (20 %) | 0.806 |
| Hirsutism | 24 (48 %) | 33 (66 %) | 0.069 |
| Acne | 16 (32 %) | 22 (44 %) | 0.216 |
| Ovulation of mature follicle | 31 (62 %) | 18 (36 %) | 0.009* |
| Chemical pregnancy | 20 (40 %) | 15 (30 %) | 0.295 |

**Table (7):** Side effects in studied groups.

| Side effects | Vitamin D group | Control groups | P.value |
|---|---|---|---|
| GIT upsets | 21 (42 %) | 26 (52) | 0.316 |
| Bluring of vision | 3 (6 %) | 1 (2 %) | 0.307 |
| Headache | 4 (8 %) | 2 (4 %) | 0.400 |
| Constipation | 5 (10 %) | 3 (6 %) | 0.461 |
| Breast tenderness | 3 (6 %) | 2 (4 %) | 0.646 |
| Hot flushes | 1 (2 %) | 2 (4 %) | 0.558 |

## 4. Discussion

One of the most frequent endocrine illnesses in women of reproductive age is polycystic ovary syndrome with variable manifestations such as menstrual disturbance, hyperandrogenism, ovulatory dysfunction, insulin resistance, metabolic syndrome and infertility. About 67–85% of PCOS patients have vitamin D levels <20 ng/ml [31].

There was no statistically significant difference in the demographic data of the patients, or the baseline hormone levels between the two groups at the time of randomization (Table 1 and 2).

After the induction cycles, 62 percent of women who got Vitamin D achieved successful ovulation, compared to 36 percent in the placebo group with P.value 0.007 (Table 6).

The vitamin D group had a higher rate of clinical and chemical pregnancy than the control group. However, there was no significant difference between studied groups according to chemical pregnancy, clinical pregnancy, oligomenorrhea, amenorrhea and acne as P-values were >0.05 (Table 6). Also, there was no significant difference between studied groups according to the side effects including GIT upsets, blurring of vision, blurring of vision, headache, constipation, breast tenderness and hot flushes as P-values were > 0.05 (Table 7).

In agreement with the present study, Radwa Rasheedy et al in 2020 concluded that studied groups didn’t show significance difference regarding baseline demographic data, hormonal levels and antral follicular count after inducion of ovulation in PCO patients [30].

Our study was supported by Ahlam Nasir in 2020 who found that absence of any significant difference between the three study groups as regards demographic data (p>0.05) [32].

Wehr et al. in 2011 found that vitamin D therapy for 12 weeks in PCOS patients showed a reduction of menstrual irregularity [29].

In agreement with our study, Radwa Rasheedy et al in 2020 concluded that taking of vitamin D increased the ovulation percentage in PCO patients with no effect on clinical or chemical pregnancy [30].

In accordance with our study, Sherif akl et al in 2019 performed a study included 300 PCO women. They concluded that

ovulation rate was elevated in the vitamin D deficient subgroup after taking of vitamin D compared to the normal vitamin D subgroup and the control group [33].
Also Shahrokhi et al. in 2016 reported that vitamin D taking might help subfertile PCOS women who are undergoing ovarian stimulation. [34].
Ansam Abdulameer Yahya et al in 2019 concluded that taking vitamin D with CoQ10 in PCOS patients with clomiphene citrate resistance improved oxidative marker, hormonal profile and ovulation outcome [35]. Ng et al. in 2017 found absence of any significant relation between vitamin D level and metabolic parameters in PCOS patients [36]. In a line with our study, Radwa Rasheedy et al in 2020 concluded that was no significant difference according to the side effects in both vitamin D and control groups including GIT upsets, bluring of vision, bluring of vision, headache, constipation, breast tenderness, hot flushes and mild OHSS as P-values are >0.05 [30]. In accordance with our study, Lili ZHUANG et al in 2019 found that side effects after treatment include nausea in 12 , 10 cases while vomiting in 10 , 11 cases , whereas diarrhea in 12 and 8 patients in study and control groups respectively ($P > 0.05$) [37]. Against our study, Sadhir et al in 2015 found that absence of significant relation between Vitamin D levels in PCOS and control group [38].
In disagreement with the present study, Vitamin D levels were not linked to the Homeostatic Model Assessment for Insulin Resistance in PCOS, according to Sahin et al in 2014 [39]. In the contrast of our study, Franasiak et al. in 2015 found that level of vitamin D had no relationship with IVF outcomes [40]. In disagreement with the present study, Low Vitamin D levels in PCOS patients are inversely connected with obesity and IR, according to Hahn et al. from 2006 [41]. Against our study, Raja-Khan et al. in 2014 found that the insulin sensitivity in PCOS patients was unchanged with high-dose vitamin D [26].

## 5. Conclusion

Vitamin D supplementation showed significant benefits according to ovulation after addining to clomiphene citrate during induction of ovulation in PCOS cases. Vitamin D supplementation is also recommended in PCO patients due to its low cost, patient convenience in the form of ease administration and other its health benefits.

## 6. Recommendation

We recommend further research with higher dose of vitamin D, other method of administration or larger size of population.

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