Section:

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Effect Of Adding Vitamin D Supplement to Clomiphene Citrate For Induction Of Ovulation In Cases Of Overweight PCOS Women

Mohamed Atef Ghanem 1* M.B.B.Ch, Ismail Mohamed El-Jarhy 2 MD and Fahd Abdel-Aal Al-Omda 2 MD.

ABSTRACT
Background: Polycystic ovary syndrome had unclear etiology, affecting 6-12% of females due to undetermined optimal treatment. Studies indulge vitamin D in pathogenesis of PCOS but without evidence demonstrating its effect on PCOS.

Aim of the work: Our objective is evaluation of vitamin D supplementation on ovulation in sub-fertile females with PCOS.

Patients and methods: Two hundred cases of PCOS at Al-Azhar University Hospital and Damanhour teaching Hospital were selected based on clinical diagnosis, ultrasonographic and laboratory findings. They were 100 cases of PCOS managed by clomifine citrate plus vitamin D and another 100 cases managed only by clomifine citrate.

Results: Our results didn't show a difference between both groups regarding demographic data, hormonal profile, fasting sugar level of both cases and controls and also, radiological findings between both groups. There was estradiol rise in serum level at time ovulation of cases; and increase pregnancy rate after induction but in doesn't reach a significant level without difference between both groups regarding complications after therapy.

Conclusion: We can concluded that addition of vitamin D to clomifine is beneficial in increasing pregnancy rate even with a non-significant effect and so, we recommend its addition to the protocol of management in cases of PCOS.

Keywords: COS; polycystic ovary syndrome; primary infertility; secondary infertility.

INTRODUCTION
Polycystic ovary syndrome (PCOS) is a common female disease, affecting 6-12% of women worldwide. It is previously called Stein-Leventhal syndrome that mostly likely represented by cystic enlargement of ovaries, menstrual disorders, signs of androgen excess and infertility. It affects approximately 4-12% of women of reproductive age globally. 1

The etiology is unknown but involves interaction between environmental and multiple genetic factors, nowadays it is attributed to obesity with insulin resistance that alters ovarian peptides responsible for enzymatic activity which increases the risk of developing metabolic syndrome characterized by obesity, type-2 diabetes, hypertension, dyslipidemia, atherosclerosis, and ischemic heart disease. It is characterized by menstrual irregularity, infertility, androgen excess, diabetes mellitus and dyslipidemia. 2

These symptoms have an impact on the quality of life and health of patients and because of unclear pathogenesis an optimal treatment has not been determined. 3

The need to admit new methods of management for this disease; recent studies found that vitamin D deficiency (VDD) is those patients and may be associated with metabolic and endocrine disorders in them. 4,7 The prevalence of VDD is relatively higher in PCOS patients. 5

Vitamin D is involved in the balance of calcium phosphate and bone mineralization. 7 Vitamin D receptors are stimulated at 2,776 genomic positions and modulate the expression of 229 genes in >30 tissues as pancreas, liver, immune cells, brain and ovaries. 8

This study aimed to evaluate vitamin D supplementation on ovulation of overweight sub-fertile PCO females undergo ovulation induction with clomiphene citrate.
PATIENTS AND METHODS

This a double blind study included 200 women of PCOS with infertility at the Department of Obstetrics and Gynecology of Al-Hussein University Hospital and Damanhour Teaching Hospital during the period from 1st Feb 2021 to 30th Sept 2021.

All cases and controls of the study underwent induction ovulation with clomifene citrate orally twice daily from the 3rd day of menstrual cycle and for 5 days to be repeated for 3 successive menstrual cycles. In regimen of patients vitamin D was supplied in addition to clomifine.

After approval of local ethics committee, all patients included in the study or their relatives were informed well about the procedure and had an informed written consent before carrying the procedure.

RESULTS

Age:
The age of cases ranged between 20-39 years with a mean of 27.47±5.07 years while in controls it ranged between 20-34 years with a mean of 26.35±4.2 years without significant statistical change regarding age (P = 0.231) (Table 1).

Duration of marriage:
The duration of marriage in cases ranged between 2-6.2 years with a mean of 3.8±1.0 years while in controls it ranged between 2.5-7 years with a mean of 4±1.1 years with a non-significant difference between both groups (P = 0.658) (Table 1).

Period of infertility:
In cases, period of infertility ranged between 1-5 years with a mean of 2.7±0.99 years while in controls it ranged between 1-6 years with a mean of 2.4±1.10 years without a significant difference between both groups (P = 0.401) (Table 1).

Type of infertility:
Fifty eight out of the cases (58%) had primary infertility and the remaining (42/100, 42%) had secondary infertility while in controls, 66 (66%) had primary infertility and the remaining (34%) had secondary infertility. There was a non-significant difference between types of infertility (P = 0.758) in cases while primary infertility was evident in controls (P = 0.02)
in addition a non-significant difference between both groups regarding primary or secondary infertility P = 748 and 0.518 respectively) (Table 1).

BMI (Kg/m2):
The weight of cases ranged between 48-87 kg with a mean of 61±8.3 kg while in controls it ranged between 51-93 kg with a mean of 65.8±8.8 kg without significant difference between both groups (P = 0.222) (Table 1).

In cases, the height ranged between 154-176 cm with a mean of 162±5.8 cm while in controls it ranged between 167-185 cm with a mean of 173±6.2 cm with a non-significant difference between both groups (P = 0.371) (Table 1).

The BMI of cases ranged between 20-25 kg/m2 with a mean of 23±1.5 kg/m2 while in controls it ranged between 21-25 kg/m2 with a mean of 22.85±1.6 kg/m2 without a significant difference between both groups (P = 0.155) (Table 1).

Serum FSH (mIU):
Serum FSH in cases ranged between 3.6-9 mIU with a mean of 6.34±1.7 mIU while in controls, the serum level of FSH ranged between 3.3-9.1 mIU with a mean of 5.62±1.5 mIU with a non-significant difference between both groups (P = 0.205) (Table 2).

Serum LH (mIU):
In cases, serum level of LH ranged between 7.2-19 mIU with a mean of 13±2.5 mIU while in controls its level ranged between 7.1-20 mIU with a mean of 12.65±3.1 mIU without a significant difference between both groups (P = 0.361) (Table 2).

LH/FSH ratio:
The LH/FSH ratio in cases ranged between 1.7-3 with a mean of 2.2±0.3 while in controls its range between 1.86-4.1 with a mean of 2.48±0.53 without a statistical difference between both groups (P1 = 0.315) (Table 2).

Free serum testosterone (Pg/ml):
The serum free testosterone of cases ranged between 2.6-4.8 Pg/ml with a mean of 3.66±0.5 Pg/ml while in controls it serum level ranged between 0.8-8 Pg/ml with a mean of 3.24±1 Pg/ml with a non-significant difference between both groups (P = 0.412) (Table 2).

Serum Prolactin (ng/ml):
Serum prolactin in cases ranged between 2.3-20 ng/ml with a mean of 10.75±3.6 ng/ml while its level in controls ranged between 6.4-21 ng/ml with a mean of 10±2.3 ng/ml without a significant difference between both groups (P = 0.218) (Table 2).

Serum Progesterone (ng/ml):
The serum level of progesterone ranged between 9.2-23 ng/ml with a mean of 15.86±3.2 ng/ml while in controls its level ranged between 8.1-30 ng/ml with a mean of 13.22±4 ng/ml with a non-significant difference between both groups (P = 0.332) (Table 2).

Serum estradiol (Pg/ml):
All women were subjected to complete history and clinical examination; laboratory hormonal assay “FSH, LH, FSH/LH ratio, Estradiol, Testosterone, Pro lactine, TSH…etc” and radiological examination by ultrasonography for exclusion of uterine factor for infertility and detection of the effect of therapy.

Follow up of patients is by ultrasonography for ovaries to detect the mature follicles, size, volume of ovaries (Fig. 1, 2, 3), level of serum hormones that detected pretreatment and comparing it with the post-therapy level. Complications were defined during 30 days post-therapy and was recorded.

Data were analyzed using corresponding statistical tests and P value <0.05 was considered significant.
Serum level of estradiol in cases ranged between 267-390 Pg/ml with a mean of 325±32 Pg/ml while its level in controls ranged between 227-375 Pg/ml with a mean of 389±35 Pg/ml without a significant difference between both groups (P = 0.122) (Table 2).

Fasting blood sugar, FBS (mg/dL):

Fasting blood sugar of cases ranged between 77-156 mg/dL with a mean of 108±22 mg/dL while in controls it ranged between 70-167 mg/dL with a non-significant difference between both groups (P = 0.178) (Table 2).

Radiological investigations "U/S examination":

Right ovary:

Volume:
The volume of right ovary in cases ranged between 13-19 gm with a mean of 16±1.5 gm while in controls it ranged between 9-19 gm with a mean of 14±2.5 gm without a significant difference between both groups (P = 0.768) (Table 3).

Number of cysts <2 mm:
The number of cysts <2 mm diameter in right ovary of cases ranged between 8-16 cysts a mean of 13±1.8 cysts while in controls its number ranged between 5-16 cysts with a mean of 11±2.2 cysts and the statistical showed a non-significant difference between both groups (P = 0.514) (Table 3).

Left ovary:

Volume:

Left ovarian volume in cases ranged between 11-19 gm with a mean of 14±1.8 gm while it ranged between 9-17 gm with a mean of 12±1.2 gm in controls without a significant difference between both groups (P = 0.655) (Table 3).

Number of cysts <2 mm:

In cases, the number of cysts <2 mm diameter in left ovary ranged between 10-17 cysts a mean of 12±1.2 cysts while in controls this number ranged between 4-16 cysts a mean of 13±1.9 cysts with a non-significant difference between both groups (P = 0.777) (Table 3).

Folliculometry at the 12th day of the last cycle "size of the most mature follicle in mm":
The size of the most mature follicle in cases ranged between 11-21 mm with a mean of 17±3.1 mm while this size ranged between 11-20 mm with a mean of 16±2.9 mm in controls with a non-significant difference between both groups at induction (P = 0.518) (Table 3).

Serum estradiol at time of ovulation (Pg/ml):

Serum estradiol at time of ovulation in cases ranged between 205-390 Pg/ml with a mean of 320±54 Pg/ml while in controls its level ranged between 201-382 Pg/ml with a significant increase of estradiol in group I than in group II at time of ovulation (P = 0.012) (Table 3).

Previous inductions:

In cases, women previous induce ovulation between 0-3 times with a mean of 1.1±0.8 times while in controls previous inductions ranged between 0-4 times with a mean of 1.4±0.9 times without a significant difference between both groups (P = 0.768) (Table 3).

Previous surgical history:

In cases, ninety (90/100, 90%) of women had negative previous surgical history and ten (10/100, 10%) women had previous surgical history "eight of the cases of positive surgical history [8/10, 80%] underwent appendectomy and two cases [2/10, 20%] underwent ovarian cystectomy while in controls, ninety-three (93/100, 93%) of women had negative previous surgical history and seven (7/100, 7%) women had previous surgical history "four of the cases of positive surgical history [4/7, 57.1%] underwent appendectomy and three cases [3/7, 42.9%] underwent ovarian cystectomy and the statistical analysis revealed a significant evidence of surgical history in cases (P = 0.021); also, appendectomy was significantly in cases (P = 0.021) while ovarian cystectomy commonly occurs in controls (P = 0.032) in addition there was a non-significant difference between surgeries in controls (P = 0.221) while appendectomy is the commonly done operation in cases (P = 0.01) (Table 4).

Fate of ovulation and pregnancy outcome:

In fifty (50/100, 50%) of cases the ovulation had succeeded and pass to pregnancy while in forty of controls (40/100, 40%) ovulation had succeeded and pass to pregnancy and the statistical analysis revealed that even with increase in the success rate of ovulation and pregnancy in cases but the increased didn't reach a significant value as shown in (Table 5).

Follow-up complications:

In both groups complications detected in a non-significant manner except nausea and vomiting that significantly occurs in cases (P = 0.021) this is clearly described in (Table 6).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group I n = 100</th>
<th>Group II n = 100</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Years):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>20-39</td>
<td>20-34</td>
<td>0.231</td>
</tr>
<tr>
<td>Mean±S.D</td>
<td>27.47±5.07</td>
<td>26.35±4.2</td>
<td>(NS)</td>
</tr>
<tr>
<td>Duration of marriage:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>2-6.2</td>
<td>2.5-7</td>
<td>0.658</td>
</tr>
<tr>
<td>Mean±S.D</td>
<td>3.8±1.0</td>
<td>4±1.1</td>
<td>(NS)</td>
</tr>
<tr>
<td>Weight (Kg):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>48-87</td>
<td>51-93</td>
<td>0.222</td>
</tr>
<tr>
<td>Mean±S.D</td>
<td>61±8.3</td>
<td>65.8±8.8</td>
<td>(NS)</td>
</tr>
<tr>
<td>Height (cm):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>154-176</td>
<td>167-185</td>
<td>0.371</td>
</tr>
<tr>
<td>Mean±S.D</td>
<td>162±5.8</td>
<td>173±6.2</td>
<td>(NS)</td>
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</table>
BMI (Kg/m²):

<table>
<thead>
<tr>
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<th>Group I</th>
<th>Group II</th>
<th>P</th>
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</thead>
<tbody>
<tr>
<td>28-39</td>
<td>30-42</td>
<td>0.155 (NS)</td>
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</tbody>
</table>

Period of infertility:

<table>
<thead>
<tr>
<th>Range</th>
<th>Mean±S.D</th>
<th>Group I</th>
<th>Group II</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-5</td>
<td>1-6</td>
<td>0.401 (NS)</td>
<td></td>
<td></td>
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</tbody>
</table>

Type of infertility:

<table>
<thead>
<tr>
<th>Primary (n/%)</th>
<th>Secondary (n/%)</th>
<th>Group I</th>
<th>Group II</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>58 (58%)</td>
<td>66 (66%)</td>
<td>0.758 (NS)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>42 (42%)</td>
<td>34 (34%)</td>
<td>0.02 (S)</td>
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BMI (Kg/m²):

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<th>Group II</th>
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<tr>
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<td>34 (34%)</td>
<td>0.02 (S)</td>
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</table>

Table 1: Demographic data of patients of both groups.

<table>
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<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 100</td>
<td>n = 100</td>
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</tbody>
</table>

Table 2: Laboratory investigations in patients of both groups.

<table>
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<tr>
<th>Variable</th>
<th>Group I</th>
<th>Group II</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 100</td>
<td>n = 100</td>
<td></td>
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</tbody>
</table>

Table 3: Radiological investigations (U/S examination) in patients of both groups.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group I</th>
<th>Group II</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td></td>
</tr>
</tbody>
</table>

Table 4: Previous surgical history in patients of both groups.
<table>
<thead>
<tr>
<th>Variable</th>
<th>Group I n (%)</th>
<th>Group II n (%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Success of ovulation (pregnancy)</td>
<td>50 (50%)</td>
<td>40 (40%)</td>
<td>0.221 (NS)</td>
</tr>
<tr>
<td>Failure of ovulation (no pregnancy)</td>
<td>50 (50%)</td>
<td>60 (60%)</td>
<td>0.201 (NS)</td>
</tr>
<tr>
<td>P</td>
<td>1.0 (NS)</td>
<td>0.031 (S)</td>
<td></td>
</tr>
</tbody>
</table>

Table 5: Fate of ovulation and pregnancy outcome in patients of both groups.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group I n (%)</th>
<th>Group II n (%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Headache</td>
<td>3 (3%)</td>
<td>5 (5%)</td>
<td>0.231 (NS)</td>
</tr>
<tr>
<td>Nausea and vomiting</td>
<td>8 (8%)</td>
<td>3 (3%)</td>
<td>0.021 (S)</td>
</tr>
<tr>
<td>Breast tenderness</td>
<td>3 (3%)</td>
<td>2 (2%)</td>
<td>0.758 (NS)</td>
</tr>
<tr>
<td>Abdominal distention</td>
<td>4 (4%)</td>
<td>6 (6%)</td>
<td>0.231 (NS)</td>
</tr>
<tr>
<td>Visual disturbances</td>
<td>3 (3%)</td>
<td>3 (3%)</td>
<td>1.00 (NS)</td>
</tr>
<tr>
<td>Ovarian hyperstimulation Syndrome</td>
<td>2 (2%)</td>
<td>3 (3%)</td>
<td>0.758 (NS)</td>
</tr>
<tr>
<td>P</td>
<td>0.254 (NS)</td>
<td>0.317 (S)</td>
<td></td>
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</tbody>
</table>

Table 6: Follow-up complications occurrence in patients of both groups.

**DISCUSSION**

Polycystic ovary syndrome (PCOS) is a common disorder affecting 6-10% of women at reproductive age accounting for 70-80% of infertility. Despite the effectiveness of pharmacologic induction of ovulation, many patients still have difficulty conceiving and have a high risk of miscarriage that might be related to reduced endometrial receptivity.¹⁰,¹⁴

Clomiphene citrate “a selective estrogen receptor modulator” is recommended as the first-line medication for inducing ovulation in PCOS. It leads to ovulation in 78–85% of the cases.¹⁵

This study conducted on two hundred women with PCOS with history of menstrual disturbance selected
from those attending the Clinic of Obstetrics and Gynecology Outpatient Clinic at Al-Hussein Hospital, Al-Azhar University and some private centers of infertility.

The mean age of cases in the study was 26.35 years and 27.47 years in controls without significant difference between both groups.

Seyedoshohadam et al. found a non-significant difference between studied groups regarding age which was agree with our study.

Akpinar et al., also found a non-significant age difference between studied groups as in our study.

In addition, Yonggang et al. didn't found a significant difference between their studied groups regarding age.

Akl et al. in a study found no difference between their studied groups regarding age which was the same we found.

Yahya et al. found no difference between both groups of their study regarding age that run in lines with our study.

Ibrahim et al. didn't found a difference between age of groups of their study (mean 31.5 and 32.7 years respectively) which agree with our results.

Our results revealed non difference between both groups regarding weight, height and BMI and this was in agree with what found by Yonggang et al.

Akl et al. found a significant increase of BMI in cases without vitamin D supplementation in their study which conflicting with our study.

In contrast, Yahya et al. found no difference between both groups of their study regarding BMI that run in lines with our study.

Our results showed non-significant difference between both groups regarding period of infertility. This is the same as Seyedoshohadam in their study.

Also, Akpinar et al. detect a non-significant difference between their studied groups regarding period of infertility.

In addition to that, Akl et al. found similar results in their study regarding duration of infertility.

In agree with our study, Yahya et al. found no difference between both groups of their study regarding duration of infertility.

Our results showed that secondary infertility was common in controls while no dominant type in cases. This was found with Seyedoshohadam et al. in their study.

Akpinar et al. also found a non-significant difference between their studied groups regarding type of infertility.

In addition to that, Yonggang et al. found a non-significant difference between groups of their study regarding type and duration of infertility.

Akl et al. found no difference between both groups of their study regarding type of infertility which was the same we found in our study. In conjunction with that, Yahya et al. found in their study the same results.

A non-significant difference between our groups regarding the duration of marriage was detected which runs in line with what found in the study of Akpinar et al. where they found a non-significant difference between groups of their study regarding the duration of marriage.

In our study we found no difference between both groups comparing FSH, LH and also LH/FSH ratio.

Chen et al. and Yuan et al. found a significant reduction of serum FSH and LH levels in cases of their study and this results were consistent to the reported results.

Akpinar et al. found in their study that the difference in the response to clomifine citrate in women with PCOS could be due to the differences in LH level, LH/FSH ratio and prolactin which disagree with our results.

Yonggang et al. found a non-significant difference between both groups of their study regarding FSH, LH and prolactin as in our results. Also, Yahya et al. found similar results in their study.

Our study revealed a non-significant difference between both groups regarding fasting blood sugar.
Sirmons et al.\textsuperscript{21} found a good relation between PCOS and DM in their study which disagree with our results.

Akl et al.\textsuperscript{12} found a significant increase in fasting blood sugar in those treated without vitamin D which conflicting with our study.

Yahya et al.\textsuperscript{17} found no difference between both groups of their study regarding FBS which run in lines with our study.

Our results revealed no difference in the serum level of testosterone in both groups similar to what found by Yahya et al.\textsuperscript{17} where they found no difference between both groups of their study regarding testosterone level pre and post-therapy which run in lines with our study.

Our results showed an increase without significant difference in serum level of progesterone in both groups. Which contradicting with Deng et al.\textsuperscript{22} where they found an increase in the level of progesterone after treatment of PCOS in their study group which conflicting with our results.

Our results revealed no significant difference in the serum level of estradiol in both groups and this agreed with what found by Chen et al.\textsuperscript{19} and Yuan et al.\textsuperscript{20} in their studies that human chorionic gonadotropin can significantly reduce serum E2 level and this results was consistent to the reported results.

Our results revealed no difference in the BMI in both groups. Jamal et al.\textsuperscript{23} revealed in their study that weight reduction is recommended as first-line therapy for the management of infertility in obese women with PCOS. Observational studies indicate that weight reduction of 5-10% can increase ovulation and pregnancies and bariatric surgery has been shown to improve cycle regularity, increase ovulation, and increase spontaneous conception which was disagree with our results. Also, Papanen et al.\textsuperscript{24} found in their study that weight reduction with diet and lifestyle changes is the treatment of choice and management of anovulation is the second step in PCOS which disagree of our results.

Our results revealed that group I has higher incidence of induction and successful pregnancy but in a non-significant manner. This similar to Brown et al.\textsuperscript{26} who reported in their study that clomiphene citrate has no difference on getting pregnancy which was in agreement with our results. Also, Rasheed et al.\textsuperscript{27} revealed in their study that addition of vitamin D to clomifine citrate significantly improve the ovulation rate which run in lines with our results but in our results the difference was not significant.

Cunha and Povoa\textsuperscript{14} documented that vitamin D supplementation may be recommended as a potential therapeutic adjunct for the ovulatory dysfunction and metabolic disorders observed in women with PCOS which run in lines with our results. Also, Yahya et al.\textsuperscript{(17)} found no difference between both groups of their study as regard pregnancy outcome which run in lines with our study.

CONCLUSION

From the previous results we can concluded that addition of vitamin D to clomifene is beneficial in increasing pregnancy rate even with a non-significant effect. And so, we recommend the addition of vitamin D to the treatment protocol of PCOS because of its beneficial effect.

Conflict of interest: none

REFERENCES


