# The Impact of Metformin Treatment on Serum Level of AMH and on Ovulation and Pregnancy Outcome in Women With PCOS

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**Abstract-** Anti-Müllerian Hormone (AMH) AMH inhibits follicle recruitment and may reduce FSH action, contributing to ovulatory issues in females. PCOS patients often receive metformin treatment. This study aimed to investigate metformin's effect on serum AMH levels in PCOS women and its impact on ovulation and pregnancy rates. This interventional study was performed between June 2023 and June 2024. 100 PCOS women attending the infertility departments of Maternity Teaching Hospital in Adiwniyah province, Iraq, were invited to participate in the study. PCOS was diagnosed based on the Rotterdam consensus statements criteria. Metformin was given to 50 women (study group) and 50 women received placebo o serve as a control group. After 3 months, comparison of mean serum AMH between study groups revealed significant variation ($P$=0.020) and this significant variation became more pronounced 6 months later ($P$<0.001). In case of placebo group, comparison of baseline mean serum AMH to those after 3 months and after 6 months revealed minimal no significant changes ($P$>0.05); whereas the use of metformin resulted in significant reduction of mean serum AMH 3 months later ($P$=0.006) and the reduction was more significant 6 months later ($P$<0.001). The rate of ovulation and the rate of positive pregnancy test were reported to be higher significantly in metformin group in contrast to placebo group ($P$=0.001 and 0.016, respectively). Metformin treatment is efficient and safe in reducing serum AMH in women with PCOS, thus indicating reduction in antral follicle count and changes in overall hormonal status that can improve ovulation and pregnancy in those women.



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## Introduction

In women of childbearing age, polycystic ovary syndrome (PCOS) is a widespread endocrine disorder and a heterogeneous array of symptoms which include among other things irregular menstrual cycles, hyperandrogenism and polycystic ovaries (1,2). The pathophysiology of Polycystic Ovary Syndrome (PCOS) remains incompletely elucidated. Among the hypothesized mechanisms underlying hyperandrogenism are abnormalities in follicular maturation, wherein the developing follicle fails to advance to a preovulatory dominant follicle (3).

In females, Anti-Müllerian Hormone (AMH) exerts an inhibitory effect on the recruitment of primordial follicles from the dormant oocyte reservoir and may attenuate the action of follicle-stimulating hormone (FSH), thereby contributing to ovulatory irregularities (4). The expression of AMH persists until follicles attain an approximate diameter of 8 mm, and its expression is markedly diminished in larger antral follicles. As a result, there exists a significant correlation between AMH levels and antral follicle count (AFC) (5).

Moreover, the anti-Müllerian hormone (AMH) levels are higher in patients diagnosed with polycystic ovary syndrome (PCOS). As such, AMH has been suggested as a PCOS marker and replacement for antral follicle count (AFC) in PCOS diagnosis, especially when the ultrasound criteria are in dispute (6). A range of AMH cutoff values have been recommended, yet the optimal

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threshold remains unknown due to differences in sensitivity and specificity (3). PCOS patients are often treated with metformin. In addition to reducing insulin resistance in these individuals, metformin has been shown to have a positive effect on menstrual cycles and induced ovulations. However, we are still far from understanding how and why metformin improves reproductive performance in PCOS patients. A lot of investigation has been done on whether metformin treatment affects AMH levels in PCOS sufferers (7).

Metformin, a widely prescribed medication for type 2 diabetes, has several contraindications related to kidney and liver diseases. For kidney function, metformin is contraindicated in patients with severely reduced kidney function, defined as an estimated glomerular filtration rate (eGFR) less than 30 mL/min/1.73 m2 (8,9). This restriction is due to the increased risk of lactic acidosis, a rare but serious complication that can occur when metformin accumulates in the body (8). However, recent guidelines have expanded metformin use to patients with moderately reduced kidney function (eGFR 30-59 mL/min/1.73 m2), with appropriate dose adjustments and monitoring (8,9).

Regarding liver disease, metformin was traditionally contraindicated in patients with chronic liver disease or significant hepatic impairment due to concerns about impaired lactate clearance (10,11). However, recent evidence suggests that metformin can be safely used in patients with stable liver disease, provided that liver function is closely monitored (12). The American Diabetes Association (ADA) guidelines recommend avoiding metformin therapy only in patients with severe hepatic impairment or in binge drinkers due to the high risk of lactic acidosis (11).

It's important to note that metformin does not appear to cause or exacerbate liver injury and may even be beneficial in patients with nonalcoholic fatty liver disease (10,13). The primary concern with metformin use in liver disease is the potential for lactic acidosis in patients with cirrhosis, particularly those with encephalopathy or arterial hypoxemia (10). Therefore, while liver disease is no longer considered an absolute contraindication for metformin use, caution is still advised in patients with severe hepatic impairment, and individual risk assessment is necessary (12,13).

.In a study, it has been found (14) an eight month course of metformin could reduce the Anti-Müllerian Hormone (AMH) levels of women who had been diagnosed with Polycystic Ovary Syndrome (PCOS). It has been shown (15) that although mean AMH levels were somewhat lower in control subjects, all PCOS patients who had undergone therapy with Metformin (850 mg administered twice daily) returned to ovulatory function and had below-normal serum AMH. On the other hand, it has been demonstrated (16) that Metformin taken for 8 months at a dose of 850 mg twice daily had no significant effect on serum AMH levels. The implementation of oral antihyperglycemic agents has been demonstrated to reduce elevated serum total androstenedione and accelerate follicular development and ovulation in patients with PCOS. Numerous studies have established that metformin promotes return of regular menstrual cycles and an increase in ovulation rate among women with PCOS. However, whether it also affects serum AMH levels in this patient population remains a matter of dispute (17). Therefore, in this research, we wanted to explore the role of metformin on serum level of AMH in women with PCOS in addition to its impact ovulation and pregnancy rate.

## Materials and Methods

### Patients and methods

This interventional study was performed between June 2023 and June 2024. 100 PCOS women attending the infertility departments of Maternity Teaching Hospital in Adiwniyah province, Iraq, were invited to participate in the study. PCOS was diagnosed based on the Rotterdam consensus statements criteria. Written informed consent was acquired from all participants. Age, weight, and height were reported. The present study was accepted by the Ethics Committee of the College of Medicine at the Province.

Inclusion criteria for this study were: PCOS, age (18-35 years). Exclusion criteria were contraindications for metformin, administration of metformin or oral contraception within the previous three months, thyroid dysfunction, hyperprolactinemia, or diabetes mellitus and allergy to metformin.

The baseline levels of FSH, and AMH were measured using 5 ml of patient’s blood plasma. For 50 patients, metformin was administrated 850 mg twice daily. The other 50 patients received placebo and served as control group. Treatment with metformin continued for 6 months. AMH level (ng/ml) was measured 3 months and 6 months later. Obtained results were compared with those of the first tests.

The exclusion criteria for the study encompassed several unfavorable conditions to ensure the safety and validity of the research. Women who were pregnant or breastfeeding at the time of recruitment were excluded from participation. The study also excluded those with a

history of thyroid disease, diabetes mellitus, liver disease, renal disease, or Cushing syndrome. Participants who had undergone bariatric surgery within the 12 months prior to the study were not eligible. Additionally, women with hyperprolactinemia, nonclassical congenital adrenal hyperplasia, or androgen-secreting tumors were excluded from the study population.

Furthermore, the exclusion criteria extended to women with severe cardiovascular diseases, alcohol abuse, or those using other insulin-sensitizing agents or ovulation induction medications. Participants with known hypersensitivity to metformin, impaired liver function, chronic or acute metabolic acidosis, or congestive heart failure were also deemed ineligible. The study excluded women who were unable to provide informed consent, those participating in other clinical trials within the three months preceding the study, and individuals with conditions that could interfere with oral medication absorption or metabolism. These comprehensive exclusion criteria helped ensure the study focused on the target population of women with PCOS without confounding factors that could affect the results or pose unnecessary risks to participants.

### Statistical analysis

It was carried out using SPSS version 16.0 (SPSS; SPSS Inc., Chicago, IL, USA). Data was presented as numbers, percentage, mean, standard deviation and range. AMH level comparison before and after intervention was carried out using paired-samples t-test. Comparison of mean serum FSH and AMH between study groups was done using independent samples t-test. Comparison of proportions of ovulation and pregnancy test was done using chi-square test. *P* of less than 0.05 was considered the cutoff value of significance.

## Results

Demographic characteristics of women with PCOS included in this research are shown in table 1. We reported no significant differences in mean age and in mean body mass index (BMI) between metformin group and placebo group ($P>0.05$). The mean age of metformin group was 25.76±4.54 years and that of placebo group was 25.84±5.16 years. The mean BMI of metformin group was 24.98±3.76 kg/m$^2$ and that of placebo group was 25.26±4.05 kg/m$^2$.

Baseline serum hormonal levels of women with PCOS enrolled in this study are shown in table 2. The mean serum follicle stimulating hormone FSH of metformin group showed no significant variation upon contrast to that of placebo group ($P=0.859$), 4.00±0.36 IU/L and 3.99±0.43 IU/L, respectively. The mean anti-mullerian hormone AMH of metformin group showed no significant variation upon contrast to that of placebo group ($P=0.854$), 8.24±3.22 ng/ml and 38.36±3.01 ng/ml, respectively.

Serum AMH levels after 3 months and after 6 months of treatment are shown in table 3. After 3 months, comparison of mean serum AMH between study groups revealed significant variation ($P=0.020$) and this significant variation became more pronounced 6 months later ($P<0.001$). In case of placebo group, comparison of baseline means serum AMH to those after 3 months and after 6 months revealed minimal no significant changes ($P>0.05$); whereas, the use of metformin resulted in significant reduction of mean serum AMH 3 months later ($P=0.006$) and the reduction was more significant 6 months later ($P<0.001$), as it is very obvious in figure 1.

Comparison of proportions of women with ovulation and positive pregnancy test between metformin group and placebo groups is shown in table 4. The rate of ovulation and the rate of positive pregnancy test were reported to be higher significantly in metformin group in contrast to placebo group ($P=0.001$ and 0.016, respectively).

**Table 1. Demographic characteristics of women with PCOS included in this research**

| Characteristic | | Metformin group *n*=50 | Placebo group *n*=50 | *P* |
|---|---|---|---|---|
| Age (years) | Mean ±SD | 25.76 ±4.54 | 25.84 ±5.16 | 0.935 I |
| | Range | 18 -34 | 18 -34 | NS |
| BMI (kg/m$^2$) | Mean ±SD | 24.98 ±3.76 | 25.26 ±4.05 | 0.721 I |
| | Range | 19.02 -31.26 | 19 -31.92 | NS |

BMI: body mass index; n: number of cases; I: independent samples t-test; NS: not significant

**Table 2. Baseline serum hormonal levels of women with PCOS enrolled in this study**

| Characteristic | | Metformin group *n*=50 | Placebo group *n*=50 | *P* |
|---|---|---|---|---|
| FSH (IU/L) | Mean±SD | 4.00 ±0.36 | 3.99 ±0.43 | 0.859 I |
| | Range | 3.33 -4.68 | 3.33 -4.7 | NS |
| AMH (ng/ml) | Mean±SD | 8.24 ±3.22 | 8.36 ±3.01 | 0.854 I |
| | Range | 3.27 -13.51 | 3.6 -13.53 | NS |

FSH: follicle stimulating hormone; AMH: anti-mullerian hormone; I: independent samples t-test; NS: not significant

**Table 3. Serum AMH levels after 3 months and after 6 months of treatment**

| Characteristic | | Metformin group *n*=50 | Placebo group *n*=50 | *p* |
|---|---|---|---|---|
| AMH (3 months) (ng/ml) | Mean±SD | 6.80 ±2.00 | 7.99 ±2.94 | 0.020 I* |
| | Range | 3.12 -9.92 | 3.13 -13.49 | |
| | Change in mean | - 1.45 | - 0.37 | |
| | *P* (Pa) | 0.006 *** | 0.520 NS | |
| AMH (6 months) (ng/ml) | Mean±SD | 5.50 ±1.39 | 8.62 ±2.84 | < 0.001 I*** |
| | Range | 3.13-7.54 | 3.21 -13.4 | |
| | Change in mean | - 2.75 | 0.64 | |
| | *P* (Pa) | <0.001 *** | 0.655 NS | |

AMH: anti-mullerian hormone; SD: standard deviation; n: number of cases; I: independent samples t-test; Pa: paired t-test; NS: not significant; *: significant at $P \leq 0.05$; ***: significant at $P \leq 0.001$

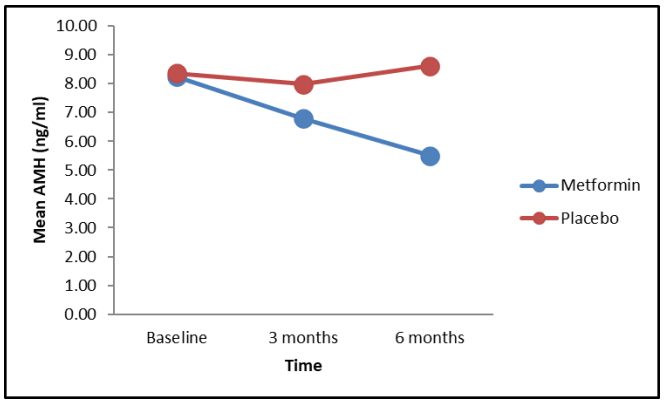


**Figure 1.** Scatter plot showing changes in mean serum AMH during 6 months of follow up in metformin and placebo groups

**Table 4. Comparison of proportions of women with ovulation and positive pregnancy test between metformin group and placebo groups**

| Characteristic | | Metformin group n=50 | Placebo group n=50 | *P* |
|---|---|---|---|---|
| Ovulation | Negative, n (%) | 24 (48.0 %) | 40 (80.0 %) | 0.001 C** |
| | Positive, n (%) | 26 (52.0 %) | 10 (20.0 %) | |
| Pregnancy | Negative, n (%) | 34 (68.0 %) | 44 (88.0 %) | 0.016 C* |
| | Positive, n (%) | 16 (32.0 %) | 6 (12.0 %) | |

C: chi-square test; **: significant at $P \leq 0.01$; *: significant at $P \leq 0.05$

## Discussion

In this study, the objective was to explore the role of metformin in treating women with PCOS taking into consideration the possible changes of AMH following six months of treatment. We found that metformin has significant impact on serum AMH by reducing its level within 3 months treatment and the reduction became more obvious following 6 months. Women with PCOS have high AMH concentrations and accordingly, AMH has been proposed as a marker of polycystic ovary syndrome and a substitute for antral follicle count (3).

In line with our observation, results of the recent meta-analysis suggested that metformin treatment could decrease serum AMH levels in patients diagnosed with PCOS (7). Therefore, the reduction of the amount of AMH in our study is reflective to the reduction of the antral follicle count and thus reducing cystic changes in women leading to improvement in overall hormonal status and improved ovulation rate and pregnancy outcome since that excessive AMH also contributes to anovulation by counteracting FSH in follicle growth and maturation (18).

As of now, the precise mechanisms by which metformin enhances reproductive functionality in patients with polycystic ovary syndrome (PCOS) remain inadequately elucidated. Numerous empirical investigations have been conducted to ascertain whether metformin therapy could modulate anti-Müllerian hormone (AMH) concentrations in individuals diagnosed

with PCOS. Certain studies indicated a reduction in AMH levels following metformin administration (19,20), whereas others noted no significant alterations in AMH concentrations (21,22).

The underlying biological pathways through which metformin augments ovarian activity continue to be incompletely understood. A prevalent hypothesis posits that metformin may enhance reproductive outcomes by alleviating insulin resistance. Nevertheless, metformin has been advocated for utilization among all individuals with PCOS irrespective of prior insulin sensitivity (23), thus suggesting an insulin-independent mechanism of action for metformin itself. In 2005, Dr. Fleming and associates published the inaugural study demonstrating the efficacy of metformin in diminishing AMH levels in patients with PCOS (14).

Metformin, a widely prescribed medication for type 2 diabetes, has multiple mechanisms of action that contribute to its glucose-lowering effects. Primarily, metformin decreases hepatic glucose production, reduces intestinal glucose absorption, and improves insulin sensitivity by increasing peripheral glucose uptake and utilization (24,25). At the molecular level, metformin inhibits mitochondrial complex I activity, which has been postulated as the primary mechanism for its antidiabetic effects (24). Additionally, metformin has been shown to inhibit mitochondrial glycerol-3-phosphate dehydrogenase, thereby reducing the contribution of glycerol to hepatic gluconeogenesis (25).

In women with polycystic ovary syndrome (PCOS), metformin treatment has been associated with a decrease in serum anti-Müllerian hormone (AMH) levels. Studies have shown that AMH levels in women with PCOS are typically 2-3 times higher than in healthy women. Metformin administration has been found to significantly reduce AMH levels in PCOS patients after 8 weeks of treatment. This reduction in AMH levels may be related to metformin's effects on insulin sensitivity, as hyperinsulinemia is a common factor in PCOS that may contribute to elevated AMH levels (20).

Metformin treatment in women with PCOS has shown promising effects on ovulation and pregnancy outcomes. It has been found to restore ovulation, improve fertility, and decrease the frequency of early pregnancy loss. A study involving 200 nondiabetic PCOS patients undergoing assisted reproduction showed that those who continued metformin use throughout pregnancy had a significantly lower rate of early pregnancy loss compared to those who discontinued the medication (11.6% vs. 36.3%) (26). Furthermore, metformin treatment throughout pregnancy in women with PCOS has been associated with increased possibilities of term delivery, vaginal delivery, and reduced risks of early pregnancy loss, preterm labor, and pregnancy complications such as gestational diabetes mellitus and pregnancy-induced hypertension (27).

In the research conducted by Tomova (28), participants who regained regular menstrual cycles post-metformin treatment exhibited a 16.27% decrease in AMH levels, while AMH levels nearly tripled in those who did not respond favorably to metformin therapy. A variety of clinical studies investigating the relationship between AMH levels and metformin have been undertaken; however, the findings have not been uniform. All these investigations were characterized by small sample sizes.

Metformin treatment is efficient and safe in reducing serum AMH in women with PCOS, thus indicating reduction in antral follicle count and changes in overall hormonal status that can improve ovulation and pregnancy in those women.

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