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ORIGINAL RESEARCH

Semaglutide Alleviates Ovary Inflammation via the AMPK/SIRT1/NF-κB Signaling Pathway in Polycystic Ovary Syndrome Mice

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Background: GLP-1 receptor agonists (GLP-1 RA) have been proven to treat several metabolic diseases; however, the effects of GLP-1 RA on polycystic ovary syndrome (PCOS) remain unclear. Here, we aimed to investigate whether semaglutide, a novel GLP-1 RA, could alleviate ovarian inflammation in PCOS mice.

Methods: Female C57BL/6J mice were subcutaneously injected with dehydroepiandrosterone for 21 days to establish the PCOS model. Then the mice were randomly divided into three groups: PCOS group (n = 6), S-0.42 group (semaglutide 0.42 mg/kg/w, n = 6), and S-0.84 group (semaglutide 0.84 mg/kg/w, n = 6). The remaining six mice were used as controls (NC). After 28 days of intervention, serum sex hormones and inflammatory cytokine levels were measured. Hematoxylin and eosin staining was used to observe the ovarian morphology. Immunohistochemical staining was used to detect the relative expression of CYP19A1, TNF-α, IL-6, IL-1β, and NF-κB in ovaries. CYP17A1 and StAR were detected using immunofluorescence staining. Finally, the relative expressions of AMPK, pAMPK, SIRT1, NF-κB, IκBα, pIκBα, TNF-α, IL-6, and IL-1β were measured using Western blotting.

Results: First, after intervention with semaglutide, the weight of the mice decreased, insulin resistance improved, and the estrous cycle returned to normal. Serum testosterone and IL-1β levels decreased significantly, whereas estradiol and progestin levels increased significantly. Follicular cystic dilation significantly improved. The expression of TNF-α, IL-6, IL-1β, NF-κB, CYP17A1, and StAR in the ovary was significantly downregulated, whereas CYP19A1 expression was upregulated after the intervention. Finally, we confirmed that semaglutide alleviates ovarian tissue inflammation and improves PCOS through the AMPK/SIRT1/NF-κB signaling pathway.

Conclusion: Semaglutide alleviates ovarian inflammation via the AMPK/SIRT1/NF-κB signaling pathway in PCOS mice.

Keywords: GLP-1 receptor agonist, polycystic ovary syndrome, semaglutide, inflammation

Introduction

Polycystic ovary syndrome (PCOS), a common reproductive endocrine disease, affects approximately 6–10% of young women.[1] Infrequent menstruation, ovulation disorders, hyperandrogenemia (HA), polycystic ovarian changes, and insulin resistance (IR) are the main characteristics of PCOS.[2] The etiology of PCOS is complex, including hypothalamic and ovarian dysfunction, chronic inflammation, IR, obesity related mechanisms, and excessive exposure to androgens.[3,4] Currently, diet adjustment, exercise intervention, and medication are the main treatment methods for PCOS. Common medications include androgen receptor antagonists, aromatase inhibitors, oral contraceptives, and insulin-sensitizing agents.[5] Despite valuable advances having been made in PCOS treatment, several barriers remain in the development of effective interventions.

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In PCOS patients, excessive androgen is the main cause of irregular ovulation, hirsutism, and acne.[6] Coding genes related to sex hormones, such as cytochrome P450 hydroxylase (CYP17A1) and cytochrome P450 family member 19A1 (CYP19A1), are all associated with the occurrence and development of PCOS.[7,8] Among them, the physiological function of CYP17A1 is mainly to encode 17-α-hydroxylase, which plays an important role in the key steps of pregnenolone conversion to 17 hydroxypregnenolone and progesterone (P) conversion to 17 hydroxyprogesterone.[9,10] Overexpression of CYP17A1 results in increased secretion of androgens, which is considered a key factor associated with PCOS. The aromatase encoded by the CYP19A1 gene is a key rate limiting enzyme in the estradiol (E2) synthesis pathway.[11] When abnormal expression of CYP19A1 or inhibition of aromatase activity occurs, the conversion of androgens to E2 is restricted.[12,13]

Chronic low-grade inflammation is considered a vital triggering factor for the development of PCOS.[14,15] Patients with PCOS exhibit pathological changes in the ovaries, mainly bilateral ovarian enlargement, thickening and sclerosis of the white membrane, cortical fibrosis, and significant interstitial hyperplasia of the ovaries. Currently, it is widely believed that this is a manifestation of chronic proliferative inflammation and one of the causes of ovulation disorders. Inflammatory cytokines, such as TNF-α, IL-18, and IL-6, are significantly elevated in patients with PCOS compared with healthy individuals.[16] Macrophages and lymphocytes in the ovarian tissue of PCOS patients secrete large amounts of TNF-α and IL-6, resulting in inhibition of the formation of dominant follicles and apoptosis of granulosa cells. After inhibiting the expression of serum TNF-α and IL-6 in PCOS rats, ovulation and luteal function were significantly improved. This suggests that chronic inflammation in both the ovaries and blood circulation can lead to ovulation disorders in PCOS.[17] The local inflammatory microenvironment of the ovary not only directly affects the function of granulosa cells but also triggers local IR in the ovary by interfering with the insulin signaling pathway. Both TNF-α and IL-6 can reduce the expression of intracellular glucose transporter 4 (GLUT4), inhibit normal phosphorylation of insulin receptors and insulin receptor substrate 1 (IRS-1), block GLUT4 translocation, and lead to IR.[18,19] IL-1 β can lead to the abnormal expression and translocation of GLUT4 in ovarian granulosa cells by reducing insulin-induced AKT phosphorylation.[20,21] Therefore, improving the chronic low-grade inflammation of the ovary may provide new strategies for the treatment of PCOS. GLP-1 receptor agonist (GLP-1RA), a novel hypoglycemic agent, not only promotes insulin synthesis and secretion, but also decreases glucagon secretion in α cells while promoting insulin secretion from β cells of the pancreas, reduces food intake, and delays gastric emptying.[22–24] The anti-inflammatory properties of GLP-1RA first originated from studies on pancreatic β cells, followed by studies on the heart, vascular system, and the liver.[25–27] In fact, GLP-1 RA can regulate several molecular pro-inflammatory factors, such as glucose toxicity, oxidative stress, and lipid toxicity.[28]

In addition to treating type 2 diabetes mellitus (T2DM), GLP-1RA has been used to explore the treatment of PCOS. A meta-analysis showed that GLP-1RA may be superior to metformin in improving insulin sensitivity in PCOS patients.[29] In terms of improving reproductive function, studies have shown that GLP-1RA can reduce total testosterone (T) and free T levels in obese PCOS patients, thereby alleviating hyperandrogenism, improving menstruation, and increasing fertility rates.[30,31] Recent studies have shown that GLP-1 can reduce ferredoxin 1 levels in the ovaries of PCOS rats.[32] Hirsch et al found that treatment with liraglutide resulted in weight loss, menstrual cycle restoration, and improved hyperandrogenism in PCOS.[33] Metformin combined with exenatide can reduce weight, BMI, and waist circumference and improve insulin sensitivity in women with PCOS.[34] Thus, the role of GLP-1RA in PCOS mainly focuses on improving body weight and IR; however, little is known about its ability to alleviate ovarian inflammation. In this study, we selected C57BL/6J mice as experimental animals and established PCOS model using dehydroepiandrosterone (DHEA). The DHEA induced PCOS model shows similar endocrine and metabolic characteristics to the PCOS patients.[35,36] Here, we investigated whether semaglutide alleviates ovarian inflammation in PCOS mice.

Materials and Methods

The Mice Used in the Experiments

Female C57BL/6J mice (10–12 g, 3 weeks old) and High-fat diet (HFD) feedstuff were obtained from Jinan Pengyue Experimental Animal Breeding Co., Ltd. (License No. SCXK (Shandong) 2023–0002). They were maintained at 22–25°C with a humidity level of 50–60% at a temperature of 22 ± 2°C and fed freely for 3 days to adapt to the new situation. The high-fat feed contains 67% mice maintenance feed, 10% lard, 20% sucrose, 2.5% cholesterol, and 0.5% sodium cholate. All experimental techniques followed the guidelines for the Care and Use of Laboratory Animals and

were conducted in compliance with the ARRIVE guidelines.[37] The Ethics Committee of Animal Experiments of Binzhou Medical University Hospital approved all animal experimental protocols (ethical approval number: 20221014–22).

PCOS Model Establishment and Intervention

After 3 days of adaptation, 24 mice were randomly divided into a normal control group (NC, n = 6) and a DHEA-induced group (n = 23). All mice in the DHEA induction group were fed HFD subcutaneously injected with DHEA (6 mg/100 g/d, dissolved in sesame oil; Macklin Biochemical Technology Co., Ltd) in the back skin for 21 days. The estrus cycle of the mice was monitored by observing the proportion of shed cells in vaginal smears. After the model was built, five mice were randomly selected to evaluate modeling success. Then the remaining 18 PCOS mice were randomly divided into three groups (n = 6): the PCOS group, PCOS+semaglutide 0.42 mg/kg/w group (S-0.42), and PCOS+semaglutide 0.84 mg/kg/w group (S-0.84). The mice in the S-0.42 and S-0.84 groups received subcutaneous injections of semaglutide once a week, whereas the mice in the NC and PCOS groups received an equal amount of normal saline. The treatment lasted for 4 weeks. The weight of the mice was measured once per week. Vaginal smears were collected daily until the end of the experiment. After the intervention, ovarian tissue and blood samples were obtained.

Identification of Estrous Cycle Stage

The vagina of the mice was exposed and the external genitalia were wiped with a cotton swab dipped in physiological saline. Then 5 μL of physiological saline was sucked using a straw, placed into the vagina, and the suction was repeated 3–5 times. Aspirated droplets were sucked onto a glass slide and stained with alkaline methylene blue. A glass slide was placed under an optical microscope to observe the estrous cycles of the mice. The cycle judgment was as follows: white blood cells were the main cells during the estrus interval; the proportion of non-keratinized nucleated epithelial cells was dominant in the early stage of estrus; during estrus, the majority of cells were a nuclear keratinocytes, and the proportion of these three types of cells was equivalent in the later stages of estrus.

Body Weight Measurement and Intraperitoneal Glucose Tolerance Tests (IPGTT)

The body weights of the mice were measured at the same time every week until the end of the mice was euthanized. The IPGTT was measured twice, at the end of the modeling and after the semaglutide intervention. Before the start of the IPGTT, the mice were fasted for 12 hours and provided with free drinking water. Tail vein blood was collected for blood glucose measurement (Bayer, Germany). After fasting glucose data were collected, the mice received an intraperitoneal glucose injection (2.0 g/kg), and blood glucose levels were determined at 15, 30, 60, 90, and 120 minutes.

Serum Inflammatory Cytokines and Sex Hormone Measurement

After anesthetizing the mice, blood was collected from the eye socket and centrifuged to obtain the serum. Serum TNF-α, IL-6, and IL-1β levels were measured using a chemiluminescence immunoassay analyzer (Spring C2200, Zhonghong Detection, China). Circulating E2, P, and T levels were determined using a chemiluminescence immunoassay analyzer (Cobas e 602; Roche, Switzerland).

H&E Staining and Immunohistochemistry

For histological analyses, 4% paraformaldehyde was used to ovarian tissue. Subsequently, the ovarian tissue was embedded in paraffin sliced into 4-micrometer-thick sections, dimethylbenzene vitrification and graded alcohol were used for dehydration and eosin and hematoxylin were used for staining. Add an appropriate amount of neutral gum onto the ovarian tissue section, and finally observed using a microscope (Olympus, Japan). For immunohistochemistry, was performed using the streptavidin biotin complex method. Ovarian tissue sections were treated with bovine serum albumin for 10 minutes and then incubated overnight with IL-1β rabbit antibodies (dilution 1:50; No. 516288, ZEN-BIOSCIENCE, China), IL-6 antibodies (dilution 1:50; No. BA4339, BOSTER, China), TNF-α antibodies (dilution 1:50; No. BA0103, BOSTER, China), CYP19A1 antibodies (dilution 1:50; No. BA3704, BOSTER, China), and NF-κB antibodies (dilution 1:50; No. BA0610, BOSTER, China). Goat anti-rabbit IgG (No. SA1020, BOSTER, China) was incubated for 1 hour and then observed using a microscope.

Immunofluorescence

To conduct immunofluorescence staining of ovarian tissues, deparaffinized and hydrated sections were first treated with bovine serum albumin to prevent nonspecific reactions. Subsequently, CYP17A1 (dilution 1:400; No. A00615-3, BOSTER, China) and StAR (dilution 1:400; No. 67130-1-Ig, Sanying, China) were applied and incubated overnight at 4°C. Following this, secondary antibodies were applied at room temperature and incubated for 1 hour, followed by 4',6-diamidino-2-phenylindole (DAPI) for 5 minutes. The sections were then sealed using anti-fade mounting medium and observed under a fluorescence microscope (Olympus, Japan). Quantification of CYP17A1 and StAR was performed using ImageJ software. The immunopositive areas of CYP17A1 and StAR in ovarian tissues were measured. Each slide contained two to four non-consecutive sections, and random microfields were selected from each slide for analysis.

Western Blot

RIPA lysate was used to lyse ovarian tissue, and the proteins were collected. A BCA protein assay reagent was used to determine the protein concentration (No. AR0146, BOSTER, China). Used an electrophoresis apparatus to electrophorese proteins (BIO-RAD, USA). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (6.5% or 10%) was used to separate proteins. Proteins were transferred onto polyvinylidene fluoride (PVDF) membranes (Millipore, USA). After blocking with skimmed milk at room temperature for 2 hours the membranes were incubated overnight at 4°C with primary antibodies against AMPK (dilution 1:1000; No. 5831S, CST, USA), pAMPK (dilution 1:1000; No. 2535S, CST, USA), SIRT1 (dilution 1:1000; No. 8469S, CST, USA), NF-κB (dilution 1:1000; No. BA0610, BOSTER, China), IκBα (dilution 1:1000; No. 4812S, CST, USA), pIκBα (dilution 1:1000; No. 2859S, CST, USA), IL-1β (dilution 1:1000; No. 516288, ZEN-BIOSCIENCE, China), and IL-6 (dilution 1:1000; No. BA4339, BOSTER, China), TNF-α (dilution 1:1000; No. BA0103, BOSTER, China). The membrane was washed three times, and diluted enzyme-labeled secondary antibody was added and incubated for 2 hours at room temperature. Relative protein expression was analyzed using β-actin (dilution 1:5000; No. BM0627, BOSTER, China).

Statistical Analysis

Each experiment was performed in a separate and independent way. The Student's *t*-test was used to compare two groups, and one-way ANOVA to compare multiple groups. Statistical analysis was performed using the SPSS software (version 26.0, IBM). Results are expressed as the mean ± SD, $P < 0.05$.

Results

Semaglutide Reduced the Body Weight of PCOS Mice

The experimental procedure is illustrated in Figure 1A. As shown in Figure 1B, compared to the NC group, PCOS mice showed a significant increase in body weight ($P < 0.05$). Compared to the models, the mice in the S-0.42 group and S-0.84 groups showed significant weight loss, with the S-0.84 group showing even more significant weight loss.

Semaglutide Alleviated IR in PCOS Mice

To further explore the effect of semaglutide on glucose tolerance, we measured the fasting blood glucose and conducted IPGTT (Figure 1C and D). The results showed that Semaglutide significantly reduced fasting glucose levels and improved glucose tolerance. The S-0.84 group showed a more significant effect. In addition, compared to the PCOS group, the area under the AUC curve of IPGTT significantly decreased after intervention with semaglutide, indicating an improvement in IR. These data suggest that semaglutide improves glucose metabolism in mice with PCOS.

Semaglutide Improved the Appearance of PCOS Mice

The estrous cycle plays an important role in the reproduction of female mice. Figure 2A shows vaginal images of mice at different stages of their estrus cycle. As presented in Figure 2B, the normal regular estrous cycle was 4–5 days, while mice in the PCOS group were all in an estrous cycle disorder. As predicted, semaglutide treatment reversed this effect. H&E staining revealed that the ovaries in the NC group had follicles at different developmental stages and no cyst-like follicles. However, the ovarian structure in PCOS mice is characterized by more cystic follicles, thinner granulosa cell layers in the follicles, and

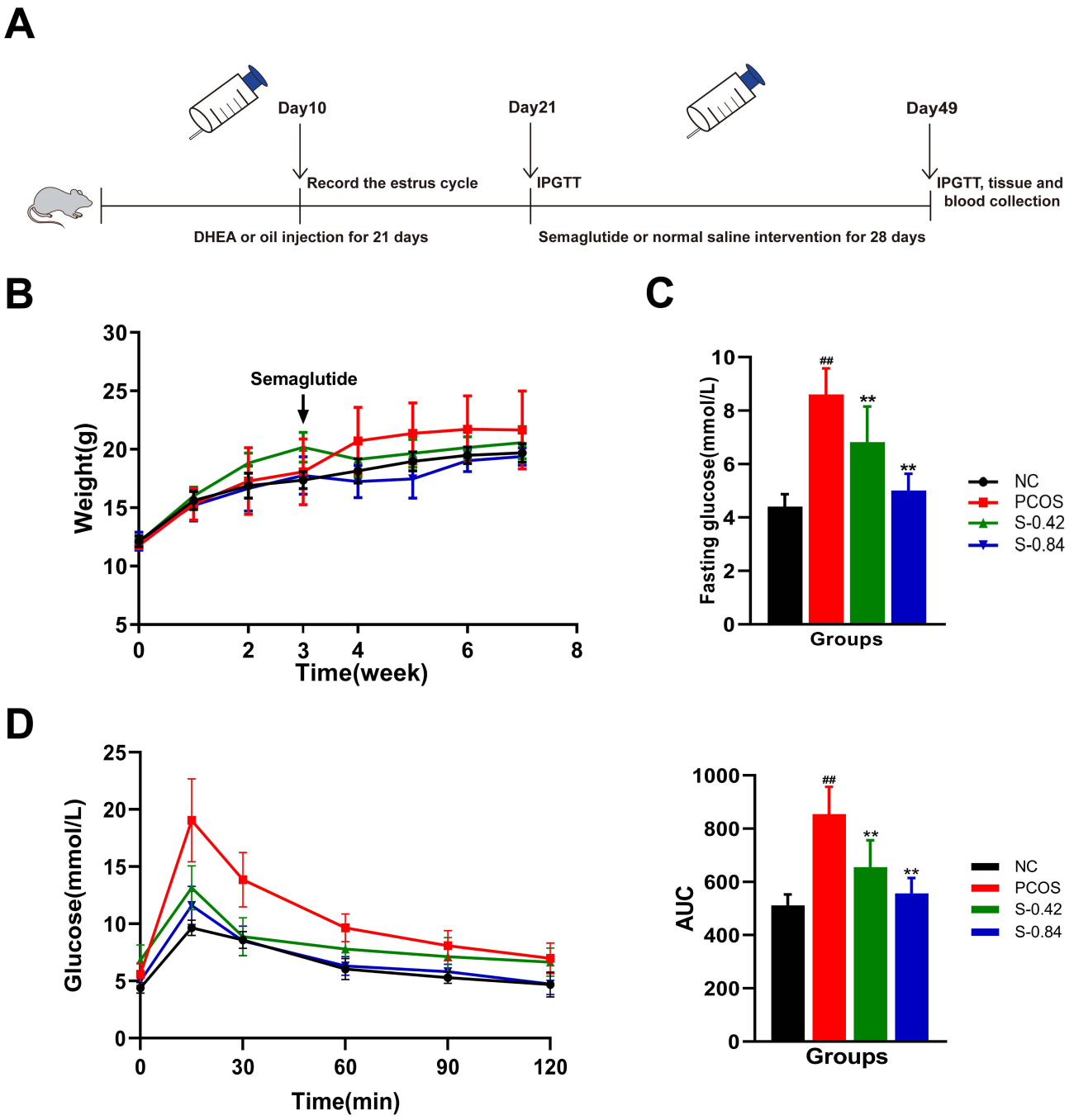

Figure 1 (**A**) Experimental flow chart; (**B**–**D**) Three weeks after modeling, PCOS mice were treated with semaglutide for 4 weeks. (**B**) Body weight; (**C**) Fasting glucose; (**D**) IPGTT and AUC. Data are presented as mean ± SD. Vs NC, $^{\#\#}P$ <0.01; vs PCOS, $^{**}P$ <0.01; n = 6 per group.

fewer corpora lutea. Importantly, semaglutide decreased the number of cystic follicles and increased the quantity of corpus luteum, suggesting that semaglutide improved the pathological development of the ovarian tissue (Figure 2C).

Semaglutide Improved Sexual Hormone Imbalance in PCOS Mice

As shown in Figure 2D, serum T in PCOS group was higher than that in NC group, while E2 and P were lower than that in NC group. After semaglutide administration, serum T decreased, whereas E2 and P levels increased. Compared to the S-0.42 group, the improvement in sexual hormone imbalance in the S-0.84 group was more significant.

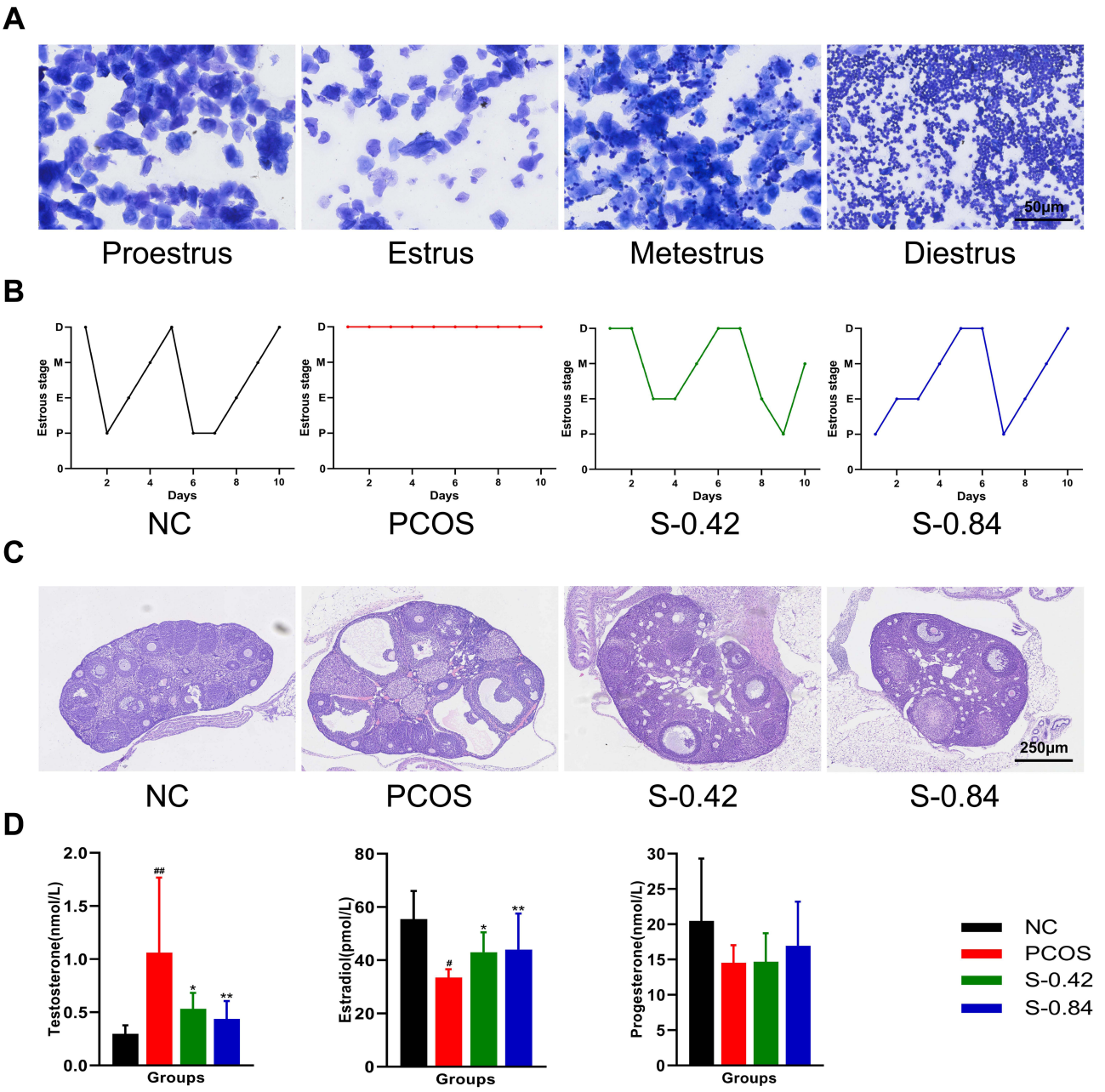

Figure 2 (**A**) Vaginal smears. The normal estrous cycle sequence is proestrus, estrus, metestrus, and diestrus; (**B**) Estrous cycle, change of the estrus cycle (10 days before the end of the experiment). P, prestrus; E, estrus; M, metestrus; D, diestrus; (**C**) Ovarian morphology by H&E staining. (**D**) Changes in peripheral blood sex hormones. Data are presented as mean ± SD. vs NC, $^{\#}P$ <0.05, $^{\#\#}P$ <0.01; Vs PCOS, $^{*}P$ <0.05, $^{**}P$ <0.01; n = 6 per group.

Semaglutide Regulated the Expression of Ovarian Steroidogenic Enzymes in PCOS Mice

As shown in Figure 3A and B, immunohistochemical staining results showed that the expression of CYP19A1 in the ovarian tissue of PCOS mice was downregulated. After semaglutide treatment, the expression of CYP19A1 increased. The immunofluorescence results showed that, compared with the NC group, the expression of StAR and CYP17A1 in the ovarian tissue of the PCOS group was significantly increased. After treatment, the expression of StAR and CYP17A1 was significantly downregulated (Figure 3C–E).

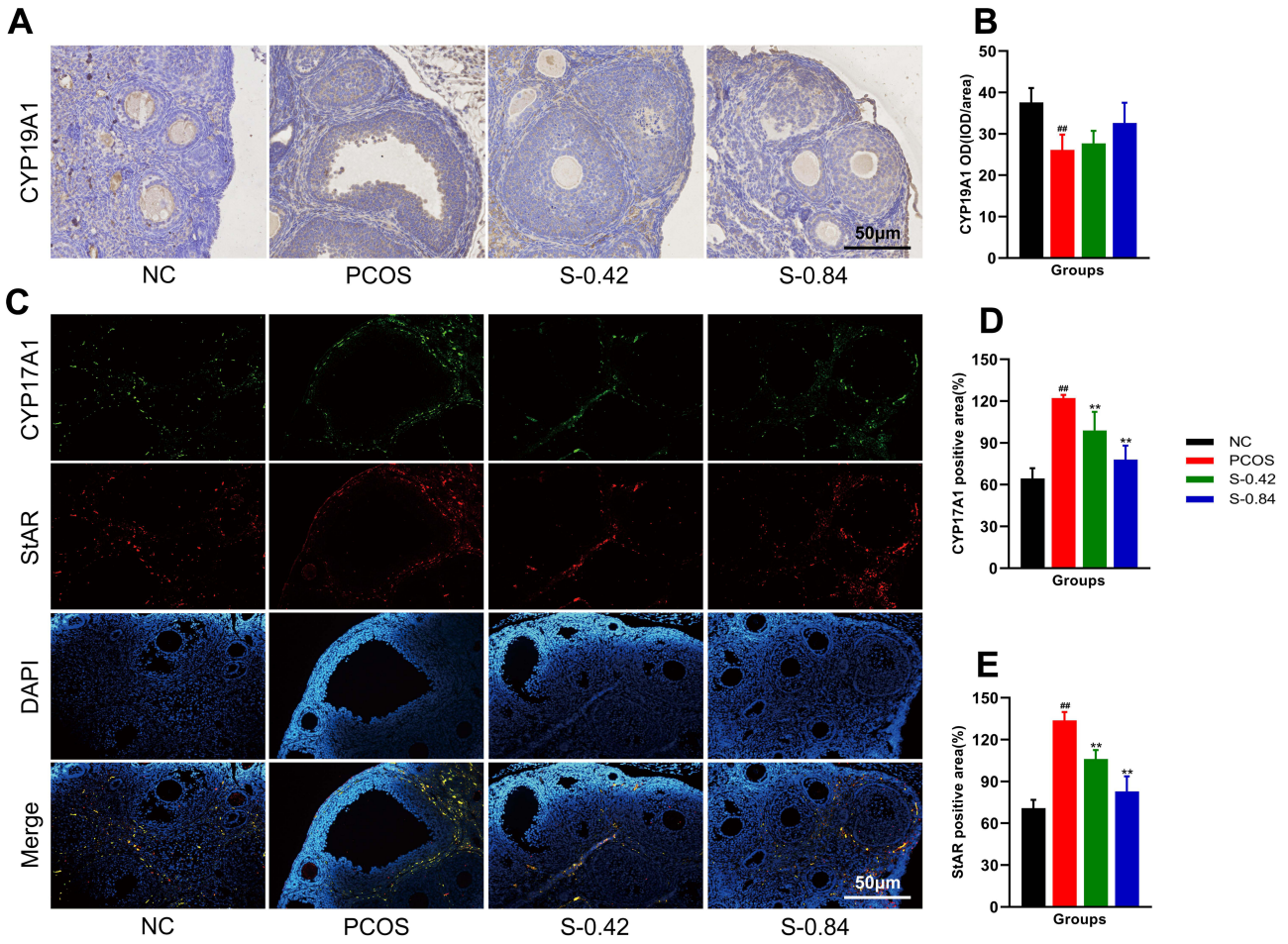

Figure 3 (**A**) Immunohistochemical images of CYP19A1; (**B**) CYP19A1 mean density; (**C**) Immunofluorescence images of CYP17A1 (green), StAR (red), and DAPI (blue); (**D**) CYP17A1 expression rate; (**E**) StAR expression rate. Data are presented as mean ± SD. vs NC, $^{\#\#}P$ <0.01; vs PCOS, $^{**}P$ <0.01; n = 6 per group.

Semaglutide Alleviated Systemic and Ovarian Inflammation

As shown in Figure 4A, immunohistochemical staining was used to determine the effect of semaglutide on ovarian tissue inflammation. The expression of IL-1β, IL-6, TNF-α, and NF-κB significantly increased in the PCOS group. Interestingly, these inflammatory cytokines decreased in a dose-dependent manner after semaglutide treatment (Figure 4B–E). We further validated these findings using Western blotting. Consistent with the immunohistochemical staining data, the expression of inflammatory cytokines was significantly increased in the ovarian tissue of the PCOS group (Figure 4F). The levels of these cytokines decreased after semaglutide treatment. As expected, the S-0.84 group showed a more significant effect in lowering inflammatory factors (P <0.01). The serum levels of inflammatory cytokines were also measured. Owing to the limited serum sample size, no data were obtained for IL-6 and TNF-α, only the data of IL-1β was obtained. Similarly, the circulating IL-1β concentration decreased after the intervention (Figure 4G).

The Effect of Semaglutide on the AMPK/SIRT1/NF-κB Signaling Pathway

To explore the anti-inflammatory mechanism of semaglutide, we detected the proteins related to the classical NF-κB inflammatory pathway. The relative expression of NF-κB phosphorylation protein and its upstream regulatory factor IκBα was upregulated in the ovaries of PCOS mice, whereas the expression of AMPK phosphorylation protein and SIRT1 was downregulated. Semaglutide improved the expression of pAMPK and SIRT1, and inhibited the expression of pIκBα and NF-κB in a dose-dependent manner (Figure 5A–E).

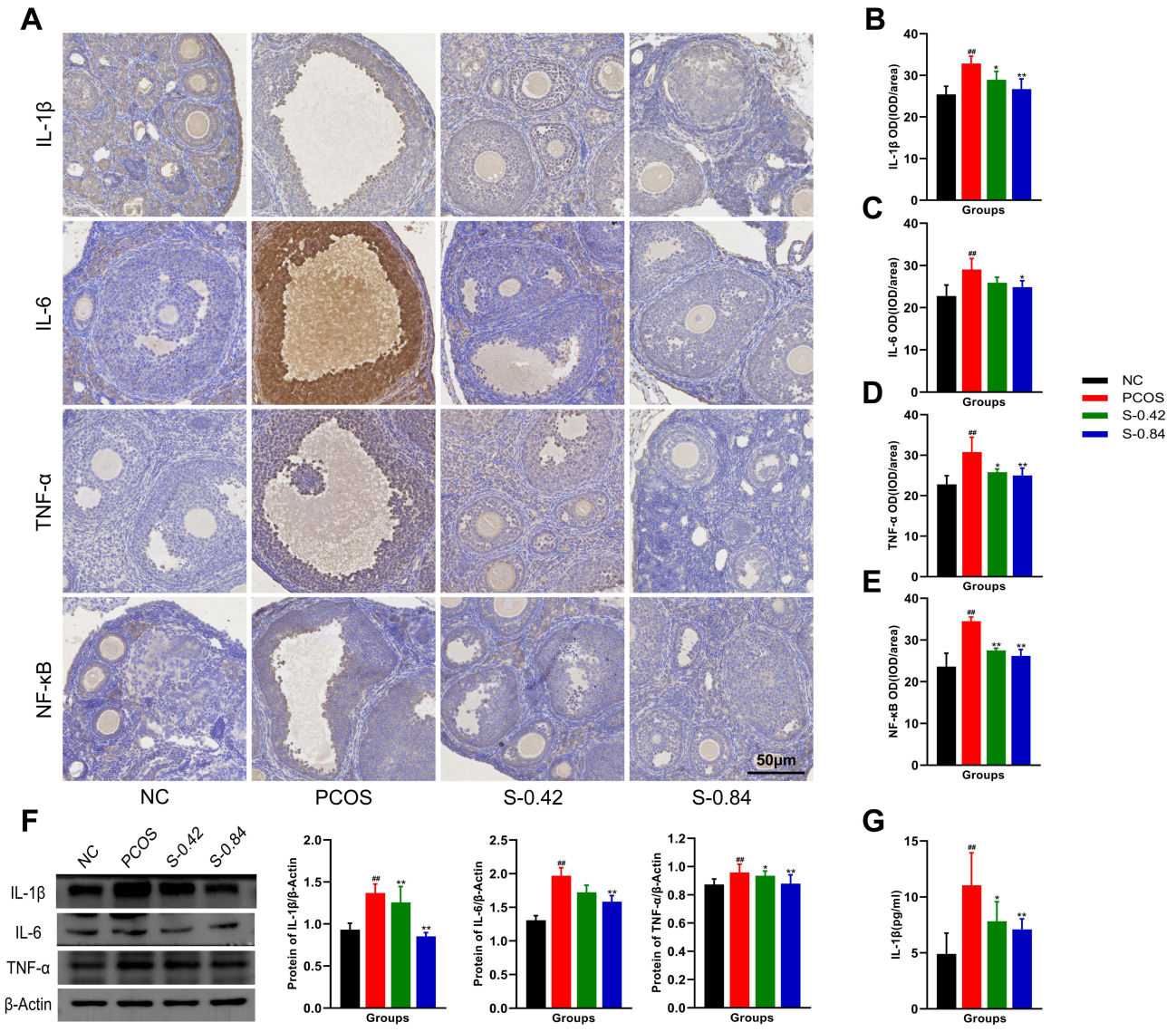

Figure 4 (**A**) Immunohistochemical images of IL-1β, IL-6, TNF-α, and NF-κB; Mean density: (**B**) IL-1β; (**C**) IL-6; (**D**) TNF-α; (**E**) NF-κB; (**F**) Western blot of IL-1β, IL-6, and TNF-α expression; (**G**) Peripheral blood IL-1β content. Data are presented as mean ± SD. Vs NC, $^{\#\#}P$ <0.01; Vs PCOS, $^{*}P$ <0.05, $^{**}P$ <0.01; n = 6 per group.

Discussion

In this study, we successfully established a PCOS mouse model. PCOS mice show hormonal imbalances, ovarian dysfunction, and persistent chronic inflammation. After semaglutide treatment, the estrous cycle of PCOS mice returned to normal and weight loss and IR improved. Simultaneously, cystic expansion of the ovarian follicles significantly improved, and systemic and ovarian inflammation were alleviated. Furthermore, the expression of sex hormone synthases CYP17A1 and StAR decreased, while that of CYP19A1 increased. Finally, we verified that semaglutide alleviated ovarian inflammation via the AMPK/SIRT1/NF-κB signaling pathway.

Obesity is a clinical feature of PCOS that can exacerbate hormone disorders and clinical symptoms.[38] Therefore, weight loss is the primary goal of PCOS treatment.[39] GLP-1RA are currently used to treat obesity and diabetes, as well as other diseases such as cardiovascular and neurodegenerative diseases. Owing to safety, GLP-1RA provides assurance of long-term use in patients with obesity and multiple comorbidities. Weight loss under the action of GLP-1RA is not only caused by acting on the hypothalamus or parasympathetic nervous system, thereby inhibiting appetite and delaying gastric emptying, but also by acting on white and brown adipocytes.[40] GLP-1RA can promote browning of white

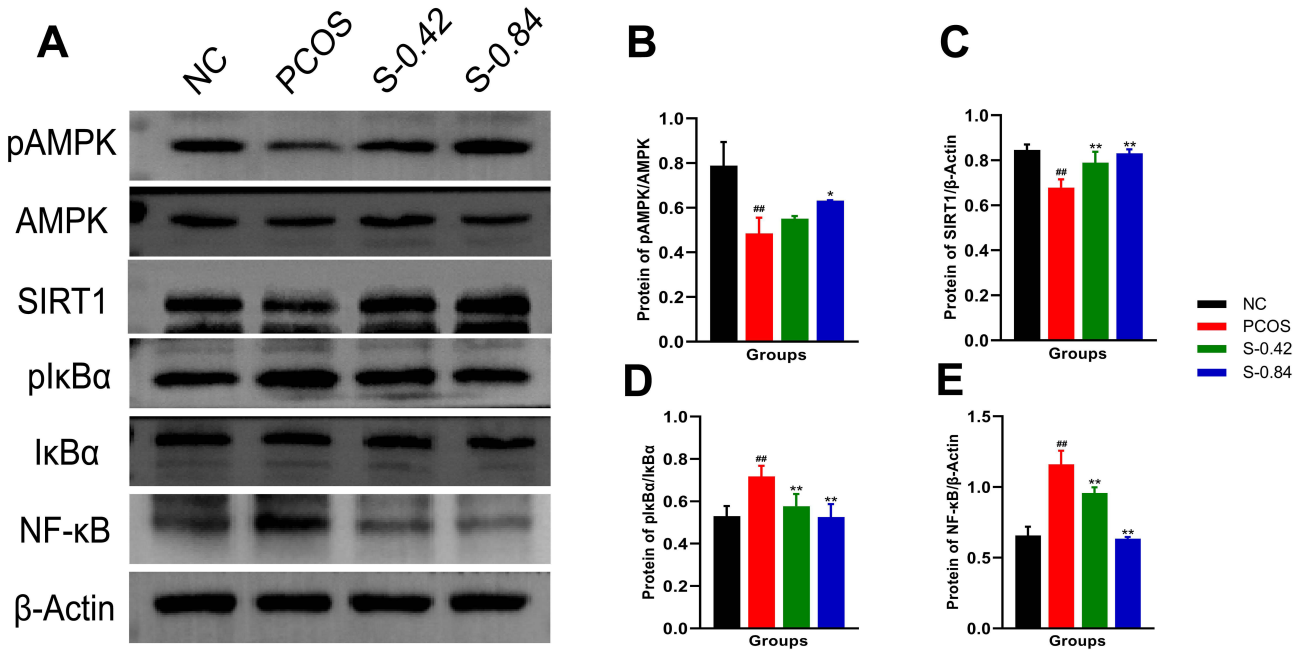

Figure 5 (**A**) Western blot of AMPK and downstream proteins; (**B–E**) Protein quantification statistics. Data are presented as mean ± SD. Vs NC, $^{\#\#}P$ <0.01; Vs PCOS, $^{*}P$ <0.05, $^{**}P$ <0.01; n = 6 per group.

adipocytes in mice through fat breakdown and oxidation, significantly reducing fat content.[41] Yahui Zhang et al found that GLP-1RA can alleviate hyperinsulinemia and hyperandrogenism in PCOS mice by reducing inflammation of white adipose tissue and stimulating browning of white adipose tissue.[42] Xiong et al confirmed that semaglutide modulated the diversity of gut microbiota in PCOS patients.[43] Previous clinical trials have shown that both metformin and metformin combined with liraglutide can improve the menstrual cycle, weight, and glucose metabolism in obese PCOS patients. The combination of metformin and liraglutide is more effective than metformin monotherapy in improving hyperandrogenism and reproductive abnormalities.[44] A meta-analysis showed that liraglutide has the strongest weight loss effect in PCOS patients compared to metformin, inositol, oseltamide, etc,[45] and 5–10% weight loss can improve the menstrual cycle and ovulation recovery in PCOS patients.[46,47] Besides, GLP-1RA can improve cardiovascular disease markers such as adiponectin in the treatment of PCOS patients, thus playing a protective role in cardiovascular disease and benefiting PCOS.[30] Currently, clinical trials of GLP-1RA in PCOS are based on weight loss effects.[33,34,48,49] Consistent with these results, in this study, PCOS mice showed significant weight loss after semaglutide treatment. However, the mechanisms underlying weight loss of GLP-1RA in PCOS are poorly understood.

In patients with PCOS, estrogen and androgen metabolism are abnormal. Excessive androgen levels are a major characteristic of PCOS.[50] Excessive production of T can increase the deposition of visceral fat, which in turn exacerbates IR and hyperinsulinemia, thereby forming a vicious cycle.[51] In our study, serum T levels increased in the PCOS group, while E2 and P levels decreased. The expression of CYP19A1 in the ovarian tissue of PCOS mice was decreased, whereas that of StAR and CYP17A1 was significantly increased. Several studies have shown that the degree of chronic inflammatory response in PCOS is closely related to obesity, IR, and hyperandrogenism.[16,52,53] On the one hand, IR not only affects normal glucose metabolism, but also promotes the synthesis of local steroid hormones in the ovaries, leading to further exacerbation of elevated circulating T levels and abnormal follicular development in PCOS patients. HA accelerates the conversion of P to androstenedione and T in PCOS, thereby increasing circulating serum T.[54] In contrast, IR and compensatory hyperinsulinemia cause abnormal activation of ovarian primordial follicles, increasing the sensitivity of pre-antral follicles to follicle-stimulating hormone (FSH), leading to excessive follicle recruitment and ultimately resulting in polycystic ovary-like changes.[55] Systemic and ovarian inflammatory factors abnormally expressed can affect the function of granulosa cells.[17,56] NF-κB is an important regulatory factor in the inflammatory response in PCOS, and is closely related to IR and granulosa cell apoptosis. The activation of NF-κB may lead to elevated androgen

levels and disrupted glucose and lipid metabolism in PCOS patients.[57] Inhibiting the NF-κB-mediated inflammatory signaling pathway significantly improves the ovarian hormone microenvironment in PCOS rats.[58] In line with these studies, the expression of TNF-α, IL-6, and IL-1β NF-κB increased in the ovaries of PCOS mice.

Owing to the wide distribution of GLP-1 receptors, the anti-inflammatory effects of GLP-1RA binding to receptors throughout the body have gradually been confirmed. With specific effects on infiltrating macrophages in streptozotocin-induced diabetes, exendin-4 attenuated adverse cardiac remodeling.[59] Exendin-4 has also been shown to moderate the enteric immune response by reducing the production of proinflammatory cytokines.[60] Furthermore, the authors validated that the production of pro-inflammatory cytokines can be reduced by exendin-4, mainly by downregulating NF-κB phosphorylation and nuclear translocation.[60] In addition, exendin-4 was demonstrated to lower hepatic production of the inflammatory markers TNF-α, IL-6, IL-1β, and macrophage markers in high-fat-diet mice.[61] To explore whether GLP-1RA improve ovarian function in PCOS mice by inhibiting inflammation, we examined the effects of semaglutide on systemic and ovarian inflammation. As expected, semaglutide treatment reversed these inflammatory conditions. Semaglutide downregulated the expression of NF-κB and upstream pIκBα.

AMPK is a key regulator of cellular energy production They maintain cellular energy homeostasis.[62] Furthermore, AMPK is significantly activated during LPS-induced lung damage, resistance to pathogen infection, and other inflammatory reactions.[63,64] Previous research has indicated that, under pathological conditions, AMPK inhibits inflammation mainly through its downstream protein SIRT1.[65–67] SIRT1 is an NAD (+)-dependent deacetylase that regulates cell differentiation and survival, inflammation, apoptosis, autophagy, and glucose and lipid metabolism.[68] SIRT1 exerts anti-inflammatory effects by inhibiting NF-κB.[69–71] GLP-1RA can upregulate SIRT1 in endothelial progenitor cells, inhibiting high-glucose-induced damage.[72] In skeletal muscle cells, GLP-1RA can activate SIRT1 through the protein kinase A and cyclic adenosine monophosphate pathways to improve IR.[73] GLP-1RA can improve the progression of nonalcoholic fatty liver disease by increasing the expression of SIRT1 in the livers of obese mice and alleviating obesity-induced renal mitochondrial dysfunction by activating SIRT1 in the kidneys.[74,75] Moreover,

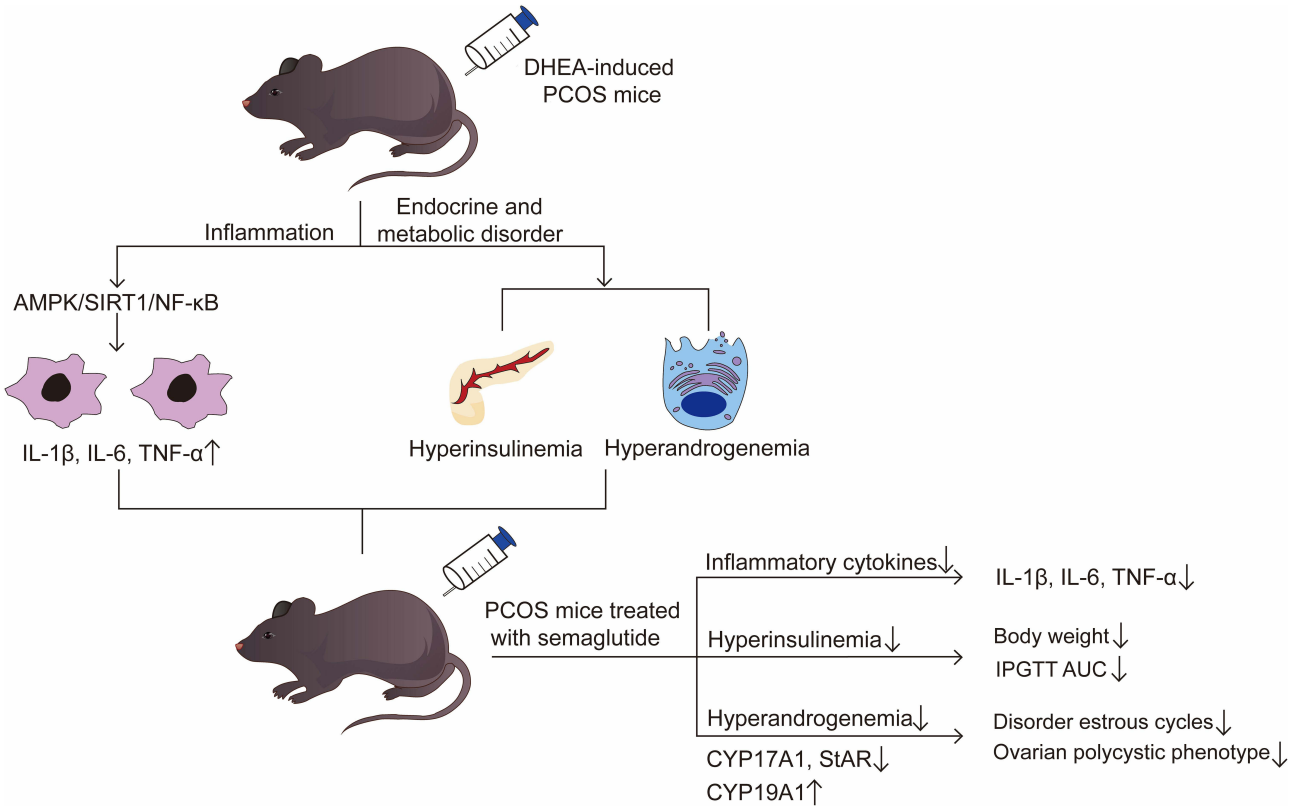

Figure 6 Summary of this research mechanism.

previous studies have confirmed that GLP-1 upregulates SIRT1 expression in adipose tissue and ameliorates IR in PCOS mice.[76] GLP-1 receptor agonists improve IR by reducing body weight in PCOS patients [31]. Exenatide upregulates the expression of SIRT1 in liver tissues and ameliorates hepatic steatosis.[77] Little research has been done on the SIRT pathway in the treatment of PCOS with GLP-1RA. A decrease in the expression of SIRT1 was found in the ovaries of PCOS rats. Treatment with exenatide increased the expression of SIRT1 in the ovaries, decreases androgen levels, and improves IR.[78] Tao et al found that exenatide increased the expression of SIRT1 in the ovarian tissues of PCOS rats.[79] Here, we found that semaglutide could also alleviate inflammation in PCOS mice by upregulating the AMPK/SIRT1/NF-κB signaling pathway (Figure 6).

This experiment preliminarily confirmed that semaglutide alleviates PCOS by alleviating inflammation, but there are still some limitations. First, these findings were not confirmed in vitro. Second, the experimental intervention lasted for 1 month, and whether PCOS rebounded after discontinuation of semaglutide has not been studied. Finally, due to the experimental conditions, we did not measure pituitary hormones such as luteinizing hormone and FSH.

In conclusion, semaglutide can regulate the expression of CYP17A1, StAR and CYP19A1, and alleviate local ovarian inflammation via the AMPK/SIRT1/NF-κB signaling pathway. This study provides a theoretical basis for the treatment of PCOS with GLP-1RA.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

The authors declare that they have no conflicts of interest in this work.

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