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Unravelling the Origins of Polycystic Ovary Syndrome: Analyzing Clinical Biomarker Profiles in Adolescent and Middle-Aged Women

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Abstract

Polycystic Ovary Syndrome (PCOS) is a complex and multifactorial condition, involving a combination of genetic, hormonal, and environmental factors, and its etiology is unknown. PCOS poses a diagnostic challenge in adolescents, and assessing the status of biomarkers in adolescents and middle-aged women will elucidate the aetiology of PCOS. To evaluate the clinical biomarkers - SHBG, testosterone, AMH, insulin, and leptin - in both adolescence and middle-aged groups. This cross-sectional study was conducted at Sree Balaji Medical College and Hospital and involved a total of 200 subjects. The participants were divided into two groups, each having two subgroups. Adolescent females with regular periods made up Group 1(a), whereas those with irregular cycles and ages ranging from 18 to 19 made up Group 1(b). Middle-aged women with PCOS made up Group 2(a), whereas control subjects between the ages of 30 and 38 made up Group 2(b). The data were presented in the form of mean ± SD. To assess the variance in the data among multiple groups, a one-way analysis of variance (ANOVA) was employed to identify the least significant difference during group-wise comparisons. Statistical Package for the Social Sciences (SPSS) was used for all statistical analyses, with a significance threshold of $P<0.05$. The values of SHBG and AMH were significantly different between the four groups, and the value of insulin was significant between controls and oligomenorrhea in the adolescent group. This study reveals the sequential expression of physiological and clinical biochemical variations, suggesting that compensatory hyperinsulinemia may play a role in initiating PCOS pathogenesis.

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Introduction

Polycystic ovary syndrome is the most common endocrine disorder in premenopausal women [1]. PCOS is notably prevalent among Indian women, with a combined occurrence nearing 10% based on both Rotterdam's and Androgen Excess Society (AES) criteria, whereas the utilization of National Institutes of Health (NIH) criteria yielded a slightly lower prevalence at 5.8%[2]. According to data from the World Health Organization (WHO), around 116 million women worldwide, constituting 3.4% of the global population, are impacted by PCOS [3,4].

The study underscores the imperative for standardized and universally accepted diagnostic criteria to enhance the accuracy and consistency of PCOS screening, emphasizing the necessity for a more cohesive approach in this regard. This syndrome is a heterogeneous disorder characterized by chronic ovulatory dysfunction in the reproductive age. Women with PCOS are generally overweight or obese. However, irrespective of their weight, PCOS subjects also have irregular cycles, acne, hirsutism, hormonal abnormalities such as higher Luteinizing Hormone (LH), Luteinizing Hormone/Follicle–Stimulating Hormone (FSH), testosterone levels, abnormal lipid profile, increased insulin level, decreased Sex Hormone Binding Globulin (SHBG), and elevated Anti-Mullerian Hormone (AMH).

From menarche to menopause, the reproductive lifespan is pathologically marked by PCOS and oligomenorrhea and physiologically characterized by an irregular cycle. The eight years from menarche to the maturation of the hypothalamic and hypophyseal axis determine the aetiology of PCOS in adulthood [5,6,7]. During puberty, irregular menstrual periods are thought to be physiological. However, in almost half of the instances, oligomenorrhea or secondary amenorrhea persists into early adulthood and is identified as a sign of polycystic ovarian syndrome (PCOS). The aetiology of PCOS remains unclear, despite its combination of reproductive, endocrine, metabolic, and psychological aspects.

A major characteristic of PCOS is hyperandrogenism and hyperinsulinemia, as these conditions can cause elevated levels of androgens, including testosterone, by lowering sex hormone binding globulin levels and increasing free androgens with unfavourable metabolic profiles [8]. The genesis of PCOS has been extensively studied, and it is widely acknowledged that insulin resistance and hyperinsulinemia have a significant impact on the molecular processes underlying the androgenic hypersecretion characteristic of this disorder [9].

The Anti Mullerian Hormone (AMH), which is a member of the transforming growth factor–B family, is produced by granulose cells of the ovary and is also proposed to be a marker for PCOS diagnosis. The granulosa cells of the preantral and small antral follicles in women produce the dimeric glycoprotein known as AMH, which is also implicated in the control of folliculogenesis. Since AMH is only secreted in the gonads, the amount of ovarian follicles in a woman's serum is assumed to be a reflection of her ovarian follicle pool size. Undoubtedly, the majority of PCOS patients appear to have significantly elevated blood AMH levels [10,11] When a trustworthy ultrasound is unavailable, elevated blood AMH in females with hyperandrogenism and/or oligo-anovulation may serve as a clinician's first clue that PCOS is present.

Polycystic Ovary Syndrome (PCOS) is a complex endocrine disorder that can manifest in adolescence, though diagnosing it in this age group can be challenging due to variations in puberty, clinical presentations, and

hormonal patterns. Laboratory assessments may reveal hormonal imbalance, and the present work, which assesses the status of clinical biomarkers like SHBG, testosterone, AMH, insulin, and leptin in adolescents and middle-aged groups, will help to hypothesize and reach a consensus on the aetiology of PCOS.

Our objective is to elucidate the aetiology of Polycystic Ovary Syndrome (PCOS) by examining clinical biomarkers and conducting a statistical analysis employing the Area Under the Receiver Operating Characteristic Curve (AUC ROC) method. This investigation will encompass both adolescent and middle-aged women to provide comprehensive insights into the condition.

Materials and Methods

This cross-sectional study was conducted at Sree Balaji Medical College and Hospital over 12 months from August 2020 to July 2021. According to the sample size calculation, a total of 200 young and middle-aged subjects who attended the Obstetrics and Gynecology OPD of Prasanth Hospital, Chennai, were recruited, and 173 subjects participated in the present study. The study received approval from the institutional ethical committee of Sree Balaji Medical College and Hospital, and informed consent was obtained from all the participants.

This study categorized participants into two main groups, each further subdivided into two subgroups. Group 1(a) comprised 46 adolescent females aged 18 to 19 years with regular menstrual cycles, while Group 1(b) consisted of 42 individuals within the same age range but with irregular cycles. Group 2(a) comprised 40 middle-aged women aged 30-38 years diagnosed with PCOS, and Group 2(b) included 45 normal subjects within the same age range. General profile measurements, encompassing age, height, weight, and BMI, were recorded for both groups.

Inclusion Criteria

The inclusion criteria for both adolescent and middle-aged women involved having a regular menstrual cycle and polycystic ovary syndrome.

Exclusion Criteria

This study excluded adolescent and middle-aged women who were using hormonal contraceptives, including oral and implanted contraceptives. Additionally, individuals with thyroid problems and those who had undergone any surgical procedures involving the ovaries were also excluded.

A menstrual cycle is considered normal if the cycle length has an average of 22 to 41 days with 12 cycles per year. When there are two or more cycles with a length of less than 22 or more than 41 days in a year, the menstrual cycle is classified as irregular or oligomenorrheic [12,13]. The subjects who met the Rotterdam diagnostic criteria [14] were classified as PCOS subjects: According to the Rotterdam criteria, polycystic ovarian syndrome is defined by the presence of two of three of the following criteria - Oligo-anovulation, hyperandrogenism, and polycystic ovaries (>12 follicles 2-9 mm in diameter or an ovarian volume >10 ml) in at least one ovary. The polycystic ovaries are confirmed based on transvaginal ultrasonography with a transducer of >8 Hz.

Clinical hyperandrogenism is defined as biochemical evidence of hyperandrogenism induced by testosterone and/or clinically by acne, androgenic alopecia, with a Ferriman Gallwey score of more than 7, which is considered a diagnosis of hirsutism. This is based on the presence of terminal hair at 11 different sites (lip, chin, chest, upper abdomen, lower abdomen, upper arm, forearm, thigh, lower leg, upper back, and lower back) were scored from 0 to 4, and the total score was calculated by a single examiner.

Biochemical Parameter Assay

Following the completion of the subjects' general profile, which included height, weight, age, and BMI for both groups, each participant had 3 millilitres of intravenous blood drawn. The specimen underwent centrifugation, and serum specimens were separated and stored at a temperature of -20°C. Hormone measurements included FSH, Estradiol on day three of the menstrual cycle, LH, testosterone, SHBG, leptin, insulin, AMH, and lipid profile measurements.

The AMH serum levels were assessed through enzyme-linked immunosorbent assay (ELISA), utilizing a direct AMH ELISA kit with a sensitivity of 0.1 ng/ml. The intra-assay and inter-assay variation coefficients were both below 5% and 10%, respectively. For testosterone, the serum levels were determined via ELISA employing a direct testosterone ELISA kit with a sensitivity of 0.022 ng/ml. The intra-assay and inter-assay variation coefficients for testosterone were less than 6% and 7.5%, respectively.

Similarly, the SHBG serum levels were measured using enzyme-linked immunosorbent assay (ELISA), utilizing an SHBG ELISA kit with a sensitivity of 0.1 nmol/L. The intra-assay and inter-assay variation coefficients for SHBG were below 7.2% and 11.6%, respectively. Additionally, the serum levels of leptin were determined through ELISA employing a leptin ELISA kit with a sensitivity of 0.42 ng/ml. The intra-assay and inter-assay variation coefficients for leptin were less than 5% and 6%, respectively. FSH levels were analyzed by MONOBIND INC, and estradiol levels were determined using ELISA kits. In the above study, the intra-assay and inter-assay variation were mentioned in percentage.

Statistical Analysis

The information was presented as mean ± SD. A one-way analysis of variance was conducted to determine the least significant difference when the data were compared between the groups more than once. To investigate the diagnostic performance of clinical biomarkers such as leptin, testosterone, SHBG, insulin, and AMH blood levels in distinguishing between oligomenorrheic and eumenorrheic individuals in group 1, as well as between PCOS patients and controls in group 2, Receiver Operating Characteristic (ROC) curves were developed. The Area Under Curve (AUC) indicates the chance of accurately distinguishing oligomenorrheic from eumenorrheic in group 1 and PCOS from controls in group 2. At every threshold level, a plot of sensitivity (y-axis) against specificity (x-axis) was created to show the noteworthy relation between clinical biomarkers. SPSS was used for all statistical analyses, with a significance threshold of $P < 0.05$.

Results

The mean age in the PCOS group was 28.33 ± 4.9 years, and in the control group was 29.45 ± 5.13 years. The BMI in the PCOS group was 25.7 ± 5.3, and in the control group was 25.1 ± 4.2, respectively Table 1. To study the status of biochemical in young and middle-aged women, both groups were age and BMI-matched. All the subjects of both groups 1 and 2 were found to be residing in urban areas from the middle socioeconomic class. In group 1a and group 1b, oligomenorrhea is confirmed by taking menstrual history, and subjects of group 2a and group 2b had an average duration of infertility less than or equal to 2 years with primary infertility as the most common type.

The value of the obesity marker leptin and hyperandrogenism markers testosterone is higher in oligomenorrheic (1.93± 2.13) subjects than in the eumenorrheic (1.76±2.81) group, as shown in Table 1. However, testosterone does not show a statistically significant difference between the four groups. The mean value of leptin shows significance

between the control (21.71 ± 0.89) and PCOS (18.21 ± 14.55) groups. In group 2, the value of insulin was higher in the PCOS (34.21 ± 7.6) group than in the eumenorrheic (12.85 group, but the mean value of insulin shows a statistically significant p-value between the four groups and also between control (13.82 ± 12.9) and PCOS (34.21 ± 7.6) in the middle-aged women group, as shown in Table/Fig 1. Regarding SHBG, its value is less in PCOS(139.8 ± 59.33) and oligomenorrheic (80.57±45.33) than in the control (224.12±128.13) group, and its mean value also shows a statistically significant difference between the four groups and also between control and oligomenorrheic and PCOS groups, as shown in Table 1.

Table 1. Comparison of Basal and Bio Clinical Parameters and Markers Between Different Groups

Basal parameter	**Eumenorrrheic**	**Oligomenorrheic**	**PCOS**	**Control**
N	46	42	40	45
Age (years)	19.28 ± 1.69^{c}	18.8 ± 1.14^{c}	28.33 ± 4.9^{c}	29.45 ± 5.13^{c}
Height (cms)	155.3 ± 5.88^{c}	155.8 ± 5.00^{c}	158.9 ± 6.6^{c}	155.0 ± 6.5
Weight (Kg)	52.27 ± 5.5^{c}	50.7 ± 5.9 ^{c}	64.45 ± 9.55	60.6 ± 9.51^{c}
BMI (Kg/m^2)	20.7±1.9^{C}	20.9±2.1^{C}	25.7±5.3^{C}	25.1±4.2^{c}
FSH(mIU/mL)	4.1 ±1.4	4.96 ± 1.96	5.2 ± 1.2	5.8 ± 1.5
LH (IU/mL)	3.4 ±0.25	3.96 ± 0.7	7.7 ± 6.5	3.9 ± 3.2
E2IU/mL)	35.8 ± 25.6	58.6 ±31.5	45 ± 22.6	43.7 ±20.9
Testosterone(nmol/L)	1.76 ± 2.81	1.93 ± 2.12	1.43 ± 0.92	1.19 ± 1.88
SHBG(nmol/L)	96.61 ± 85.4^{ac}	80.57 ± 45.33^{ac}	139.8 ± 59.33^{bc}	224.12±128.13^{bc}
Leptin(ng/mL)	31.13 ± 56.65	74.44 ± 178.23	18.21 ± 14.55^{b}	21.7 ± 10.89^{b}
AMH(ng/mL)	5.3 ± 5.3^{ac}	6.8 ± 5.4^{ac}	4.4 ± 3.43^{bc}	1.03 ± 1.2^{bc}
Insulin	12.85±6.7^{ac}	14.18 ± 9.1^{ac}	34.2±17.6^{bc}	13.82±12.9^{bc}

(Values are represented in Mean ± SD and represent the value is significant at p <0 .05 level)

*^{A} values represent a test of significance between groups **and 1b.**

*^{B} values represent a test of significance between groups **2a and 2b**

*^{C} analysis of variance between group **1a, 1b, 2a, 2b**

The value of AMH is also higher in PCOS (4.4 ± 3.43), and their mean value is statistically significant in group 2, and the value of AMH is more in oligomenorrheic (6.8 ± 5.4) of group 1, but their mean value is also statistically significant, as shown in Table 1. The AUC of leptin level was 0.434 in group 1 and 0.359 in group 2, and the optimal leptin cut-off level was 40.6 in group 1 and 25.69 in group 2, yielding sensitivity and specificity of 44% and 33% in group 1 and sensitivity and specificity of 28% and 21% in group 2, respectively (Table 2, Figures 1 and 2).

Table 2. Diagnostic Power of Biochemical Markers for Oligomenorrheic and PCOS Subjects.

Groups	**AUC ROC**	**Threshold value**	**Sensitivity**	**Specificity**
AMH				
Oligomenorrheic	0.457 (0.256-0.612)	5.07	42%	29%
PCOS	0.923(0.852-0.993)	3.07	86%	68%
SHBG				
Oligomenorrheic	0.485(0.312-0.659)	79.5	45%	31%
PCOS	0.310(0.167-0.452)	328.15	26%	18%
TESTOSTERONE				
Oligomenorrheic	0.475(0.269-0.680)	1.26	42%	29%
PCOS	0.683(0.539-0.828).	1.6	76%	62%
INSULIN				
Oligomenorrheic	0.505(0.329-0.682)	13.6	59%	51%
PCOS	0.563(0.400-0.726)	17.23	68%	58%
LEPTIN				
Oligomenorrhiec	0.434(0.256-0.612)	40.6	44%	33%
PCOS	0.359(0.296-0.556)	25.69	28%	21%

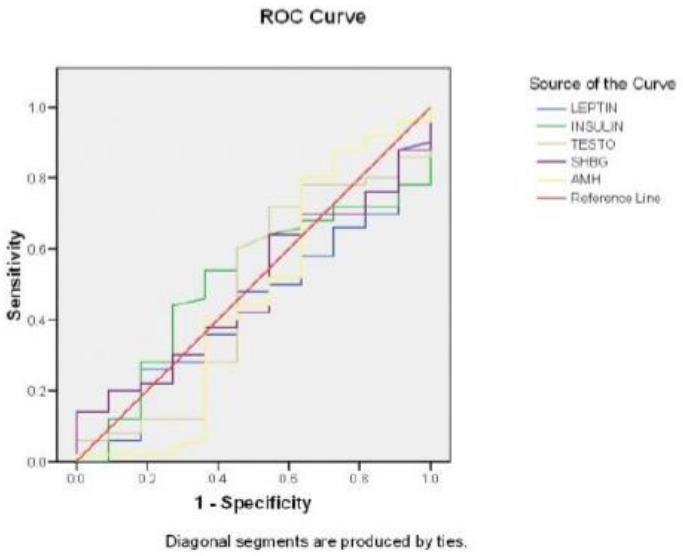

Figure 1. Showing AUC Roc of Group 1A and 1B

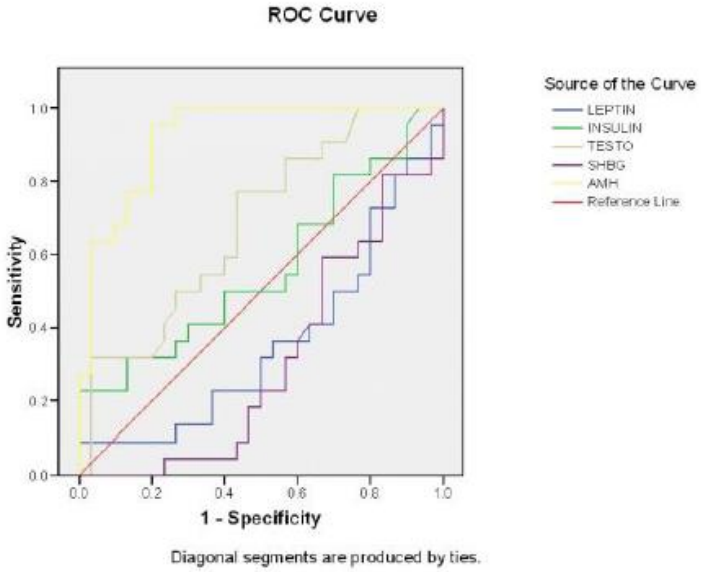

Figure 2. Showing AUC Roc of Group 2A and 2B

The AUC of SHBG level was 0.485 in group 1 and 0.350 in group 2, as shown in Fig.2, and the optimal SHBG cut-off level was 79.5 in group 1 and 328.15 in group 2, yielding a sensitivity of 45% and specificity of 35% in group 1 and sensitivity of 26% and specificity of 18%, respectively.

Compared to the oligomenorrheic group and the control group, the AMH levels in the PCOS groups were considerably greater. Fig.2 summarizes the oligomenorrheic group from the control and the diagnostic power of PCOS. In group 2, the diagnosis of PCOS from the control had an area under the ROC curve of 0.923, a cut-off value of 3.07 ng/ml, 86% sensitivity, and 68% specificity. When distinguishing between oligomenorrheic and eumenorrheic, the area under the ROC curve was 0.457, with a cutoff value of 5.07 ng/ml, 42% sensitivity, and 29% specificity, as shown in Fig.1 and Fig.2.

Discussion

Intra-physiological overload, obesity, hyperinsulinemia, and hyperandrogenism, along with their reflections through clinical biomarkers in a cascade manner, elucidate the pathogenesis of PCOS [15]. The study reveals that the value of AMH is higher in PCOS patients as well as in oligomenorrheic subjects. The AUC ROC of AMH indicates that it is a good marker in middle-aged women for distinguishing PCOS from controls, and in young adolescent girls, it is more effective in distinguishing oligomenorrhea from controls.

The pathogenesis of ovarian dysfunction is connected to elevated levels of LH, hyperandrogenemia, hyperinsulinemia, and obesity [16]. The excess androgen exposure throughout pregnancy, childhood, and adolescence may promote a body fat distribution that is mostly visceral and abdominal, potentially leading to insulin resistance and hyperinsulinemia later in life. The vicious cycle of insulin resistance-hyperinsulinemia in PCOS patients can be broken by the acceleration of insulin resistance through androgen excess, leading to the formation of abdominal obesity. Significant evidence of hirsutism in the evaluation of teenage PCOS is crucial because adolescents with PCOS are more insulin resistant, have hyperinsulinemia, and exhibit lower SHBG levels. This results in a greater fraction of physiologically active androgen [17].

The development of hyperinsulinemia as a result of insulin resistance appears to be a key component of the pathophysiologic process connecting PCOS and its associated metabolic disorders. In addition to its role in the development of metabolic problems, compensatory hyperinsulinemia also contributes to the elevated levels of testosterone found in women with PCOS [18]. The primary issue in PCOS individuals is androgen excess, although additional variables also play a role in its initiation, including obesity and insulin resistance. The main cause of PCOS is hypothalamic-pituitary dysfunction, likely exacerbated by androgen synthesis and secretion.

For the clinical marker insulin, eumenorrheic and oligomenorrheic females exhibited statistically significant discriminating power. Similarly, middle-aged women with PCOS and eumenorrhea demonstrated a statistically significant discriminative power for therapeutic biomarkers like insulin. The aetiology of PCOS is generally accepted to be hyperinsulinemia initially, followed by hyperandrogenism [19]. Clinical evidence suggests that elevated insulin levels are more likely to cause hyperandrogenism than the reverse. This is substantiated by the observed reduction in serum androgen levels when circulating insulin concentrations are decreased through the administration of insulin-sensitizing drugs or substances inhibiting insulin secretion, such as diazoxide or somatostatin analogues. However, it has not been established that reducing serum androgen levels, even though extreme measures like bilateral oophorectomy or the use of GnRH agonists, significantly impacts blood insulin levels in the short term [20].

PCOS is characterized by a high incidence of obesity, which makes assessing leptin and ovarian function important. According to Student T's test, there was a significant difference in leptin concentration between teenage females who menstruated frequently and those who did not. Additionally, leptin concentration was greater in PCOS and oligomenorrheic patients than in the control group. On the other hand, there is disagreement regarding leptin levels in the PCOS and control groups. The results of studies by Rouru et al and Laughlin et al show no difference between PCOS and controls [21,22].

The formation of antral follicles may be stimulated by hyperinsulinemia, leading to

increased granulose cell sensitivity to follicle-stimulating hormone. This process could result in a higher number and size of follicular cysts and an increase in ovarian volume. Consequently, granulose cells synthesize more AMH, leading to elevated AMH release in polycystic ovaries [23]. Granulose cells from polycystic ovaries typically exhibit higher average levels of AMH compared to cells from normal ovaries.

Disturbance in folliculogenesis might also be the cause of elevated AMH levels in PCOS, leading to an excess build-up of preantral and tiny antral follicles [24]. PCOS is commonly characterized by an excessive number of developing follicles (2–3 times that of normal ovaries) progressing to tiny antral follicles sized between 2–5 mm. Our findings also show that adolescents with oligomenorrhea had higher blood AMH levels compared to controls with normal cycles. Additionally, it has been discovered that AMH levels are higher in prepubertal and peripubertal girls with PCOS who are teenage PCOS females with regular menstrual cycles. Our research suggests that abnormal follicle formation may exist before clinical symptoms of ovarian dysfunction appear in infancy and early adulthood. As AMH is produced by granulose cells from preantral and small antral follicles [25], and follicles in the 2mm to 5 mm range, it is related to androgen serum levels. Excess androgen levels for many years disturb folliculogenesis, contributing to the mechanisms of oligoovulation and anovulation. Oligomenorrhea or amenorrhea is observed in PCOS patients with hyperandrogenism but is also seen in patients without hyperandrogenism [26].

Folliculogenesis may be disrupted in PCOS individuals with hyperandrogenism due to an excess of AMH production. Even though AMH is now recommended for PCOS diagnosis, there are significant differences in diagnostic capacity in actual practice. However, the current study's findings cast doubt on AMH's ability to accurately diagnose PCOS in young women by showing that its diagnostic sensitivity and specificity were not high enough in this population. However, as demonstrated by ROC and AUC in middle-aged women, AMH exhibited a strong diagnostic capacity to distinguish PCOS in these individuals.

Limitation(s)

This study employed a cross-sectional design, allowing it to capture only a snapshot of the biomarker profiles at a particular point in time. This limitation makes it challenging to establish causal relationships between clinical biomarkers and the development of PCOS, as it cannot account for temporal changes or the dynamic nature of the condition.

Conclusion(s)

This study establishes that the variation in physiology, clinical biochemistry, and the etiopathogenesis of PCOS (polycystic ovary syndrome) changes as it progresses from infancy to adulthood, depending on genetic and lifestyle factors. Various clinical biomarkers can help identify the progression of PCOS in a cascade manner, as proven by various statistical approaches. The research emphasizes that compensatory hyperinsulinemia may initiate the pathogenesis of PCOS during the growth of the hypothalamo-hypophyseal axis.

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Conflicts of Interest

The authors declare no conflicts of interest.

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