Evaluation of Anti-Müllerian Hormone (AMH) and Insulin Resistance as Biomarkers of Infertility in Polycystic Ovary Syndrome

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Abstract

Background: The most prevalent endocrine disorder in women of reproductive age, and the major cause of infertility caused by anovulatory, is polycystic ovary syndrome (PCOS). Anti-Mullerian hormone (AMH) and insulin resistance are also considered crucial pathophysiological traits of PCOS, and their roles as the biomarkers of infertility are not yet fully understood.

Design: A cross-sectional group cohort study designed to assess the AMH and insulin resistance (measured by HOMA-IR) levels in infertile and fertile PCOS patients, and to find out whether they can be used as predictive biomarkers of infertility in PCOS.

Methods: The case-control study was carried out in January 20, May first, 2025 in Samarra General Hospital, Iraq. One hundred and five women of reproductive age (18-40 years) were recruited and separated into three groups, PCOS patients with infertility (n=35), PCOS patients without infertility (n=35), and healthy fertile controls (n=35). The diagnosis of PCOS was done using Rotterdam criteria. The AMH serum levels, fasting glucose levels, fasting insulin levels, follicle-stimulating hormone (FSH) levels, luteinizing hormone (LH) levels, and testosterone levels were measured. The Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) was used in the evaluation of insulin resistance. Body mass index (BMI) and waist circumference were anthropometric measurements. The identification of independent predictors of infertility relied on binary logistic regression.

Findings: PCOS infertile patients were found to have much higher AMH levels than PCOS infertile and healthy controls (8.94 ± 2.31 ng/mL vs. 6.82 ± 1.95 ng/mL vs. 3.45 ± 1.12 ng/mL, p=0.001). The PCOS patients with infertility (4.76 +/-1.84) had a significantly higher value in HOMA-IR than PCOS without infertility (3.21 +/-1.45), and controls (1.89 +/-0.67) (p<0.001). The LH/FSH was also found to be much greater in infertile patients with PCOS (2.84 ± 0.92) than in the other groups (p<0.001). The analysis of ROC curves showed that AMH level above 7.5 ng/mL (sensitivity 82.9, specificity 77.1, AUC=0.812) and HOMA-IR level above 3.8 (sensitivity 74.3, specificity 71.4, AUC=0.768) were the best cut-off points in predicting infertility in PCOS patients. The independent variables that AMH (OR=2.84, 95% CI: 1.62-4.98, p<0.001) and HOMA-IR (OR=2.13, 95% CI: 1.38-3.29, p=0.001) were confirmed as independent predictors of infertility in binary logistic regression. The AMH and HOMA-IR had a strong positive correlation in the infertile PCOS group (r=0.68, p<0.001).

Conclusion: AMH and insulin resistance levels are strongly connected to infertility among PCOS patients and this is an independent predictor of fertility. These biomarkers can be effective pointers of fertility prospects in PCOS and can be discussed as complements to overall fertility assessment in order to implement therapeutic measures.

Keywords: Polycystic ovary syndrome, Anti-Mullerian hormone, Insulin resistance, HOMA-IR, Infertility, Biomarkers.

1. Introduction

Poly cystic ovary syndrome (PCOS) is a heterogeneous disorder of endocrine and metabolic systems of the body, and is a problem that afflicts about 6-20 percent of women of reproductive age, all around the world, making it one of the most common endocrinopathies among this group of people (1, 2). According to the Rotterdam consensus criteria, PCOS is a set of clinical and biochemical changes, such as hyperandrogenism, ovulatory dysfunction, and polycystic ovarian morphology (3). In addition to its reproductive expression, PCOS is linked to severe metabolic imbalances especially insulin resistance which occurs in 50-70% of its patients irrespective of body mass (4).

One of the most painful effects of PCOS is infertility that affects women with this condition up to 40 percent (5). PCOS-related infertility has a multifactorial pathophysiology, which includes the disrupted folliculogenesis, anovulation, poor oocyte quality, and endometrial dysfunction (6). In spite of the current research, it is still clinically difficult to identify credible biomarkers that can be used to predict fertility outcomes of PCOS patients. This would make such biomarkers invaluable in risk stratification, individual treatment planning and prognostic counseling.

The AMH is an anti-Mullerian hormone secreted by granulosa cells of preantral and small antral follicles that has become a promising option as a biomarker in PCOS (7). The AMH levels in women with PCOS are usually expanded 2-3 times in comparison with the age-matched controls due to the high prevalence of small follicles inherent in the syndrome (8). Although AMH is currently being extensively applied in measuring ovarian reserve, the predictive use of AMH in terms of fertility after PCOS remains controversial. There is some evidence that extreme AMH concentrations are possibly associated with worse fertility and more severe anovulation (9, 10), and some studies do not indicate the same (11).

Insulin resistance and compensatory hyperinsulinemia are major players in the pathogenesis of PCOS because they stimulate ovarian and adrenal secretion and androgen production, disruption of gonadotropin secretion, and endometrial receptivity (12). Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) is a clinical, cost-effective, and validated technique, which can be used to assess insulin resistance (13). Even though insulin resistance is identified to worsen metabolic and reproductive dysfunction in PCOS, how exactly it serves as a predictive factor of infertility has not been clearly determined (14).

PCOS in Iraq and the Middle East, in general, is a major social health issue, and the rates of its prevalence might be even higher than in the Western world as a result of genetic factors, eating habits, and rapidly growing rates of obesity (15, 16). Nevertheless, there is a lack of regional data on the correlation between AMH, insulin resistance and infertility in PCOS patients. This knowledge of these relationships among the population in Iraq may be used to develop more effective and culturally suitable management practices.

The current research was aimed at carrying out an in-depth assessment of the serum AMH and insulin resistance (HOMA-IR) of PCOS patients who are fertile and infertile and comparing them to the healthy fertile controls. We hypothesized that the two markers would be significantly increased among infertile PCOS patients and may be used as predictors of fertility independence. We also sought to identify the best cut-off values of these biomarkers that can be used in clinical

decision-making. These clarified relationships mean that this study will help to develop better fertility evaluation and management initiatives that the Iraqi PCOS women will appreciate.

2. Materials and Methods

2.1 Study Design and Setting

The present case-control study was carried out in the Department of Obstetrics and Gynecology, Samarra General Hospital, Samarra, Iraq, during the period of about 3.5 months (January 20 to May 1, 2025). The ethical approval of the study protocol by the Institutional Ethics Committee of the Samarra General Hospital was received (approval number: SGH-IRB-2025-012), and all the procedures were carried out according to the ethical principles of the Declaration of Helsinki. All the participants had signed informed consent following sufficient explanation of the objectives, procedures, possible risks, and benefits of the study.

2.2 Study Population and Sample Size

A total of 105 women of reproductive age (18-40 years) were enrolled in the study through consecutive sampling from patients attending the gynecology and infertility clinics. Sample size calculation was based on previously reported differences in AMH levels between PCOS phenotypes and controls (effect size=0.8, α=0.05, power=80%), resulting in a minimum of 30 participants per group (17), which provided adequate statistical power for the planned between-group comparisons in the present study. To account for potential dropouts and strengthen statistical power, 35 participants were recruited for each group.

Participants were divided into three groups:

- **Group 1 (Infertile PCOS):** PCOS patients with primary or secondary infertility (n=35)
- **Group 2 (Fertile PCOS):** PCOS patients without infertility who had at least one spontaneous conception (n=35)
- **Group 3 (Healthy Controls):** Healthy fertile women with regular menstrual cycles and at least one spontaneous conception (n=35)

2.3 Diagnostic Criteria

2.3.1 PCOS Diagnosis

PCOS was diagnosed according to the Rotterdam 2003 consensus criteria, requiring the presence of at least two of the following three features (3):

1. Oligo-ovulation or anovulation (menstrual cycles >35 days or <8 cycles per year)
2. Clinical hyperandrogenism (hirsutism, acne, male-pattern alopecia) or biochemical hyperandrogenism (elevated serum testosterone)
3. Polycystic ovaries on transvaginal ultrasound (≥12 follicles measuring 2-9 mm in diameter, or ovarian volume >10 mL in at least one ovary)

Other endocrine disorders including congenital adrenal hyperplasia, Cushing's syndrome, and androgen-secreting tumors were excluded through appropriate clinical and biochemical evaluation.

2.3.2 Infertility Definition

Infertility was defined as the inability to conceive after 12 months or more of regular unprotected sexual intercourse. Only women with ovulatory dysfunction-related infertility were included; those with male factor infertility, tubal factor, or other identifiable causes of infertility were

excluded after appropriate evaluation including partner semen analysis and hysterosalpingography when indicated.

2.4 Inclusion and Exclusion Criteria

Inclusion Criteria:

- Women aged 18-40 years
- For Groups 1 and 2: Diagnosed with PCOS according to Rotterdam criteria
- For Group 1: Primary or secondary infertility attributed to PCOS
- For Group 2: At least one spontaneous pregnancy despite PCOS diagnosis
- For Group 3: Regular menstrual cycles (25-35 days), at least one spontaneous pregnancy, no clinical or biochemical signs of PCOS

Exclusion Criteria:

- Current use of hormonal medications (combined oral contraceptives, metformin, or ovulation induction agents) within the past 3 months
- Pregnancy or lactation
- Diagnosis of diabetes mellitus, thyroid disorders, hyperprolactinemia, or other endocrine disorders
- History of ovarian surgery or chemotherapy
- Chronic systemic diseases (cardiovascular, hepatic, or renal disease)
- Male factor infertility, tubal factor infertility, or endometriosis in patients being evaluated for infertility
- Use of medications affecting glucose or insulin metabolism

2.5 Data Collection and Clinical Assessment

2.5.1 Anthropometric Measurements

Trained nursing staff measured all anthropometric measurements and had standardized protocols. The weight of the body was taken as the closest weight (0.1 kg) and a digital scale (Seca 803, Germany) was used and the weight recorded. A wall-mounted stadiometer was used to measure height to the nearest 0.1 cm (Seca 206, Germany). The body mass index (BMI) was estimated as the weight (kg)/height (m 2) and categorized based on the WHO standards. The waist circumference was obtained as the midline between lower costal margin and iliac crest with the help of a non stretchable measuring tape whose measurement was taken to the nearest one centimeter.

2.5.2 Clinical Evaluation

All the participants provided a comprehensive medical history, including menstrual history, reproductive history, months of infertility (when it is applicable), prior pregnancies, use of contraceptives, family history of PCOS or diabetes, and lifestyle. An evaluation of clinical hyperandrogenism utilized the modified Ferriman-Gallwey scoring system of hirsutism with a score of 8 and above being significant (18).

2.6 Laboratory Investigations

2.6.1 Sample Collection

Blood samples were obtained during the period of 2-5 days of the menstrual cycle (early follicular phase) or at least 45 days of amenorrhea in anovulatory patients. Every participant was asked to fast between 10 and 12 hours prior to the collection of blood. To eliminate circadian variation venous blood samples (10 mL) were collected between 8:00 and 10:00 AM, drawing samples through the antecubital vein. The blood was taken in plain tubes where hormonal tests were to be carried out and fluoride oxalate tubes where glucose was to be measured. Samples were left at the room temperature to clot and then centrifuged at 3000 rpm to 15 minutes. Serum was spun and stored at -80o C till analysis. Within 2 months of collection all samples were analysed.

2.6.2 Biochemical Assays

Hormonal Assays: The concentration of AMH, FSH, LH and total testosterone in serum was analyzed using an enzyme-linked immunosorbent assay (ELISA) kit (Bioassay Technology Laboratory, Shanghai, China) under the instructions of the manufacturer. The intra-assay and the inter-assays coefficients of variation were less than 5 percent and less than 10 percent respectively. The AMH, FSH, and LH and testosterone assays had a sensitivity of 0.1 ng/mL, 0.5 mIU/mL and 0.05 ng/mL respectively.

Metabolic Measures: Fasting plasma glucose (FPG) was determined by the glucose oxidase procedure (Glucolab, Egypt). ELISA (Monobind Inc., USA) with a sensitivity of 0.5 0IU/mL was used to measure fasting serum insulin. Each of the assays was done in duplicate and the average was analyzed.

2.6.3 Calculation of Insulin Resistance

Insulin resistance was assessed using the HOMA-IR index, calculated using the following formula (13):

HOMA-IR = [Fasting Insulin (μIU/mL) × Fasting Glucose (mg/dL)] / 405

A HOMA-IR value ≥2.5 was considered indicative of insulin resistance based on established cut-offs for reproductive-aged women (19).

2.6.4 Ultrasonographic Evaluation

Transvaginal ultrasound examination was performed using a 7.5 MHz transvaginal probe (GE Voluson S6, USA) by experienced sonographers blinded to the clinical and biochemical data. Ovarian volume was calculated using the simplified formula for an ellipsoid (length × width × thickness × 0.523). Antral follicle count (AFC) was determined by counting all follicles measuring 2-9 mm in diameter in both ovaries.

2.7 Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA). Normality of continuous variables was assessed using the Shapiro-Wilk test and visual inspection of Q-Q plots. Descriptive statistics were presented as mean ± standard deviation (SD) for normally distributed continuous variables, median and interquartile range (IQR) for non-normally distributed variables, and frequencies and percentages for categorical variables.

Comparisons between the three groups were performed using one-way analysis of variance (ANOVA) with post-hoc Tukey's honestly significant difference (HSD) test for normally distributed variables, or Kruskal-Wallis test with post-hoc Dunn's test for non-normally distributed

variables. Categorical variables were compared using chi-square test or Fisher's exact test when appropriate.

The results between AMH and HOMA-IR with other clinical/biochemical parameters were evaluated by Pearson correlation coefficient. The independent predictors of infertility status (infertile vs. fertile PCOS) were analyzed using binary logistic regression analysis adjusted by the possibility of confounders such as age, BMI, testosterone and LH/FSH ratio. In univariate analysis, the variables whose p-values were below 0.10 were added to the multivariate model. To measure the model fit, Hosmer-Lemeshow goodness-of-fit test was conducted and to measure the strength of association Nagelkerke R2 was used.

Analysis of receiver operating characteristic (ROC) curve was performed to identify the best cut-off values of AMH and HOMA-IR used to predict infertility in PCOS patients and the area under the curve (AUC), sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated.

All analyses were statistically significant at a two tailed p-value less than 0.05.

3. Results

The basic characteristics of study participants in 3.1 will be clarified One hundred and fifteen women were recruited and fulfilled the research. The baseline demographic and clinical characteristics of the three study groups are given in Table 1. The age was similar between all groups (infertile PCOS: 28.6 ± 4.3 years; fertile PCOS: 29.1 ± 4.8 years; controls: 28.9 ± 4.5 years; p=0.864), which made age-matching between groups.

Body Mass Index and Anthropometric Measurements: Mean BMI was significantly higher in both PCOS groups compared to controls (infertile PCOS: 29.7 ± 5.4 kg/m²; fertile PCOS: 27.9 ± 4.8 kg/m²; controls: 24.3 ± 3.2 kg/m²; p<0.001). Infertile PCOS patients had the highest BMI, though the difference between the two PCOS groups was not statistically significant (p=0.152). Similarly, waist circumference was significantly greater in infertile PCOS patients (94.8 ± 11.6 cm) compared to fertile PCOS (89.3 ± 10.2 cm) and controls (79.4 ± 8.7 cm) (p<0.001).

Clinical Hyperandrogenism: Ferriman-Gallwey scores were significantly higher in infertile PCOS patients (12.4 ± 4.6) compared to fertile PCOS patients (9.8 ± 4.1) and controls (2.3 ± 1.8) (p<0.001). The prevalence of clinical hyperandrogenism (FG score ≥8) was 82.9% in infertile PCOS, 68.6% in fertile PCOS, and 0% in controls.

Menstrual Characteristics: Mean menstrual cycle length was significantly longer in infertile PCOS patients (48.7 ± 18.4 days) compared to fertile PCOS patients (38.6 ± 12.7 days) and controls (28.4 ± 2.1 days) (p<0.001). Oligomenorrhea was present in 88.6% of infertile PCOS patients and 71.4% of fertile PCOS patients.

Table 1. Baseline Demographic and Clinical Characteristics of Study Participants

Characteristic	Infertile PCOS (n=35)	Fertile PCOS (n=35)	Healthy Controls (n=35)	p-value
Demographics				
Age (years)	28.6 ± 4.3	29.1 ± 4.8	28.9 ± 4.5	0.864
Anthropometric measurements				
BMI (kg/m²)	29.7 ± 5.4[a]	27.9 ± 4.8[a]	24.3 ± 3.2[b]	<0.001

Waist circumference (cm)	94.8 ± 11.6[a]	89.3 ± 10.2[b]	79.4 ± 8.7[c]	<0.001
Clinical features				
Ferriman-Gallwey score	12.4 ± 4.6[a]	9.8 ± 4.1[b]	2.3 ± 1.8[c]	<0.001
Clinical hyperandrogenism, n (%)	29 (82.9)[a]	24 (68.6)[a]	0 (0)[b]	<0.001
Menstrual characteristics				
Menstrual cycle length (days)	48.7 ± 18.4[a]	38.6 ± 12.7[b]	28.4 ± 2.1[c]	<0.001
Oligomenorrhea, n (%)	31 (88.6)[a]	25 (71.4)[b]	0 (0)[c]	<0.001
Duration of infertility (months)	26.4 ± 14.8	N/A	N/A	-

The data is given in the form of mean SD or n%. The statistically significant differences between the groups are denoted by different superscript letters (a, b, c) in condition of the post-hoc analysis ($p<0.05$). BMI: body mass index, PCOS: polycystic ovary syndrome, N/A: not applicable.

3.2 Hormonal Profile

The parameters of the hormones are summarized in Table 2 in three study groups.

Anti-Mullerian Hormone (AMH): Serum AMH levels were found to be significantly higher in both PCOS groups than in the controls with the highest levels being found in infertile PCOS patients (8.94 +/- 2.31 ng/mL) when compared to fertile PCOS patients (6.82 +/- 1.95 ng/mL) and normal controls (3.45 +/- 1.12 ng/mL) ($p=0.001$). The infertile and fertile patients with PCOS had a significantly high difference ($p=0.001$), showing that the AMH level is increased even higher in a situation of infertility.

Gonadotropins and LH/FSH Ratio: No significant differences in the mean FSH levels were found between groups (infertile PCOS: 5.284823 mIU/mL; fertile PCOS: 5.440546 mIU/mL; controls: 5.881892 mIU/mL; $p=0.187$). Nevertheless, the LH level was found to be significantly greater in infertile PCOS patients (14.8 ± 4.6 mIU/mL) than in fertile PCOS (11.2 ± 3.8 mIU/mL) and controls (5.9 ± 2.1 mIU/mL) ($p<0.001$). Therefore, LH/FSH ratio was much higher in infertile patients with PCOS (2.84 ± 0.92) than the fertile PCOS (2.07 ± 0.71) and the controls (1.02 ± 0.36) ($p<0.001$).

Androgens: Infertile PCOS patients (1.89 +/-0.64 ng/mL) had significantly high levels of serum total testosterone than fertile PCOS (1.43 +/-0.52 ng/mL) and controls (0.48 +/-0.21 ng/mL) ($p<0.001$). The value of difference between the two PCOS groups was also significant ($p=0.003$).

Table 2. Hormonal Profile of Study Groups.

Parameter	Infertile PCOS (n=35)	Fertile PCOS (n=35)	Healthy Controls (n=35)	p-value
AMH (ng/mL)	8.94 ± 2.31[a]	6.82 ± 1.95[b]	3.45 ± 1.12[c]	<0.001
FSH (mIU/mL)	5.2 ± 1.4	5.4 ± 1.3	5.8 ± 1.5	0.187
LH (mIU/mL)	14.8 ± 4.6[a]	11.2 ± 3.8[b]	5.9 ± 2.1[c]	<0.001
LH/FSH ratio	2.84 ± 0.92[a]	2.07 ± 0.71[b]	1.02 ± 0.36[c]	<0.001

Total testosterone (ng/mL)	1.89 ± 0.64[a]	1.43 ± 0.52[b]	0.48 ± 0.21[c]	<0.001

Data are presented as mean ± SD. Different superscript letters (a, b, c) indicate statistically significant differences between groups based on post-hoc analysis ($p<0.05$). AMH: anti-Müllerian hormone; FSH: follicle-stimulating hormone; LH: luteinizing hormone; PCOS: polycystic ovary syndrome

3.3 Metabolic Parameters and Insulin Resistance

Table 3 presents the metabolic parameters and insulin resistance indices.

Fasting Glucose and Insulin: Fasting plasma glucose was significantly higher in infertile PCOS patients (98.7 ± 12.4 mg/dL) compared to fertile PCOS (91.3 ± 10.8 mg/dL) and controls (84.2 ± 8.6 mg/dL) ($p<0.001$). Fasting insulin levels showed even more pronounced differences, with infertile PCOS patients having the highest levels (19.4 ± 7.8 µIU/mL) compared to fertile PCOS (13.6 ± 5.9 µIU/mL) and controls (7.8 ± 2.9 µIU/mL) ($p<0.001$).

HOMA-IR: Insulin resistance, as measured by HOMA-IR, was significantly elevated in infertile PCOS patients (4.76 ± 1.84) compared to fertile PCOS (3.21 ± 1.45) and controls (1.89 ± 0.67) ($p<0.001$ for all pairwise comparisons). The prevalence of insulin resistance (HOMA-IR ≥2.5) was 85.7% in infertile PCOS, 62.9% in fertile PCOS, and 14.3% in controls.

Table 3. Metabolic Parameters and Insulin Resistance Indices

Parameter	Infertile PCOS (n=35)	Fertile PCOS (n=35)	Healthy Controls (n=35)	p-value
Fasting glucose (mg/dL)	98.7 ± 12.4[a]	91.3 ± 10.8[b]	84.2 ± 8.6[c]	<0.001
Fasting insulin (µIU/mL)	19.4 ± 7.8[a]	13.6 ± 5.9[b]	7.8 ± 2.9[c]	<0.001
HOMA-IR	4.76 ± 1.84[a]	3.21 ± 1.45[b]	1.89 ± 0.67[c]	<0.001
Insulin resistance (HOMA-IR ≥2.5), n (%)	30 (85.7)[a]	22 (62.9)[b]	5 (14.3)[c]	<0.001

Data are presented as mean ± SD or n (%). Different superscript letters (a, b, c) indicate statistically significant differences between groups based on post-hoc analysis ($p<0.05$). HOMA-IR: Homeostatic Model Assessment for Insulin Resistance; PCOS: polycystic ovary syndrome

3.4 Ovarian Morphology

Antral Follicle Count and Ovarian Volume: Transvaginal ultrasound examination revealed significantly higher AFC in infertile PCOS patients (28.4 ± 8.7 follicles) compared to fertile PCOS (22.6 ± 6.9 follicles) and controls (9.3 ± 3.2 follicles) ($p<0.001$). Similarly, mean ovarian volume was greater in infertile PCOS (14.8 ± 4.2 mL) compared to fertile PCOS (12.1 ± 3.6 mL) and controls (7.2 ± 2.1 mL) ($p<0.001$).

Table 4. Ovarian Morphology by Transvaginal Ultrasound

Parameter	Infertile PCOS (n=35)	Fertile PCOS (n=35)	Healthy Controls (n=35)	p-value
Antral follicle count (total)	28.4 ± 8.7[a]	22.6 ± 6.9[b]	9.3 ± 3.2[c]	<0.001
Mean ovarian volume (mL)	14.8 ± 4.2[a]	12.1 ± 3.6[b]	7.2 ± 2.1[c]	<0.001

PCOM (≥12 follicles/ovary), n (%)	35 (100)[a]	35 (100)[a]	0 (0)[b]	<0.001

Data are presented as mean ± SD or n (%). Different superscript letters (a, b, c) indicate statistically significant differences between groups based on post-hoc analysis ($p<0.05$). PCOS: polycystic ovary syndrome; PCOM: polycystic ovarian morphology

3.5 Correlation Analysis

Pearson correlation analysis was performed to examine relationships between AMH, HOMA-IR, and other clinical and biochemical parameters (Table 5).

In the infertile PCOS group:

- AMH showed strong positive correlations with HOMA-IR ($r=0.68$, $p<0.001$), LH ($r=0.54$, $p<0.001$), LH/FSH ratio ($r=0.57$, $p<0.001$), testosterone ($r=0.51$, $p=0.002$), AFC ($r=0.62$, $p<0.001$), and BMI ($r=0.43$, $p=0.009$)
- HOMA-IR correlated positively with testosterone ($r=0.59$, $p<0.001$), LH ($r=0.48$, $p=0.003$), BMI ($r=0.71$, $p<0.001$), and waist circumference ($r=0.64$, $p<0.001$)
- Negative correlations were observed between AMH and FSH ($r=-0.38$, $p=0.024$)

In the fertile PCOS group:

- Similar but weaker correlations were observed between AMH and HOMA-IR ($r=0.42$, $p=0.012$) and other metabolic parameters

In controls, no significant correlations were found between AMH and HOMA-IR ($r=0.18$, $p=0.298$).

Table 5. Correlation Analysis Between AMH, HOMA-IR, and Clinical/Biochemical Parameters in Infertile PCOS Group

Variable	AMH	HOMA-IR
	r (p-value)	**r** (p-value)
HOMA-IR	0.68 (<0.001)	-
FSH	-0.38 (0.024)	-0.21 (0.229)
LH	0.54 (<0.001)	0.48 (0.003)
LH/FSH ratio	0.57 (<0.001)	0.52 (0.002)
Total testosterone	0.51 (0.002)	0.59 (<0.001)
Antral follicle count	0.62 (<0.001)	0.46 (0.005)
BMI	0.43 (0.009)	0.71 (<0.001)
Waist circumference	0.39 (0.021)	0.64 (<0.001)
Fasting glucose	0.37 (0.029)	0.73 (<0.001)
Fasting insulin	0.52 (0.002)	0.89 (<0.001)

AMH: anti-Müllerian hormone; HOMA-IR: Homeostatic Model Assessment for Insulin Resistance; FSH: follicle-stimulating hormone; LH: luteinizing hormone; BMI: body mass index; PCOS: polycystic ovary syndrome

3.6 ROC Curve Analysis and Optimal Cut-off Values

ROC curve analysis was performed to evaluate the diagnostic accuracy of AMH and HOMA-IR in predicting infertility among PCOS patients (comparing infertile PCOS vs. fertile PCOS) (Figure 1).

AMH as a Predictor:

- AUC: 0.812 (95% CI: 0.714-0.910, p<0.001)
- Optimal cut-off: >7.5 ng/mL
- Sensitivity: 82.9%
- Specificity: 77.1%
- PPV: 78.4%
- NPV: 81.8%
- Accuracy: 80.0%

HOMA-IR as a Predictor:

- AUC: 0.768 (95% CI: 0.662-0.874, p<0.001)
- Optimal cut-off: >3.8
- Sensitivity: 74.3%
- Specificity: 71.4%
- PPV: 72.2%
- NPV: 73.5%
- Accuracy: 72.9%

Combined Model (AMH + HOMA-IR): A combined model incorporating both AMH and HOMA-IR showed improved diagnostic performance:

- AUC: 0.856 (95% CI: 0.771-0.941, p<0.001)
- Sensitivity: 85.7%
- Specificity: 80.0%
- Accuracy: 82.9%

When both biomarkers were elevated above their respective cut-offs, the positive likelihood ratio for infertility was 4.29, while negative likelihood ratio was 0.18.

Table 6. ROC Curve Analysis for Predicting Infertility in PCOS Patients

Biomarker/Model	AUC (95% CI)	Optimal Cut-off	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)	p-value
AMH	0.812 (0.714 - 0.910)	>7.5 ng/mL	82.9	77.1	78.4	81.8	80.0	<0.001

HOMA-IR	0.768 (0.662 - 0.874)	>3.8	74.3	71.4	72.2	73.5	72.9	<0.001
Combined (AMH + HOMA-IR)	0.856 (0.771 - 0.941)	-	85.7	80.0	81.1	84.8	82.9	<0.001

AUC: area under the curve; CI: confidence interval; PPV: positive predictive value; NPV: negative predictive value; AMH: anti-Müllerian hormone; HOMA-IR: Homeostatic Model Assessment for Insulin Resistance; PCOS: polycystic ovary syndrome

3.7 Binary Logistic Regression Analysis

To determine the independent predictors of infertility status (infertile vs. fertile) among the PCOS patients with the potential confounders (adjusted logistic regression) binary logistic regression analysis was done (Table 7). The dependent variable was the fertility status (1 = infertile, 0= fertile) and independent variables were AMH, HOMA-IR, total testosterone, BMI, LH/FSH ratio and age. The ones whose p had a value lower than 0.10 in the univariate analysis were included in the multivariate model through the enter method.

The last logistic regression equation displayed the following independent predictors of infertility:

- **AMH** (OR=2.84, 95% CI: 1.62-4.98, p<0.001) - Each 1 ng/mL increase in AMH was associated with a 2.84-fold increase in the odds of infertility
- **HOMA-IR** (OR=2.13, 95% CI: 1.38-3.29, p=0.001) - Each unit increase in HOMA-IR was associated with a 2.13-fold increase in the odds of infertility
- **Total testosterone** (OR=1.89, 95% CI: 1.24-2.88, p=0.003) - Each 1 ng/mL increase in testosterone was associated with an 89% increase in the odds of infertility
- **BMI** (OR=1.12, 95% CI: 1.01-1.24, p=0.033) - Each unit increase in BMI was associated with a 12% increase in the odds of infertility

The LH/FSH ratio did not remain a significant independent predictor after adjustment for other variables (OR=1.47, 95% CI: 0.88-2.45, p=0.142). Similarly, age was not significantly associated with fertility status (OR=0.98, 95% CI: 0.91-1.06, p=0.621).

The overall model was statistically significant (χ^2=58.42, df=6, p<0.001), indicating that the set of predictor variables reliably distinguished between infertile and fertile PCOS patients. The model demonstrated good fit according to the Hosmer-Lemeshow test (χ^2=6.73, p=0.566), with a Nagelkerke R^2 of 0.691, suggesting that approximately 69% of the variance in fertility status was explained by the independent variables. The model correctly classified 84.3% of cases overall (sensitivity: 82.9%, specificity: 85.7%).

When AMH and HOMA-IR were dichotomized using their optimal ROC-derived cut-off values (AMH >7.5 ng/mL and HOMA-IR >3.8), patients with both markers elevated had significantly higher odds of infertility compared to those with both markers below the cut-offs (OR=14.67, 95% CI: 5.21-41.33, p<0.001).

Table 7. Binary Logistic Regression Analysis of Independent Predictors of Infertility in PCOS Patients

Variable	Univariate Analysis			Multivariable Analysis		
	OR	95% CI	p-value	Adjusted OR	95% CI	p-value
AMH (per 1 ng/mL)	3.24	1.89-5.56	<0.001	2.84	1.62-4.98	<0.001
HOMA-IR (per unit)	2.67	1.76-4.05	<0.001	2.13	1.38-3.29	0.001
Total testosterone (per 1 ng/mL)	2.45	1.67-3.59	<0.001	1.89	1.24-2.88	0.003
BMI (per kg/m²)	1.18	1.06-1.31	0.003	1.12	1.01-1.24	0.033
LH/FSH ratio	2.13	1.34-3.38	0.001	1.47	0.88-2.45	0.142
Age (per year)	1.02	0.95-1.09	0.618	0.98	0.91-1.06	0.621

Model statistics: Overall χ^2=58.42 (df=6, p<0.001); Hosmer-Lemeshow χ^2=6.73 (p=0.566); Nagelkerke R^2=0.691; Overall classification accuracy=84.3% (sensitivity 82.9%, specificity 85.7%)

OR: odds ratio; CI: confidence interval; AMH: anti-Müllerian hormone; HOMA-IR: Homeostatic Model Assessment for Insulin Resistance; BMI: body mass index; LH: luteinizing hormone; FSH: follicle-stimulating hormone; PCOS: polycystic ovary syndrome

4. Discussion

The current research presents sufficient data that indicate a high level of AMH and high insulin resistance, measured using HOMA-IR, is strongly linked with infertility in PCOS women. This is the first study that was carried out in Iraq and has juxtaposed the biomarkers of infertile and fertile PCOS patients systematically including a healthy control group, which provides some valuable information on the pathophysiology of PCOS-infertility in the Middle Eastern population.

4.1 AMH as a Biomarker of infertility in PCOS is presented

We have found that the serum AMH levels among infertile PCOS patients (8.94 ± 2.31 ng/mL) were significantly higher than those of fertile PCOS patients (6.82 ± 1.95 ng/mL) and healthy control patients (3.45 ± 1.12 ng/mL). This high AMH in PCOS patients as compared to controls is in line with the known observation that PCOS is typified by high follicular pool and stunted follicular growth (7, 20). More to the point, the large disparity between the AMH levels of infertile and fertile patients with PCOS indicates that overly high AMH levels can be used as a predictor of more serious ovulatory dysfunction and lower fertility performance.

These results are consistent with some of the past research. According to Piouka et al. (10), the spontaneous conception rate among PCOS patients with AMH level exceeding 7.0 ng/mL was

much lower than the spontaneous conception rate among PCOS patients with AMH levels of lower values. Also, La Marca et al. (21) have determined that the highest AMH levels were linked to more severe oligo-anovulation and lower response to ovulation induction therapy. The suggested mechanisms of this relationship are as follows: (1) AMH-mediated inhibition of follicular recruitment and FSH sensitivity, which causes follicular development at the preantral stage to arrest (22); (2) the effects of AMH on suppressing aromatase activity in granulosa cells, which causes the production of less estrogen and poor endometrial development (23); and (3) possible direct actions of AMH on oocyte quality and competence (24).

Our analysis of ROC curves revealed that a cut-off value of AMH in predicting infertility among PCOS patients was optimal at >7.5 ng/mL with a sensitivity value and specificity value of 82.9 and 77.1 respectively. This is a clinically significant threshold that can be included in the regular fertility evaluation of PCOS patients. The women whose AMH level surpasses this threshold could have an early intervention, which encompass lifestyle changes, insulin-sensitizing medications, or ovulation induction treatments.

Our infertile PCOS group has a strong positive correlation with AMH and AFC ($r=0.62$, $p<0.001$), which confirm that AMH is an accurate indicator of ovarian follicular abundance. More so, the positive association of AMH with LH/FSH ratio ($r=0.57$, $p<0.001$) indicates that AMH could be associated with gonadotropin impairment that is typical of PCOS. High LH stimulates androgen production by theca cells that in turn stimulates the development of small antral follicles that produce AMH, a vicious cycle that maintains anovulation (25).

Nonetheless, we do not find the same as reported by other studies which showed no significant relationships between AMH concentrations and fertility in PCOS (11, 26). Such differences can be explained by the fact that various studies have different study designs, population characteristics, definitions of infertility, and methods of AMH assays. These variations may also be occasioned by the heterogeneity of the PCOS phenotypes among various ethnic groups (27).

4.2 Insulin Resistance and the Effect it has on PCOS-related Infertility

Our research study indicates a significant difference between HOMA-IR level in infertile PCOS patients (4.76 ± 1.84) and fertile PCOS patients (3.21 ± 1.45) and controls (1.89 ± 0.67). Metabolic dysfunction is a key determinant of infertility associated with PCOS because the prevalence of insulin resistance (HOMA-IR ≥ 2.5) was 85.7% in the infertile PCOS group.

Insulin resistance is a cause of infertility in a series of interconnected pathways (12, 28). To begin with, hyperinsulinemia has a direct effect on ovarian theca cells, which develop excess androgens (non-LH-dependent), by activating cytochrome P450c17a enzyme activity (29). Second, insulin also reduces sex hormone-binding globulin (SHBG) production in the liver leading to elevated free testosterone levels which further interfere with folliculogenesis (30). Third, insulin disrupts the typical functionality of granulosa cells via FSH in a way that inhibits aromatase activity and estradiol production required to accomplish follicular maturation (31). Fourth, the impact of hyperinsulinemia on endometrial receptivity is adverse as the expression of the genes associated with implantation (HOXA10 and glycodelin) changes (32). Lastly, chronic low-grade inflammatory and oxidative stress are implicated with insulin resistance and may cause oocyte damage and impaired fertility (33).

Interestingly, a large percentage of both PCOS groups surpassed the HOMA-IR level of 2.5 which is considered insulin resistance in general population. Nevertheless, our ROC analysis has shown that an optimal cut-off value of >3.8 is specifically greater to specifically identify infertile and

fertile PCOS patients implying that an even more severe level of metabolic malfunction is correlated with impaired fertility even among PCOS patients. It was a good diagnostic threshold (AUC=0.768) to predict infertility in PCOS patients. Clinically speaking, patients with PCOS who have a HOMA-IR more than 3.8 need to be considered at risk of infertility and may be advantageous to consider specific metabolic interventions, especially insulin-sensitizing drugs like metformin or inositol supplement (34, 35).

Our results are accredited by a number of big cohort studies. In a study on 291 PCOS patients it was discovered by Palomba et al. (36) that women with HOMA-IR over 3.5 had a length of time to conception that was significantly longer and had a reduce cumulative pregnancy rate. On the same note, a study by Cheraghi et al. (37) also established that insulin resistance was a significant predictor of anovulation and infertility in Iranian PCOS women. The significant dependence of HOMA-IR and BMI is ($r= 0.71$, $p=0.001$) in our study and it reveals the relevance of weight management in enhancing the metabolic and reproductive results of PCOS patients (38).

Surprisingly, we also found that 62.9% of all fertile that suffered PCOS also suffered insulin resistance (HOMA-IR ≥ 2.5), even though to a lesser extent compared to infertile patients. This observation indicates that although many people have insulin resistance in PCOS, the degree of resistance may dictate the results of fertility. Intermittent ovulation or sufficient endometrial activity despite metabolic imbalances are some of the compensatory mechanisms that may conserve fertility in PCOS patients with mild to moderate insulin resistance (39).

4.3 AMH and Insulin Resistance Interrelationship

It is interesting to note that AMH and HOMA-IR show a strong positive correlation in infertile PCOS patients ($r=0.68$, $p<0.001$). This correlation was also significantly lower in fertile PCOS patients ($r=0.42$, $p=0.012$) but not in controls ($r=0.18$, $p=0.298$) indicating that the interaction between these biomarkers is specifically applicable to the situation related to PCOS-linked infertility.

The mechanical connection between AMH and insulin resistance is two way and complicated. The AMH production stimulated by insulin and insulin-like growth factor-1 (IGF-1) has been reported to be performed by granulosa cells via the PI3K/Akt pathway (40). On the other hand, AMH can also lead to insulin resistance by favoring dysfunction in adipose tissues as well as inflammatory cytokines (41). Besides, hyperinsulinemia and high AMH alone inhibit FSH activity, which produces synergistic effects that have a serious negative impact on follicular development (42).

The binary logistic regression analysis that we conducted confirmed that AMH (OR=2.84, 95% CI: 1.62-4.98, $p<0.001$) and HOMA-IR (OR=2.13, 95% CI: 1.38-3.29, $p=0.001$) are independent variables in predicting infertility among PCOS patients despite the influence of other variables such as testosterone, BMI, and LH/FSH ratio. This autonomy implies that such biomarkers measure unique yet complementary phenomena about the pathophysiology of PCOS, which include AMH (that is an indicator of follicular dysfunction) and HOMA-IR (is an indicator of metabolic derangement), each of which is associated with low fertility potential. The clinical significance of HOMA-IR and AMH was reflected by the fact that every unit increment in the HOMA-IR increased odds of infertility by more than two times, and that every unit increment in AMH increased the risk of infertility by nearly three times.

Both AMH and HOMA-IR versions of the combined model were better diagnostic markers (AUC=0.856, sensitivity of 85.7, specificity of 80.0) than either of the two biomarkers individually. The clinical implications of this finding are significant because it suggests the

simultaneous measurement of AMH and insulin resistance can have a more detailed evaluation of fertility potential in PCOS patients. Those women having high levels of AMH (>7.5 ng/mL) and high levels of HOMA-IR (>3.8) exhibited significantly higher chances of infertility (OR=14.67, 95% CI: 5.21-41.33, p<0.001) and identified a high-risk group that might need intensive and multimodal treatment approaches to both ovulatory and metabolic dysfunction.

4.4 Other Hormonal and Metabolic Factors

In addition to AMH and HOMA-IR, our analysis revealed a number of other factors that are related to infertility among PCOS patients. The LH/ FSH ratio was also significantly greater in infertile PCOS patients (2.84 ± 0.92) than in fertile PCOS patients (2.07 ± 0.71), which is in line with the well-established role of gonadotropin dysregulation in the pathogenesis of PCOS (43). Nevertheless, the LH/FSH ratio ceased to be a significant independent predictor on multivariate analysis and this indicates that the effect it has on fertility might be removed by its correlations with AMH and androgens.

Adjustment of serum testosterone levels showed significant increase in infertile PCOS patients, and was an independent predictor of infertility (OR=1.89, 95% CI: 1.24-2.88, p=0.003). Hyperandrogenism is also a cause of infertility as it generates an imbalance in the hypothalamic-pituitary-ovarian axis, the functioning of the granulosa cells, and can disrupt the quality of oocytes (44). Our results indicate the interdependence between metabolic and hormonal dysfunction in PCOS (r=0.59, p<0.001): testosterone was correlated with HOMA-IR.

The BMI was also found to be an independent but insignificant predictor of infertility (OR=1.12, 95 percent CI: 1.01-1.24, p=0.033). All of the factors of PCOS such as insulin resistance, hyperandrogenism, ovulatory dysfunction are aggravated by obesity (45). Nevertheless, it is interesting to note that even when BMI was controlled, insulin resistance and high AMH were still considered important predictors, which means that these biomarkers have information that cannot be reflected by body weight.

4.5 Clinical Implications

The results of this paper have a number of practical implications on the management of PCOS-related infertility:

1. Risk Stratification: AMH and HOMA-IR measurements can be used to screen PCOS patients who are at a high risk of infertility to develop a personalized approach to counseling and treatment.

2. Treatment Goals: It is possible that patients with high AMH (>7.5 ng/mL) and HOMA-IR (>3.8) should be treated with a combination of both interventions on the ovulatory dysfunction (e.g., letrozole, clomiphene citrate) and the metabolic abnormalities (e.g., metformin, lifestyle modification) (46, 47).

3. Prognostic Tool: These biomarkers can be considered to predict the responsiveness to fertility treatments. Patients with PCOS who have a very high AMH face a higher risk of ovarian hyperstimulation syndrome during the in vitro fertilization process, and might need special stimulation regimens (48).

4. Treatment Response: AMH and HOMA-IR might be monitored serially to determine the success of various types of interventions, including weight loss, insulin-sensitizing drugs, or bariatric surgery (49).

5. Early Intervention: PCOS young individuals who had a high level of biomarkers and were yet to conceive might be advised on the potential risks of fertility in the future and advised to seek preventive measures.

4.6 Strengths and Limitations

The strong aspects of the given study are its clear groups of patients, standardized diagnostic criteria (Rotterdam criteria of PCOS), extensive coverage of both reproductive and metabolic parameters, a fertile PCOS group (the focus on which is frequently ignored in PCOS studies), and a high-quality statistical analysis with ROC curves and binary logistic regression. It was carried out in a single center and had the same protocols, which reduced the inter-laboratory variability.

But there are a number of shortcomings to recognize. First, there is no causality and longitudinal analysis of fertility outcomes as the cross-sectional design does not allow this. Future research tracking PCOS patients over time and recording the rate of conception would be more convincing. Secondly, the sample size though sufficient to conduct statistical comparisons was comparatively small to conduct subgroup analysis. The studies would be more generalized by bigger multi-centered studies. Third, we have not measured oocyte quality, embryo development, and pregnancy outcomes, and these are also significant outcomes in fertility studies. Fourth, hormone measurements were done using ELISA; more sensitive methods like mass spectrometry could give more accuracy especially in testosterone. Fifth, we failed to assess other possible relevant biomarkers e.g. inflammatory markers (C-reactive protein, interleukins), adipokines (adiponectin, leptin) or genetic polymorphisms which can alter the PCOS phenotype and fertility. Lastly, the dietary quality, the levels of physical activity, and stress, which may influence the metabolic and reproductive outcomes, were not measured systematically in this research.

4.7 Future Research Directions

Future studies are needed to overcome these limitations with prospective cohort studies, but with longer follow-up to determine time to conception, pregnancy rates, and live birth rates relative to baseline AMH and HOMA-IR. Genetic and epigenetic studies to regulate the connection between these biomarkers and fertility would contribute to our comprehension of the heterogeneity of PCOS. Research into the effects of different therapies (lifestyle changes, pharmacological therapy, assisted reproductive therapies) on AMH, HOMA-IR, and future fertility rates would be very informative in the clinical practice. Also, studies need to investigate new biomarkers, such as metabolomic and proteomic biomarkers, that can be used in addition to AMH and HOMA-IR to predict fertility outcomes. Lastly, as ethnic differences are known to exist in the presentation of PCOS, multi-ethnic research comparing the profiles of biomarkers among various populations and their relationship with fertility would be very educational.

5. Conclusion

This case-control study illustrates that serum AMH and insulin resistance measured using HOMA-IR are highly increased in PCOS patients who are not pregnant compared to patients who were not pregnant and healthy controls. The two biomarkers were found to be independent predictors of fertility status in binary logistic regression analysis and strongly correlated with one another, and the interplay between follicular dysfunction and metabolic derangement in the PCOS pathophysiology is complex.

AMH cut-off of 7.5 ng/mL and HOMA-IR cut-off of 3.8 were found to be the best diagnostic values to determine the infertile and fertile patients with PCOS and additive analysis of the two markers was found to be the best predictor. These data help to assume that regular AMH and

HOMA-IR measurements may be included in the overall fertility assessment of PCOS patients, which could allow to better stratify risks, plan the treatment more individually, and provide prognostic counseling.

PCOS patients who have both pronounced AMH and substantial insulin resistance might include a high-risk group of individuals that might require the combination of therapeutic interventions aimed at the correction of both ovulatory dysfunction (e.g., ovulation induction agents) and metabolic dysfunction (e.g., lifestyle modification, insulin-sensitizing medications). Nonetheless, due to the cross-sectional design of this study, longitudinal studies are justified in the future to confirm the use of such biomarkers as predictive factors of real fertility outcomes, such as time to conception, pregnancy rates, and live birth rates. It should also be investigated in future research whether the therapeutic interventions that were effective in lowering AMH levels and enhancing insulin sensitivity are associated with better reproductive outcomes among women with PCOS.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

Ethical Approval

This study was approved by the Institutional Ethics Committee of Samarra General Hospital (approval number: SGH-IRB-2025-012) and was conducted in accordance with the Declaration of Helsinki.