SIRT3 protects endometrial receptivity in patients with polycystic ovary syndrome

Zhonghong Zeng[1,2,3,4,5], Hongying Shan[1,2,3,4,6], Mingmei Lin[1,2,3,4], Siyu Bao[1,2,3,4], Dan Mo[1,2,3,4,5], Feng Deng[1,2,3,4], Yang Yu[1,2,3,4], Yihua Yang[5], Ping Zhou[1,2,3,4], Rong Li[1,2,3,4]

[1]Department of Obstetrics and Gynecology, Center for Reproductive Medicine, Peking University Third Hospital, Beijing 100191, China;
[2]Key Laboratory of Assisted Reproduction (Peking University), Ministry of Education, Beijing 100191, China;
[3]Beijing Key Laboratory of Reproductive Endocrinology and Assisted Reproductive Technology, Beijing 100191, China;
[4]National Clinical Research Center for Obstetrics and Gynecology (Peking University Third Hospital), Beijing 100191, China;
[5]Guangxi Reproductive Medical Center, The First Affiliated Hospital of Guangxi Medical University, Nanning, Guangxi 530021, China;
[6]Reproductive Medical Center, The First Affiliated Hospital of Shihezi University, Shihezi, Xinjiang 832000, China.

Abstract

Background: The sirtuin family is well recognized for its crucial involvement in various cellular processes. Nevertheless, studies on its role in the human endometrium are limited. This study aimed to explore the expression and localization of the sirtuin family in the human endometrium, focusing on sirtuin 3 (SIRT3) and its potential role in the oxidative imbalance of the endometrium in polycystic ovary syndrome (PCOS).

Methods: Endometrial specimens were collected from both patients with PCOS and controls undergoing hysteroscopy at the Center for Reproductive Medicine, Peking University Third Hospital, from July to August 2015 and used for cell culture. The protective effects of SIRT3 were investigated, and the mechanism of SIRT3 in improving endometrial receptivity of patients with PCOS was determined using various techniques, including cellular bioenergetic analysis, small interfering ribonucleic acid (siRNA) silencing, real-time quantitative polymerase chain reaction, Western blot, immunofluorescence, immunohistochemistry, and flow cytometry analysis.

Results: The sirtuin family was widely expressed in the human endometrium, with SIRT3 showing a significant increase in expression in patients with PCOS compared with controls (P <0.05), as confirmed by protein and gene assays. Concurrently, endometrial antioxidant levels were elevated, while mitochondrial respiratory capacity was reduced, in patients with PCOS (P <0.05). An endometrial oxidative stress (OS) model revealed that the downregulation of *SIRT3* impaired the growth and proliferation status of endometrial cells and reduced their receptivity to day 4 mouse embryos. The results suggested that SIRT3 might be crucial in maintaining normal cellular state by regulating antioxidants, cell proliferation, and apoptosis, thereby contributing to enhanced endometrial receptivity.

Conclusions: Our findings proposed a significant role of SIRT3 in improving endometrial receptivity in patients with PCOS by alleviating OS and regulating the balance between cell proliferation and apoptosis. Therefore, SIRT3 could be a promising target for predicting and improving endometrial receptivity in this patient population.

Keywords: Apoptosis; Endometrial receptivity; Oxidative stress; Polycystic ovary syndrome; Sirtuin 3

Introduction

Infertility is defined as the inability to achieve a clinical pregnancy after regular unprotected sexual intercourse for more than 1 year, affecting approximately 10% of the population.[1,2] This complex condition arises from various etiological factors, including female, male, and unexplained factors. Ovulation disorders, accounting for approximately 25–35% of infertility cases, are particularly significant. Notably, polycystic ovary syndrome (PCOS) stands out as the most prevalent ovulation disorder. Patients with PCOS often seek medical assistance and undergo assisted reproductive technology (ART) to achieve pregnancy.[2] Successful pregnancy outcomes

Access this article online

Quick Response Code:

Website: www.cmj.org

DOI: 10.1097/CM9.0000000000003127

Zhonghong Zeng, Hongying Shan, Mingmei Lin, and Siyu Bao contributed equally to this work.

Correspondence to: Rong Li, Department of Obstetrics and Gynecology, Peking University Third Hospital, No. 49 HuaYuan North Road, Haidian District, Beijing 100191, China
E-Mail: roseli001@sina.com;
Ping Zhou, Department of Obstetrics and Gynecology, Peking University Third Hospital, No. 49 HuaYuan North Road, Haidian District, Beijing 100191, China
E-Mail: zhoup0520@163.com;
Yihua Yang, Guangxi Reproductive Medical Center, The First Affiliated Hospital of Guangxi Medical University, No. 6 Shuangyong Road, Qingxiu District, Nanning 530021, China
E-Mail: workyyh@163.com

Chinese Medical Journal 2025;138(10)
Received: 05-12-2023; **Online:** 09-05-2024 **Edited by:** Yanjie Yin

depend largely on embryo quality and endometrial environment.[3] The use of oocytes donated by the patients with PCOS did not decrease the overall pregnancy success rate.[4] This finding suggested that the reduced fertility in patients with PCOS was associated with not only factors such as internal physiological disorders and diminished oocyte quality but also compromised endometrial receptivity.[5,6] Previous studies focused on the connection between compromised endometrial receptivity in patients with PCOS and disrupted regulatory mechanisms, including imbalances in endometrial oxidative stress (OS), chronic low-grade inflammation, metabolic disorders, endocrine dysregulation, and abnormalities in endometrial hormone receptors. Among these factors, OS imbalance plays a prominent role.[7]

OS is characterized by the overproduction of reactive oxygen species (ROS), wherein the oxidative burden surpasses the capacity of antioxidant defense systems to neutralize these radicals. This imbalance leads to the chronic accumulation of ROS in various cell types, including vascular endothelial cells, endometrial epithelial cells, and stromal cells. Consequently, this aberrant redox state triggers internal signaling pathways that directly or indirectly induce cellular and tissue damage, affecting components such as deoxyribonucleic acid (DNA), lipid membranes, and protein.[8–10] Within the context of OS, mitochondrial respiration plays a crucial role in ROS generation. Disruptions in mitochondrial function can have serious consequences, such as a significant reduction in the activities of cellular respiratory chain complexes I and IV, impairments in oxidative phosphorylation, reduced adenosine triphosphate (ATP) synthesis, changes in cell membrane permeability, and an abnormal increase in cellular OS levels.[7] Increasing evidence supports significantly elevated OS levels in patients with PCOS compared with normal individuals, as evaluated by circulating markers including malondialdehyde, catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx).[6,11,12] Previous studies identified increased concentrations of OS markers in the follicular fluid from patients with PCOS compared with controls, potentially leading to a decline in oocyte quality and affecting subsequent steps of the reproductive process.[13,14] However, studies on the correlation between endometrial OS and PCOS remain inadequate.

Sirtuins are a family of nicotinamide adenine dinucleotide (NAD+)-dependent protein deacetylases playing a vital role in oxidative metabolism and lifespan extension in lower organisms.[15–19] In mammals, this family consists of seven members (SIRT1–7), each exhibiting distinct subcellular localization. Specifically, SIRT1, SIRT6, and SIRT7 are predominantly localized in the nucleus; SIRT2 in the cytoplasm; and SIRT3, SIRT4, and SIRT5 in the mitochondria.[20,21] These versatile enzymes participate in various biological processes. SIRT1 primarily participates in energy metabolism, gene transcription, and OS; SIRT2 in cell cycle regulation; SIRT3 in energy metabolism and apoptosis; SIRT4 in the tricarboxylic acid cycle and insulin secretion; SIRT5 in the ornithine cycle; SIRT6 in DNA repair and inflammation; and SIRT7 in nucleolus stress and rRNA transcription.[22] Although existing studies investigating the role of sirtuins in reproductive functions have predominantly focused on SIRT1 and SIRT3 in ovarian function, energy metabolism, and OS,[23] studies exploring the role of sirtuins in the endometrium are limited. A previous study showed that resveratrol, a SIRT1 activator, played a crucial role in endometrial receptivity by upregulating E-cadherin expression.[24] Additionally, several studies have suggested distinctive roles for sirtuins in endometrial cancer, with SIRT1 shown to accelerate the proliferation of endometrial carcinoma cell lines.[25–28] In the context of PCOS, numerous studies have reported the presence of OS in affected patients.[29] However, the relationship between the imbalanced oxidative endometrium of PCOS and sirtuins remains poorly understood.

This study aimed to investigate the expression and localization of sirtuins in the human endometrium, focusing on SIRT3 and its potential role in the imbalanced oxidative endometrium of PCOS.

Methods

Study participants and tissue collection

In this study, 71 participants were enrolled from the Reproductive Medicine Center of Peking University Third Hospital, including 34 patients with PCOS and 37 controls. All specimens were acquired with the informed consent of patients after approval from the Ethics Committee of Peking University Third Hospital (No. S2018042). The diagnostic criteria for PCOS were based on the presence of at least two of the following three features: (1) oligomenorrhea or amenorrhea, (2) clinical and/or laboratory-confirmed hyperandrogenism, and (3) polycystic ovaries observed via ultrasonography, with the exclusion of related disorders.[30] The control group consisted of women experiencing infertility due to male factors, excluding those with endometriosis, tubal effusion, endometrial cavity fluid, endometrial polyps, submucosal uterine fibroids, and pelvic tuberculosis. Eligible participants were 35 years of age or younger and had not received any hormonal treatment in the last 3 months. The endometrial samples were obtained during hysteroscopic examination in the hyperplasia phase, as confirmed by histopathological analysis. Fresh specimens were collected, preserved in phosphate-buffered saline (PBS; Gibco, NY, USA) on ice, washed three times with PBS, and subsequently stored in liquid nitrogen for long-term preservation.

Cell culture and induction of OS

The endometrial tissue samples obtained from the hysteroscopic examination were dissociated into single cells using mechanical and enzymatic digestion as described previously.[31,32] The endometrial cells obtained from both the PCOS and control groups were cultured in Dulbecco's Modified Eagle Medium (DMEM)/F12 medium (Gibco) containing 10% fetal bovine serum (Hyclone, Loga, USA), penicillin (100 U/mL, Gibco), and streptomycin (100 μg/mL, Gibco). The cells were subjected to 250 μmol/L hydrogen peroxide (H_2O_2) (FreeMoreBio, Beijing, China) treatment

for 12 h to induce OS, and then cultured in a CO_2 incubator (Thermo Fisher Scientific, Waltham, MA, USA) under a 5% CO_2 atmosphere at 37°C. The following experiments and measurements were performed in parallel for both the control and OS groups.

Cellular bioenergetic analysis

The Seahorse Bioscience XF analyzer (Billerica, MA, USA) was employed to measure the real-time energy metabolism of the cells to assess mitochondrial function. Endometrial cells derived from groups with either OS or PCOS, as well as the control group, were cultured in the same energy metabolism orifice plate, with a cell density of 4–6 × 10^4 (24-well plate, 250 μL per well). Subsequently, the cells in the orifice plate were washed three times with the cell culture medium and subsequently treated with oligomycin (1 μg/mL; Sigma-Aldrich, St. Louis, MO, USA), carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone (FCCP) (0.7 μmol/L; Sigma-Aldrich), antimycin A (1 μmol/L; Sigma-Aldrich), and rotenone (1 μmol/L; Sigma-Aldrich) in the dosing wells surrounding each cell hole on the second day. The oxygen consumption rate (OCR) of the cells was measured using the Seahorse Bioscience XF analyzer following incubation under a 5% CO_2 atmosphere at 37°C for 20 min.

Small interfering ribonucleic acid (siRNA) silenced the gene expression of SIRT3

The *SIRT3* siRNA lipid complex was co-cultured with the endometrial cells to downregulate the expression of the *SIRT3* gene. Specifically, the cells were cultured with RPMI-1640 culture medium (Invitrogen, Carlsbad, CA, USA) for 24 h. Then, a mixture of *SIRT3* siRNA (600 pmol) and RNA interference (RNAi; GenePharma, Shanghai, China) mate (Lipofectamine 2000, 10 μL; Invitrogen) reagents was added to the cells in a 60-mm Petri dish containing RPMI-1640 (400 μL) on the following day. The RNAi-treated cells were then incubated under a 5% CO_2 atmosphere at 37°C for 24–48 h.

RNA extraction and real-time quantitative polymerase chain reaction (RT-qPCR)

Total RNA was extracted from endometrial tissues using the TRIzol reagent (Ambion, Austin, TX, USA) following the manufacturer's protocol. Subsequently, complementary DNA was synthesized from total RNA using the Thermo Fisher Scientific kit (Thermo Fisher Scientific). RT-qPCR was performed to analyze the expression of sirtuin genes using the SYBR Select Master Mix (Thermo Fisher Scientific). Each 20-μL qPCR reaction included 0.4 μmol/L of forward and reverse primers for sirtuin genes, and the reactions were performed in triplicate. The specific oligonucleotide primers used in this study were represented in Supplementary Table 1, http://links.lww.com/CM9/C6. The PCR cycling conditions were as follows: an initial denaturation step of 2 min at 50°C, followed by another denaturation step of 2 min at 95°C. Subsequently, 40 amplification cycles were performed, consisting of 15 s at 95°C, 15 s at 56°C, and 40 s at 72°C on the ABI 7500 Real-Time PCR System (Invitrogen).

Western blot analysis

Endometrial tissue protein extraction was performed using a protein lysate (250 mg tissues/mL) (CWBIO, Jiangsu, China), protease inhibitor (1:100; CWBIO), and phenylmethanesulfonyl fluoride (1:100; CWBIO). The concentration of extracted protein was determined using the BCA protein assay kit (Beyotime, Shanghai, China) and a microplate reader. Gels with appropriate concentrations of 10% and 5% for separation and concentration, respectively, were prepared using the Western blot kit (CWBIO). Electrophoresis was performed at 90 V for 30 min, followed by 110 V for 120 min. The resulting protein samples were transferred onto a polyvinylidene fluoride (PVDF) membrane through electrotransfer at 300 mA for 2–3 h. After washing the membrane three times with 0.1% Tris-buffered saline/Tween (TBST; Amresco, Solon, OH, USA), it was blocked with 5% skimmed milk powder in TBST for 1 h. The membrane was then incubated with a SIRT3 antibody (1:1000; Abcam, Cambridge, UK) overnight at 4°C, followed by incubation with peroxidase-conjugated donkey anti-goat IgG secondary antibody (1:2000; Beyotime) at room temperature for 1 h. Finally, the Western blot strips were detected after being washed three times with TBST.

Immunofluorescence

The endometrial cells were fixed with 4% paraformaldehyde (Sigma-Aldrich) for 30 min, followed by treatment with 1% Triton-X-100 (Sigma-Aldrich) for 7 min and incubated with 1% bovine serum albumin (BSA) blocking solution (Sigma-Aldrich) for 1 h at room temperature. The specimens were then incubated with primary antibodies for sirtuins (SIRT1 1:500; SIRT2 1:100; SIRT3 1:100; SIRT4 1:100; SIRT5 1:100; SIRT6 1:100; and SIRT7 1:50; Abcam) in blocking solution at room temperature for 1 h, followed by incubation with secondary antibodies (1:200 dilution, ZF-0314, ZF-0317; ZSGB-BIO, Beijing, China) at room temperature for 1 h. The nuclei were labeled with Hoechst 33342 (1:100 dilution, H3570; Invitrogen) in the last 5 min. Immunofluorescence was observed using a confocal microscope (Zeiss LSM 710; Zeiss, Oberkochen, Germany).

Immunohistochemical analysis

In this study, the endometrial tissues were fixed with 4% paraformaldehyde, embedded in paraffin, sectioned, and dewaxed. Antigen retrieval involved boiling the sections in citrate buffer at pH 6.0 for 25 min, followed by three washes with PBS. To eliminate endogenous peroxidase, we treated the sections with a 3% H_2O_2 solution at room temperature for 15 min. Subsequently, the sections were incubated with the primary antibody of SIRT3 (1:200; Abcam), followed by incubation with the horseradish peroxidase–labeled secondary antibody (ZSGB-BIO), to visualize the protein expression. The immunohistochemical images were captured using a light microscope (Nikon, Tokyo, Japan).

Apoptosis detection

The endometrial cells were collected and processed for flow cytometry analysis (BD, Franklin Lakes, NJ, USA).

Briefly, the cells were digested and collected in a 15-mL centrifuge tube. Each sample contained approximately 1–5 × 10^6 cells, which were then centrifuged at 1000 rpm (r = 10 cm) for 5 min and resuspended after discarding the medium. Annexin V labeling solution (100 μL; BD) and fluorescein isothiocyanate (FITC) (5 μL; BD) were added to the cells, and the mixture was incubated in the dark at room temperature for 25 min. After centrifugation and one wash with incubation buffer, fluorescent 7-aminoactinomycin D (AAD) (10 μL; BD) was added and the cells were incubated again in the dark at room temperature for 20 min. Following the final centrifugation and wash, the cells were resuspended in 300 μL of incubation buffer. Flow cytometry analysis was performed using a fluorescence wavelength of 488 nm, with FITC fluorescence detected using a bandpass filter with a wavelength of 515 nm and propidium iodide detected using a wavelength greater than 560 nm.

Bioinformatics analysis

To examine the impact of *SIRT3* siRNA, we employed principal component analysis (PCA) to assess the differential expression of genes across various sample types. The identification of differentially expressed genes (DEGs) in endometrial samples between individuals treated with *SIRT3* siRNA and controls was conducted using the "limma" package of R software (version 3.54.2; R Foundation, Vienna, Austria). The screening criteria for DEGs were set as a P value <0.05 and $|\log_2$ fold change (FC)| >1. Subsequently, the DEGs were visually represented through a heatmap and a volcano plot using the "ggplot2" package (version 3.4.2). To gain a comprehensive understanding of the biological pathways associated with the identified DEGs, gene set enrichment analysis (GSEA) was performed. The GSEA software (GSEA_4.1.0, CapitalBio Technology, Beijing, China) was employed to analyze the data, with gene sets exhibiting an adjusted P-value (FDR) <0.05 deemed statistically significant.

Statistical analysis

The statistical analysis of the data was performed using SPSS software (version 26.0; IBM, Armonk, NY, USA). Each experiment was replicated at least thrice, and the results were presented as mean ± standard deviation (or mean ± standard error). The independent-samples t-test was employed to evaluate the differential gene expression between the two groups. One-way analysis of variance was employed to compare two or more datasets. The χ^2 test was used to analyze the embryo count and other relevant data. A P value <0.05 indicated a statistically significant difference.

Results

Clinical data

The endometrial specimens were obtained from 37 patients diagnosed with PCOS and 34 patients with male factor infertility as controls. The clinical data, including the mean age, endometrial thickness, and levels of luteinizing hormone in the PCOS group and control group with no significant difference. The body mass index (BMI) of patients with PCOS was significantly higher than that of the controls (26.04 ± 3.47 kg/m^2 *vs*. 22.37 ± 3.62 kg/m^2, t = –4.353, P <0.001), indicating that most patients with PCOS in this study were overweight or obese, possibly as a result of metabolic dysfunction.[33] Consistent with the diagnosis of PCOS, the antral follicle count (23.11 ± 7.72 *vs*. 11.82 ± 3.38, t = –7.863, P <0.001) and the basal testosterone hormone of patients with PCOS were significantly higher than that of the controls. However, despite the abnormal metabolic state of patients with PCOS, no significant difference in the cumulative clinical pregnancy rate was observed between the PCOS and controls (54.54% *vs*. 56.25%, χ^2 = 0.006, P >0.05) after ART [Supplementary Table 2, http://links.lww.com/CM9/C6], which was consistent with previous findings.[34]

Expression of sirtuin family member SIRT1-7 in the human endometrium

This study reported the expression and subcellular localization of seven sirtuin family members in endometrial cells, with SIRT1 and SIRT6 showing localization to both cytoplasm and nucleus, SIRT2 mainly localizing to cytoplasm, SIRT3–5 mainly localizing to cytoplasm (mitochondria), and SIRT7 showing localization to both nucleus and nuclear membrane [Figure 1A]. Additionally, we performed qRT-PCR to examine the mRNA expression of *SIRT1–7* in all human endometrial specimens and found that *SIRT3–5* mRNA expression in endometrial specimens of patients with PCOS was higher than that in the control group, with *SIRT3* mRNA expression showing a statistically significant increase (P <0.05) [Figure 1B]. To verify the elevated SIRT3 expression, we conducted immunofluorescence, immunohistochemistry, and Western blot analysis and found significantly higher SIRT3 protein expression in the PCOS group compared with the control group [Figures 1C–E]. These results suggested that SIRT3 was overexpressed in the endometrium of patients with PCOS and localized not only to mitochondria but also to the nucleus in patients with PCOS compared with the control group.

Activation of anti-OS and a decrease in mitochondrial respiratory function in the endometrium of patients with PCOS

Due to the established association between SIRT3 and OS, we conducted further investigations to assess the markers of antioxidants and mitochondrial energy metabolism, which served as the primary source of ROS. We observed an upregulation in the expression of antioxidant genes, including *SOD*, *CAT*, and *GPx*, in the endometrium of patients with PCOS compared with the control group, although these differences were not statistically significant. Considering the upregulation of *SIRT3*, we inferred that the endometrium of patients with PCOS experienced chronic OS, potentially affecting mitochondrial function. We observed a decrease in the energy metabolism of mitochondria in the endometrium of patients with PCOS compared with the control group

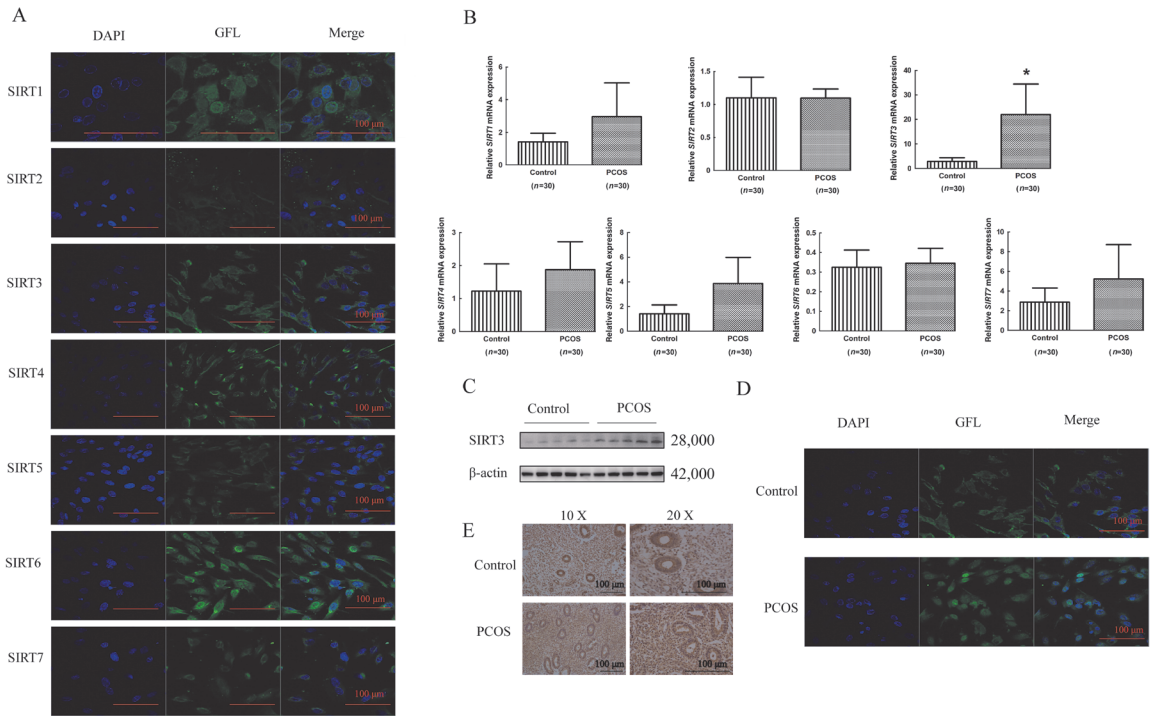

Figure 1: Expression of sirtuin family member SIRT1–7 in the human endometrium. (A) Sirtuin proteins were expressed in endometrial cells. The green fluorescence visualized these proteins. DAPI was used to visualize the nuclei. Original magnification × 63; scale bar = 100 μm. (B) Expression of mRNA of *SIRT1–7*. (C) Protein levels of SIRT3 in the PCOS and control groups as assessed using Western blotting. (D) Distribution of SIRT3 in the nucleus of PCOS endometrial cells was significantly higher than that in the control group. Scale bar = 100 μm. (E) Immunohistochemical analysis in the PCOS and control groups. Scale bar = 100 μm (*P <0.05 *vs.* the control group). DAPI: Diamidino-phenyl-indole; GFL: Green fluorescence; mRNA: Messenger RNA; PCOS: Polycystic ovary syndrome; SIRT: Sirtuin.

using Seahorse Bioscience XF, particularly a significant reduction in basal respiration (P <0.05; Figure 2). Consistent with previous findings, our results also indicated that the endometrium of patients with PCOS was in a state of chronic OS, resulting in mitochondrial dysfunction.

Modeling chronic OS in endometrial cells

To establish an OS model, we cultured the endometrial cells with a culture medium containing H_2O_2, based on the hypothesis that it upregulates SIRT3 expression. Previous studies suggested treatment durations of 4–12 h and H_2O_2 concentrations ranging from 125 μmol/L to 500 μmol/L to induce chronic OS in endometrial cells.[35,36] However, in this study, we observed that treatment with H_2O_2 at a concentration of 125 μmol/L for 12 h could not induce obvious chronic OS, whereas a concentration of 500 μmol/L for 4 h resulted in a high number of cell deaths [Figure 3]. Therefore, based on our findings, we identified that treatment with H_2O_2 at a concentration of 250 μmol/L for 12 h was the most suitable condition for inducing chronic OS in endometrial cells.

Expression of related factors and functions after the exposure of endometrial cells to 250 μmol/L H_2O_2

Upon exposure to 250 μmol/L H_2O_2, OS was induced in endometrial cells, leading to the upregulation of antioxidant markers and the expression of *SIRT3*, *SIRT4*, and *SIRT5* [Figures 4A, B, D, E]. It was observed that the expression of SIRT3 did not show a significant increase after treatment with 250 μmol/L H_2O_2 for 4 h. However, after 12 h of treatment, a noticeable difference in the expression levels of SIRT3 was evident compared to the control group [Figures 4C, 4F]. The assessment of mitochondrial energy and metabolism, conducted using the Seahorse Bioscience XF assay, revealed a noticeable impairment in mitochondrial function, including basal respiration, ATP production, maximal respiration, and spare capacity [Figure 5]. Therefore, our findings suggested that chronic OS could upregulate the expression of SIRT3 and lead to mitochondrial dysfunction, indicating a potential pathogenic mechanism of PCOS and suggesting therapeutic strategies. However, we did not observe a significant decrease in the adhesion rate in the chronic OS group compared with the control group [Figure 5]. This result was consistent with the comparable cumulative pregnancy rate observed among patients with PCOS [Supplementary Table S2, http://links.lww.com/CM9/C6]. Therefore, we believed that SIRT3 might play a vital role in maintaining endometrial receptivity by providing protection against OS.

SIRT3 protected the endometrial receptivity by anti-OS

To elucidate the role of SIRT3 in endometrial receptivity, we employed siRNA-mediated knockdown of the *SIRT3* gene in endometrial cells, followed by co-culturing with mouse embryos. The adhesion rate was used to evaluate the endometrial receptivity in *SIRT3* knocked-down cells, revealing a significantly lower adhesion rate in these cells compared with the control group (58.89% *vs.* 73.86%,

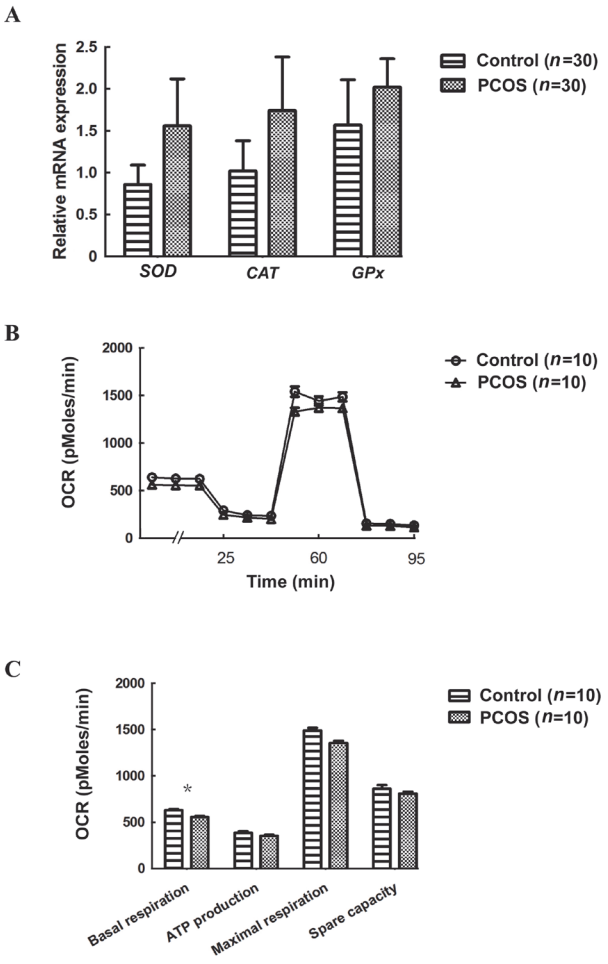

Figure 2: Elevated levels of antioxidants and decreased mitochondrial respiratory capacity in the endometrium of patients with PCOS. (A) Genes of anti-OS were upregulated in PCOS endometrial tissues. (B,C) OCR of PCOS endometrial cells decreased compared with that in the control group (*P <0.05 *vs.* the control group). ATP: Adenosine triphosphate; CAT: Catalase; GPx: Glutathione peroxidase; mRNA: Messenger RNA; OCR: Oxygen consumption rate; OS: Oxidative stress; PCOS: Polycystic ovary syndrome; SOD: Superoxide dismutase.

P <0.05) after 48 h of co-culture [Figures 6A–C]. Morphological abnormalities, including cell shrinkage and small bubble-like vacuoles, were observed in *SIRT3* knocked-down endometrial cells [Figures 6D–F]. These observations were confirmed by flow cytometry analysis, indicating that the cells progressed to apoptosis [Figure 6G]. We also found significant alterations in the expression of genes associated with apoptosis, including *P53* (P = 0.053), *P21* (P = 0.052), and *BCL2* (P <0.05), in *SIRT3* knocked-down endometrial cells [Figure 6H]. The expression of genes involved in the anti-OS pathway, such as *CAT* (P <0.05), *SOD* (P <0.01), and *GPx*, was markedly reduced in *SIRT3* knocked-down endometrial cells [Figure 6H]. Collectively, these findings suggested that SIRT3 was a key factor in the anti-OS pathway that influenced the proliferation and apoptosis of endometrial cells, resulting in improved endometrial receptivity.

PCA and differential gene expression analysis

The PCA indicated no notable differences between samples from the two groups [Supplementary Figure 1A, http://links.lww.com/CM9/C6]. Moreover, employing siRNA-mediated knockdown of the *SIRT3* gene in endometrial cells resulted in a significant reduction in *SIRT3* expression (P = 0.0063; Supplementary Figure 1B, http://links.lww.com/CM9/C6). Subsequently, different types of samples were screened for DEGs based on the PCA results. The outcome was visualized through a volcano map and a heat map [Supplementary Figures 1C, D, http://links.lww.com/CM9/C6]. Overall, 180 DEGs were identified, with 94 genes upregulated and 86 genes downregulated. The top 10 most significant DEGs were selected and plotted accordingly [Supplementary Figure 1D, http://links.lww.com/CM9/C6]. Further investigations using GSEA–KEGG pathway enrichment analysis of the DEGs revealed 24 enrichment pathways, particularly including the oxidative phosphorylation and apoptosis pathways

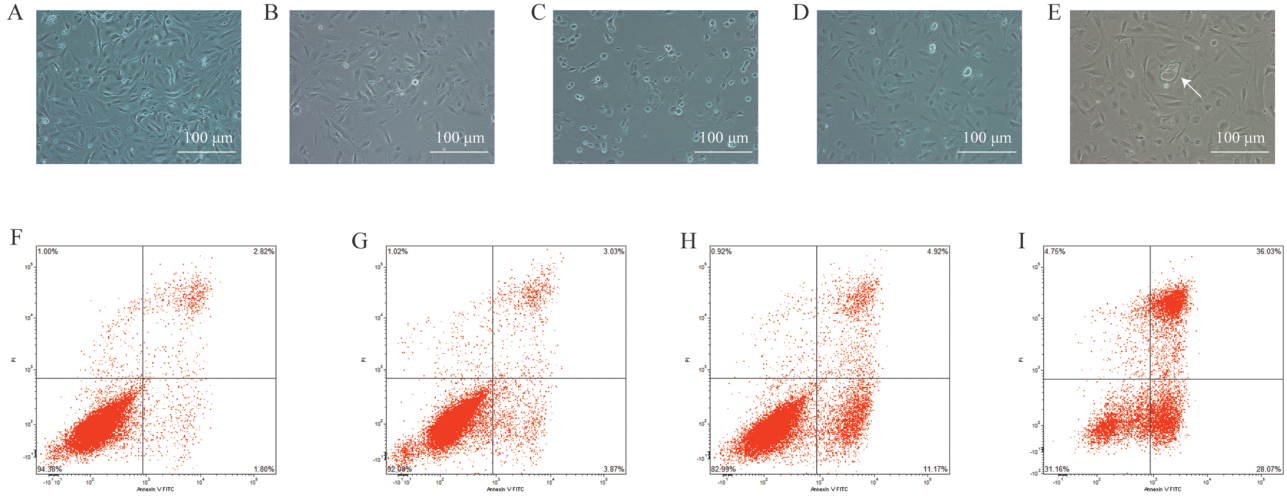

Figure 3: Model of chronic OS in endometrial cells. (A) Control group of endometrial cells. (B) State of the cells after culturing with 125 μmol/L H_2O_2 for 12 h. No significant abnormality was observed. (C) A large number of cells died after culturing with 500 μmol/L H_2O_2 for 4 h. (D,E) State of the cells after culturing with 250 μmol/L H_2O_2 for 4 h and 12 h, respectively. No significant abnormality was observed. Scale bar = 100 μm. The arrow shows cellular oncosis. (F) Apoptosis in the control group. (G,H) Apoptosis of endometrial cells cultured with 250 μmol/L H_2O_2 for 4 h and 12 h. The apoptosis percentage was 3.03% and 4.92%, respectively. (I) A higher percentage of cell apoptosis was observed when the cells were cultured with 500 μmol/L H_2O_2 for 4 h, reaching 36.03%. H_2O_2: Hydrogen peroxide; OS: Oxidative stress.

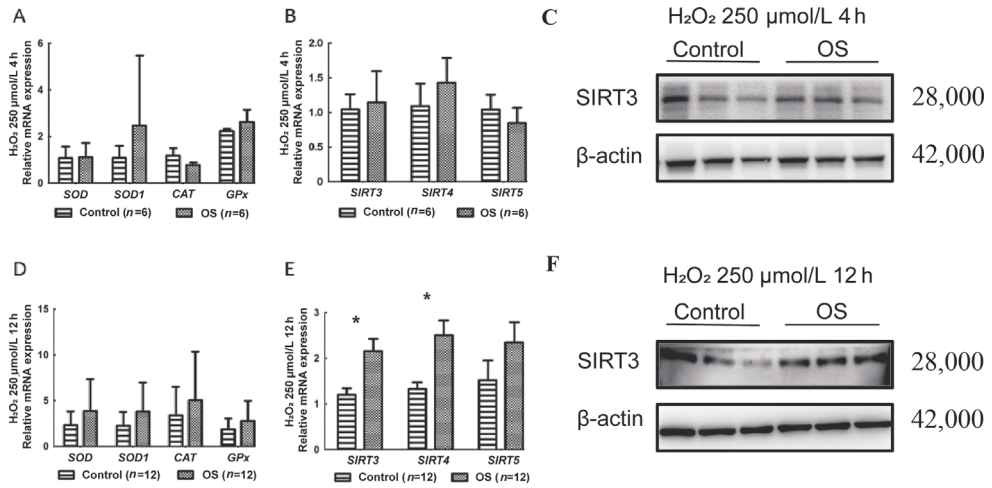

Figure 4: Expression of related factors was evaluated after exposure of endometrial cells to 250 μmol/L H_2O_2 for 4 h and 12 h. (A–C) Endometrial cells cultured using 250 μmol/L H_2O_2 for 4 h. The expression of anti-OS markers and *SIRT3–5* was not significantly different between the OS and control groups. (D–F) Endometrial cells cultured with 250 μmol/L H_2O_2 for 12 h. The expression of anti-OS markers was upregulated, and the expression of *SIRT3* significantly increased in the OS group compared with the control group (*P <0.01 *vs.* the control group). CAT: Catalase; GPx: Glutathione peroxidase; H_2O_2: Hydrogen peroxide; OS: Oxidative stress; SOD: Superoxide dismutase.

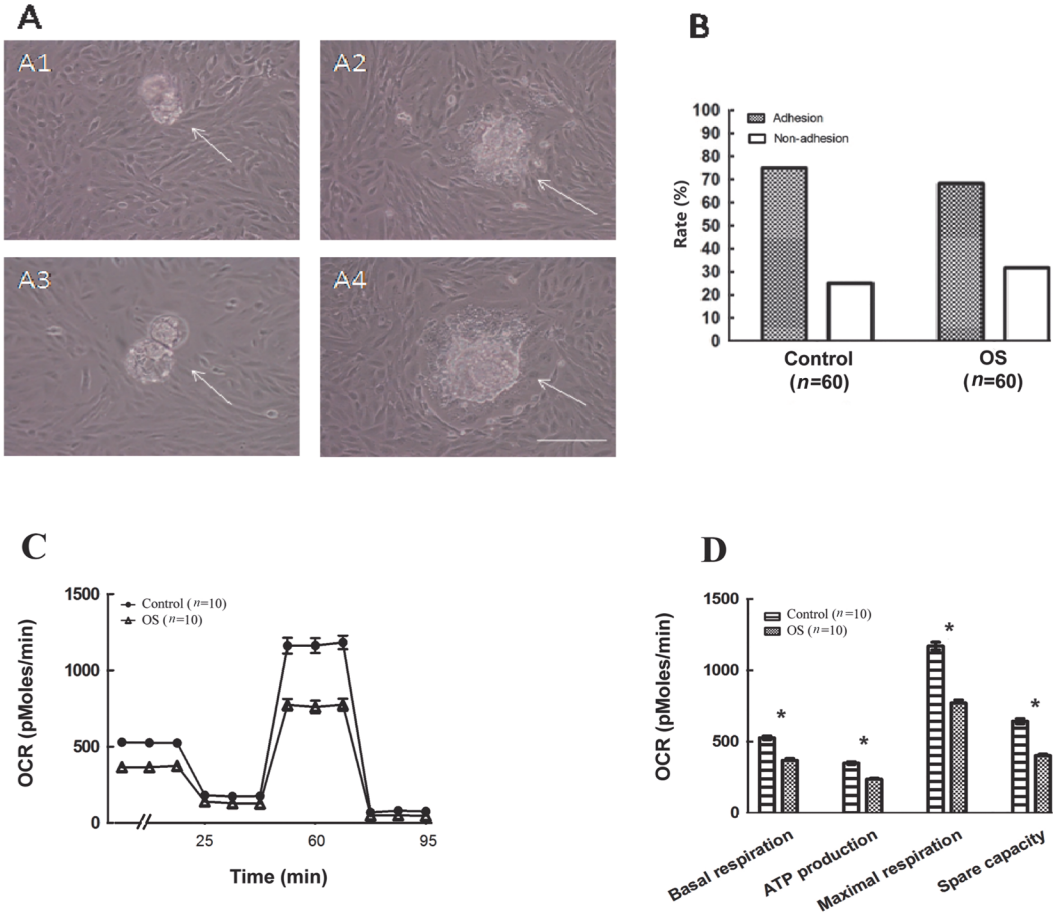

Figure 5: Expression of related functions was evaluated after exposure of endometrial cells to 250 μmol/L H_2O_2 for 12 h. Embryos from both the non-adhesion (A1) and adhesion groups (A2) interacting with endometrial cells in the control group. Embryos of non-adhesion (A3) and adhesion groups (A4) interacting with OS endometrial cells. (B) Adhesion rate of embryos to OS endometrial cells was not significantly different from that in the control group. (C) OCR curve of endometrial cells in the OS and control groups. (D) Basal respiration, ATP production, maximal respiration, and spare capacity of the OS group significantly decreased compared with that in the control group (*P <0.001 *vs.* the control group). ATP: Adenosine triphosphate; H_2O_2: Hydrogen peroxide; OCR: Oxygen consumption rate; OS: Oxidative stress.

[Supplementary Figure 1E, http://links.lww.com/CM9/C6]. These findings hold the potential for elucidating the underlying mechanisms by which SIRT3 regulates OS in the endometrium.

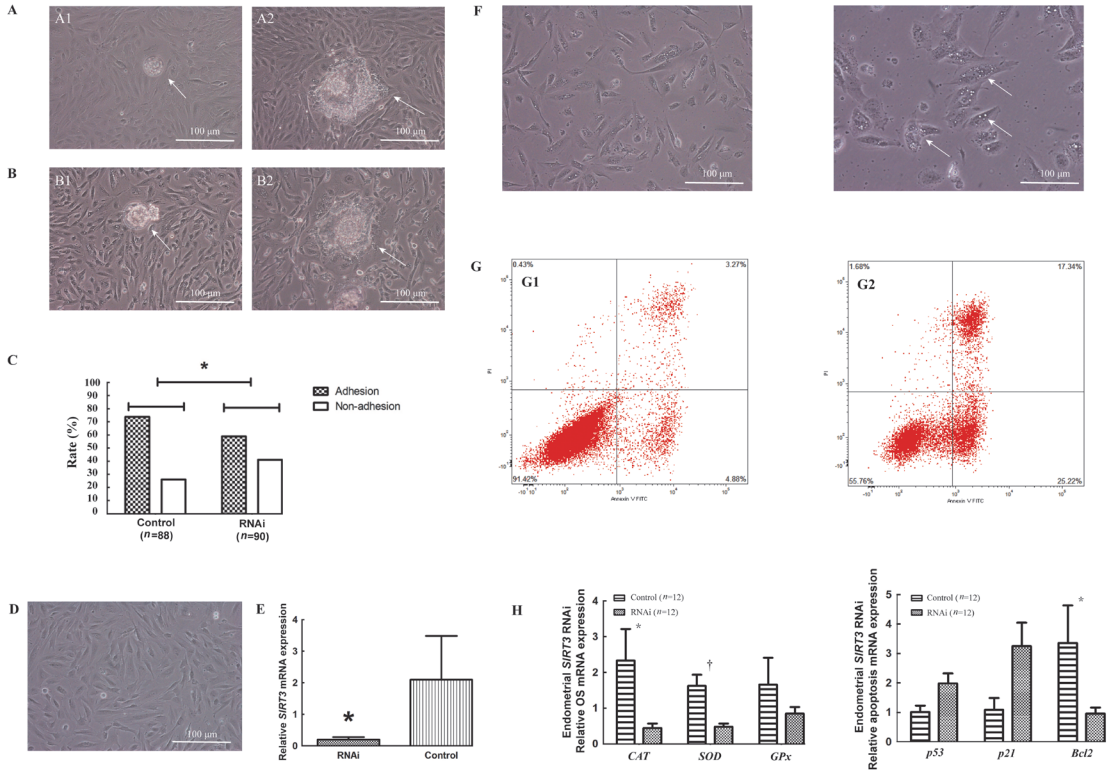

Figure 6: SIRT3 protected the endometrial receptivity by anti-OS. (A) Control group with non-implanted (A1) and implanted embryos (A2). (B) *SIRT3* RNAi endometrial cells with non-implanted (B1) and implanted embryos (B2). (C) Embryonic adhesion rate of endometrial cells in the *SIRT3* RNAi group was 58.89%, which was significantly lower than the adhesion rate of 73.86% in the control group. (D) Endometrial cells in the control group. (E) Expression of *SIRT3* in endometrial cells was significantly inhibited by siRNA. (F) Endometrial cells of *SIRT3* RNAi. (G) Apoptotic rate of *SIRT3* RNAi endometrial cells significantly increased compared with that in the control group. (G1) Control group. (G2) *SIRT3* RNAi group. (H) Expression of markers of anti-OS, such as CAT, SOD, and GPx, significantly decreased after treatment with *SIRT3* RNAi. The apoptosis pathway was significantly activated after treatment with *SIRT3* RNAi. *P53* and *p21* were the factors promoting apoptosis, whereas *Bcl2* was an anti-apoptotic factor (scale bar = 100 μm, $^{*}P<0.05$, $^{\dagger}P<0.01$ *vs.* the control group). Bcl2: B-Cell lymphoma 2; CAT: Catalase; GPx: Glutathione peroxidase; OS: Oxidative stress; RNAi: RNA interference; SIRT3: Sirtuin 3; siRNA: Small interfering ribonucleic acid; SOD: Superoxide dismutase.

Discussion

PCOS is a prevalent gynecological endocrine disorder that manifests with clinical or biochemical indications of hyperandrogenism, sporadic ovulation or anovulation, and polycystic ovarian changes. It commonly co-occurs with obesity and dysregulation of glucose and lipid metabolism. Although the precise etiology and pathogenesis of PCOS remain incompletely understood, current research suggests a multifactorial origin encompassing both genetic and environmental factors. Importantly, patients with PCOS face an elevated risk of developing endometrial hyperplasia and endometrial cancer. Therefore, long-term and standardized management strategies are imperative to optimize patient care and mitigate these risks. The administration of medications to regulate menstruation, enhance fertility, and protect the endometrium of patients with PCOS can be further augmented through synergistic approaches, including the supplementation of antioxidants such as resveratrol, melatonin, quercetin, and coenzyme Q, to improve therapeutic efficacy.[37]

OS is a pathological state implicated in various diseases including PCOS.[38] Sirtuins, a group of deacetylase proteins, play a crucial role in anti-OS, metabolic regulation, and epigenetic modification.[22] In this study, we investigated the expression of sirtuin genes and proteins in human endometrium and observed a significant increase in the expression of SIRT3 in patients with PCOS compared with the control group, which could be attributed to the chronic OS state. Using *SIRT3* knockdown and H_2O_2-induced OS cell models, we further demonstrated the protective role of SIRT3 in endometrial receptivity by regulating anti-OS responses and mediating the apoptosis pathway. Our findings emphasized the significance of SIRT3 in maintaining endometrial function and suggested its potential as a therapeutic target for managing PCOS.

Liu *et al*[39] showed that although the total antioxidant capacity (TAC) did not exhibit a significant alteration compared with the control group, a slight increase was observed in individual antioxidant markers such as TAC, glutathione (GSH), and SOD. This increase could potentially be attributed to a compensatory response aimed at maintaining redox homeostasis. Consistent with this finding, our study also demonstrated elevated expression levels of oxidation markers, such as *CAT*, *GPx*, and *SOD*, in the endometrium in the PCOS group compared with the control group. Moreover, we observed impaired basal respiration in the mitochondria in the PCOS group. Mitochondria serve as both the primary source of ROS production and a highly vulnerable organelle prone to ROS-induced damage. This vulnerability arises from several factors. First, mitochondrial DNA is located close to its own oxidative phosphorylation machinery, rendering it

particularly susceptible to ROS attack and subsequent mutagenesis. Unlike nuclear DNA, mitochondrial DNA lacks histone protection, further increasing its vulnerability. Additionally, mitochondrial DNA regulates the expression of 13 subunits within the respiratory chain complex and the expression of various transfer RNAs and ribosomal RNAs within the mitochondria. Consequently, mutations affecting these genes can potentially disrupt mitochondrial function.[40] In addition, our RT-qPCR results demonstrated a significant upregulation of *SIRT3*, an antioxidant, in patients with PCOS compared with controls. This led us to further investigate the expression characteristics of SIRT3 protein using immunohistochemical and Western blotting techniques. Our results confirmed a significant increase in SIRT3 protein expression in the endometrial cells of patients with PCOS and suggested that SIRT3 was located in not only the mitochondria but also the nucleus. Interestingly, previous studies indicated that full-length SIRT3 was translocated to the nucleus under cellular stress conditions,[41] which was consistent with our findings. Moreover, previous studies reported that human SIRT3 was a soluble mitochondrial matrix protein that primarily regulated energy metabolism processes, influenced the flow of mitochondrial oxidative pathways, and ultimately controlled the rate of ROS production.[42–44]

In this study, we aimed to investigate the potential causes of differences in SIRT3 expression between the PCOS and control groups. The retrospective analysis of the clinical data of the two groups revealed no significant differences in basic endocrine levels. In contrast, BMI was significantly higher in patients with PCOS than in the control group. Previous studies have established a close association between obesity or overweight and OS, leading to several diseases such as metabolic syndrome.[45] Numerous studies have emphasized the role of OS in the pathogenesis of obesity and its associated risk factors, underscoring the importance of regulating body weight for better health benefits.[46] In this study, we observed a higher expression of SIRT3 in the endometrial cells of women with PCOS, suggesting a chronic imbalance in the oxidation state that impacted pregnancy outcomes and contributed to a higher clinical infertility rate. To validate this finding, we induced an endometrial oxidative state with H_2O_2 and found significantly upregulated expression of SIRT3 and classical anti-OS enzymes, similar to the PCOS endometrial specimen test results. These preliminary findings suggested that OS was one of the main pathological sources affecting SIRT3 expression. This aligned with previous studies demonstrating increased SIRT3 expression and activation of downstream targets associated with increased cell survival and decreased apoptosis, whether induced by oxidants or arising under pathological conditions.[47,48]

Over the past two decades, sirtuins have been extensively studied in mammals. These ubiquitously expressed deacetylase enzymes play a vital role in various processes associated with antioxidants and OS.[49] SIRT3–5 are primarily localized to the mitochondria, with SIRT3 displaying robust deacetylase activity.[44] To investigate the role of SIRT3 in anti-OS and endometrial receptivity, we knocked down *SIRT3* in endometrial cells using siRNA. Our results revealed significantly reduced antioxidant levels in *SIRT3* RNAi endometrial cells, indicating a critical role of *SIRT3* in the anti-OS pathway. Additionally, *SIRT3* knockdown resulted in cellular abnormalities, including the accumulation of vacuoles in the cytoplasm and an increased rate of cell apoptosis. The embryo adhesion experiment using *SIRT3* RNAi endometrial cells demonstrated significant damage to endometrial receptivity. These findings provided direct evidence supporting the protective role of SIRT3 in endometrial receptivity through its involvement in the anti-OS pathway. Furthermore, our study revealed significant differences in the expression pattern of nuclear SIRT3 between endometrial cells in the PCOS and control groups, indicating the upregulation of nuclear SIRT3 in endometrial cells of patients with PCOS experiencing chronic OS. Previous studies indicated that SIRT3 underwent rapid degradation under cellular stress, including OS and ultraviolet irradiation, thereby influencing the expression of stress-related mitochondrial genes encoded in the nucleus.[41] Therefore, the rapid removal of SIRT3 from chromatin could induce the expression of genes required for the stress response.[50] Earlier findings indicated that SIRT3 localized not only to the mitochondria but also to the nucleus, deacetylating acetyl-Lys 9 and acetyl-Lys 16 of histones H3 and H4, respectively.[51] This finding was significant because histone H3 acetylation was implicated in OS related to alcoholism, and SIRT1 activation resulted in H3 deacetylation, attenuating metabolic disease OS and complications.[52,53] Recent studies have also elucidated the close association between epigenetic modifications of H3 and the activation of the ERK pathway, which is involved in OS-mediated DNA damage and cell death.[54] Consistent with previous findings, our results also confirmed the significance of SIRT3 in anti-OS and the increased expression of SIRT3 in the endometrial cells of patients with PCOS, particularly in the nuclear SIRT3 of endometrial cells with chronic OS, emphasizing its critical role as a key protective factor for maintaining endometrial receptivity.

Endometrial receptivity, which involves complex physiological processes including OS, plays a vital role in successful blastocyst attachment, penetration, and localized stromal changes. In this study, we demonstrated that patients with PCOS exhibited a persistent OS, with upregulated antioxidants, such as SIRT3, resulting in mitochondrial respiratory damage and impaired endometrial receptivity. Previous studies have shown that ROS levels can stimulate normal physiological functions (e.g., cell growth and proliferation), but excessive ROS can cause cellular injury (e.g., damage to DNA, lipid membranes, and proteins), which negatively affects female fertility and *in vitro* fertilization outcomes.[6] Gupta *et al*[29] extensively reviewed the role of OS in altering protein pathways, thereby contributing to the pathophysiology of female infertility. Different from the classic pathways reported in previous studies, we identified SIRT3 as an endometrial receptivity protective factor that mediated anti-OS and apoptosis pathways. The knockdown of *SIRT3* using siRNA weakened anti-OS pathways while activating apoptosis pathways, leading to a significant increase in proapoptotic factors p53 and p21 and a notable decrease

in the antiapoptotic factor Bcl2 (P <0.05). Previous studies have established the involvement of leukemia inhibitory factor (LIF) and vascular endothelial growth factor (VEGF) in facilitating the entry of endometrial cells into the window of implantation.[55] Recent research has identified p53 as a key regulator of maternal reproduction and blastocyst implantation through regulating LIF and VEGF expression,[56,57] inducing endometrial cells to prepare for embryo implantation.[58] Therefore, we believe that the SIRT3-SOD-p53 pathway is crucial for endometrial receptivity during the pathophysiological process of PCOS, but further investigations are needed to verify this observation.

The limitation of this study should be acknowledged. This study aimed to investigate the potential enhancement of endometrial receptivity in patients with PCOS through the involvement of SIRT3, which mitigated OS while regulating cellular proliferation and apoptosis. However, due to the limited time and funding, we did not further explore the precise mechanism by which SIRT3 modulated endometrial OS.

In conclusion, this study proposed a significant role of SIRT3 in enhancing endometrial receptivity in the adverse uterine environment of patients with PCOS and also elucidated the underlying mechanisms. Specifically, SIRT3 alleviated OS damage and regulated the balance between cell proliferation and apoptosis in patients with PCOS. Therefore, SIRT3 could be a promising target for predicting and improving endometrial receptivity in this patient population.

Acknowledgments

We would like to thank all the patients and staff of the Reproductive Medicine Center, Department of Obstetrics and Gynecology, Peking University Third Hospital, for their valuable contributions to this study.

Funding

This study was supported in part by grants from the National Key Research and Development Project of China (Nos. 2021YFC2700303 and 2022YFC2702500) and the National Natural Science Foundation of China (Nos. 81925013, 82288102, 82101714, and 82201806).

Conflicts of interest

None.

References

1. Kessler LM, Craig BM, Plosker SM, Reed DR, Quinn GP. Infertility evaluation and treatment among women in the United States. Fertil Steril 2013;100:1025–1032. doi: 10.1016/j.fertnstert.2013.05.040.
2. Chen H, Zeng R, Zeng X, Qin L. Cluster analysis reveals a homogeneous subgroup of PCOS women with metabolic disturbance associated with adverse reproductive outcomes. Chin Med J 2023. doi: 10.1097/CM9.0000000000002787.
3. Zhang S, Lin H, Kong S, Wang S, Wang H, Wang H, *et al*. Physiological and molecular determinants of embryo implantation. Mol Aspects Med 2013;34:939–980. doi: 10.1016/j.mam.2012.12.011.
4. Ashkenazi J, Farhi J, Orvieto R, Homburg R, Dekel A, Feldberg D, *et al*. Polycystic ovary syndrome patients as oocyte donors: The effect of ovarian stimulation protocol on the implantation rate of the recipient. Fertil Steril 1995;64:564–567. doi: 10.1016/s0015-0282(16)57793-0.
5. Fiorentino G, Cimadomo D, Innocenti F, Soscia D, Vaiarelli A, Ubaldi FM, *et al*. Biomechanical forces and signals operating in the ovary during folliculogenesis and their dysregulation: Implications for fertility. Hum Reprod Update 2023;29:1–23. doi: 10.1093/humupd/dmac031.
6. Qiao J, Feng HL. Extra- and intra-ovarian factors in polycystic ovary syndrome: Impact on oocyte maturation and embryo developmental competence. Hum Reprod Update 2011;17:17–33. doi: 10.1093/humupd/dmq032.
7. Shan H, Luo R, Guo X, Li R, Ye Z, Peng T, *et al*. Abnormal endometrial receptivity and oxidative stress in polycystic ovary syndrome. Front Pharmacol 2022;13:904942. doi: 10.3389/fphar.2022.904942.
8. Dalle-Donne I, Rossi R, Colombo R, Giustarini D, Milzani A. Biomarkers of oxidative damage in human disease. Clin Chem 2006;52:601–623. doi: 10.1373/clinchem.2005.061408.
9. De Bont R, van Larebeke N. Endogenous DNA damage in humans: A review of quantitative data. Mutagenesis 2004;19:169–185. doi: 10.1093/mutage/geh025.
10. Dong C, Zhang NJ, Zhang LJ. Oxidative stress in leukemia and antioxidant treatment. Chin Med J 2021;134:1897–1907. doi: 10.1097/CM9.0000000000001628.
11. Murri M, Luque-Ramirez M, Insenser M, Ojeda-Ojeda M, Escobar-Morreale HF. Circulating markers of oxidative stress and polycystic ovary syndrome (PCOS): A systematic review and meta-analysis. Hum Reprod Update 2013;19:268–288. doi: 10.1093/humupd/dms059.
12. Zuo T, Zhu M, Xu W. Roles of oxidative stress in polycystic ovary syndrome and cancers. Oxid Med Cell Longev 2016;2016:8589318. doi: 10.1155/2016/8589318.
13. Bausenwein J, Serke H, Eberle K, Hirrlinger J, Jogschies P, Hmeidan FA, *et al*. Elevated levels of oxidized low-density lipoprotein and of catalase activity in follicular fluid of obese women. Mol Hum Reprod 2010;16:117–124. doi: 10.1093/molehr/gap078.
14. Piomboni P, Focarelli R, Capaldo A, Stendardi A, Cappelli V, Cianci A, *et al*. Protein modification as oxidative stress marker in follicular fluid from women with polycystic ovary syndrome: The effect of inositol and metformin. J Assist Reprod Genet 2014;31:1269–1276. doi: 10.1007/s10815-014-0307-z.
15. Imai S, Armstrong CM, Kaeberlein M, Guarente L. Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. Nature 2000;403:795–800. doi: 10.1038/35001622.
16. Kaeberlein M, McVey M, Guarente L. The SIR2/3/4 complex and SIR2 alone promote longevity in Saccharomyces cerevisiae by two different mechanisms. Genes Dev 1999;13:2570–2580. doi: 10.1101/gad.13.19.2570.
17. Rogina B, Helfand SL. Sir2 mediates longevity in the fly through a pathway related to calorie restriction. Proc Natl Acad Sci U S A 2004;101:15998–16003. doi: 10.1073/pnas.0404184101.
18. Tissenbaum HA, Guarente L. Increased dosage of a sir-2 gene extends lifespan in *Caenorhabditis elegans*. Nature 2001;410:227–230. doi: 10.1038/35065638.
19. Viswanathan M, Kim SK, Berdichevsky A, Guarente L. A role for SIR-2.1 regulation of ER stress response genes in determining *C. elegans* life span. Dev Cell 2005;9:605–615. doi: 10.1016/j.devcel.2005.09.017.
20. Chang HC, Guarente L. SIRT1 and other sirtuins in metabolism. Trends Endocrinol Metab 2014;25:138–145. doi: 10.1016/j.tem.2013.12.001.
21. Sauve AA, Wolberger C, Schramm VL, Boeke JD. The biochemistry of sirtuins. Annu Rev Biochem 2006;75:435–465. doi: 10.1146/annurev.biochem.74.082803.133500.
22. Haigis MC, Sinclair DA. Mammalian sirtuins: Biological insights and disease relevance. Annu Rev Pathol 2010;5:253–295. doi: 10.1146/annurev.pathol.4.110807.092250.
23. Tatone C, Di Emidio G, Vitti M, Di Carlo M, Santini S Jr, D'Alessandro AM, *et al*. Sirtuin functions in female fertility: Possible role in oxidative stress and aging. Oxid Med Cell Longev 2015;2015:659687. doi: 10.1155/2015/659687.
24. Shirane A, Wada-Hiraike O, Tanikawa M, Seiki T, Hiraike H, Miyamoto Y, *et al*. Regulation of SIRT1 determines initial step of endometrial receptivity by controlling E-cadherin expression. Bio-

chem Biophys Res Commun 2012;424:604–610. doi: 10.1016/j.bbrc.2012.06.160.
25. Asaka R, Miyamoto T, Yamada Y, Ando H, Mvunta DH, Kobara H, *et al*. Sirtuin 1 promotes the growth and cisplatin resistance of endometrial carcinoma cells: A novel therapeutic target. Lab Invest 2015;95:1363–1373. doi: 10.1038/labinvest.2015.119.
26. Bartosch C, Monteiro-Reis S, Almeida-Rios D, Vieira R, Castro A, Moutinho M, *et al*. Assessing sirtuin expression in endometrial carcinoma and non-neoplastic endometrium. Oncotarget 2016;7:1144–1154. doi: 10.18632/oncotarget.6691.
27. Chen X, Lai X, Wu C, Tian Q, Lei T, Pan J, *et al*. Decreased SIRT4 protein levels in endometrioid adenocarcinoma tissues are associated with advanced AJCC stage. Cancer Biomark 2017;19:419–424. doi: 10.3233/CBM-160419.
28. Lin L, Zheng X, Qiu C, Dongol S, Lv Q, Jiang J, *et al*. SIRT1 promotes endometrial tumor growth by targeting SREBP1 and lipogenesis. Oncol Rep 2014;32:2831–2835. doi: 10.3892/or.2014.3521.
29. Gupta S, Ghulmiyyah J, Sharma R, Halabi J, Agarwal A. Power of proteomics in linking oxidative stress and female infertility. Biomed Res Int 2014;2014:916212. doi: 10.1155/2014/916212.
30. Legro RS, Arslanian SA, Ehrmann DA, Hoeger KM, Murad MH, Pasquali R, *et al*. Diagnosis and treatment of polycystic ovary syndrome: An Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab 2013;98:4565–4592. doi: 10.1210/jc.2013-2350.
31. Chan RW, Schwab KE, Gargett CE. Clonogenicity of human endometrial epithelial and stromal cells. Biol Reprod 2004;70:1738–1750. doi: 10.1095/biolreprod.103.024109.
32. Rajaraman G, White J, Tan KS, Ulrich D, Rosamilia A, Werkmeister J, *et al*. Optimization and scale-up culture of human endometrial multipotent mesenchymal stromal cells: Potential for clinical application. Tissue Eng Part C Methods 2013;19:80–92. doi: 10.1089/ten.TEC.2011.0718.
33. Rosenfield RL, Ehrmann DA. The pathogenesis of polycystic ovary syndrome (PCOS): The hypothesis of PCOS as functional ovarian hyperandrogenism revisited. Endocr Rev 2016;37:467–520. doi: 10.1210/er.2015-1104.
34. Vembu R, Reddy NS. Serum AMH level to predict the hyper response in women with PCOS and non-PCOS undergoing controlled ovarian stimulation in ART. J Hum Reprod Sci 2017;10:91–94. doi: 10.4103/jhrs.JHRS_15_16.
35. Dell'Orco M, Milani P, Arrigoni L, Pansarasa O, Sardone V, Maffioli E, *et al*. Hydrogen peroxide-mediated induction of SOD1 gene transcription is independent from Nrf2 in a cellular model of neurodegeneration. Biochim Biophys Acta 2016;1859:315–323. doi: 10.1016/j.bbagrm.2015.11.009.
36. Thanan R, Techasen A, Hou B, Jamnongkan W, Armartmuntree N, Yongvanit P, *et al*. Development and characterization of a hydrogen peroxide-resistant cholangiocyte cell line: A novel model of oxidative stress-related cholangiocarcinoma genesis. Biochem Biophys Res Commun 2015;464:182–188. doi: 10.1016/j.bbrc.2015.06.112.
37. Azziz R, Carmina E, Chen Z, Dunaif A, Laven JS, Legro RS, *et al*. Polycystic ovary syndrome. Nat Rev Dis Primers 2016;2:16057. doi: 10.1038/nrdp.2016.57.
38. Kajihara T, Ishihara O, Brosens JJ. Oxidative stress and its implications in endometrial function. In: Uterine endometrial function; 2016: 105–123. doi: 10.1007/978-4-431-55972-6_7.
39. Liu Y, Yu Z, Zhao S, Cheng L, Man Y, Gao X, *et al*. Oxidative stress markers in the follicular fluid of patients with polycystic ovary syndrome correlate with a decrease in embryo quality. J Assist Reprod Genet 2021;38:471–477. doi: 10.1007/s10815-020-02014-y.
40. Yakes FM, Van Houten B. Mitochondrial DNA damage is more extensive and persists longer than nuclear DNA damage in human cells following oxidative stress. Proc Natl Acad Sci USA 1997;94:514–519. doi: 10.1073/pnas.94.2.514.
41. Iwahara T, Bonasio R, Narendra V, Reinberg D. SIRT3 functions in the nucleus in the control of stress-related gene expression. Mol Cell Biol 2012;32:5022–5034. doi: 10.1128/MCB.00822-12.
42. Schwer B, Bunkenborg J, Verdin RO, Andersen JS, Verdin E. Reversible lysine acetylation controls the activity of the mitochondrial enzyme acetyl-CoA synthetase 2. Proc Natl Acad Sci U S A 2006;103:10224–10229. doi: 10.1073/pnas.0603968103.
43. Schwer B, North BJ, Frye RA, Ott M, Verdin E. The human silent information regulator (Sir)2 homologue hSIRT3 is a mitochondrial nicotinamide adenine dinucleotide-dependent deacetylase. J Cell Biol 2002;158:647–657. doi: 10.1083/jcb.200205057.
44. Lombard DB, Alt FW, Cheng HL, Bunkenborg J, Streeper RS, Mostoslavsky R, *et al*. Mammalian Sir2 homolog SIRT3 regulates global mitochondrial lysine acetylation. Mol Cell Biol 2007;27:8807–8814. doi: 10.1128/MCB.01636-07.
45. Savini I, Catani MV, Evangelista D, Gasperi V, Avigliano L. Obesity-associated oxidative stress: Strategies finalized to improve redox state. Int J Mol Sci 2013;14:10497–10538. doi: 10.3390/ijms140510497.
46. Manna P, Jain SK. Obesity, oxidative stress, adipose tissue dysfunction, and the associated health risks: Causes and therapeutic strategies. Metab Syndr Relat Disord 2015;13:423–444. doi: 10.1089/met.2015.0095.
47. Dai SH, Chen T, Wang YH, Zhu J, Luo P, Rao W, *et al*. Sirt3 protects cortical neurons against oxidative stress via regulating mitochondrial Ca2+ and mitochondrial biogenesis. Int J Mol Sci 2014;15:14591–14609. doi: 10.3390/ijms150814591.
48. Wang XQ, Shao Y, Ma CY, Chen W, Sun L, Liu W, *et al*. Decreased SIRT3 in aged human mesenchymal stromal/stem cells increases cellular susceptibility to oxidative stress. J Cell Mol Med 2014;18:2298–2310. doi: 10.1111/jcmm.12395.
49. Singh CK, Chhabra G, Ndiaye MA, Garcia-Peterson LM, Mack NJ, Ahmad N. The role of sirtuins in antioxidant and redox signaling. Antioxid Redox Signal 2018;28:643–661. doi: 10.1089/ars.2017.7290.
50. Ansari A, Rahman MS, Saha SK, Saikot FK, Deep A, Kim KH. Function of the SIRT3 mitochondrial deacetylase in cellular physiology, cancer, and neurodegenerative disease. Aging Cell 2017;16:4–16. doi: 10.1111/acel.12538.
51. Scher MB, Vaquero A, Reinberg D. SirT3 is a nuclear NAD+-dependent histone deacetylase that translocates to the mitochondria upon cellular stress. Genes Dev 2007;21:920–928. doi: 10.1101/gad.1527307.
52. Choudhury M, Park PH, Jackson D, Shukla SD. Evidence for the role of oxidative stress in the acetylation of histone H3 by ethanol in rat hepatocytes. Alcohol 2010;44:531–540. doi: 10.1016/j.alcohol.2010.06.003.
53. Bagul PK, Deepthi N, Sultana R, Banerjee SK. Resveratrol ameliorates cardiac oxidative stress in diabetes through deacetylation of NFkB-p65 and histone 3. J Nutr Biochem 2015;26:1298–1307. doi: 10.1016/j.jnutbio.2015.06.006.
54. Wang J, Hao M, Liu Y, Liu R. Cadmium induced apoptosis in mouse primary hepatocytes: The role of oxidative stress-mediated ERK pathway activation and the involvement of histone H3 phosphorylation. RSC Adv 2015;5:31798. doi: 10.1039/c5ra03210e.
55. Wang X, Yu Q. An update on the progress of transcriptomic profiles of human endometrial receptivity. Biol Reprod 2018;98:440–448. doi: 10.1093/biolre/ioy018.
56. Farhang Ghahremani M, Goossens S, Haigh JJ. The p53 family and VEGF regulation: "It's complicated". Cell Cycle 2013;12:1331–1332. doi: 10.4161/cc.24579.
57. Vagnini LD, Nascimento AM, Canas Mdo C, Renzi A, Oliveira-Pelegrin GR, Petersen CG, *et al*. The relationship between vascular endothelial growth factor 1154G/A polymorphism and recurrent implantation failure. Med Princ Pract 2015;24:533–537. doi: 10.1159/000437370.
58. von Grothusen C, Lalitkumar S, Boggavarapu NR, Gemzell-Danielsson K, Lalitkumar PG. Recent advances in understanding endometrial receptivity: Molecular basis and clinical applications. Am J Reprod Immunol 2014;72:148–157. doi: 10.1111/aji.12226.

How to cite this article: Zeng ZH, Shan HY, Lin MM, Bao SY, Mo D, Deng F, Yu Y, Yang YH, Zhou P, Li R. SIRT3 protects endometrial receptivity in patients with polycystic ovary syndrome. Chin Med J 2025;138:1225–1235. doi: 10.1097/CM9.0000000000003127