Association betweenReproductive Hormone Levels and ATG5 gene Expression with Polycystic Ovary Syndrome (PCOS) in Pre- and Postmenopausal Iraqi Women

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ABSTRACT
The most common endocrine condition in women of reproductive age is polycystic ovary syndrome (PCOS). Syndrome of Polycystic Ovaries in premenopausal women is characterized by polycystic ovaries, dysmenorrhea, and hyperandrogenism.Women often experience infertility due to the hormonal imbalance linked to polycystic ovarian syndrome, which has detrimental effects on their physical, mental, and social well-being.This study aims to survey the biochemical markers of some hormones in polycystic ovary syndrome, such as follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin, estradiol, and testosterone in the blood of residents of Dhi Qar city with polycystic ovary syndrome (polycystic ovary syndrome). Bioinformatics software predicted the ATG5-binding microRNA, and the dual-luciferase reporter assay was used to determine their targeting relationship. Furthermore, quantitative real-time polymerase chain reaction (qRT-PCR) was used to evaluate the expression of ATG5 in the ovary. For this purpose, the blood serum consisting of 106 samples will be divided into (55, 25, and 26) pre-menopausal patients, post-menopausal patients, and a healthy control group, respectively.According to the results, the average serum LH levels were higher in both groups of patients compared with healthy controls, and the difference was significant ($P = 0.001$). In postmenopausal patients, the mean serum FSH levels were significantly greater ($P = 0.001$) than in the other groups. While both patient groups' average serum prolactin levels were lower than those of the healthy controls, the difference was not statistically significant ($P = 0.396$). The average serum estradiol levels were significantly lower ($p<0.01$) in postmenopausal patients compared to the other groups. The average ATG5 gene expression was significantly increased ($p<0.01$) in both groups of patients compared with healthy controls.

1. Introduction

A complex condition affecting fertile women, polycystic ovarian syndrome, or PCOS, is inherited. Women who have oligomenorrhea, amenorrhea, acne, hirsutism, insulin resistance, obesity, hyperandrogenism, or infertility may be diagnosed with this disorder (1). PCOS is an extremely prevalent endocrine disorder among premenopausal women.Genetic susceptibility, neuroendocrine problems, hyperinsulinemia, hormone imbalance, chronic inflammation, and autoimmune illnesses are among the most complicated and poorly understood environmental factors that cause it (2).

Insulin resistance (IR), obesity, anovulation, hyperandrogenism, as well as PCOS, are the hallmarks of the most common causes of chronic anovulatory infertility, which affects 6-8% of women of reproductive age(3).Infertility in women is the hormonal imbalance linked to polycystic ovarian syndrome, which has detrimental effects on social, psychological, and physical well-being (4). A healthy menstrual cycle and ovarian function are essential for maintaining fertility. These processes are mediated by hormones acting normally.An ovarian cyst may develop when there is a persistent

disruption in females' hormone levels, which affects the function of the ovaries.. However, in females with polycystic ovary syndrome, the male hormone androgen rises at a pace higher than usual (5). Obesity and type 2 diabetes are caused by insulin resistance. Irregular menstruation is a sign ofinfertility in PCOS (5). Numerous hormonal and metabolic changes take place during the menopause. It is well known that while estrogen levels drop sharply as women approach menopause, androgen levels stay steady or even rise during this period. Many characteristics, including insulin resistance, chronic inflammation, abdominal obesity, and dyslipidemia, tend to deteriorate as women become more androgenic (6, 7). Primary ovarian insufficiency (POI) is caused by problems in cell apoptosis and follicular atresia, which can result from various genes involved in the generation and maturation of germ cells. One of the principal pathways for the ovary's removal of germ cells is follicular atresia (8). Anovulatory dysfunction is significantly influenced by autophagy. Nonetheless, it is unclear how hyperandrogenism and autophagy in human granulosa cells relate to PCOS pathophysiology (9). Granulosa cells in PCOS patients have significantly higher levels of the autophagy-related genes ATG5, ATG7, and BECN1 mRNA (10). The ability of healthy women and women with PCOS to secrete androgens decreases with age, but in PCOS patients, this capacity is augmented until the later stages of reproductive life. It is also possible that women with PCOS are exposed to hyperandrogenism for an extended amount of time because adrenal androgen production is visible in these women even after menopause (7). The aim of this study was to determine the effect of hormone levels and their relationship to gene expression in patients with polycystic ovary syndrome.

2. Materials and methods

2.1. Experimental and participant design

The total number of patients and healthy individuals was split up into three groups: the premenopausal women's group (55 patients), the postmenopausal women's group (25 patients), and the healthy group (26 samples). This study was carried out from October 2022 to September 2023 at the Nasiriyah Teaching Hospital, namely in the infertility unit. Mean ages of premenopausal, postmenopausal women, and control group were specified as 26.18±7.55, 48.40±2.78, and 31.11±6.23 years, and there was a significant difference in mean age between the groups ($P = 0.001$). Age, BMI, marital status, and other sociodemographic factors are provided for pre-menopausal patients, post-menopausal patients, and control groups.

2.2 Hormones measurement method

The CL-series of (LH, FSH, PRL, TESTO, and E2) assay is a two-site sandwich assay to determine the level of its. In the first step, sample, paramagnetic microparticle coated with monoclonal anti hormone antibody and monoclonal anti-antibody alkaline phosphatase conjugate are added into a reaction vessel. After incubation, (LH, FSH, PRL, TESTO, and E2) present in the sample binds to both anti hormone antibody coated microparticle and anti-hormone antibody alkaline phosphatase-labeled conjugate to form a sandwich complex. Microparticle is magnetically captured while other unbound substances are removed by washing. In the second step, the substrate solution is added to the reaction vessel. It is catalyzed by anti-hormone antibody alkaline phosphatase conjugate in the immunocomplex retained on the microparticle.

The resulting chemiluminescent reaction is measured as relative light units (RLUS) by a photomultiplier built into the system. The amount of (LH, FSH, PRL, TESTO and E2) present in the sample is proportional to the light units (RLUS) generated during the reaction. The concentration of hormones can be determined via a calibration curve.

2.3. Geneexpression study

Total RNA was isolated from cells using TRIZOL reagent.By measuring the optical density (OD) of 260/280 nm (the absorption wavelength of nucleic acid and protein), which is determined by spectrophotometry, the concentration and purity of the extracted RNA are determined. The concentration is expressed in mg/μL. Acceptable RNA purity is 1.7-1.9(11).

The concentration of RNA samples is normalized according to the lowest sample concentration using the following equation:

$$V_n = \frac{C_0 \times V_0}{C_n} \qquad \dots (4.2)$$

Where Cn is the current sample concentration, Vn is the current sample volume that needs to be diluted with tdH2O to produce precisely 100 μL, and C0 is the minimum concentration. cDNA synthesis and amplification were performed using EasyScript one-step gDNA removal and the DNA synthesis supermix.

2.4. qRT-PCR data analysis

The target and housekeeping gene q RT-PCR data findings were processed using the Livak technique (12) to determine the relative quantitative gene expression levels (fold change). Comparative quantification technique (CQT) was used to normalize the quantities from a q RT-PCR experiment so that the results have biological significance. The following equations were used to apply the ΔCT method to the reference gene. One of the experimental samples acts as the calibrator (control samples) in this method, and each of the measured target values (CT values) is divided by the measured target value of the calibrator to generate relative expression levels:

ΔCT(calibrator) = CT(target, calibrator) – CT(reference, calibrator)

Ratio(target/reference) = 2CT(reference) – CT(target)

The CT values measured during the thermal reaction are recorded to calculate the following measurements (Table 1) (13).

Table 1. Sequence-specific primers were used in qRT-PCR analysis.

Name	Sequence (5′–3′)	TM	PCR product
Fw- Atg5	5- TGGATGGGATTGCAAAATGACAG-3	65 °C	77 bp
Rv- Atg5	5- TCTTCTGCAGGATATTCCATGAGT-3		
Fw-B-actin	5- GGACTTCGAGCAGGAGATGG-3	65 °C	93 bp
Rv-B-actin	5- TTGCCGATGGTGATGACCTG-3		

3. Statistical analysis

By using Excel and SPSS version 26, data were collected, compiled, evaluated, and presented as Mean ± SD following the Kolmogorov-Smirnov normality test and classification as normal or abnormal. Normally distributed variables. The independent sample t-test was used to study the difference in means between any two groups provided that the variable was normally distributed. The chi-square test was utilized to investigate the relationship between any two categorical variables, and one of the ANOVA testing methods was employed to investigate the mean differences between more than two groups, given that the variable is normally distributed**.**The level of significance was considered with a threshold of $p<0.05$ and a high level of significance at $p<0.001$.

4. Results

4.1. Hormone markers (LH, FSH, prolactin, testosterone, and estradiol) in patients and healthy controls.

Comparison of hormone indices (LH, FSH, prolactin, TESTO, and E2) was performed in patients and healthy control groups, and the results are shown in Table 2. The mean serum luteinizing hormone levels were 8.42±0.85, 16.18±1.71 and 4.07±0.30, in premenopausal patients, postmenopausal patients, and healthy controls, respectively; The average levels were higher in both groups of patients compared to healthy controls, and the difference was significant ($P = 0.001$). The mean levels were also a significant difference between premenopausal patients and postmenopausal patients ($P > 0.05$) . Mean levels of follicle-stimulating hormone in serum were 7.95 ± 0.78, 42.46 ± 5.05, and 7.22 ± 0.34, in premenopausal patients. Menopause and postmenopausal patients. and a healthy control group, respectively; The mean levels were higher in postmenopausal patients compared to the other groups, and the difference was significant ($P = 0.001$). However, the mean levels were not significantly different between premenopausal patients and the control group ($P< 0.05$) . The average serum prolactin levels were 16.83±1.40, 18.84±4.90, and 23.39±5.76, in premenopausal, postmenopausal patients, and the healthy control group, respectively. The mean level was lower in both patient groups compared with healthy controls ; however, the difference was not found to be significant ($P = 0.396$).The average levels of testosterone in the blood were significantly higher ($P<0.01$) in pre-menopausal patients compared to the other groups, while a non-significant difference ($p>0.05$) was found between post-menopausal patients and the control group. The serum estradiol level was significantly lower ($P<0.01$) in

postmenopausal patients compared to the other groups, while a non-significant difference (P>0.05) was found between premenopausal patients and the control group .

Table 2: Hormone contents (LH, FSH, prolactin, TESTO, and E2) in patients and healthy controls.

Hormones	*Premenopausal* n=55	*Postmenopausal* n=25	*Control n=26*	*P-value*
Luteinizing Hormone (LH)IU/L				
Mean ± SE	**8.42 ± 0.85**	**16.18 ± 1.71**	**4.07±0.30**	**< 0.001**
Range	1.80- 30.70	2.59 – 33.56	1.16 – 8.56	
Follicle-stimulating hormone (FSH) IU/L				
Mean ± SE	**7.95 ± 0.78**	**42.46 ± 5.05**	**7.22±0.34**	**< 0.001**
Range	3.07- 43.90	4.91 – 98.76	3.90 – 10.63	
Prolactinng/mL				
Mean ± SE	**16.83±1.40**	**18.84 ± 4.90**	**23.39±5.76**	**0.396**
Range	5.90- 55.0	5.48 – 128.80	5.43 – 120.50	
Testosteroneng/mL				
Mean ± SE	**1.26 ± 0.132**	**0.73 ± 0.12**	**0.67 ± 0.12**	**0.003**
Range	0.29- 4.26	0.08 – 1.83	0.08 – 2.20	
Estradiolpg/mL				
Mean ± SE	**45.89 ± 2.56**	**21.13 ± 5.29**	**45.60 ± 3.62**	**< 0.001**
Range	17.20- 89.40	0.90 – 154.84	24.19 – 107.90	
Different letters denote the significant differences at p< 0.05				

n: number of cases; SD: standard deviation.

4.2. Gene expression analysis

4.2.1. ATG5 gene expression in patients and healthy controls.

The comparison between the results of ATG5 gene expression between patients and healthy individuals is shown in Table 3Mean values of*ATG5 gene expression* were 68.78±11.79, 3.95±1.27, and 1.0, in premenopausal patients, postmenopausal patients, and healthy controls, respectively. Therewas asignificant increase (P<0.01) in the expression of *ATG5* A in both groups of patients compared to healthy controls. A significant difference (P<0.05) was also found between the patient groups.

Table 3: ATG5 gene expression in patients and healthy controls.

Gene expression	*Premenopausal* n=55	*Postmenopausal* n=25	*Control n=26*	*P-value*
ATG5 gene expression				
Mean ± SE	**68.78 ± 11.79^{A}**	**3.95 ± 1.27^{B}**	**1.0 ^{C}**	**> 0.001**
Range	0.14- 249.0	0.03 – 16.22		
Different letters denote the significant differences at p< 0.05				

n: number of cases; SD: standard deviation.

4.2.2. Evaluationof *ATG5 gene* expression in premenopausal patients.

To assess the cut-off value for the ATG5 gene The receiver operator characteristic (ROC) curve analysis was carried out to predict premenopausal PCOS as diagnostic tests or auxiliary diagnostic tests. The results are displayed in Figures 4 and 7. The threshold value was greater than 1.05 times, and the AUC, sensitivity, specificity, PPV, and NPV values were obtained to be 92.7%, 100.0%, 100.0%, 86.7%, and 0.920 (0.814-1.000). The present data suggest the ATG5 gene is a good diagnostic marker for PCOS.

Table 4: Sensitivity and specificity of *ATG5 gene* (> 1.05-fold) in premenopausal PCOS

ATG5 gene	Premenopausal patients	Healthy control
> 1.05	51	0
<1.05	4	26
Sensitivity %	92.7 %	
Specificity %	100.0%	
PPV %	100.0 %	
NPV %	86.7%	
AUC (95% CI)	0.920 (0.814- 1.000)	

CI: Confidence interval, AUC: Area under curve.

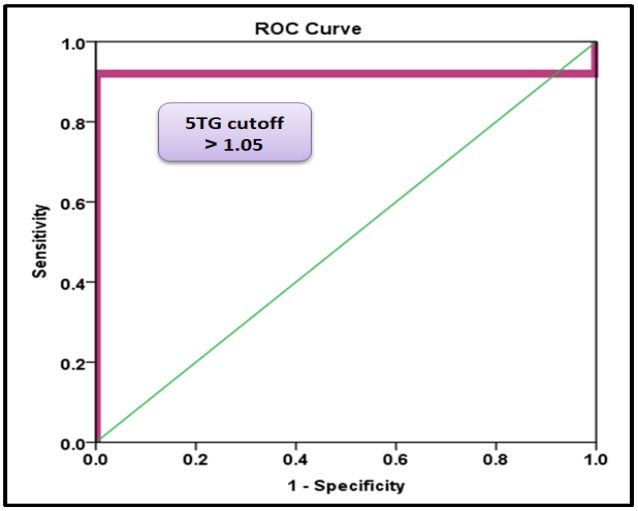

Figure 7: Receiver operator characteristic curve analysis of *ATG5 gene* for the calculation of possible diagnostic cutoff value.

4.2.3. Evaluation of *ATG5 gene* expression in postmenopausal patients

To evaluate the *ATG5 gene* cut-off for predicting postmenopausal PCOS, as the main or auxiliary diagnostic test, receiver operator characteristic (ROC) curve analysis was performed and the results are shown in Figure 8 (5). The cutoff value of the *ATG5 gene*was >0.95-fold with sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and area under the curve 76.0%, 76.9%, 76.0%, 76.9%, 0.762 (0.526-0.998), respectively. The current results indicate *ATG5 gene* is considered an acceptable diagnostic sign.

Table 5: Sensitivity and specificity of *ATG5 gene* (> 2.24-fold) in postmenopausal PCOS

5TG gene	**Postmenopausal patients**	**Healthy control**
> 0.95	19	6
<0.95	6	20
Sensitivity %	76.0 %	
Specificity %	76.9%	
PPV %	76.9 %	
NPV %	76.0%	
AUC (95% CI)	0.762 (0.526- 0.998)	

CI: Confidence interval, AUC: Area under curve.

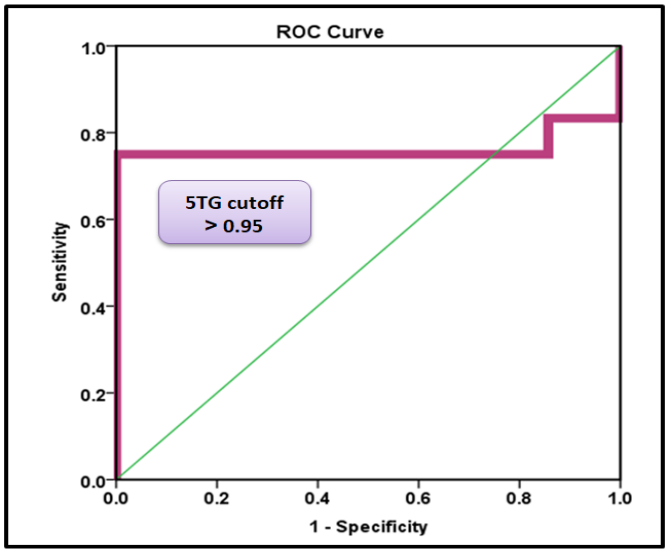

Figure 8: Receiver operator characteristic curve analysis of *ATG5 gene* for the calculation of possible diagnostic cutoff value.

4.2.4. The relationship between *ATG5 gene* expression and hormones

Correlations between *ATG5 gene expression*and other clinical variables in PCOS patients, such as hormones, are presented in Table 6. The results show a significant positive relationship between *ATG5 expression* and estradiol (r = 0.454, P= 0.019), in patients withpremenopausal and LH (r = 0.401, P= 0.039) in patients with postmenopausal. Other results show a non-significant relationship between other hormones in both groups.

Characteristic	*ATG5 gene expression*			
	Premenopausal patients		Postmenopausal patients	
	r	*P*	*r*	*P*
LH	0.155	0.459	-0.401	0.039*
FSH	0.260	0.210	0.239	0.455
Prolactin	-0.248	0.194	0.140	0.664
Testosterone	0.153	0.464	0.111	0.732
Estradiol	0.454	0.019*	-0.179	0.577

r: correlation coefficient.

5. Discussion

The reason for the highly significant increase in LH in the PCOS group is still unknown, but it appears that androgens block the negative feedback effects of progesterone and estrogen on pulsatile LH release. FSH increases LH receptors'

expression, which in turn causes an increase in estrogen receptors. More granulosa cell proliferation results from an increase in LH receptors and enhanced estrogen and FSH receptors, which in turn led toan increase in estrogen secretion(14). There will be a shift in estrogen's feedback from negative to positive if it reaches a particular threshold. Theca and follicular cells in the follicles are responsible for producing estrogen. When FSH is low and large amounts of LH are released, ovarian follicles will not develop and there will be no production of estrogen(15). In women with PCOS, the frequency of the LH pulse is increased by about 40% when compared to healthy subjects, resulting in higher levels of LH and a relative deficiency of FSH (16).Low FSH levels result in anovulation, but high LH levels seems to stimulate the synthesis of androgen in follicular theca cells(17). The S.LH mean values at which the number of patients from the premenopausal and postmenopausal groups increased significantly in contrast to the group under control. This study is in line with previous research since the average S.LH values in the patients were also highly significant, and there was a significant difference (P = 0.001) when compared to the control group.FSH tests can be misleading because hormones rise and fall irregularly during perimenopause (18). A persistently high level of FSH can indicate menopause. The mean FSH level was significantly lower in premenopausal patients than in postmenopausal women, and there was no significant difference between premenopausal patients and the controls (P>0.05) (19).From premenopausal to old age, women with PCOS exhibited persistently reduced FSH levels in comparison with controls. This result is in line with earlier research done on perimenopausal women with PCOS from a different cohort that received a later diagnosis in 1992 (20).When a postmenopausal woman exhibits symptoms of hyperandrogenism, particularly virilization symptoms (such as clitoral enlargement, deepening of the voice, and breast atrophy), she should be evaluated by an endocrine examination, preferably using serum levels of FSH, LH, testosterone, SHBG, and estradiol (21).Because of follicle depletion, ovarian theca cells produce less testosterone after menopause; however, this loss is offset by an increase in LH stimulation of testosterone production by stroma cells. Therefore, there is little to no change in ovarian androgen production with menopause (21). After menopause, there is an unknown mechanism causing decreasing residual FSH levels. Because the rate at which gonadotropin-releasing hormone (GnRH) pulses in the hypothalamus varies in fertile women with PCOS compared to healthy controls (22). Between the premenopausal and postmenopausal patient groups, mean FSH levels increased dramatically around menopause, rising from 7.95 ± 0.78 (unit???) to 42.46 ± 5.05 (unit???), respectively. For women without PCOS, therefore, values at or above 40 IU/L were suggestive of menopause (22). Serum prolactin (PRL) concentrations in PCOS-affected women showed mixed results. Some studies have found that women with PCOS have higher PRL levels (23), while other studies have found no discernible variations in PRL levels between PCOS and healthy populations (23).It is important to note that most of the studies that are currently available had limited sample sizes and only included women with PCOS who had normal prolactin levels, hospitalized women, infertile women, or women who were referred to the clinic(24).Studies involving patients diagnosed using the Androgen Excess Society (AES) or NIH criteria did not find any significant differences in PRL levels between the two groups(25). Nevertheless, a few individual investigations have discovered statistically lower PRL levels in PCOS-affected women compared to their contemporaries without PCOS, even within this

meta-analysis (25, 26). Sex hormones including testosterone and estradiol have been linked to depression in studies conducted on clinical populations. Women diagnosed with severe depressive illness have consistently low levels of estradiol (27). Furthermore, with steady estradiol levels being restored, clinical depression recovery occurs during the postpartum, perimenopausal, and postmenopausal phases. Comparing premenopausal PCOS women with the control group, the basal serum levels of E2 were higher in the former group, and as soon as PCOS women went through menopause, the levels dramatically dropped (6). Statistically substantial elevations in circulating testosterone were the most prevalent observation in postmenopausal women with PCOS when compared to women of the same age without PCOS (28).The recruitment of follicles and the process of ovulation depend on the ovary's synthesis of androgen and its conversion to estradiol insufficient synthesis of testosterone in the follicular phase(FP). Anovulation can result from elevated androgen levels(29). Females with anovulatory menstrual periods had considerably greater testosterone levels during FP(30). Serum testosterone starts to decline with age at age 25, while serum estradiol levels drop by 90% after menopause (30). It is believed that premenopausal estrogen levels cause pituitary insensitivity to the negative feedback from estrogen, which is why LH is raised above basal levels (31). A regular length cycle results from anovulatory cycles that happen during the late transitional period of menopause. Indicating that there may be a fertile time up till the final menstrual period (FMP), it may appear hormonally normal. With FSH rising and estrogen declining until it stabilizes after about two years of FMP, the ovarian reserve is very low (i.e., undetectable) at this point (32).BECN1 and ATG5, two genes associated with autophagy, have considerably greater expression in ovarian granulosa cells from PCOS patients (3). Autophagy has been linked to a range of metabolic and endocrine abnormalities in PCOS. PCOS patient ovarian granulosa cells (GCs) had considerably higher levels of the autophagy-specific genes becn1 and ATG5. There is a favorable correlation between the basal serum total testosterone level and the mRNA abundance of becn1(3). Oocyte maturation is directly inhibited by a decrease in the levels of autophagy markers (ATG7 and BECN1) (39). Autophagy regulates the production of steroid hormones, and atg5 in steroid-producing cells is linked to a lower rate of steroid hormone production. This reduction in steroid hormone production was also discovered to be caused by lower cholesterol as a result of lower autophagy (33). A substantial decline in fertility, higher blood FSH levels, decreased ultrasonography number of antral follicles, decreased antimüllerian hormone (AMH) levels, and decreased suppressed secretion in the early follicular phase are all signs of ontogenesis failure (34). FSH levels start to rise several years before menopause, but spike in the final two years and stabilize to steady-state values two years following menopause (35).Additionally, there is a gradual decrease in androgen status (i.e., testosterone and androstenedione), which is not noticeable enough after menopause (34). Compared to younger women, older ovulatory women had higher levels of FSH throughout the entire menstrual cycle. A woman entering perimenopause also experiences a decrease in the ovaries' functional ability. Women over the age 40, produce less total inhibit by granulosa cells in response to stimulation, even though estradiol (E2) levels do not differ considerably between younger and older women(34). As discussed earlier, genetic abnormalities cause structural alterations in FSH receptors.Age-related declines in testosterone levels are caused by the ovaries' incapacity to offset the decline in the generation of testosterone prohormones by the

adrenal glands. Because the adrenal gland participates in the regulation of testosterone levels following menopause(36).FSH steadily increases throughout menopause, despite the continuation of menstruation. The main causes of this are lower levels of inhibin B and estradiol (37). Indeed, inhibit B similarly suppresses pituitary FSH secretion, while estradiol decreases gonadotropin responsiveness to GnRH at the pituitary level and reduces GnRH output via negative feedback.Several endocrine and metabolic problems in PCOS patients are linked to autophagy. First and foremost, increased androgen levels are linked to autophagy. In PCOS patients' ovarian GCs, there was a substantial increase in the mRNA abundance of the autophagy-specific genes becn1, ATG5, and ATG7 (18). ATG5ν, ATG7Ο, BECLIN1, and the ratio of autophagy marker protein 3B II/I (LC3 II/I) light chain can be strongly stimulated by excess androgens in PCOS patients (3, 38). Estradiol, progesterone, and testosterone were considerably lowered when autophagy was genetically disrupted by silencing autophagy genes (Beclin1 and ATG5) using siRNA and shRNA methods (34). In order to explore the possibility that 17β-estradiol controls autophagy during spheroid formation through the traditional oncogenic pathwaythe impact of these activators on the expression of genes linked to autophagosome formation and autophagy induction (39). There is probably an estrogen response element (ERE) in the bovine ATG5 gene's promoter regions.Additionally, the transcription factors AP1, Sp1, and CREB have many binding sites on beclin-1. These proteins are known to interact with steroid receptors, allowing sex hormones to indirectly activate gene expression. Expression of four genes, ATG5, BECN1, and ATG5, which are essential for autophagy induction and autophagosome formation, and are probably controlled by E2(39).

Autophagy has been shown to impact granule cell survival as well. Oocyte formation and granulosa cell survival have been observed to be affected by modifications in normal autophagy in PCOS ovaries(40, 41). Increased amounts of inflammatory cytokines like IGF-1, appear to have an impact on autophagy in PCOS. Similar to numerous other molecules found in the ovary, IGF1 is necessary for a proper balance; an excess or deficiency can cause issues. Insulin resistance, inflammation, and high androgens are among the causes that raise IGF-1 (3). The endometrium's autophagic condition is regulated by a number of other hormones. Following menopause, follicle-stimulating hormone (FSH) usually rises as ovarian function declines, while estrogen and progesterone levels decrease .According to a publication, postmenopausal women with high levels of FSH phosphorylate Smad2/Smad3 via transforming growth factor beta receptor II (TGFBR-II) (42).Reduced autophagic activity and ovarian aging have been linked, as evidenced by the markedly lower expression levels of the Atg5 gene, Beclin1, in the ovaries of aging patients. Beclin1 is strongly tied to the start and maintenance of autophagy (43).Apoptosis can occur either directly or indirectly as a result of persistently activated cell autophagy. Accordingly, FSH may encourage endometrial glandular cells to undergo autophagy (44). Furthermore, higher androgen availability in PCOS may be linked to autophagy. Autophagy-related genes were revealed to be adversely correlated with the free androgen index and considerably reduced in ovulatory PCOS compared to healthy endometrium (45). Because increased androgen availability in PCOS is linked to downregulation of endometrial autophagy and overexpression of FSH, the effect of androgens on autophagy is adversely correlated.Since transforming growth factor beta (TGFβ) activates the phosphorylation of Smad2/Smad3 in part (42).Follicle-stimulating hormone, or FSH, is likewise strictly prohibited from causing follicular atresia. FSH is good for follicle

development. The follicle secretes estrogen once it has grown, and when this production is large, it decreases FSH outputs. Thus, follicular growth and atresia are regulated by hormones in a threshold-dependent manner (46).

6. Conclusion

Infertility, diabetes, insulin resistance, hirsutism, and other serious health issues are caused by the complex disease known as polycystic ovarian syndrome (PCOS), which is very common in women and has multiple causes. Treatment for PCOS is essentially symptomatic. It is important to consider not only the intricate clinical picture involving various tissue modifications but also the psychological effects on female patients. Because PCOS is linked to several endocrine and metabolic issues in PCOS patients, PCOS, and its symptoms/consequences may contribute to the development of premenopausal autophagy. The endometrium's autophagic condition is regulated by a number of other hormones. Overall, mRNAs and hormones have a mutual regulatory interaction; however, more research is needed to clear up some of the misunderstandings.The ATG5 gene expression and hormone levels have a reciprocal regulatory interaction that needs more research to clear up certain ambiguities and may also be explored as a potential treatment target for PCOS.When compared to healthy controls, the average serum LH levels were significantly greater in both patient groups. The mean serum FSH levels in postmenopausal patients were notably higher than those in the other groups. The average serum prolactin levels in both patient groups were lower than in the healthy controls, but the difference was not statistically significant. Compared to the other groups, postmenopausal patients had considerably lower average serum estradiol levels. When comparing the two sick groups' average ATG5 gene expression to that of the healthy controls, a substantial increase was seen.

Reference

1. Nautiyal H, Imam SS, Alshehri S, Ghoneim MM, Afzal M, Alzarea SI, et al. Polycystic ovarian syndrome: a complex disease with a genetics approach. Biomedicines. 2022;10(3):540.
2. Ożegowska K, Plewa S, Mantaj U, Pawelczyk L, Matysiak J. Serum metabolomics in PCOS women with different body mass index. Journal of Clinical Medicine. 2021;10(13):2811.
3. Li X, Qi J, Zhu Q, He Y, Wang Y, Lu Y, et al. The role of androgen in autophagy of granulosa cells from PCOS. Gynecological Endocrinology. 2019;35(8):669-72.
4. Wawrzkiewicz-Jałowiecka A, Kowalczyk K, Trybek P, Jarosz T, Radosz P, Setlak M, et al. In search of new therapeutics—molecular aspects of the PCOS pathophysiology: genetics, hormones, metabolism and beyond. International Journal of Molecular Sciences. 2020;21(19):7054.
5. Ajmal N, Khan SZ, Shaikh R. Polycystic ovary syndrome (PCOS) and genetic predisposition: A review article. European journal of obstetrics & gynecology and reproductive biology: X. 2019;3:100060.
6. Puurunen J, Piltonen T, Morin-Papunen L, Perheentupa A, Järvelä I, Ruokonen A, et al. Unfavorable hormonal, metabolic, and inflammatory alterations persist after menopause in women with PCOS. The Journal of Clinical Endocrinology & Metabolism. 2011;96(6):1827-34.

7. Sharma S, Mahajan N. Polycystic ovarian syndrome and menopause in forty plus women. Journal of Mid-life Health. 2021;12(1):3.
8. Yatsenko SA, Rajkovic A. Genetics of human female infertility. Biology of reproduction. 2019;101(3):549-66.
9. Azziz R, Carmina E, Dewailly D, Diamanti-Kandarakis E, Escobar-Morreale HF, Futterweit W, et al. The Androgen Excess and PCOS Society criteria for the polycystic ovary syndrome: the complete task force report. Fertility and sterility. 2009;91(2):456-88.
10. Weng Y-M, Ke C-R, Kong J-Z, Chen H, Hong J-J, Zhou D-S. The significant role of ATG5 in the maintenance of normal functions of Mc3T3-E1 osteoblast. European Review for Medical & Pharmacological Sciences. 2018;22(5).
11. Sambrook J, Russell DW. Molecular Cloning: Ch. 8. In Vitro amplification of DNA by the polymerase chain reaction: Cold Spring Harbor Laboratory Press; 2001.
12. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. methods. 2001;25(4):402-8.
13. Kadhim WA. Detection of Gene Expression of Efflux Pumps in Escherichia coli Isolated from Children with Urinary Tract Infections. 2023.
14. Ramaswamy S, Weinbauer GF. Endocrine control of spermatogenesis: Role of FSH and LH/testosterone. Spermatogenesis. 2014;4(2):e996025.
15. Joham AE, Norman RJ, Stener-Victorin E, Legro RS, Franks S, Moran LJ, et al. Polycystic ovary syndrome. The lancet Diabetes & endocrinology. 2022;10(9):668-80.
16. Garg A, Patel B, Abbara A, Dhillo WS. Treatments targeting neuroendocrine dysfunction in polycystic ovary syndrome (PCOS). Clinical endocrinology. 2022;97(2):156-64.
17. Ding H, Zhang J, Zhang F, Zhang S, Chen X, Liang W, et al. Resistance to the insulin and elevated level of androgen: A major cause of polycystic ovary syndrome. Frontiers in endocrinology. 2021;12:741764.
18. McNamara M, Batur P, DeSapri KT. Perimenopause. Annals of internal medicine. 2015;162(3):ITC1-ITC16.
19. Onizuka Y, Nagai K, Ideno Y, Kitahara Y, Iwase A, Yasui T, et al. Association between FSH, E1, and E2 levels in urine and serum in premenopausal and postmenopausal women. Clinical Biochemistry. 2019;73:105-8.
20. Forslund M, Landin-Wilhelmsen K, Schmidt J, Brännström M, Trimpou P, Dahlgren E. Higher menopausal age but no differences in parity in women with polycystic ovary syndrome compared with controls. Acta Obstetricia et Gynecologica Scandinavica. 2019;98(3):320-6.
21. Hirschberg AL. Approach to investigation of hyperandrogenism in a postmenopausal woman. The Journal of Clinical Endocrinology & Metabolism. 2023;108(5):1243-53.
22. Forslund M, Schmidt J, Brännström M, Landin-Wilhelmsen K, Dahlgren E. Reproductive hormones and anthropometry: a follow-up of PCOS and controls from perimenopause to older than 80 years. The Journal of Clinical Endocrinology & Metabolism. 2021;106(2):421-30.
23. Mahboobifard F, Rahmati M, Amiri M, Azizi F, Tehrani FR. To what extent does polycystic ovary syndrome influence the cut-off value of prolactin? Findings of a community-based study. Advances in Medical Sciences. 2022;67(1):79-86.
24. Jiang Y, Liu Y, Yu Z, Yang P, Zhao S. Serum asprosin level in different subtypes of polycystic ovary syndrome: a cross-sectional study. Revista da Associação Médica Brasileira. 2021;67:590-6.
25. Mastnak L, Herman R, Ferjan S, Janež A, Jensterle M. Prolactin in polycystic ovary syndrome: metabolic effects and therapeutic prospects. Life. 2023;13(11):2124.

26. Yang H, Di J, Pan J, Yu R, Teng Y, Cai Z, et al. The association between prolactin and metabolic parameters in PCOS women: a retrospective analysis. Frontiers in Endocrinology. 2020;11:263.
27. Stanikova D, Zsido RG, Luck T, Pabst A, Enzenbach C, Bae YJ, et al. Testosterone imbalance may link depression and increased body weight in premenopausal women. Translational psychiatry. 2019;9(1):160.
28. Millán-de-Meer M, Luque-Ramírez M, Nattero-Chávez L, Escobar-Morreale HF. PCOS during the menopausal transition and after menopause: a systematic review and meta-analysis. Human reproduction update. 2023;29(6):741-72.
29. Rosenfield RL, Ehrmann DA. The pathogenesis of polycystic ovary syndrome (PCOS): the hypothesis of PCOS as functional ovarian hyperandrogenism revisited. Endocrine reviews. 2016;37(5):467-520.
30. Johansen N, Linden Hirschberg A, Moen MH. The role of testosterone in menopausal hormone treatment. What is the evidence? Acta Obstetricia et Gynecologica Scandinavica. 2020;99(8):966-9.
31. Weiss G, Skurnick JH, Goldsmith LT, Santoro NF, Park SJ. Menopause and hypothalamic-pituitary sensitivity to estrogen. Jama. 2004;292(24):2991-6.
32. Santoro N, Roeca C, Peters BA, Neal-Perry G. The menopause transition: signs, symptoms, and management options. The Journal of Clinical Endocrinology & Metabolism. 2021;106(1):1-15.
33. Kumariya S, Ubba V, Jha RK, Gayen JR. Autophagy in ovary and polycystic ovary syndrome: role, dispute and future perspective. Autophagy. 2021;17(10):2706-33.
34. Esmaeilian Y, Hela F, Bildik G, İltumur E, Yusufoglu S, Yildiz CS, et al. Autophagy regulates sex steroid hormone synthesis through lysosomal degradation of lipid droplets in human ovary and testis. Cell Death & Disease. 2023;14(5):342.
35. Randolph Jr JF, Zheng H, Sowers MR, Crandall C, Crawford S, Gold EB, et al. Change in follicle-stimulating hormone and estradiol across the menopausal transition: effect of age at the final menstrual period. The Journal of Clinical Endocrinology & Metabolism. 2011;96(3):746-54.
36. Marina L, Sojat A, Maseroli E, Spaggiari G, Pandurevic S, Santi D. Hormonal profile of menopausal women receiving androgen replacement therapy: a meta-analysis. Journal of endocrinological investigation. 2020;43:717-35.
37. Sansone A, Romanelli F. Hormones in aging. Human Aging: Elsevier; 2021. p. 207-17.
38. Ye W, Xie T, Song Y, Zhou L. The role of androgen and its related signals in PCOS. Journal of cellular and molecular medicine. 2021;25(4):1825-37.
39. Zielniok K, Motyl T, Gajewska M. Functional Interactions between 17β-Estradiol and Progesterone Regulate Autophagy during Acini Formation by Bovine Mammary Epithelial Cells in 3D Cultures. BioMed research international. 2014;2014(1):382653.
40. Roche J, Ramé C, Reverchon M, Mellouk N, Cornuau M, Guerif F, et al. Apelin (APLN) and apelin receptor (APLNR) in human ovary: expression, signaling, and regulation of steroidogenesis in primary human luteinized granulosa cells. Biology of Reproduction. 2016;95(5):104, 1-12.
41. Li D, You Y, Bi F-F, Zhang T-N, Jiao J, Wang T-R, et al. Autophagy is activated in the ovarian tissue of polycystic ovary syndrome. Reproduction. 2018;155(1):85-92.
42. Yang S, Wang H, Li D, Li M. Role of endometrial autophagy in physiological and pathophysiological processes. Journal of Cancer. 2019;10(15):3459.
43. Wang X, Wang L, Xiang W. Mechanisms of ovarian aging in women: a review. Journal of Ovarian Research. 2023;16(1):67.

44. Zhang D, Li J, Xu G, Zhang R, Zhou C, Qian Y, et al. Follicle-stimulating hormone promotes age-related endometrial atrophy through cross-talk with transforming growth factor beta signal transduction pathway. Aging cell. 2015;14(2):284-7.
45. Sumarac-Dumanovic M, Apostolovic M, Janjetovic K, Jeremic D, Popadic D, Ljubic A, et al. Downregulation of autophagy gene expression in endometria from women with polycystic ovary syndrome. Molecular and cellular endocrinology. 2017;440:116-24.
46. Zhou J, Peng X, Mei S. Autophagy in ovarian follicular development and atresia. International journal of biological sciences. 2019;15(4):726.