Postprint: Er Chen Tang Improves Follicular Function in Phlegm-Dampness Type Polycystic Ovary Syndrome via the ATG7/FTH1 Pathway

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Abstract

Background Polycystic ovary syndrome (PCOS) is a gynecological endocrine metabolic disease of unclear etiology, with increasing prevalence among younger populations. Therefore, investigating the molecular pathogenesis of PCOS provides evidence for clinical treatment and protects women' s health. Objective Based on Traditional Chinese Medicine theory that "phlegm-dampness" constitution causes infertility, this study explores the ameliorative effect of Erchen Decoction on mitophagy-dependent ferroptosis in PCOS ovarian granulosa cells. Methods The Laboratory Animal Center of Liaoning University of Traditional Chinese Medicine provided an SPF-grade laboratory and 32 female SD rats. After one week of acclimatization, the rats were randomly divided into a control group (n=10) and a model group (n=22). The control group received normal maintenance diet for 12 weeks; the model group received high-fat diet throughout, with daily intragastric letrozole solution during modeling. After modeling, rats from the model group with disordered estrous cycles were selected as the treatment group (n=12) and administered Erchen Decoction daily for 4 weeks. Post-sacrifice, ovarian tissues were examined via HE staining for pathological changes, transmission electron microscopy for mitochondrial morphology in granulosa cells, and Western Blotting for protein levels of ATG7, P62, anti-autophagy effector protein 1 (Beclin1), LC3 , LC3 , FTH1, and ISCU2 in rat ovarian granulosa cells. The O2K mitochondrial function assay system was used to detect changes in mitochondrial oxygen consumption rate in ovarian granulosa cells. Results The model group showed increased ovarian cystic follicles, decreased corpora lutea, and mitochondrial autophagy/ferroptosis in granulosa cells, with partial recovery after treatment. Compared with the control group, the model group exhibited elevated protein expression of ATG7, P62, Beclin1, and LC3 ($P<0.05$), increased LC3 /LC3 ratio ($P<0.05$), and reduced protein expression of LC3 , FTH1, and ISCU2 ($P<0.05$). Compared with the model

group, the treatment group showed decreased protein expression of ATG7, P62, Beclin1, and LC3 (P<0.05), reduced LC3 /LC3 ratio (P<0.05), and increased FTH1 protein expression (P<0.05). Comparison of oxygen consumption rates for ETS, C , and C among the three groups showed statistically significant differences (P<0.05); specifically, the model group had lower ETS, C , and C oxygen consumption rates than the control group, while the treatment group had higher ETS, C , and C oxygen consumption rates than the model group, with all differences being statistically significant (P<0.05). Conclusion Erchen Decoction reverses autophagy-dependent ferroptosis in ovarian granulosa cells through the ATG7/FTH1 pathway, thereby improving follicular function in phlegm-dampness type PCOS rats.

Full Text

Study on the Mechanism of Erchen Decoction Intervening in ATG7/FTH1 Pathway to Improve Follicular Function in Phlegm-Damp Type Polycystic Ovary Syndrome

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Abstract

Background: Polycystic ovary syndrome (PCOS) is a gynecologic endocrine and metabolic disease with no clearly defined etiology, and its prevalence is becoming increasingly younger and more widespread. Therefore, investigating the molecular biological pathogenesis of PCOS is essential for providing evidence for clinical treatment and protecting women' s health.

Objective: Based on the traditional Chinese medicine theory that "phlegm-dampness" constitution leads to infertility, this study explored the ameliorating effect of Erchen Decoction on autophagy-dependent ferroptosis in ovarian granulosa cells of PCOS.

Methods: The Experimental Animal Center of Liaoning University of Traditional Chinese Medicine provided SPF-grade laboratory facilities and 32 female SD rats. After one week of adaptive feeding, the rats were randomly divided into a control group (n=10) and a model group (n=22). The control group received regular maintenance feed for 12 weeks, while the model group received high-fat feed throughout the entire period and daily gavage of letrozole solution during modeling. After modeling completion, rats with disordered estrous cycles from the model group were selected as the treatment group (n=12) and received daily gavage of Erchen Decoction for 4 consecutive weeks. After sample collection, hematoxylin-eosin (HE) staining was used to observe pathological changes in rat ovaries, transmission electron microscopy captured mitochondrial morphological changes in ovarian granulosa cells, and Western Blotting detected protein level changes of autophagy-related gene 7 (ATG7), P62, anti-autophagy effector protein 1 (Beclin1), microtubule-associated protein I light chain 3 (LC3 I), microtubule-associated protein II light chain 3 (LC3 II), ferritin heavy chain 1 (FTH1), and iron-sulfur cluster assembly enzyme 2 (ISCU2). The O2K mitochondrial function assay system measured changes in mitochondrial oxygen consumption rate in ovarian granulosa cells.

Results: The model group showed increased ovarian cystic follicles, decreased corpora lutea, and mitochondrial autophagy and ferroptosis in ovarian granulosa cells, with partial morphological recovery observed after treatment. Compared with the control group, the model group exhibited increased protein expression of ATG7, P62, Beclin1, and LC3 II ($P < 0.05$), elevated LC3 II/LC3 I ratio ($P < 0.05$), and decreased expression of LC3 I, FTH1, and ISCU2 proteins ($P < 0.05$). Compared with the model group, the treatment group showed decreased protein expression of ATG7, P62, Beclin1, and LC3 II ($P < 0.05$), reduced LC3 II/LC3 I ratio ($P < 0.05$), and increased FTH1 protein expression ($P < 0.05$). Statistically significant differences were observed among the three groups in oxygen consumption rates of electron transfer system (ETS), complex I (C I), and complex II (C II) ($P < 0.05$), with the model group showing lower rates than the control group and the treatment group showing higher rates than the model group ($P < 0.05$).

Conclusion: Erchen Decoction can reverse autophagy-dependent ferroptosis in ovarian granulosa cells through the ATG7/FTH1 pathway, thereby improving follicular function in phlegm-damp type PCOS rats.

Keywords: Polycystic ovary syndrome; Erchen Decoction; Autophagy-dependent ferroptosis; Phlegm-dampness infertility; Mitochondria

Introduction

Polycystic ovary syndrome (PCOS) is a common gynecological disease closely associated with endocrine and metabolic disorders such as insulin resistance (IR), hyperandrogenism, and chronic low-grade inflammation. In traditional Chinese medicine (TCM), PCOS falls under the categories of menstrual irregularities, amenorrhea, and infertility, with "phlegm-dampness obstructing the Chong and Ren meridians" considered a key pathological mechanism. Clinical investigations have shown that phlegm-damp type PCOS has become a prevalent syndrome among PCOS patients, often accompanied by obesity and IR. Our research group previously confirmed that ferroptosis occurs in granulosa cells of phlegm-damp type PCOS rats [2-3], which may be related to oxidative stress induced by long-term high-fat feeding.

Recent studies have found that excessive ferritin autophagy can elevate ferroptosis levels [4], and overactivation or increased expression of autophagy factors can cause ferritin autophagy, increase free iron levels, and induce oxidative damage in ovarian granulosa cells, thereby affecting dominant follicle formation [5]. However, whether ferroptosis in PCOS ovarian granulosa cells depends on excessive autophagy elevation remains unclear. Furthermore, whether Erchen Decoction, the fundamental formula for "resolving phlegm and strengthening the spleen," can improve granulosa cell energy metabolism by intervening in this process requires investigation. This study aims to explore these mechanisms to provide new therapeutic targets and clear mechanistic evidence for clinical PCOS treatment.

Materials and Methods

1.1 Experimental Animals and Ethics Thirty-two female SD rats (specific pathogen-free grade, weighing 100-120 g) were obtained from the Experimental Animal Center of Liaoning University of Traditional Chinese Medicine (license number: SCXK (Liao) 2020-0001). This study was approved by the Animal Experimental Ethics Committee of Liaoning University of Traditional Chinese Medicine (license number: 21000042021124).

1.2 Reagents and Materials Erchen Decoction herbal slices were purchased from the Second Affiliated Hospital of Liaoning University of Traditional Chinese Medicine, composed of 12 g Banxia (Pinelliae Rhizoma), 9 g Chenpi (Citri Reticulatae Pericarpium), 15 g Fuling (Poria), and 6 g Zhigancao (Glycyrrhizae Radix Praeparata). The decoction was prepared using an automatic herbal medicine machine, then concentrated and filtered under high pressure to contain 0.88 g/mL of crude drug [6]. Letrozole was purchased from Vendetta Biotechnology Co., Ltd. (Beijing, batch number: JM0614). Primary antibodies against ferritin heavy chain 1 (FTH1, batch number: A19544, Abclonal), iron-sulfur cluster assembly enzyme 2 (ISCU2, batch number: 14812-1-AP, Proteintech), glyceraldehyde-3-phosphate dehydrogenase (GAPDH, batch number: ab9485, Abcam), autophagy-related gene 7 (ATG7, batch number: ab133528, Abcam),

P62 (batch number: ab109012, Abcam), Beclin1 (batch number: ab62557, Abcam), microtubule-associated protein I light chain 3 (LC3 I, batch number: ab52768, Abcam), and microtubule-associated protein II light chain 3 (LC3 II, batch number: ab63817, Abcam) were used.

1.3 Instruments The following instruments were used: LKB-1 ultramicrotome (LKB Biochrom, Sweden), Nikon optical microscope (Nikon Corporation, Japan), JEM-1400 transmission electron microscope (JEOL, Japan), Multiskan FC microplate reader (Thermo Fisher Scientific, USA), 5200 chemiluminescence imaging system (Tanon, Shanghai), and Oxygraph-2k (O2K) mitochondrial function assay system (Oroboros, Austria).

1.4 Experimental Methods

1.4.1 Animal Grouping and Drug Intervention After one week of adaptive feeding, 32 rats were randomly divided into a control group (n=10) and a model group (n=22). The control group received regular maintenance feed for 12 weeks. The model group received high-fat feed (60% fat-derived energy) throughout the study, and from week 4 to week 12 received daily gavage of 1 $mg \cdot kg^{-1} \cdot d^{-1}$ letrozole solution dissolved in 0.5% sodium carboxymethyl cellulose (1 mL) at the same time each morning, with unrestricted water access [7]. After modeling, rats with disordered estrous cycles from the model group were selected as the treatment group (n=12) and received daily gavage of 44.2 $g \cdot kg^{-1} \cdot d^{-1}$ Erchen Decoction (50 mL) for 4 consecutive weeks [8]. During this period, the model group received letrozole solution every 2 days to maintain the disease state until the end of the experiment.

1.4.2 Ovarian Morphological Observation Two days after the final administration, samples were collected. Unilateral ovaries were randomly selected from each group (n=3 per group), fixed in 4% paraformaldehyde for 24 hours, then dehydrated with xylene and graded ethanol. After paraffin embedding, 4 m sections were cut, dewaxed, rehydrated, stained with hematoxylin for 15 minutes, briefly immersed in 1% hydrochloric acid ethanol, counterstained with 1% eosin solution for 1 minute, dehydrated, cleared, and mounted for microscopic observation.

1.4.3 Electron Microscopy of Ovarian Granulosa Cell Mitochondria Unilateral ovaries were randomly selected from each group (n=3 per group) and cut into approximately 1 mm^3 pieces. Tissues were fixed in 2.5% glutaraldehyde at room temperature for 1 hour, then stored at 4°C. For examination, samples were washed with phosphate-buffered saline (PBS), post-fixed with 1% osmium tetroxide, dehydrated with graded ethanol and acetone, embedded in epoxy resin, stained with 3% uranyl acetate and lead citrate, sectioned at 50-60 nm thickness, and examined under transmission electron microscopy to analyze and photograph mitochondrial ultrastructure in ovarian granulosa cells.

1.4.4 Western Blotting Analysis of Autophagy and Ferroptosis Proteins Ovarian granulosa cells were extracted to prepare single-cell suspensions. Total protein concentration was determined using the BCA method, and sample loading volumes were calculated. SDS-PAGE gel electrophoresis, membrane transfer, and blocking were performed sequentially. Primary antibodies against ATG7, P62, Beclin1, LC3 I, LC3 II, FTH1, ISCU2, and GAPDH (4 mL each) were incubated overnight at 4°C on a shaker. On the following day, HRP-labeled goat anti-rabbit secondary antibody was incubated for 2 hours at 37°C in a constant temperature water bath shaker. After washing the membrane three times, protein bands were visualized and analyzed using Image J software for grayscale values. GraphPad Prism 9.5.1 software was used to calculate relative protein expression levels as the ratio of target protein to GAPDH grayscale values.

1.4.5 O2K Measurement of Oxygen Consumption in Rat Ovarian Granulosa Cells Following the "uncoupler-respiratory chain I inhibitor-electron transport inhibitor" titration protocol, reagents were added to the chamber for detection. Initially, 10 L glutamic acid (G), malic acid (M), and 5 L pyruvic acid (P) were added, followed by 4 L adenosine diphosphate (ADP). After the oxygen consumption rate curve stabilized, the oxygen consumption rate of mitochondrial complex I (C I) was obtained. Subsequently, 20 L succinate (SCC), the substrate for mitochondrial complex II (C II), was added to obtain the oxygen consumption rate of both C I and C II. The uncoupler FCCCP (U) was then added to achieve maximum mitochondrial respiration. Finally, rotenone (ROT), the inhibitor of C I, and antimycin A (AMA), the inhibitor of mitochondrial complex III (C III), were added to block all mitochondria-related respiration, leaving only non-mitochondrial respiration [9]. The experiment was terminated, the O2K metabolic analyzer was cleaned, data were exported and saved, and curve graphs were generated.

1.5 Statistical Analysis All experimental data were normally distributed measurement data and expressed as ($\bar{x}\pm s$). GraphPad Prism 9.5.1 software was used for analysis. One-way ANOVA was used for comparisons among multiple groups, with $P < 0.05$ considered statistically significant.

Results

2.1 Ovarian Morphological Changes Histological examination revealed that the control group had well-developed and abundant corpora lutea and antral follicles. In contrast, the model group showed fewer mature follicles, numerous large vacuolated cystic cavities, increased cystic follicles, and decreased mature follicles and corpora lutea compared with the control group, confirming successful PCOS modeling. The treatment group demonstrated relative recovery in corpus luteum numbers and reduced cystic follicle counts compared with the model group [Figure 1: see original paper].

2.2 Mitochondrial Electron Microscopy of Ovarian Granulosa Cells In the control group, ovarian granulosa cell mitochondria were abundant with normal rod-shaped or spherical morphology, intact double membranes, and clearly identifiable cristae structures. The model group exhibited characteristic features of ferroptosis (significant mitochondrial swelling, increased membrane density of outer and inner membranes, reduced or absent cristae, and darker staining) and autophagy (decreased mitochondrial numbers, membrane condensation, and formation of autophagosomes with double membranes surrounding mitochondria). The treatment group showed partial recovery of mitochondrial cristae, reduced vacuoles, alleviated mitochondrial swelling, and relatively restored mitochondrial numbers [Figure 2: see original paper].

2.3 Protein Expression in Ovarian Granulosa Cells Significant differences were observed among the three groups in protein expression levels of ATG7, P62, Beclin1, LC3 I, LC3 II, FTH1, ISCU2, and LC3 II/LC3 I ratio ($P < 0.05$). Compared with the control group, the model group showed increased expression of ATG7, P62, Beclin1, and LC3 II proteins, elevated LC3 II/LC3 I ratio, and decreased expression of LC3 I, FTH1, and ISCU2 proteins ($P < 0.001$). Compared with the model group, the treatment group exhibited decreased expression of ATG7, P62, Beclin1, and LC3 II proteins, reduced LC3 II/LC3 I ratio, and increased FTH1 protein expression ($P < 0.05$) [Figure 3: see original paper] and .

2.4 Oxygen Consumption Rate Detected by O2K Statistically significant differences were found among the three groups in oxygen consumption rates of ETS, C I, and C II ($P < 0.05$). The model group showed lower oxygen consumption rates for ETS, C I, and C II compared with the control group, while the treatment group demonstrated higher rates than the model group ($P < 0.05$) [Figure 4: see original paper] and .

Discussion

Traditional Chinese medicine posits that spleen deficiency leads to fluid metabolism dysfunction, causing fluid accumulation that transforms into "phlegm," a pathological product that obstructs the uterus and causes female infertility. Therefore, the key pathogenesis of PCOS ovarian pathology is spleen deficiency with phlegm-dampness obstructing the uterus [10]. TCM interventions have shown excellent clinical efficacy in treating PCOS, with Erchen Decoction being an essential basic formula for phlegm-damp type PCOS patients. Originating from the Song Dynasty pharmacopeia "Taiping Huimin Heji Jufang," Erchen Decoction consists of Banxia as the sovereign drug to dry dampness and resolve phlegm, Chenpi as the minister drug to regulate qi and strengthen the spleen, Fuling as the adjuvant to resolve phlegm and promote diuresis, and Zhigancao as the messenger drug to tonify the spleen and resolve phlegm. However, in-depth molecular biological research on Erchen Decoction' s intervention in PCOS remains limited.

Moderate autophagy is essential for normal physiological processes such as oocyte development and follicular growth and differentiation. Oocytes are highly susceptible to oxidative environments, and maintaining a normal physiological hypoxic microenvironment within the ovary requires appropriate autophagy to balance energy metabolism [11]. However, excessive autophagy significantly reduces oocyte quality, clinically manifesting as polycystic ovaries and infertility [12]. Research has identified ATG7 as the protein most closely associated with IR in the autophagy process, and its expression may affect the autophagy level required for normal follicular growth and development [13]. Our study confirmed that excessive autophagy occurred in ovarian granulosa cells of PCOS model rats. Electron microscopy revealed autophagic characteristics including incomplete mitochondrial outer membranes, disappearance of mitochondrial cristae, endoplasmic reticulum uncoupling, fusion of autophagosomes with lysosomes, and digestion of sequestered material. Beclin1, a crucial indicator for evaluating elevated autophagy levels, showed significantly enhanced expression, with ATG7 protein overexpression mediated by Beclin1 and abnormal LC3 II/LC3 I conversion. These findings suggest substantially increased autophagy levels in PCOS rats, which Erchen Decoction can ameliorate by improving the expression of these proteins to varying degrees.

Our electron microscopy results of ovarian granulosa cells in phlegm-damp type PCOS rats also revealed ferroptosis phenomena, including mitochondrial contraction and rounding, cristae fragmentation, and increased membrane density. Ferroptosis is a form of cell death triggered by iron metabolism disorders that induce lipid peroxidation and excessive accumulation of reactive oxygen species (ROS). This led us to investigate whether ferroptosis in PCOS granulosa cells is related to autophagy. By examining the expression level of FTH1, a selective degradation target of iron ion regulatory factors during excessive autophagy, we found significantly reduced FTH1 expression in granulosa cells of model group rats, which Erchen Decoction could improve. These results suggest that Erchen Decoction may reverse autophagy-dependent ferroptosis (ADF) by ameliorating high-fat diet-induced excessive autophagy, mediating iron storage homeostasis within cells, reducing iron accumulation, and consequently decreasing Fenton reactions and oxidative stress-induced energy metabolism damage in granulosa cells.

Furthermore, we found that normal mitochondrial respiratory chain function in ovarian granulosa cells is the molecular basis for ensuring stable energy metabolism in both granulosa cells and oocytes. During ferroptosis, oxidative stress may disrupt FTH1, which maintains iron homeostasis, leading to significantly reduced FTH1 protein expression. Fe^{3+} that can stably bind to transferrin is reduced to unstable Fe^{2+}, which enters mitochondria through mitochondrial membrane proteins, causing mitochondrial respiratory chain damage [14]. O2K results demonstrated decreased oxygen consumption in ovarian granulosa cells of model group rats, with mitochondrial respiratory chain complexes showing damage, while Erchen Decoction improved oxygen consumption levels. We therefore hypothesize that ADF in PCOS may damage the mitochon-

drial respiratory chain, reduce mitochondrial oxidative phosphorylation levels in the already hypoxic environment, and cause more severe energy metabolism impairment. Further detection of ISCU2 protein expression, an auxiliary group of respiratory chain electron transport proteins, revealed significantly reduced expression in granulosa cells of model group rats, suggesting mitochondrial respiratory chain damage and reduced ATP transfer, which further confirms our hypothesis. Additionally, excessive autophagy can cause mitochondrial energy metabolism disorders, leading to increased ROS, which in turn causes mitochondrial damage and creates a vicious cycle [15-16].

In conclusion, Erchen Decoction treats PCOS by intervening in the ATG7/FTH1 pathway to mitigate mitochondrial respiratory chain damage in ovarian granulosa cells, alleviating autophagy-dependent ferroptosis, thereby reducing oxidative stress-induced damage to ovarian granulosa cells, ensuring energy supply from granulosa cells to oocytes, and promoting dominant follicle formation in clinical patients. This study aims to provide new therapeutic targets and clear mechanistic evidence for clinical PCOS treatment. The specific interaction mechanisms of Erchen Decoction' s intervention in the ATG7/FTH1 pathway will be further investigated at the cellular level.

Author Contributions: LYU Xiaojiao was primarily responsible for manuscript writing, animal experiments, figure preparation, and data processing. BAI Zihan and MA Yan were mainly responsible for experimental animal care. CHEN Wei contributed to TCM clinical treatment concepts. QU Yang and SUI Guoyuan revised the manuscript. SHANG Bing provided TCM theoretical guidance. CONG Peiwei was responsible for the overall research concept, complete design of animal and cell experiments, manuscript review, and overall responsibility for the article.

Conflict of Interest: The authors declare no conflict of interest.

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References

[1] TU Haoyan, LEI Xiaocan, HUO Peng, et al. Research progress on energy metabolism requirements and regulatory mechanisms during folliculogenesis [J]. Acta Academiae Medicinae Sinicae, 2019, 41(3): 408-414. DOI:10.3881/j.issn.1000-503X.10774.

[2] LI Jia, WANG Yanan, WU Zhaoli, et al. Analysis of the pathogenesis

of polycystic ovary syndrome based on "phlegm-dampness infertility" [J]. Chinese Archives of Traditional Chinese Medicine, 2024, 42(10): 123-126. DOI:10.13193/j.issn.1673-7717.2024.10.027.

[3] CONG Peiwei, ZHANG Lina, WANG Dan, et al. Effect and mechanism of Erchen Decoction on regulating GPX4/cyclooxygenase-mediated oxidative damage and ferroptosis to improve polycystic ovary syndrome [J]. China Journal of Traditional Chinese Medicine and Pharmacy, 2023, 38(2): 615-619.

[4] BATTAGLIA A M, CHIRILLO R, AVERSA I, et al. Ferroptosis and cancer: mitochondria meet the "iron maiden" cell death [J]. Cells, 2020, 9(6): 1505. DOI:10.3390/cells9061505.

[5] LIU J, KUANG F M, KROEMER G, et al. Autophagy-dependent ferroptosis: machinery and regulation [J]. Cell Chemical Biology, 2020, 27(4): 420-435. DOI:10.1016/j.chembiol.2020.02.005.

[6] CONG Peiwei, ZHANG Lina, WANG Dan, et al. Effect of Erchen Decoction on endocrine and glucose-lipid metabolism in phlegm-damp type polycystic ovary syndrome rats [J]. Chinese Archives of Traditional Chinese Medicine, 2021, 39(8): 58-61, 264. DOI:10.13193/j.issn.1673-7717.2021.08.013.

[7] LIN Ying, ZHANG Hong, HUANG Mingqing, et al. Experimental study on letrozole combined with high-fat diet to induce metabolic phenotypes in insulin-resistant polycystic ovary syndrome rats [J]. China Journal of Modern Medicine, 2016, 26(3): 9-13. DOI:10.3969/j.issn.1005-8982.2016.03.002.

[8] CONG Peiwei, WU Zhaoli, ZHANG Lina, et al. Mechanism study on Guben Huayu method regulating insulin resistance by affecting GLUT4/Akt expression in polycystic ovary syndrome rats [J]. Chinese Archives of Traditional Chinese Medicine, 2019, 37(8): 1872-1876, 2052. DOI:10.13193/j.issn.1673-7717.2019.08.018.

[9] GÓMEZ-VALADÉS A G, POZO M, VARELA L, et al. Mitochondrial cristae-remodeling protein OPA1 in POMC neurons couples Ca^{2+} homeostasis with adipose tissue lipolysis [J]. Cell Metabolism, 2021, 33(9): 1820-1835.e9. DOI:10.1016/j.cmet.2021.07.008.

[10] CONG Peiwei, ZHANG Lina, ZHAO Danyu, et al. Study on Erchen Decoction improving uterine glucose transport function in phlegm-damp type PCOS rats by enhancing autophagy [J]. Journal of Basic Chinese Medicine, 2021, 27(8): 1270-1274. DOI:10.19945/j.cnki.issn.1006-3250.2021.08.020.

[11] KUMARIYA S, UBBA V, JHA R K, et al. Autophagy in ovary and polycystic ovary syndrome: role, dispute and future perspective [J]. Autophagy, 2021, 17(10): 2706-2733. DOI:10.1080/15548627.2021.1938914.

[12] WANG L, TANG J H, WANG L, et al. Oxidative stress in oocyte aging and female reproduction [J]. Journal of Cellular Physiology, 2021, 236(12): 7966-7983. DOI:10.1002/jcp.30468.

[13] SONG Z H, YU H Y, WANG P, et al. Germ cell-specific Atg7 knockout results in primary ovarian insufficiency in female mice [J]. Cell Death & Disease, 2015, 6(1): e1589. DOI:10.1038/cddis.2014.559.

[14] ZHANG J, ZHENG Y P, WANG Y, et al. YAP1 alleviates sepsis-induced acute lung injury via inhibiting ferritinophagy-mediated ferroptosis [J]. Frontiers in Immunology, 2022, 13: 884362. DOI:10.3389/fimmu.2022.884362.

[15] ZHAO Na, SUI Guoyuan, LIU Yuedong, et al. Discussion on mitochondrial dysfunction and liver cancer from the perspective of spleen deficiency and impaired transportation [J]. Liaoning Journal of Traditional Chinese Medicine, 2025, 52(1): 41-44. DOI:10.13192/j.issn.1000-1719.2025.01.012.

[16] MANCIAS J D, WANG X X, GYGI S P, et al. Quantitative proteomics identifies NCOA4 as the cargo receptor mediating ferritinophagy [J]. Nature, 2014, 509(7498): 105-109. DOI:10.1038/nature13148.

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Note: Figure translations are in progress. See original paper for figures.