doi.org/10.36721/PJPS.2025.38.6.REG.14133.1

Quercetin protects the survival and decidualization of endometrial stromal cells in PCOS mice by enhancing autophagy through PI3K/Akt/FoxO1

Jinglu Yu[1#], Xiaoling Feng[2#], Wei Jiang[3], Ying Huang[3], Miao Sun[2], Yongwei Du[3] and Ge Yu[4]*

[1]Department of Obstetrics and Gynecology, First Affiliated Hospital, Heilongjiang University of Chinese Medicine, China
[2]Department of Gynecology, First Affiliated Hospital, Heilongjiang University of Chinese Medicine, China
[3]Department of Gynecology, First People's Hospital of Bei'an City, China
[4]Department of Gynecology, Harbin Medical University Cancer Hospital, China

Abstract: Polycystic ovary syndrome (PCOS) can lead to increased abortion rates. Quercetin treats PCOS, but its specific mechanism has not been fully clarified. PCOS was induced in mice by dehydroepiandrosterone, and decidualization was induced by corn oil. Mice were treated with quercetin, autophagy inhibitor 3-MA, autophagy inducer rapamycin, PI3K inhibitor LY294002, and PI3K inducer 740Y-P. Pathological damage in the ovary and uterus was observed by HE staining. The levels of sex hormones, metabolism, and inflammatory factors were detected using ELISA. The survival and decidualization of endometrial stromal cells were identified by immunohistochemistry, immunofluorescence, qRT-PCR, and Western blot. Autophagy and the PI3K/Akt/FoxO1 pathway-related protein levels were detected by Western blot. The theca cell layer and endometrium of PCOS mice were significantly thinner. The levels of sex hormone, pro-inflammatory factors, COX-2, integrin αvβ3, and autophagy-related proteins were obviously raised, while Vimentin, IGFBP-1, PRL, and PI3K/Akt/FoxO1 pathway expression were significantly decreased. The above indices were reversed considerably after quercetin treatment. 3-MA could reduce the level of autophagy, LY294002 could reduce the levels of PI3K/Akt/FoxO1 pathway, Vimentin, and PRL, and increase the level of autophagy. In conclusion, quercetin enhanced autophagy through the PI3K/Akt/FoxO1 pathway; thereby protecting endometrial stromal cells and improving decidualization disorders.

Keywords: Autophagy; Decidualization; PCOS; PI3K/Akt/FoxO1 pathway; Quercetin

Submitted on 17-02-2025 – Revised on 14-04-2025– Accepted on 01-07-2025

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a prevalent hormonal disorder affecting women of reproductive age. After women enter the childbearing age, reduced ovulation or anovulation can lead to female infertility. Even if there is a pregnancy, there will still be signs of abortion (Song *et al.*, 2022). Therefore, PCOS has become a key factor in infertility symptoms in women of childbearing age (Fernandez *et al.*, 2021; Tay *et al.*, 2023). Because the etiology and pathogenesis of PCOS are inconclusive, there is no effective treatment at present. The treatment focuses on symptom management (Singh *et al.*, 2023; Walter, 2022). Therefore, we need to understand the biological mechanism of PCOS development further and then explore effective PCOS treatment strategies.

The endometrium is a dynamic development of human tissue characterized by periodicity. It is a key component of the female reproductive system. It is composed of a functional layer and a basal layer. The basal layer is mainly composed of endometrial stromal cells (Shi *et al.*, 2021), which are essential for the production of menstruation, embryo implantation, and fetus development. Under the strict regulation of estrogen and progesterone, the process of endometrial stromal cells with strong proliferation ability to differentiate into decidual stromal cells with epithelioid secretion function is called decidualization. Endometrial decidualization disorder is also the root cause of various abnormal phenomena in early pregnancy (Ashary *et al.*, 2020). Therefore, improving endometrial homeostasis is one of the crucial breakthroughs to enhance the pregnancy rate and reduce abortion risk in PCOS patients.

Autophagy is a balanced mechanism for eukaryotic cells to maintain homeostasis, which can remove defective proteins, organelles, and pathogens in cells (Kirkin, 2020). In terms of reproduction, a normal autophagy level contributes to the development and maturation of oocytes and regulate endometrial function, which is an indispensable part of endometrial decidualization. The phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt)/forkhead box protein O1 (FoxO1) pathway is an essential regulatory signaling pathway for autophagy. FoxO1 marks the initiation of decidualization, which can directly bind to the promoter to regulate the transcription of insulin-like growth factor binding protein-1 (IGFBP-1) and prolactin (PRL) and promote decidualization (Jozaki *et al.*, 2019). The PI3K/Akt pathway is the direct upstream

**Corresponding author:* e-mail: yuhenren@163.com
#Jinglu Yu and Xiaoling Feng are co-first authors.

regulator of FoxO1 protein (Calissi *et al.*, 2021). Studies have shown that Microcystin-LR can damage the decidualization of endometrial cancer through the PI3K/Akt/FoxO1 axis (Yan *et al.*, 2024). The PI3K/Akt signaling pathway is also a key pathway involved in autophagy (Tong *et al.*, 2022). Therefore, this axis is vital for endometrial autophagy and decidualization of PCOS.

Quercetin has high biological activity and extensive pharmacological effects (hypoglycemic, anti-inflammatory, and anti-oxidation effects) (Batiha *et al.*, 2020). Studies have shown that quercetin can treat PCOS by improving ovulation disorders, reducing insulin resistance, improving the ovarian environment, reducing androgens, regulating lipid metabolism, and intestinal flora (Chen *et al.*, 2022; Jian *et al.*, 2024; Ma *et al.*, 2022). Quercetin can also play a protective role in ovarian granulosa cells through autophagy, thereby alleviating ovarian aging and damage (Cai *et al.*, 2024). However, it is not clear whether quercetin can improve the uterine environment of PCOS.

Therefore, this research explores whether quercetin improves endometrial homeostasis and autophagy in PCOS mice by regulating the PI3K/Akt/FoxO1 axis and clarifies its molecular mechanism of PCOS treatment, providing novel ideas for future study on PCOS.

MATERIALS AND METHODS

Preparation of the animal model

Forty-eight specific pathogen-free (SPF) 4-week-old female C57BL/6 mice, weighing about 15 g, were purchased from Junke Bioengineering Co., Ltd. (Nanjing, China). The mice were maintained in a SPF environment at a room temperature of 24°C with a 12 h light/dark cycle. Adequate food and water were available ad libitum.

We randomly divided the mice into eight groups: Control group, DHEA group, DHEA + 125 mg/kg quercetin (Quercetin) group, DHEA + 300 mg/kg metformin positive control (Metformin) group, DHEA + 125 mg/kg quercetin + autophagy inhibitor 3-MA (Quercetin+3-MA) group, DHEA + 125 mg/kg quercetin + autophagy inducer rapamycin (Quercetin+Rapa) group, DHEA + 125 mg/kg quercetin + PI3K inhibitor LY294002 (Quercetin+LY) group, DHEA + 125 mg/kg quercetin + PI3K inducer 740Y-P (Quercetin+740Y-P) group. There were 6 in each group. Mice were treated with DHEA to establish a PCOS mouse model (Di Emidio *et al.*, 2020). Mice in the Control group were subcutaneously injected with 0.1 mL sesame oil every day, and the remaining mice were subcutaneously injected with dehydroepiandrosterone (DHEA, 60 mg/kg, 100 μL/mouse, dissolved in sesame oil) every day for 20 days. The estrous cycle, hormone levels, and body weight of mice in the DHEA group were screened. The results showed that their estrous cycle was disordered and remained in diestrus, indicating that the PCOS mice were successfully modeled(Liu *et al.*, 2024; Peng *et al.*, 2023). Then the successful model mice were randomly divided into groups. The mice were given 125 mg/kg quercetin and 300 mg/kg metformin by gavage, respectively. At the same time, 0.5 mg/kg 3-MA, 3 mg/kg Rapa, 0.3 mg/kg LY294002, and 10 mg/kg 740Y-P were injected intraperitoneally for 20 days.

The female mice after the intervention were mated with male C57BL/6 mice with vasectomy to induce pseudopregnancy. The vagina of female mice was examined at 8 am the next day, and the morning after vaginal obstruction was defined as the first day of pseudopregnancy. On the fourth day of pseudopregnancy, female mice were anesthetized with 5% lidocaine, and 25 μL corn oil was injected into the uterine horn on one side to induce uterine decidualization. In contrast, the other side served as the control. The artificially induced decidualized mice were sacrificed on the 8th day of pseudopregnancy (Hong *et al.*, 2024; Wu *et al.*, 2023). Fig.1 illustrates the experimental procedure.

Observation of the estrous cycle

From the 11th day to the 20th day after the establishment of the PCOS mouse model and 10 days before the end of the experiment (31st to 40th day), the vaginal fluid smear of the mice was performed every morning: a sterile cotton swab infiltrated with normal saline entered the vagina about 0.5 cm and gently rotated for one circle. After being taken out, it was thoroughly smeared on the glass slide, naturally air-dried, fixed in 70% ethanol for 8 min, taken out, and dried and stained with 0.1% methylene blue (G1300, Solarbio, Beijing, China) for 3 min (Ji *et al.*, 2021). The smear was slowly rinsed with water and observed under a microscope after being dried. The number of nuclear epithelial cells, epithelial keratinocytes, and white blood cells on the smear was counted, and the estrus period of the mice was judged and recorded. The mice were then monitored for 10 days.

Ovarian and uterine index

After the modeling, the mice were weighed and then sacrificed. Subsequently, their bilateral ovaries and uterus were collected and weighed. Ovarian index = ovarian weight/mouse weight, uterine index = uterine weight/mouse weight.

HE staining

The ovaries and uterus were fixed in 4% paraformaldehyde (PFA), respectively. After dehydration and embedding, tissue sections were cut at a thickness of four μm and stretched and adhered to the slides. After soaking in xylene for 10 min, dewaxing, hematoxylin (G1004, Servicebio) staining for 5 min, eosin (G1001, Servicebio) staining for 2 min, conventional dehydration and transparency, neutral gum (WG10004160, Servicebio) sealing. A microscope (OLYMPUS, Tokyo, Japan) was used for image acquisition.

The pathological damage of the ovaries and the uterine morphology of mice in each group were observed, and the thickness of endometrium was quantified.

ELISA

The collected blood of mice was allowed to clot for 60 minutes, after which the serum was collected. The levels of follicle-stimulating hormone (FSH, ml104030, mlbio, Shanghai, China), luteinizing hormone (LH, ml063366, mlbio), estradiol (E_2, ml103526, mlbio), testosterone (T, ml001948, mlbio), progesterone (P, ml103523, mlbio), fasting blood glucose (FBG, YT-H14575, Yueteng, Tianjin, China), fasting insulin (FINS, ml022831, mlbio), interleukin (IL)-1β (ml106733, mlbio), tumor necrosis factor-α (TNF-α, ml002095, mlbio), interferon-γ (IFN-γ, ml027464, mlbio), IL-10 (mIC50274-1, mlbio), IL-4 (ml064310, mlbio) in serum were measured by ELISA. A total of 90 μL of sample serum and 90 μL standard were added to the reaction hole, and 10 μL of horseradish peroxidase-labeled antibody was incubated. After mixing, the plate was covered with a sealing membrane and incubated for two h in the dark. Wash with detergent 3 times and dry on absorbent paper. 100 μL TMB substrate solution was added to each well. Then, the plate was sealed and incubated for 20 min. Add 50 μL stop solution, measure the OD value immediately after mixing, and calculate the corresponding concentration of the sample following the standard curve. HOMA-IR = (FBG (mmol/L) × FINS (μU/mL)) / 22.5.

qRT-PCR

TransZol (ET101-01-V2, TRANS, Beijing, China) was used to extract total RNA from the uterine tissues of each group. Using the TransScript® Green One-Step qRT-PCR SuperMix kit (AQ211-01, TRANS), RNA was reverse transcribed into cDNA and subjected to qRT-PCR amplification. The data were quantified by the $2^{-\Delta\Delta Ct}$ method. GAPDH served as an internal reference.

Primer sequence

IGFBP-1: F: GCCCAACAGAAAGCAGGAGATG; R: GTAGACACACCAGCAGAGTCCA; PRL: F: CTGGCTACACCTGAAGACAAGG; R: TCACTCGAGGACTGCACCAAAC; GAPDH: F: 5'-TGGCCTTCCGTGTTCCTAC-3'; R: 5'-GAGTTGCTGTTGAAGTCGCA-3'.

Immunohistochemistry (IHC)

After the paraffin section of the uterus was dewaxed and hydrated, the sodium citrate buffer (C1010, Solarbio) was employed for antigen retrieval for 25 min, and the membrane was blocked with 5% bovine serum albumin (SW3015, Solarbio) for 30 min. Cyclooxygenase 2 (COX-2, ab283574, abcam), integrin αvβ3 (bs-1310R, Bioss Antibodies, Woburn, MA, USA), and IGFBP-1 (13981-1-AP, proteintech, Wuhan, China) primary antibodies were added and incubated overnight at 4°C. HRP-labeled goat anti-rabbit IgG (GB23303, Servicebio) was added dropwise and incubated for 30 min. After DAB staining for 5 min, the tissue sections were put into distilled water to terminate the color development. Hematoxylin (G1004, Servicebio) was added dropwise and counterstained for 1 min, and the sections were mounted with neutral gum (WG10004160, Servicebio). The images were observed and collected under the microscope. The brown content in the tissue was the amount of protein expression.

Transmission electron microscope

Fresh uterine tissue was quickly fixed with electron microscope fixative (G1102, Servicebio) and placed at 4 °C. Then samples were post-fixed in 1% osmic acid following PBS rinsing at room temperature in the dark for two h. The tissue was dehydrated with gradient ethanol and then infiltrated overnight in a mixture of equal proportions (acetone: 812 embedding agent). The ultra-thin sections were prepared, and then double stained with 3% uranyl acetate-lead citrate. Finally, the ultrastructure of uterine tissue in each group was observed under a transmission electron microscope and photographed.

Immunofluorescence

Uterine tissue sections were subjected to dewaxing, protease K (P9460, Solarbio) incubation, sodium citrate buffer (C1010, Solarbio) repair antigen, and bovine serum albumin (SW3015, Solarbio) blocking. Vimentin (ab20346, abcam), cytokeratin 19 (CK19, ab52625, abcam), Microtubule-associated protein 1 light chain 3 (LC3, 14600-1-AP, proteintech, Wuhan, China) primary antibodies were added and incubated overnight at 4°C. Fluorescence-labeled goat anti-rabbit IgG (GB28301, Servicebio) was applied. After incubation for 45 min under dark conditions, DAPI was used to counterstain the nucleus after washing, and the fluorescent signal of the protein was observed under a fluorescence microscope. Three non-overlapping fields of vision were randomly selected from each uterine tissue section, and the fluorescence intensity was analyzed using Image J software.

Western blot (WB)

Appropriate amount of mouse endometrial tissue was minced and homogenized for total protein extraction. The BCA kit (PC0020, Solarbio) was used to detect the protein concentration of each group, and the protein was denatured at high temperature. The appropriate concentration of the gel was prepared.

After loading the samples, electrophoresis was performed. Subsequently, the gel was immersed in transfer buffer and the proteins were transferred onto a PVDF membrane at low temperature. At the end of the incubation, the membrane was blocked with a rapid blocking solution for 30 min. COX-2 (ab283574, abcam), integrin αvβ3 (bs-1310R, Bioss Antibodies), PRL (ab183968, abcam), IGFBP-1 (13981-1-AP, proteintech), bone morphogenetic

protein 2 (BMP2, ab284387, abcam), p62 (ab314504, abcam), Beclin-1 (ab302669, abcam), autophagy related 5 homolog (Atg5, ab109490, abcam), LC3 II (ab63817, abcam), PI3K (4292S, Cell Signaling, Danvers, MA, USA), p-PI3K (17366T, Cell Signaling), p-Akt (9271S, Cell Signaling), Akt (9272S, Cell Signaling), FoxO1 (9454S, Cell Signaling), p-FoxO1 (9461T, Cell Signaling) and β-actin (ab8224, abcam) primary antibodies were incubated overnight at 4°C. Then the corresponding secondary antibodies were incubated. After PBST washing, the membrane was developed using a light-emitting liquid (G2161, Servicebio), the gel imaging system (SCG-W1000, Servicebio) was used for imaging, and the protein gray value was analyzed by Image J software. Finally, the results were saved and analyzed.

The nucleoprotein was extracted from fresh endometrial tissue using a nuclear protein extraction kit (P0027, Beyotime), and then the protein expression levels of FoxO1 and Lamin B1 (ab229025, abcam) were quantified.

Statistical analysis

Statistical analysis used Prism software (Graphpad 9.0), with normally distributed data expressed as mean ± standard deviation. Multiple groups were compared using one-way ANOVA analysis. $P<0.05$ was considered statistically significant.

RESULTS

Quercetin improved ovarian pathological changes and endocrine and metabolic disorders in PCOS mice

From the 11th day to the 20th day of the establishment of the PCOS mouse model, the estrous cycle in mice was detected via vaginal smear, and the mice were in diestrus (D), indicating that the PCOS mouse model was successfully established (fig. S1). After that, the estrous cycle of mice was detected again 10 days before the end of the experiment.

The results of vaginal smear showed that the proestrus (P) was dominated by nucleated epithelial cells, the estrus (E) was dominated by keratinocytes, the metestrus (M) was dominated by keratinocytes and white blood cells and the D was dominated by white blood cells (fig.2A). Normal mice proestrus, estrus, metestrus, diestrus appear in order; PCOS mice lost the normal estrous cycle, long-term in the diestrus, lack of proestrus, which can be speculated that PCOS mice due to ovulation disorders affect the estrous cycle, so that sexual function decreased; after treatment with quercetin, the estrous cycle was improved and basically returned to normal levels, that is, ovulation function was restored; although the estrous cycle was also enhanced after metformin treatment, the recovery effect was not as good as that of quercetin (fig. 2B).

Simultaneously, HE staining of the ovaries showed that normal mice showed complete ovarian structure and PCOS mice showed bigger cystic follicles, thinner granular layer and theca cell layer and reduced luteal cells. Although quercetin and metformin treatment improved ovarian histomorphology, a substantial number of atretic follicles and cysts remained (fig.2C). Furthermore, while DHEA induction significantly increased body weight, ovarian weight, and ovarian index, both quercetin and metformin attenuated these increases (fig.2D-F). The levels of serum sex hormones were detected using ELISA. The results found that FSH, LH, FSH/LH, and T contents in PCOS mice markedly raised, E_2 and P contents obviously lessened, suggesting endocrine disorders in PCOS mice.

After treatment with quercetin and metformin, the endocrine disorders were improved, FSH, LH, FSH/LH, and T contents decreased, while E_2 and P contents increased (fig.2G-L). FBG, FINS, and HOMA-IR are essential indicators of insulin resistance. They were also notably elevated after DHEA induction and significantly decreased after treatment with quercetin and metformin (fig.2M-O). Quercetin and metformin had similar effects in improving endocrine and metabolic disorders, but metformin was superior to quercetin in reducing ovarian index, FSH, and FBG levels. This finding indicates that quercetin can improve DHEA-induced insulin resistance and improve metabolic abnormalities in mice.

Quercetin attenuated the inflammatory response in PCOS mice

DHEA can cause changes in the level of inflammatory factors and appear in an inflammatory state (Yu *et al.*, 2023). In this study, IL-1β, TNF-α and IFN-γ contents of PCOS mice significantly increased, the anti-inflammatory factors IL-10 and IL-4 significantly decreased. After treatment with quercetin and metformin, the pro-inflammatory factor contents notably reduced, the anti-inflammatory factor contents obviously elevated (fig. 3A-E). The balance between inflammatory factors improved, and the function of the immune system was also restored. However, the anti-inflammatory effect of metformin was slightly better than that of quercetin, particularly in terms of IL-4.

Quercetin improved endometrial receptivity and promoted stromal cell survival in PCOS mice

COX-2 and integrin αvβ3 are regulatory factors of endometrial receptivity, and their expression decreased in PCOS patients. The results of IHC and WB showed that the positive rate and protein level of COX-2 in PCOS mice endometrium notably elevated, the positive rate and protein level of integrin αvβ3 significantly decreased, suggesting that DHEA could reduce endometrial receptivity. Quercetin and metformin reduced COX-2 expression and increased integrin αvβ3 expression (fig.4A-F), indicating that quercetin could improve PCOS by improving endometrial receptivity, but the effect of quercetin on reducing COX-2 and increasing integrin αvβ3 is not as good as that of metformin.

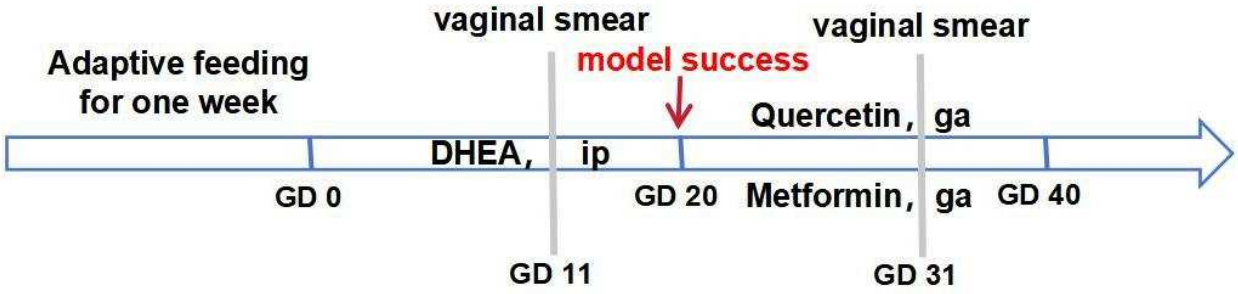


Fig. 1: Experimental process

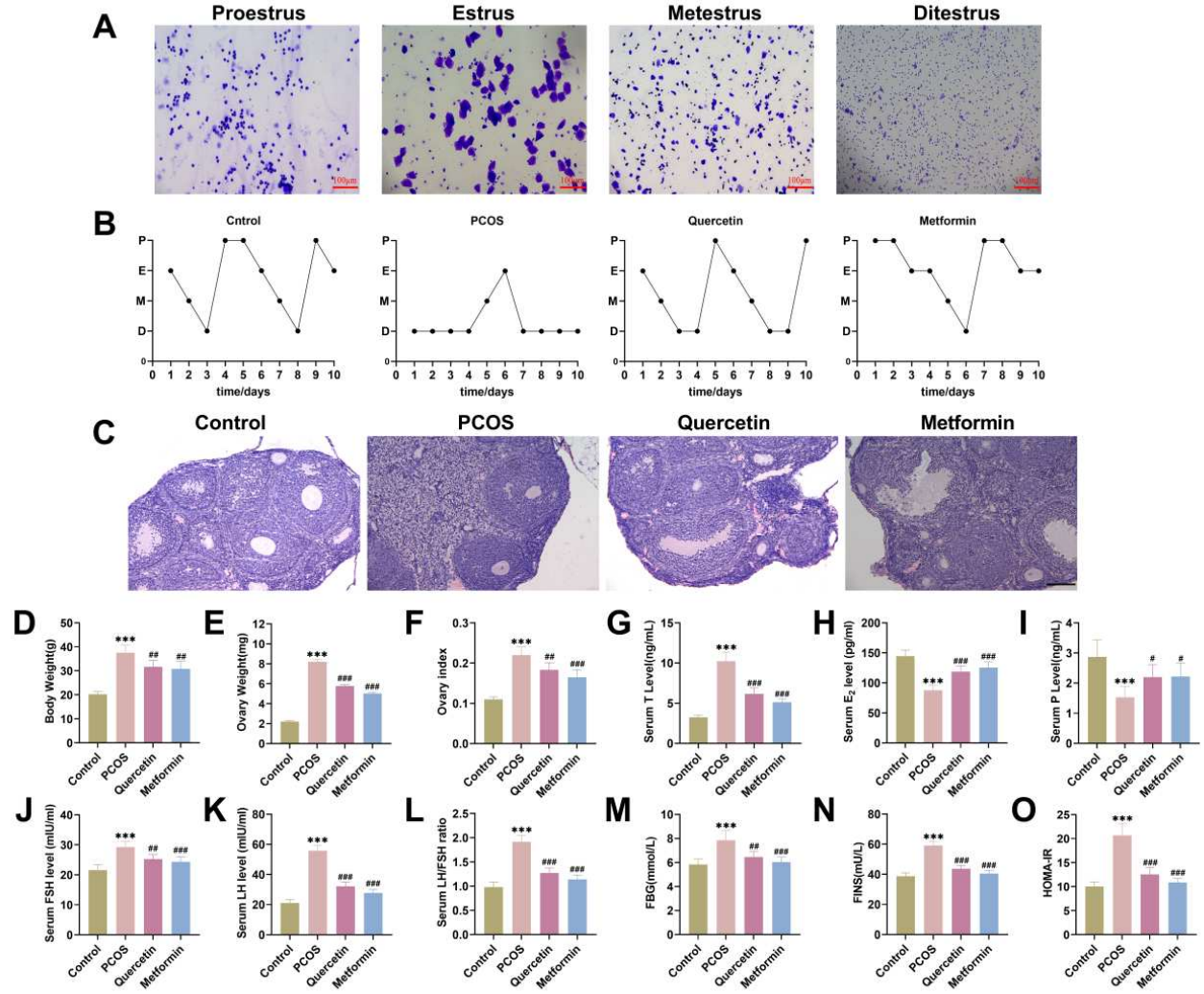


Fig. 2: Quercetin improved ovarian pathological changes and endocrine and metabolic disorders in PCOS mice

A-B: 10 days before the end of the experiment, the vaginal smear cytology was used to monitor the estrous cycle (×20, 100 μm). C: The pathological damage of the ovaries was observed by HE staining (×20, 100 μm). D-F: The body weight, ovarian weight, and ovarian index. G-L: The serum sex hormone (FSH, LH, FSH/LH, T, E_2, and P) levels were measured using ELISA.

M-O: The metabolic (FBG, FINS, and HOMA-IR) levels were measured using ELISA.

n=6, ***$P < 0.001$ vs Control group; #$P < 0.05$, ##$P < 0.01$, ###$P < 0.001$ vs PCOS group.

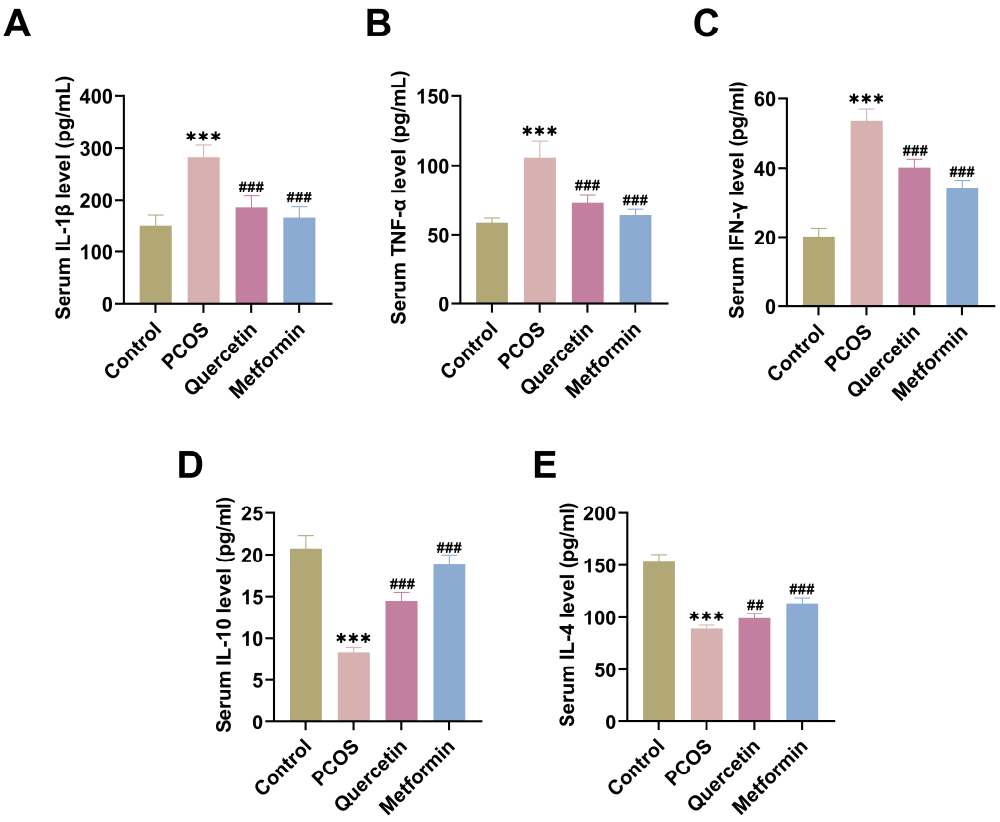


Fig. 3: Quercetin attenuated the inflammatory response in PCOS mice
A-C: ELISA discovered the contents of serum pro-inflammatory factors (IL-1β, TNF-α, and IFN-γ). D-E: ELISA discovered the contents of serum anti-inflammatory factors (IL-10 and IL-4). n=6, ***$P < 0.001$ vs Control group; ##$P < 0.01$, ###$P < 0.001$ vs PCOS group.

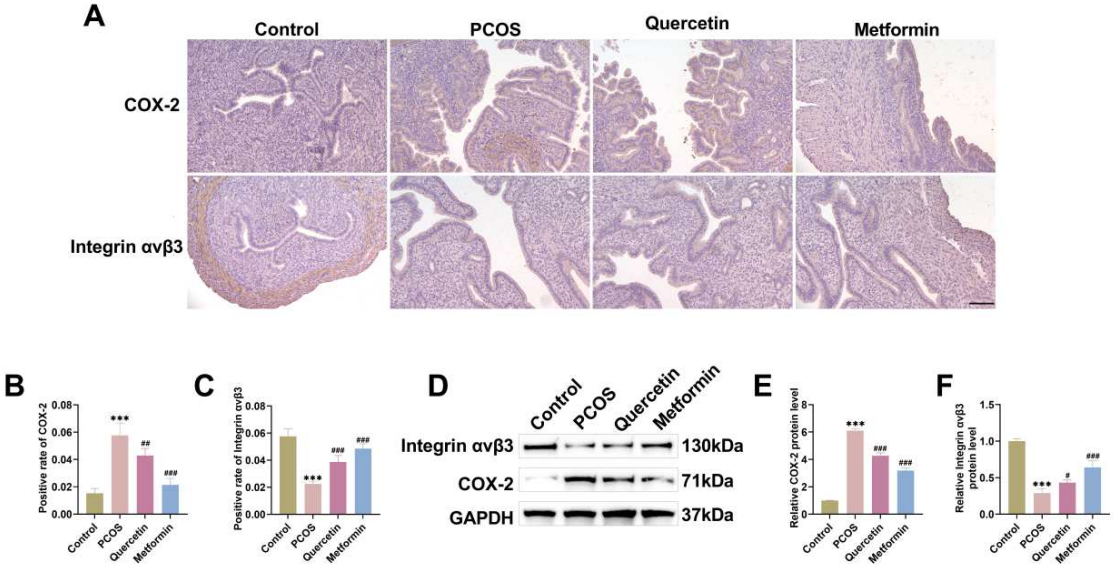


Fig. 4: Quercetin improved endometrial receptivity in PCOS mice
A-C: IHC detected the levels of COX-2 and integrin αvβ3 (×20, 100 μm). D-F: WB detected COX-2 and integrin αvβ3 levels. n=6, ***$P < 0.001$ vs Control group; #$P < 0.05$, ##$P < 0.01$, ###$P < 0.001$ vs PCOS group.

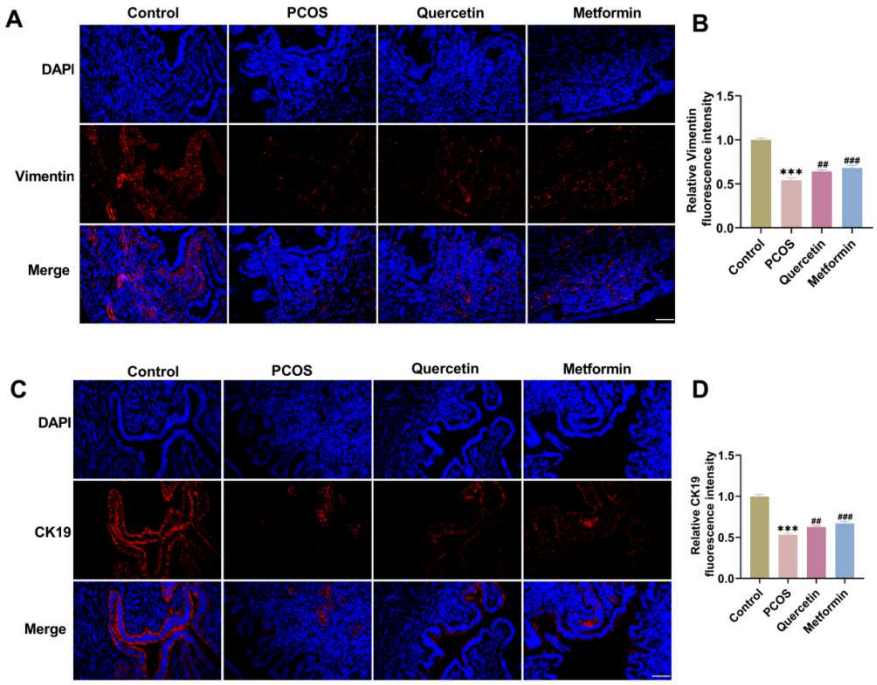


Fig. 5: Quercetin promoted stromal cell survival in PCOS mice
A-D: Immunofluorescence detected the levels of Vimentin and CK19 (×40, 50 μm).
n=6, ***$P < 0.001$ vs Control group; ##$P < 0.01$, ###$P < 0.001$ vs PCOS group

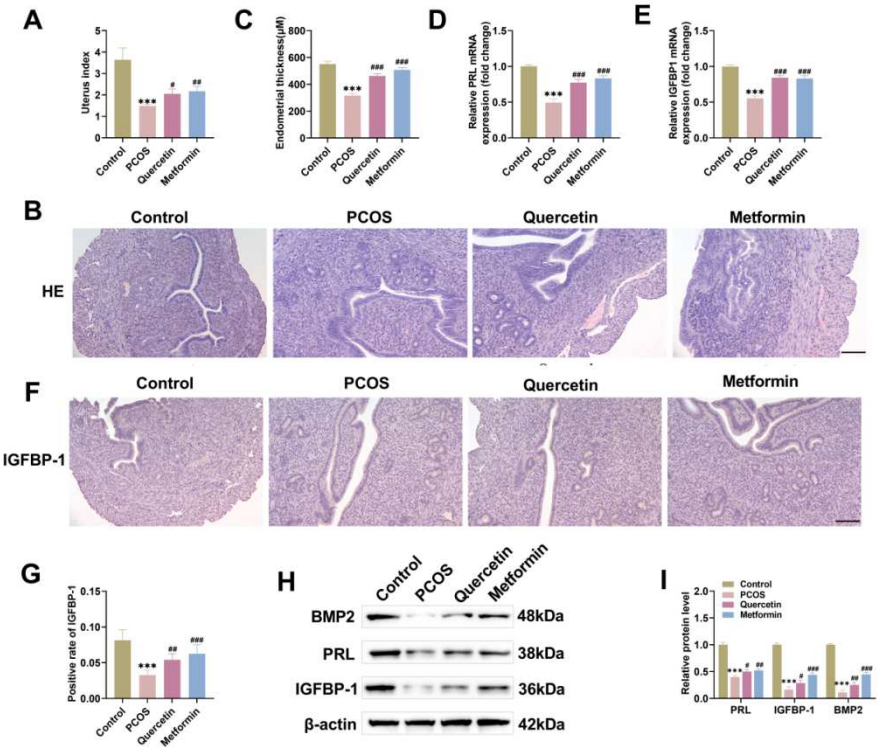
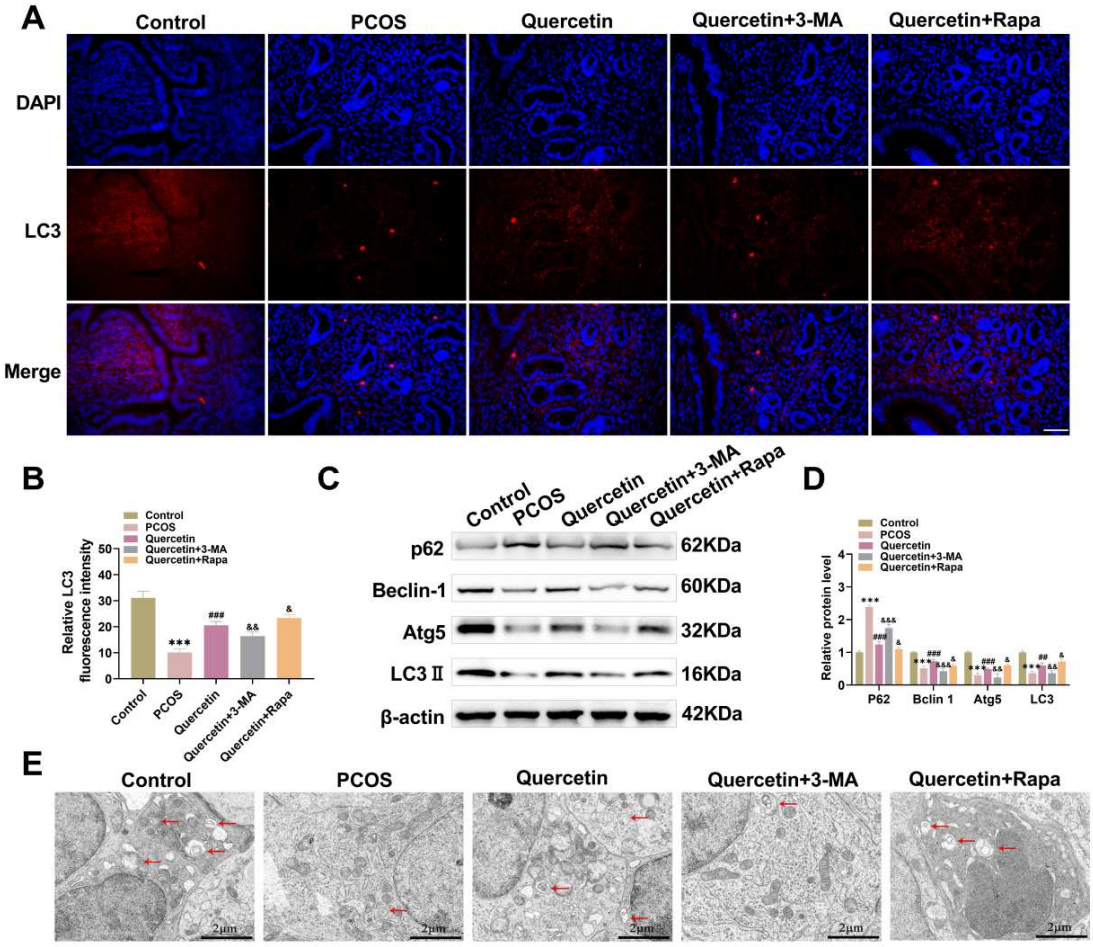


Fig. 6: Quercetin improved endometrial decidualization disorder in PCOS mice
A: The uterine index of mice. B-C: HE staining observed the uterine morphology of mice in each group (×20, 100 μm).
D-E: IGFBP-1 and PRL levels were detected using qRT-PCR. F-G: IHC detected the level of IGFBP-1 in endometrial tissue (×20, 100 μm). H-I: WB detected the levels of endometrial decidualization marker proteins (IGFBP-1, PRL, and BMP2).
n=6, ***$P < 0.001$ vs Control group; #$P < 0.05$, ##$P < 0.01$, ###$P < 0.001$ vs PCOS group.



A-B: Immunofluorescence detected LC3 level in endometrial tissue (×40, 50 μm).
C-D: WB detected the levels of autophagy proteins (P62, Beclin-1, Atg5, and LC3 II) in endometrial tissue.
E: The transmission electron microscope observed the ultrastructure of endometrium (×7.0k, 2 μm).
n=6, ***$P < 0.001$ vs Control group; ###$P < 0.001$ vs PCOS group; &$P < 0.05$, &&$P < 0.01$ vs Quercetin group.
Fig. 7: Quercetin enhanced the level of endometrial autophagy in PCOS mice

Vimentin and CK19 were markers of endometrial stromal cells and epithelial cells, respectively. The fluorescence intensity of Vimentin and CK19 markedly decreased in PCOS endometrium compared to controls, indicating a loss of stromal and epithelial cells. Quercetin therapy could significantly increase the fluorescence intensity of Vimentin and CK19 (fig.5A-D), suggesting that impaired endometrial development in PCOS mice, characterized by this cell loss, underlies the associated morphological defects and reduced receptivity. Quercetin and metformin can improve this, and the improvement effect of metformin was better than that of quercetin. The above results indicated that quercetin improved endometrial receptivity and promoted the survival of stromal cells, thereby improving the reproductive function of PCOS mice, restoring normal pregnancy and avoiding abortion.

Quercetin improved endometrial decidualization disorder in PCOS mice

The uterine index of PCOS mice were significantly increased after DHEA induction and significantly decreased after treatment with quercetin and metformin (fig. 6A). Concurrently, HE staining found that the endometrial structure of normal mice was complete, the level boundary was clear, the columnar epithelial cells were distributed throughout the uterine cavity and glandular cavity and the glands were located in the stroma, oval or round, occasionally in clusters, with normal morphology and clear blood vessels. The endometrial structure of PCOS mice was utterly destroyed, the boundary with the muscular layer was blurred, the epithelial cells were abnormal and scattered, the number of glands and blood vessels reduced, the interstitial edema observed, many inflammatory cells infiltration found and the endometrium was significantly thinner.

After treatment with quercetin and metformin, the endometrial damage of mice was improved, the boundary with the muscular layer was gradually clear, the gland morphology returned to normal, the interstitial edema and

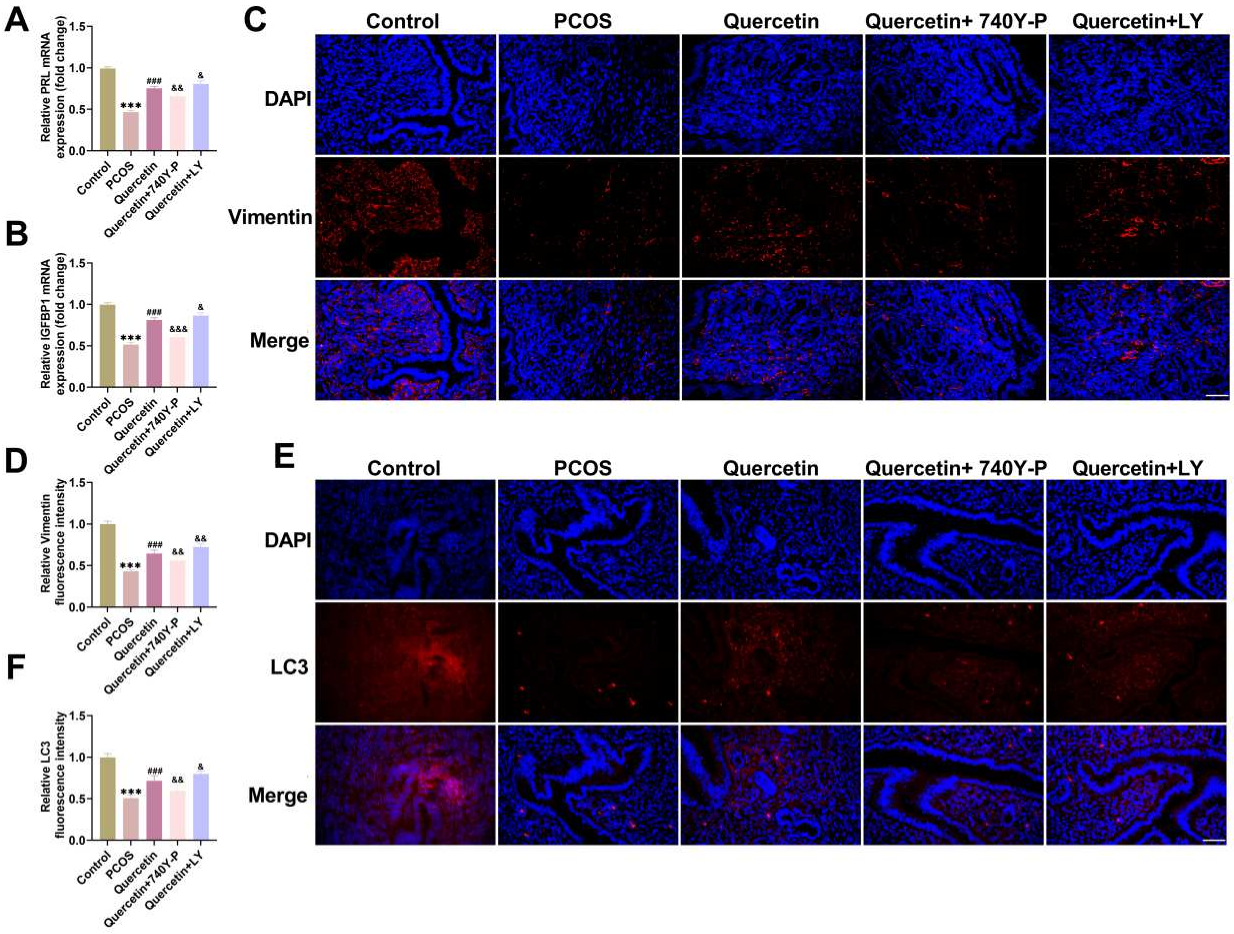


Fig. 8: Quercetin enhanced autophagy to protect the survival and decidualization of endometrial stromal cells in PCOS mice

A-B: qRT-PCR identified IGFBP1 and PRL mRNA levels.

C-F: Immunofluorescence detected the expression of Vimentin and LC3 in endometrial tissue (×40, 50 μm).

n=6, ***$P < 0.001$ vs Control group; ###$P < 0.001$ vs PCOS group; &$P < 0.05$, &&$P < 0.01$, &&&$P < 0.001$ vs Quercetin group.

inflammatory cell infiltration were reduced, and the endometrial thickness was increased (fig.6B-C). The improvement effect of metformin was better than that of quercetin. IGFBP-1 and PRL were endometrial decidualization markers. They were decreased in PCOS mice, while quercetin could increase IGFBP-1 and PRL mRNA expression (fig.6D-E). They were consistent with the previous narrative. The brown positive rate and protein level of IGFBP-1 in endometrial tissue of PCOS mice lessened, PRL and BMP2 protein contents were also significantly decreased (fig.6F-I), but after treatment with quercetin and metformin, IGFBP-1, PRL, and BMP2 expression raised considerably. Metformin had a better effect on improving endometrial decidualization disorder in PCOS mice. Finally, quercetin promoted the formation of uterine blood vessels and endometrium, improved uterine pathological damage and endometrial decidualization disorder. This therapy was administered to restore the normal physiological state of endometrium.

Quercetin enhanced the level of endometrial autophagy in PCOS mice

Normal autophagy level can regulate endometrial function. We used the autophagy inhibitor 3-MA and the autophagy inducer Rapa to intervene in PCOS mice. LC3 is a standard autophagy marker. Through immunofluorescence, the fluorescence intensity of LC3 of PCOS mice notably lessened, it significantly elevated after quercetin treatment. Compared with quercetin treatment, LC3 level was significantly decreased after the application of 3-MA and increased dramatically after Rapa intervention (fig.7A-B).

WB detected the autophagic flux to detect the expression of autophagy-related proteins. P62 protein level was significantly increased in PCOS mice, Beclin-1, Atg5, and LC3 II levels were markedly lowered. The levels of autophagy protein obviously reversed after treatment with quercetin; compared with quercetin therapy, 3-MA further inhibited the autophagy level, lessened P62 expression,

increased Beclin-1, Atg5, and LC3 II levels, while Rapa promoted the level of autophagy (fig. 7C-D). In addition, this study also observed that the number of autophagic vesicles in PCOS mice decreased by transmission electron microscopy, and the number of autophagic vesicles increased after quercetin intervention, indicating that quercetin could restore the impaired autophagic flux caused by PCOS. However, after co-treatment with 3-MA, the accumulation of autophagic vesicles induced by quercetin was significantly reduced, while Rapa further promoted the accumulation of autophagic vesicles (fig.7E). In conclusion, quercetin could improve the endometrial environment of PCOS by restoring autophagic flux and enhancing autophagy.

Quercetin enhanced autophagy to protect the survival and decidualization of endometrial stromal cells in PCOS mice via regulating the PI3K/Akt/FoxO1 pathway

The PI3K/Akt/FoxO1 pathway is an essential upstream regulatory mechanism of autophagy. We used PI3K inhibitor LY294002 and inducer 740Y-P to intervene in PCOS mice. Consistent with previous findings, the levels of IGFBP1, PRL, Vimentin, and LC3 were decreased in PCOS mice, while quercetin treatment markedly raised them. Compared with quercetin treatment, 740Y-P decreased the levels of IGFBP1, PRL, Vimentin, and LC3, while LY294002 increased the levels of IGFBP1, PRL, Vimentin, and LC3 (fig.8A-F). Hence, the PI3K/Akt/FoxO1 axis was vital for the survival and decidualization of endometrial stromal cells. p-PI3K, p-Akt, and p-FoxO1 proteins were markedly increased in PCOS mice and significantly decreased after quercetin intervention. On this basis, the PI3K/Akt/FoxO1 proteins significantly increased after 740Y-P intervention and decreased considerably after LY294002 intervention (fig. 9A-B), indicating that quercetin regulated the PI3K/Akt/FoxO1 axis. FoxO1 nuclear exclusion is due to FoxO1 phosphorylation mediated by PI3K/Akt pathway activation. The results showed that the level of p-FoxO1 protein in the cytoplasm was consistent with that detected previously. The level of p-FoxO1 protein decreased significantly after quercetin and LY294002 intervention and increased considerably after 740Y-P intervention. The trend of FoxO1 protein level in the nucleus was opposite to that of p-FoxO1 protein level (fig.9C-E). Quercetin can inhibit the PI3K/Akt/FoxO1 pathway and enhance autophagy to protect the survival and decidualization of endometrial stromal cells.

DISCUSSION

PCOS is a heterogeneous reproductive disease, mainly based on ovarian morphological changes. Hyperandrogenism caused by PCOS can reduce estrogen and progesterone in the patient's body. It can also increase the secretion of androgen through direct or indirect channels, thereby interfering with follicular development, causing premature follicular atresia or anovulation and ultimately making it difficult for the patient to conceive, even if the pregnancy is also prone to abortion (Siddiqui *et al.*, 2022). Obesity can aggravate hyperandrogenism and insulin resistance in PCOS patients, which are also the primary defects of PCOS (Wang *et al.*, 2019). In this study, the PCOS model was induced with DHEA, and the decidualization was artificially induced, disrupting the estrous cycle in mice. Most of them were in the diestrus, and the body weight, ovarian weight, and index increased. The ovarian tissue showed bigger cystic follicles and thinner granular layer and theca cell layer, suggesting that there was ovarian ovulation dysfunction, indicating that the model was successfully constructed. This study also found that PCOS mice serum sex hormones FSH, LH, FSH/LH, and T levels increased, E_2 and P levels decreased, FBG, FINS, and HOMA-IR levels increased, indicating that PCOS mice serum sex hormones and metabolic disorders, hyperandrogenism, and insulin resistance. After treatment with quercetin, the estrous cycle of mice recovered, the body weight decreased, and the histomorphological characteristics, sex hormones, and metabolic levels of the ovaries improved. Quercetin has a practical therapeutic effect on PCOS and is vital in preventing abortion caused by PCOS.

The pathogenesis of PCOS and its complications is closely related to inflammation (Shamsi *et al.*, 2022; Wang *et al.*, 2023), and the inflammatory state can be directly reflected by inflammatory factor levels. The imbalance between pro-inflammatory and anti-inflammatory factors can damage ovarian function. Therefore, balancing inflammatory factors is key for maintaining ovarian function (Shamsi et al., 2022). TNF-α, IL-6, and IFN-γ of PCOS patients raised (Kumariya *et al.*, 2021; Qin *et al.*, 2016). The level of sex hormones is affected by IL-1β, which is too high to cause sex hormone disorders, thus hindering the normal growth and development of follicles (Orisaka *et al.*, 2023). In contrast, IL-10 and IL-4 were decreased in PCOS patients (Dantas *et al.*, 2019). IL-10 inhibited other cytokine synthesis in T cells, including IL-6. IL-10 maintains pregnancy through progesterone synthesis and luteal maturation and is anti-inflammatory for PCOS. Echoing the previous studies, this research also showed that the pro-inflammatory factors IL-1β, TNF-α, and IFN-γ contents of PCOS mice increased, the anti-inflammatory factors IL-10 and IL-4 levels decreased, and the inflammatory factors were imbalanced. Quercetin can inhibit pro-inflammatory factor expression, promote anti-inflammatory factor expression, restore cytokine balance, thereby treating PCOS.

The endometrial thickness of PCOS patients obviously thinned in clinical practice. Moreover, the high abortion rate of PCOS is correlated with poor endometrial receptivity (Yang *et al.*, 2021), and the expression of endometrial receptivity regulatory factors, including COX-

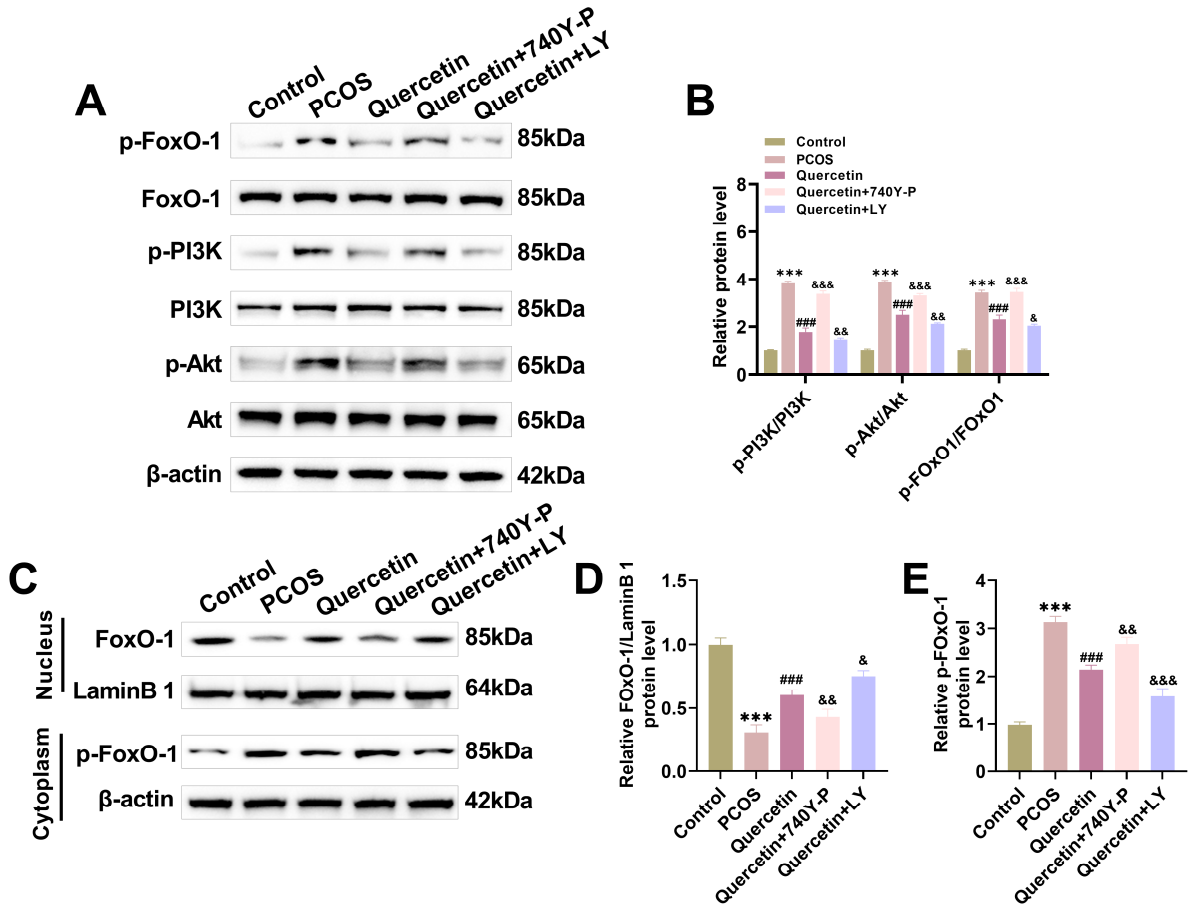


Fig. 9: Quercetin regulated PI3K/Akt/FoxO1 pathway

A-B: WB detected the expression of pathway-related proteins (PI3K, p-PI3K, Akt, p-Akt, FoxO1, and p-FoxO1). C-E: WB detected the expressions of FoxO1 protein in the nucleus and p-FoxO1 in the cytoplasm.

n=6, ***$P < 0.001$ vs Control group; ###$P < 0.001$ vs PCOS group; &$P < 0.05$, &&$P < 0.01$, &&&$P < 0.001$ vs Quercetin group.

2 and integrin αvβ3, is reduced. Endometrial receptivity is essential for successful embryo implantation in mammals (Dong *et al.*, 2022). To achieve successful implantation, the endometrium will undergo morphological and structural changes according to physiological characteristics, including endometrial stromal cell proliferation and epithelial cell differentiation, which will eventually be transformed into a state that can accept embryo adhesion under the synergistic effect of estrogen and progesterone, making embryo adhesion and successful implantation possible (Shekibi *et al.*, 2022). Vimentin is mainly expressed in stromal cells' cytoplasm, its expression level reflects the growth status in endometrial stromal cells.

CK19 is expressed primarily in endometrial glandular epithelium and luminal epithelial cells, which can effectively maintain the morphological integrity of epithelial cells, and its expression level reflects the growth of endometrial epithelial cells (Bardag-Gorce *et al.*, 2018). Decidualization is closely related to endometrial receptivity. Abnormal decidualization can lead to embryo implantation failure or adverse pregnancy outcomes, such as abortion, premature delivery, etc. For humans, during decidualization, endometrial fibroblast transform into secretory cells and secrete high levels of PRL and IGFBP-1 (Ng *et al.*, 2020; Okada *et al.*, 2018). IGFBP-1 and PRL are unique biological indicators of decidualization. IGFBP-1 can regulate the role of insulin-like growth factor, mobilize trophoblast cells' growth, invasion, and the differentiation of endometrium; pRL can also restrict the growth and invasion of trophoblasts, promote the formation of new blood vessels, regulate immunity, control fluid exchange between mother and fetus, and inhibit P catabolism (Ezoe *et al.*, 2021; Rana *et al.*, 2022). In this research, COX-2 content in PCOS mice's endometrium markedly elevated, and the levels of integrin αvβ3, Vimentin, CK19, IGFBP-1, and PRL significantly decreased. The endometrial structure was destroyed, the epithelial cells were abnormal and scattered, the number of glands and blood vessels was reduced, and the endometrium was considerably thinner. After treatment with quercetin, the endometrial damage improved, the endometrial thickness increased, COX-2 level lessened, integrin αvβ3, Vimentin, CK19, IGFBP-1, and PRL levels

increased, indicating that quercetin can improve uterine pathological damage, promote endometrial thickening, improve endometrial receptivity and decidualization disorders, promote stromal cell survival and thus effectively promote endometrial repair.

Autophagy is an intricate metabolic pathway governed by various genes. Beclin-1 and LC3 are autophagy markers involved in autophagosome formation and maturation. Atg5, as one of the essential autophagy-related regulatory proteins, can participate in autophagosome formation. P62 is a negative regulator. P62 can promote autophagy degradation through ubiquitination. Therefore, Beclin-1, LC3, Atg5, and P62 levels serve as an evaluation of autophagy activity. Studies have shown that autophagy is crucial for preserving endometrial balance and is involved in early pregnancy and decidualization (Su *et al.*, 2020). After intervention with autophagy inhibitors, the expression of decidualization markers and progesterone receptors decreased significantly, and endometrial decidualization was impaired (Su et al., 2020), indicating that autophagy is involved in the occurrence of endometrial decidualization in early pregnancy. If the level of autophagy abnormally reduced during implantation, it will not be conducive to the formation of decidualization, which will affect embryo implantation and lead to abortion. The autophagy of endometrial cells is regulated by ovarian hormones. The study found that the serum free androgen index of PCOS patients was negatively related to endometrial autophagy level, suggesting that the increase of androgen utilization rate in PCOS patients would down-regulate the level of endometrial autophagy (Sumarac-Dumanovic *et al.*, 2017). It is speculated that this is a key factor in the reduction of endometrial receptivity in PCOS patients. The abnormal autophagy in PCOS patients' endometrium can harm the endometrial receptivity, participate in embryo implantation failure, and early abortion occurrence. Therefore, this study further explored the impact of autophagy on the uterus and whether quercetin could affect PCOS mice by regulating autophagy. Autophagy was induced by intraperitoneal injection of Rapa and inhibited by 3-MA. Rapa is a lipophilic macrolide antibiotic that promotes autophagy by inhibiting mTOR activity (Sato *et al.*, 2019). 3-MA is an autophagy inhibitor, which inhibits the formation of autophagosomes membrane via inhibiting PI3K activity, thereby inhibiting the occurrence of autophagy (Dikic and Elazar, 2018). Studies have shown that Rapa or 3-MA can regulate the levels of autophagy in the endometrium (Zhang *et al.*, 2021).

This experiment found that P62 expression in the endometrial tissue of PCOS elevated, Beclin-1, LC3, Atg5, and LC3 II expression decreased, and the number of autophagic vesicles also decreased, indicating that inhibition of autophagy. Quercetin could enhance the level of autophagy in the endometrium, the autophagy protein levels obviously reversed, and the number of autophagic vesicles increased. After 3-MA application, autophagy level was significantly inhibited, while autophagy was elevated considerably after the application of Rapa, indicating that quercetin can improve the endometrial environment of PCOS by enhancing autophagy. At the same time, this study also found that quercetin can reduce p-PI3K, p-Akt and p-FoxO1 protein and increase the level of nuclear FoxO1 protein; however, after PI3K inhibitor LY294002 application, LC3, Vimentin, IGFBP-1 and PRL protein in the uterus of mice increased, that is, the intervention improved autophagy, endometrial stromal cell survival and decidualization disorders; in contrast, the application of PI3K inducer 740Y-P inhibited autophagy, endometrial stromal cell survival and decidualization. In conclusion, quercetin can enhance autophagy to protect decidualization and endometrial stromal cell survival in PCOS mice, which is involved with the PI3K/Akt/FoxO1 axis.

CONCLUSION

Quercetin can restore the estrous cycle of PCOS rats, regulate serum sex hormone levels, correct blood lipid disorders, significantly improve the pathological changes of ovary and uterus, reduce the level of inflammation, regulate the PI3K/Akt/FoxO1 pathway to enhance autophagy and improve endometrial stromal cells and decidualization (Fig.10). Quercetin has a specific effect on PCOS. The results suggest that quercetin is expected to be a therapeutic drug to improve the adverse pregnancy outcomes (abortion, etc.) of PCOS patients in the future and provide a theoretical basis for clinical PCOS treatment. Unfortunately, this study only preliminarily explored the mechanism of quercetin improving PCOS through the PI3K/Akt/FoxO1 pathway and did not further study the specific mechanism. We will consider using in vivo experiments, network pharmacology, and other methods to further explore.

Acknowledgement
Not applicable.

Authors' contributions
[Jinglu Yu, Xiaoling Feng]: Developed and planned the study, performed experiments and interpreted results. Edited and refined the manuscript with a focus on critical intellectual contributions.
[Wei Jiang, Ying Huang, Miao Sun]: Participated in collecting, assessing and interpreting the date. Made significant contributions to date interpretation and manuscript preparation.
[Yongwei Du, Ge Yu]: Provided substantial intellectual input during the drafting and revision of the manuscript.

Funding
BeiJing Heart To Heart Foundation (HXXT2022ktyj002).
National Natural Science Foundation of China (82174195)
The Scientific Research Project of Traditional Chinese

Medicine of Heilongjiang Province (ZHY2024-258)
National Natural Science Foundation of China Cultivation and Support Program of the First Affiliated Hospital of Heilongjiang University of Chinese Medicine (PYQN202501007)

Data availability statement

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Ethical approval

This study was approved by First Affiliated Hospital, Heilongjiang University of Chinese Medicine Committee (Approval No. HZYLLKY201800601).

Conflict of interest

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

Consent to publish

The manuscript has neither been previously published nor is under consideration by any other journal. The authors have all approved the content of the paper.

Supplementary data

https://www.pjps.pk/uploads/2025/11/SUP1763121129.pdf

REFERENCES

Ashary N, Laheri S and Modi D (2020). Homeobox genes in endometrium: From development to decidualization. *Int. J. Dev. Biol.,* **64**(1-2-3): 227-237.

Bardag-Gorce F, Makalinao A, Meepe I, Hoft R H, Cortez D, Oliva J, Laporte A, Stark J, Gorce A, Di Lorenzo M, French S W, Lungo W and Niihara Y (2018). Corneal keratin aggresome (CKAGG) formation and clearance by proteasome activation. *Heliyon,* **4**(12): e01012.

Batiha GE, Beshbishy AM, Ikram M, Mulla ZS, El-Hack MEA, Taha AE, Algammal AM and Elewa YHA (2020). The pharmacological activity, biochemical properties and pharmacokinetics of the major natural polyphenolic flavonoid: Quercetin. *Foods,* **9**(3): 374.

Cai M, Li Q, Cao Y, Huang Y, Yao H, Zhao C, Wang J and Zhu H (2024). Quercetin activates autophagy to protect rats ovarian granulosa cells from H(2)O(2)-induced aging and injury. *Eur. J. Pharmacol.,* **966**: 176339.

Calissi G, Lam E W and Link W (2021). Therapeutic strategies targeting FOXO transcription factors. *Nat. Rev. Drug Discov.,* **20**(1): 21-38.

Chen T, Jia F, Yu Y, Zhang W, Wang C, Zhu S, Zhang N and Liu X (2022). Potential role of quercetin in polycystic ovary syndrome and its complications: A review. *Molecules,* **27**(14): 4476.

Dantas WS, Neves WD, Gil S, Barcellos CRG, Rocha MP, de Sá-Pinto AL, Roschel H and Gualano B (2019). Exercise-induced anti-inflammatory effects in overweight/obese women with polycystic ovary syndrome. *Cytokine,* **120**: 66-70.

Di Emidio G, Rea F, Placidi M, Rossi G, Cocciolone D, Virmani A, Macchiarelli G, Palmerini MG, D'Alessandro AM, Artini PG and Tatone C (2020). Regulatory functions of L-carnitine, acetyl and propionyl L-carnitine in a PCOS mouse model: Focus on antioxidant/antiglycative molecular pathways in the ovarian microenvironment. *Antioxidants (Basel),* **9**(9): 867.

Dikic I and Elazar Z (2018). Mechanism and medical implications of mammalian autophagy. *Nat. Rev. Mol. Cell Biol.,* **19**(6): 349-364.

Dong G, Sun R, Zhang R, Qin Y, Lu C, Wang X, Xia Y and Du G (2022). Preimplantation triclosan exposure alters uterine receptivity through affecting tight junction protein. *Biol. Reprod.,* **107**(1): 349-357.

Ezoe K, Miki T, Ohata K, Fujiwara N, Yabuuchi A, Kobayashi T and Kato K (2021). Prolactin receptor expression and its role in trophoblast outgrowth in human embryos. *Reprod. Biomed. Online,* **42**(4): 699-707.

Fernandez RC, Moore VM, Rumbold AR, Whitrow MJ, Avery JC and Davies MJ (2021). Diagnosis delayed: health profile differences between women with undiagnosed polycystic ovary syndrome and those with a clinical diagnosis by age 35 years. *Hum. Reprod.,* **36**(8): 2275-2284.

Hong L, Xiao S, Diao L, Lian R, Chen C, Zeng Y and Liu S (2024). Decreased AMPK/SIRT1/PDK4 induced by androgen excess inhibits human endometrial stromal cell decidualization in PCOS. *Cell Mol. Life Sci.,* **81**(1): 324.

Ji C, Xu W, Zhang Z, Cui S and Yi W (2021). Effect of electroacupuncture on reproductive disorders and insulin resistance in a murine polycystic ovary syndrome model. *Evid. Based Complement Alternat. Med.,* **2021**: 9968463.

Jian X, Shi C, Luo W, Zhou L, Jiang L and Liu K (2024). Therapeutic effects and molecular mechanisms of quercetin in gynecological disorders. *Biomed. Pharmacother.,* **173**: 116418.

Jozaki K, Tamura I, Takagi H, Shirafuta Y, Mihara Y, Shinagawa M, Maekawa R, Taketani T, Asada H, Sato S, Tamura H and Sugino N (2019). Glucose regulates the histone acetylation of gene promoters in decidualizing stromal cells. *Reproduction,* **157**(5): 457-464.

Kirkin V (2020). History of the selective autophagy research: How did it begin and where does it stand today? *J. Mol. Biol.,* **432**(1): 3-27.

Kumariya S, Ubba V, Jha RK and Gayen JR (2021). Autophagy in ovary and polycystic ovary syndrome: Role, dispute and future perspective. *Autophagy,* **17**(10): 2706-2733.

Liu M, Guo S, Li X, Tian Y, Yu Y, Tang L, Sun Q, Zhang T, Fan M, Zhang L, Xu Y, An J, Gao X, Han L and Zhang L (2024). Semaglutide alleviates ovary inflammation via the AMPK/SIRT1/NF-κB signaling pathway in

polycystic ovary syndrome mice. *Drug Des. Devel. Ther.,* **18**: 3925-3938.

Ma C, Xiang Q, Song G and Wang X (2022). Quercetin and polycystic ovary syndrome. *Front Pharmacol.,* **13**: 1006678.

Ng SW, Norwitz GA, Pavlicev M, Tilburgs T, Simón C and Norwitz ER (2020). Endometrial decidualization: The Primary Driver of Pregnancy Health. *Int. J. Mol. Sci.,* **21**(11): 4092.

Okada H, Tsuzuki T and Murata H (2018). Decidualization of the human endometrium. *Reprod. Med. Biol.,* **17**(3): 220-227.

Orisaka M, Mizutani T, Miyazaki Y, Shirafuji A, Tamamura C, Fujita M, Tsuyoshi H and Yoshida Y (2023). Chronic low-grade inflammation and ovarian dysfunction in women with polycystic ovarian syndrome, endometriosis and aging. *Front Endocrinol. (Lausanne),* **14**: 1324429.

Peng Q, Chen X, Liang X, Ouyang J, Wang Q, Ren S, Xie H, Wang C, Sun Y, Wu X, Liu H, Hei C, Sun M, Chang Q, Liu X, Li G and He R (2023). Metformin improves polycystic ovary syndrome in mice by inhibiting ovarian ferroptosis. *Front Endocrinol. (Lausanne),* **14**: 1070264.

Qin L, Xu W, Li X, Meng W, Hu L, Luo Z, Wang Y, Luo S and Li S (2016). Differential expression profile of immunological cytokines in local ovary in patients with polycystic ovarian syndrome: Analysis by flow cytometry. *Eur. J. Obstet. Gynecol. Reprod. Biol.,* **197**: 136-141.

Rana M, Jain S and Choubey P (2022). Prolactin and its significance in the placenta. *Hormones (Athens),* **21**(2): 209-219.

Sato M, Seki T, Konno A, Hirai H, Kurauchi Y, Hisatsune A and Katsuki H (2019). Rapamycin activates mammalian microautophagy. *J. Pharmacol. Sci.,* **140**(2): 201-204.

Shamsi M, Ghazavi A, Saeedifar AM, Mosayebi G, Pour SK and Ganji A (2022). The immune system's role in PCOS. *Mol. Biol. Rep.,* **49**(11): 10689-10702.

Shekibi M, Heng S and Nie G (2022). MicroRNAs in the Regulation of endometrial receptivity for embryo implantation. *Int. J. Mol. Sci.,* **23**(11): 6210.

Shi Q, Wang D, Ding X, Yang X and Zhang Y (2021). Exosome-shuttled miR-7162-3p from human umbilical cord derived mesenchymal stem cells repair endometrial stromal cell injury by restricting APOL6. *Arch. Biochem. Biophys.,* **707**: 108887.

Siddiqui S, Mateen S, Ahmad R and Moin S (2022). A brief insight into the etiology, genetics and immunology of polycystic ovarian syndrome (PCOS). *J. Assist. Reprod. Genet.,* **39**(11): 2439-2473.

Singh S, Pal N, Shubham S, Sarma DK, Verma V, Marotta F and Kumar M (2023). Polycystic ovary syndrome: Etiology, current management and future therapeutics. *J. Clin. Med.,* **12**(4): 1454.

Song L, Yu J, Zhang D, Li X, Chen L, Cai Z and Yu C (2022). Androgen excess induced mitochondrialabnormality in ovarian granulosa cells in a rat model of polycystic ovary syndrome. *Front Endocrinol. (Lausanne),* **13**: 789008.

Su Y, Zhang JJ, He JL, Liu XQ, Chen XM, Ding YB, Tong C, Peng C, Geng YQ, Wang YX and Gao RF (2020). Endometrial autophagy is essential for embryo implantation during early pregnancy. *J. Mol. Med. (Berl),* **98**(4): 555-567.

Sumarac-Dumanovic M, Apostolovic M, Janjetovic K, Jeremic D, Popadic D, Ljubic A, Micic J, Dukanac-Stamenkovic J, Tubic A, Stevanovic D, Micic D and Trajkovic V (2017). Downregulation of autophagy gene expression in endometria from women with polycystic ovary syndrome. *Mol. Cell Endocrinol.,* **440**: 116-124.

Tay CT, Garrad R, Mousa A, Bahri M, Joham A and Teede H (2023). Polycystic ovary syndrome (PCOS): international collaboration to translate evidence and guide future research. *J. Endocrinol.,* **257**(3): e220232.

Tong C, Wu Y, Zhang L and Yu Y (2022). Insulin resistance, autophagy and apoptosis in patients with polycystic ovary syndrome: Association with PI3K signaling pathway. *Front Endocrinol. (Lausanne),* **13**: 1091147.

Walter K (2022). What Is polycystic ovary syndrome? *JAMA,* **327**(3): 294.

Wang J, Wu D, Guo H and Li M (2019). Hyperandrogenemia and insulin resistance: The chief culprit of polycystic ovary syndrome. *Life Sci.,* **236**: 116940.

Wang J, Yin T and Liu S (2023). Dysregulation of immune response in PCOS organ system. *Front Immunol.,* **14**: 1169232.

Wu H, Zhao B, Yao Q and Kang J (2023). Dehydroepiandrosterone-induced polycystic ovary syndrome mouse model requires continous treatments to maintain reproductive phenotypes. *J. Ovarian. Res.,* **16**(1): 207.

Yan P, Guo M, Gan Y, Zhu M, Han X and Wu J (2024). Early pregnancy exposure to Microcystin-LR compromises endometrial decidualization in mice via the PI3K/AKT/FOXO1 signaling pathway. *Chemosphere,* **366**: 143466.

Yang AM, Xu X, Han Y, Wei JJ, Hao GM, Cui N, Zhao Z M, Wang W and Huang X (2021). Risk Factors for Different types of pregnancy losses: Analysis of 15,210 pregnancies after embryo transfer. *Front Endocrinol. (Lausanne),* **12**: 683236.

Yu F, Xue Y, Zhao Y, Zhang L, He X and Liu Z (2023). Isorhamnetin inhibits inflammatory response to alleviate DHEA-induced polycystic ovary syndrome in rats. *Gynecol. Endocrinol.,* **39**(1): 2183045.

Zhang Y, Gao R, Zhang L, Geng Y, Chen Q, Chen X, Liu X, Mu X, Ding Y, Wang Y and He J (2021). AMPK/mTOR downregulated autophagy enhances aberrant endometrial decidualization in folate-deficient pregnant mice. *J. Cell Physiol.,* **236**(11): 7376-7389.