Proteomic alteration of endometrial tissues during secretion in polycystic ovary syndrome may affect endometrial receptivity

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Abstract
Embryo implantation is a complex developmental process that requires coordinated interactions among the embryo, endometrium, and the microenvironment of endometrium factors. Even though the impaired endometrial receptivity of patients with polycystic ovary syndrome (PCOS) is known, understanding of endometrial receptivity is limited. A proteomics study in three patients with PCOS and 3 fertile women was performed to understand the impaired endometrial receptivity in patients with PCOS during luteal phases. Through isobaric tags for relative and absolute quantitation (iTRAQ) analyses, we identified 232 unique proteins involved in the metabolism, inflammation, and cell adhesion molecules. Finally, our results suggested that energy metabolism can affect embryo implantation, whereas inflammation and cell adhesion molecules can affect both endometrial conversion and receptivity. Our results showed that endometrial receptive damage in patients with PCOS is not a single factor. It is caused by many proteins, pathways, systems, and abnormalities, which interact with each other and make endometrial receptive research more difficult.

Keywords: Endometrial receptivity, Endometrium, PCOS, Proteomics

Introduction
Polycystic ovary syndrome (PCOS) is the most common endocrine disorder among women of reproductive age and perplexes researchers and doctors globally [1]. Even though many researchers focus on the pathophysiology of PCOS, the etiology underlying PCOS is still unknown. Many present studies mainly focused on improving clinical symptoms, such as insulin resistance, obesity, metabolic derangements, and increase in androgen, to achieve successful conceiving, reduce pregnancy-related complications, and enhance pregnancy outcomes [2, 3]. Ovulation disorders were previously considered the main cause of infertility in patients with PCOS. The pregnancy rates are still low in patients with PCOS and the high risk of biochemical abortion after ovulation disorders have been reduced. Many factors may lead to this situation, and impaired endometrial receptivity could be a responsible reason for adverse pregnancy outcomes in patients with PCOS. Unfortunately, only a few studies have elucidated the molecular mechanisms underlying impaired endometrial receptivity. Some important proteins involved in embryo implantation, such as forkhead box protein O1 (FOXO1), homeobox A10 (HOXA10), insulin-like growth factor-binding protein 1 (IGFBP-1), and inhibiting insulin growth factor 1 (IGF-1) are known to be abnormal in patients with PCOS compared with healthy individuals [4]. Single protein changes do not reflect the function of the endometrial microenvironment due to protein–protein interactions; therefore, the ongoing studies have increasingly focused on proteomic analyses.

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Proteomics-based analyses are not limited by previous information on the problem and can help discover the potential advantage of revealing novel associations with unexpected molecules that can lead to new mechanistic explanations for impaired endometrial implantation.

In the present years, proteomics analyses have been used to elucidate the potential mechanisms underlying adverse pregnancy outcomes in patients with PCOS. To the best of our knowledge, no research has been performed on the secretory endometrial proteome in patients with PCOS to date. To elucidate the molecular basis underlying infertility related to endometrium implantation in patients with PCOS, we compared the secretory endometrial proteomic profile of patients with PCOS with that of healthy fertile women using isobaric tags for relative and absolute quantitation (iTRAQ).

**Materials and methods**

**Clinical sample preparation methods**

The endometrial tissues were obtained from 3 patients with PCOS and 3 healthy volunteers who already had children. The patients with PCOS took letrozole on the 3rd day of menstruation; their ovulation was continuously monitored, starting from the 10th day of menstruation; and the endometrium was obtained on the 5th day of ovulation.

These patients were also screened for their glucose metabolism and endocrine normality through serum determinations of the levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol, glucose, and insulin on day 3 of the menstrual cycle. No participants demonstrated any evidence of chromosomal abnormality, pathological uterine disorder, or endometrial hyperplasia. None of the patients had used oral contraception or had undergone hormonal therapy during the past 3 months. The diagnosis of PCOS was made in accordance with the 2003 Rotterdam criteria, which included any two or all three of the following features: (1) oligo-/anovulation; (2) clinical or biochemical signs of hyperandrogenism; and (3) polycystic ovary morphology on ultrasound examination [5]. The main demographic characteristics of the patient and the control groups are summarized in Table 1. The results for the PCOS and control groups did not differ in terms of age, body mass index (BMI), FSH, LH, and testosterone, albeit it differed for the levels of insulin and glucose. Each biopsy was dry frozen at −80 °C for protein extraction.

The patients were recruited at the Reproductive Medicine Center, Department of Obstetrics and Gynecology, The First Affiliated Hospital of Anhui Medical University, approved by the Institutional Ethics Committee (No: 20170609). All patients provided their informed consent prior to their participation in the study. Figure 1 displays the basic principle of iTRAQ quantitative proteomics and the main steps involved in the quantitative techniques.

**Protein extraction**

We used the lysis buffer 3 (8 M urea, TEAB or 40 mM Tris–HCl with 1 mM PMSF, 2 mM EDTA and 10 mM DTT; pH 8.5) and two magnetic beads to extract the proteins. Then, we removed the mixtures into a tissue lyser for 2 min at 50 Hz to release the proteins. Next,
the supernatant was transferred into a new tube after centrifugation at 25,000×g at 4 °C for 20 min, reduced with 10-mM dithiothreitol (DTT) at 56 °C for 1 h, and alkylated with 55-mM iodoacetamide (IAM) in the dark at room temperature for 45 min. Following centrifugation, the supernatant containing the proteins was quantified by Bradford assay.

**QC of protein extraction**

**Protein quantitation by Bradford assay**

First, we added 0, 2, 4, 6, 8, 10, 12, 14, 16, and 18 μL of the BSA solution, separately, into a 96-well plate, and to the corresponding wells, we added 20, 18, 16, 14, 12, 10, 8, 6, 4, and 2 μL of pure water, separately. Meanwhile, we prepared serial dilutions (20 μL/well) of the unknown sample for enumeration. Next, we added 180 μL of Coomassie blue to each well and mixed the contents of each well. The absorbance of each standard and sample well were read at 595 nm. Each sample had at least two duplicates. Then, the absorbance of the standards vs. their concentration was plotted. Finally, we calculated the extinction coefficient and the concentrations of the unknown samples.

**Protein digestion**

The protein solution (100 μg) containing 8 M urea was diluted 4 times with 100 mM TEAB. We then applied trypsin gold (Promega, Madison, WI, USA) to digest the proteins (protein: trypsin = 40:1) at 37 °C overnight. Next, we used the Strata X C18 column (Phenomenex) and vacuum-dried the specimens to desalt the peptides with triethylammonium bicarbonate (TEAB). In the labeling reaction, the isopropanol concentration was maintained at >75%, and the labeling process was stopped by adding trifluoroacetic acid (TFA) at the end of the incubation period at the ambient temperature for 2 h. Then, we combined and desalted the labeled peptides on the Strata X C18 column and vacuum-dried them as per the manufacturer’s protocol.

**Peptide fractionation**

We separated the peptides through the Shimadzu LC-20AB HPLC Pump System coupled with a high-pH RP column. Next, we reconstituted the peptides with buffer A (5% ACN, 95% H2O, adjusted the pH to 9.8 with ammonia) to 2 mL and loaded them onto a column (5 μm, 20 cm×180 μm; Gemini C18) containing 5-μm particles (Phenomenex). Then, we separated the peptides at the flow rate of 1 mL/min with a gradient of 5% buffer B (5% H2O, 95% ACN, adjusted pH to 9.8 with ammonia) for 10 min, 5–35% buffer B for 40 min, and 35–95% buffer B for 1 min. Then, the system was maintained in 95% buffer B for another 3 min and decreased to 5% within 1 min before equilibration with 5% buffer B for 10 min. Next, we monitored the elution by measuring the absorbance at 214 nm and collected the fractions every minute. Finally, we divided the eluted peptides into 20 fractions and vacuum-dried them for further analyses.

**HPLC**

First, each fraction was resuspended in buffer A (2% ACN, 0.1% FA) and centrifuged at 20,000×g for 10 min. Then, the supernatant was loaded on the Thermo Scientific™ UltiMate™ 3000 UHPLC system equipped with a trap and an analytical column. We loaded the samples on the trap column (PEPMAP 100 C18 5UM 0.3×5MM 5PK) at 5 μL/min for 8 min and eluted it into the homemade nanocapillary C18 column (ID 75 μm×25 cm, 3-μm particles) with a 300 nL/min flow rate. The gradient of buffer B (98% ACN, 0.1% FA) was raised from 5 to 25% in 40 min, raised to 35% in 5 min, followed by a 2-min linear gradient to 80%, maintained at 80% B for another 2 min, returned to 5% in 1 min, and then equilibrated for 6 min.

**Mass spectrometer detection**

We subjected the peptides separated from nanoHPLC to tandem mass spectrometry Q EXACTIVE HF X (Thermo Fisher Scientific, San Jose, CA) for data-dependent acquisition (DDA) detection by nanoelectrospray ionization. The relevant parameters of the MS analysis were as follows: precursor scan range: 350–1500 m/z at the resolution of 60,000 in Orbitrap; electrospray voltage: 2.0 kV; MS/MS fragment scan range: in HCD mode with a 100 m/z scan, resolution at 15,000; normalized collision energy setting: 30%; dynamic exclusion time: 30 s; automatic gain control (AGC) for full MS target and MS2 target: 3e6 and 1e5, respectively; the number of MS/MS scans following one MS scan: 20 most abundant precursor ions above a threshold ion count of 10,000.
**Protein quantification**

We used an automated software called IQuant to quantitatively analyze the labeled peptides with isobaric tags. This software integrates the Mascot Percolator [6] to provide reliable significance measurements. To assess the confidence of peptides, the PSMs were prefiltered at 1% PSM-level FDR. Then, based on the “simple principle” (the parsimony principle), the identified peptide sequences were assembled into a set of confident proteins. To control the rate of false positives at the protein level, a protein FDR of 1%, which is based on the selected protein FDR strategy [7], was estimated after protein inference (protein-level FDR ≤ 0.01). The process of protein quantification comprised the following steps: protein identification, tag impurity correction, data normalization, missing value imputation, protein ratio calculation, statistical analysis, and result presentation [7]. Data normalization: We selected variance stabilization normalization (VSN) [8, 9] as our preferred normalization strategy. Protein ratio calculation: nonunique peptides and outlier peptide ratios were removed prior to their quantification [10]. The weight approach proposed elsewhere [11] was employed to evaluate the ratios of protein quantity based on the reporter ion intensities. Statistical analysis: Permutation tests were widely applied in the fields of microarray and RNA-Seq data analysis [12, 13]. To estimate the statistical significance of the protein quantitative ratios, IQuant adopted the permutation test, a nonparametric approach, as reported by Nguyen et al. [14]. For each protein, IQuant provided a significance evaluation that was corrected for multiple hypothesis testing by the Benjamini–Hochberg method [15].

**Results**

**Altered levels of proteins in the endometrium of women with PCOS**

We quantitatively identified 6524 proteins in samples from the PCOS group and the control group. We used CV to evaluate the reproducibility. CV is defined as the ratio of the standard deviation (SD) and the mean. Lower CV indicates better reproducibility. The mean CV (0.12) showed that the proteins identified in this study have good reproducibility. (Additional file 1: Fig. S1). Proteins with a 1.2-fold change and Q value less than 0.05 were determined as differentially expressed proteins (DEPs) in a single replicate. Compared with the control group, 232 proteins showed significant changes in their levels in the PCOS group. Of these, 108 proteins were increased and 124 proteins were decreased. The list of significantly regulated proteins along with their log 2 changes, corresponding p-values, and relevant biological processes are shown in Fig. 2 and Table 2.
**Table 2** List of significantly regulated proteins in PCOS and control groups

| No. | Protein_ID | Description | P       | Mean_Ratio_treated-VS-control |
|-----|------------|-------------|---------|-------------------------------|
| 1   | sp|Q7Z680|CCD91_HUMAN | Coiled-coil domain-containing protein 91 (CCDC91) | 0.00 | 0.82 |
| 2   | sp|Q8NH9|DDX55_HUMAN | ATP-dependent RNA helicase DDX55 (DDX55) | 0.00 | 0.82 |
| 3   | sp|Y9621|CAN6_HUMAN | Calpain-6 (CAPN6) | 0.00 | 0.79 |
| 4   | sp|QNYC9|DYH9_HUMAN | Dynein heavy chain 9, axonemal (DNAH9) | 0.00 | 0.33 |
| 5   | sp|Q9BZV7|TSG10_HUMAN | Testis-specific gene 10 protein (TSGA10) | 0.00 | 0.8 |
| 6   | sp|Q9NS5Y|NRFP2_HUMAN | Nuclear receptor-binding protein 2 (NRP2P) | 0.02 | 0.8 |
| 7   | sp|O60331|PI51C_HUMAN | Phosphatidylinositol 4-phosphate 5-kinase type-1 gamma (PIPSK1C) | 0.03 | 0.7 |
| 8   | sp|Q9Y4X5|ARI1_HUMAN | E3 ubiquitin-protein ligase ARI1 (ARIH1) | 0.03 | 0.74 |
| 9   | sp|P1602|KV105_HUMAN | Immunoglobulin kappa variable 1-5 (IGKV1-5) | 0.01 | 0.74 |
| 10  | sp|Q8N6U8|GP161_HUMAN | G-protein coupled receptor 161 (GPR161) | 0.04 | 0.83 |
| 11  | sp|P05543|THBG_HUMAN | Thrombospondin 2 (THBG) | 0.02 | 0.82 |
| 12  | sp|Q9NX55|HYPK_HUMAN | Hsp70-interacting protein K (HSPK) | 0.00 | 0.74 |
| 13  | sp|P35058|PLTP_HUMAN | Phospholipid transfer protein (PLTP) | 0.04 | 0.78 |
| 14  | sp|Q7SO15|FCG3B_HUMAN | Low affinity immunoglobulin gamma Fc region receptor III-B (FCG3B) | 0.04 | 0.82 |
| 15  | sp|Q9HCJ0|TNRC6C_HUMAN | Trinucleotide repeat-containing gene 6C protein (TNRC6C) | 0.03 | 0.76 |
| 16  | sp|P04439|A010_HUMAN | HLA class I histocompatibility antigen, A-3 alpha chain (HLA-A) | 0.05 | 0.65 |
| 17  | sp|Q9H8V3|ECT2_HUMAN | Protein ECT2 (ECT2) | 0.03 | 0.81 |
| 18  | sp|O43174|CP26A_HUMAN | Cytochrome P450 26A1 (CYP26A1) | 0.02 | 0.83 |
| 19  | sp|Q9P3F6|HRG20_HUMAN | Rho GTPase-activating protein 20 (ARHGAP20) | 0.00 | 0.75 |
| 20  | sp|Q9NVQ4|FAIM1_HUMAN | Fas apoptotic inhibitory molecule 1 (FAIM1) | 0.00 | 0.71 |
| 21  | sp|Q8NAN2|MIGA1_HUMAN | Mitogargin 1 (MIGA1) | 0.00 | 0.56 |
| 22  | sp|Q9ND83|SLA1_HUMAN | SLA1-motif-containing protein 1 (SLA11) | 0.01 | 0.83 |
| 23  | sp|Q9UP85|S12A4_HUMAN | Solute carrier family 12 member 4 (SLC12A4) | 0.01 | 0.76 |
| 24  | sp|Q96D05|FAM241B_HUMAN | Uncharacterized protein FAM241B (FAM241B) | 0.00 | 0.77 |
| 25  | sp|Q13009|TIA1_HUMAN | T-lymphoma invasion and metastasis-inducing protein 1 (TIA1M) | 0.03 | 0.65 |
| 26  | sp|A0A04D2H29|HV103_HUMAN | Immunoglobulin heavy variable 1-3 (IGHV1-3) | 0.04 | 0.72 |
| 27  | sp|P01597|KV139_HUMAN | Immunoglobulin kappa variable 1-39 (IGKV1-39) | 0.00 | 0.79 |
| 28  | sp|A0A0758660|LV861_HUMAN | Immunoglobulin lambda variable 8-61 (IGL8-61) | 0.03 | 0.77 |
| 29  | sp|Q99699|RARR2_HUMAN | Retinoic acid receptor responder protein 2 (RARRR2) | 0.02 | 0.72 |
| 30  | sp|Q8NN98|EIF1A_HUMAN | Probable RNA-binding protein EIF1AD (EIF1AD) | 0.03 | 0.8 |
| 31  | sp|P0DOX3|IGD_HUMAN | Immunoglobulin delta heavy chain | 0.01 | 0.83 |
| 32  | sp|Q15751|HERC1_HUMAN | Probable E3 ubiquitin-protein ligase HERC1 (HERC1) | 0.00 | 0.81 |
| 33  | sp|P58873|UB2D2_HUMAN | Ubiquitin-conjugating enzyme E2 D2 (UBE2D2) | 0.00 | 0.81 |
| 34  | sp|A0A0411YV94_HUMAN | Immunoglobulin lambda variable 9-49 (IGLV9-49) | 0.00 | 0.82 |
| 35  | sp|P0DP01|HV108_HUMAN | Immunoglobulin heavy variable 1-8 (IGHV1-8) | 0.01 | 0.64 |
| 36  | sp|P56962|STX17_HUMAN | Syntaxin-17 (STX17) | 0.00 | 0.69 |
| 37  | sp|P09601|HMOX1_HUMAN | Heme oxygenase 1 (HMOX1) | 0.02 | 0.75 |
| 38  | sp|A0A075685T|TVA18_HUMAN | T cell receptor alpha variable 18 (TRAV18) | 0.00 | 0.66 |
| 39  | sp|P10643|CO7_HUMAN | Complement component C7 (C7) | 0.00 | 0.79 |
| 40  | sp|Q93933|HSF2_HUMAN | Heat shock factor protein 2 (HSF2) | 0.00 | 0.64 |
| 41  | sp|A0A04C1B3H8|HVS51_HUMAN | Immunoglobulin heavy variable 5-51 (IGHV5-51) | 0.03 | 0.78 |
| 42  | sp|Q15139|KPCD1_HUMAN | Serine/threonine-protein kinase D1 (PRKD1) | 0.00 | 0.81 |
| 43  | sp|Q9H1X3|DJC25_HUMAN | DnaJ homolog subfamily C member 25 (DNAJC25) | 0.00 | 0.6 |
| 44  | sp|A4UGR9|XIRP2_HUMAN | X-linked insulator repeat-containing protein 2 (XIRP2) | 0.00 | 0.68 |
| 45  | sp|Q8N6N6|NATD1_HUMAN | Protein NATD1 (NATD1) | 0.00 | 0.76 |
| 46  | sp|A0P2Z3|GXL12_HUMAN | Glucoside xylosyltransferase 2 (GXYLT2) | 0.00 | 0.81 |
| 47  | sp|P15169|CBPN_HUMAN | Carboxypeptidase N catalytic chain (CPN1) | 0.02 | 0.71 |
| 48  | sp|Q94952|FBX21_HUMAN | F-box only protein 21 (FBXO21) | 0.00 | 0.83 |
| No. | Protein_ID | Description                                                                 | P  | Mean_Ratio_treated-VS-control |
|-----|------------|------------------------------------------------------------------------------|----|-------------------------------|
| 49  | sp|Q4U2R6|RM51_HUMAN | 39S ribosomal protein L51, mitochondrial (MRPL51) | 0.02 | 0.83 |
| 50  | sp|P50749|RASF2_HUMAN | Ras association domain-containing protein 2 (RASSF2) | 0.02 | 0.82 |
| 51  | sp|Q66P3|AR6P4_HUMAN | ADP-ribosylation factor-like protein 6-interacting protein 4 (ARL6IP4) | 0.01 | 0.8 |
| 52  | sp|Q94868|FCSK2_HUMAN | F-BAR and double SH3 domains protein 2 (FCHSD2) | 0.03 | 0.7 |
| 53  | sp|Q9YSU8|MPC1_HUMAN | Mitochondrial pyruvate carrier 1 (MPC1) | 0.00 | 0.75 |
| 54  | sp|Q96NT0|CC115_HUMAN | Coiled-coil domain-containing protein 115 (CCDC115) | 0.01 | 0.78 |
| 55  | sp|Q9UGI0|AAG02_HUMAN | S'-AMP-activated protein kinase subunit-gamma-2 (PRKAG2) | 0.00 | 0.81 |
| 56  | sp|Q0P749|CB080_HUMAN | Uncharacterized protein C2orf80 (C2orf80) | 0.00 | 0.69 |
| 57  | sp|Q96GM8|TOE1_HUMAN | Target of EGR1 protein 1 (TOE1) | 0.01 | 0.8 |
| 58  | sp|P01825|H4V59_HUMAN | Immunoglobulin heavy variable 4-59 (IGHV4-59) | 0.02 | 0.78 |
| 59  | sp|Q9854|ATG1_A_HUMAN | Autophagy-related protein 101 (ATG101) | 0.04 | 0.81 |
| 60  | sp|Q53FV1|ORML2_HUMAN | ORM1-like protein 2 (ORMDL2) | 0.03 | 0.81 |
| 61  | sp|P20742|PZP_HUMAN | Pregnancy zone protein (PZP) | 0.00 | 0.8 |
| 62  | sp|Q15213|WDR46_HUMAN | WD repeat-containing protein 46 (WDR46) | 0.01 | 0.83 |
| 63  | sp|Q9P1P5|TAAR2_HUMAN | Trace amine-associated receptor 2 (TAAR2) | 0.00 | 0.72 |
| 64  | sp|P0CG29|GST2_HUMAN | Glutathione S-transferase theta-2 (GSTT2) | 0.01 | 0.75 |
| 65  | sp|Q960Z8|NSD2_HUMAN | Histone-lysine N-methyltransferase NSD2 (NSD2) | 0.05 | 0.82 |
| 66  | sp|Q9NX3|DNC28_HUMAN | DnaJ homolog subfamily C member 28 (DNAJC28) | 0.00 | 0.7 |
| 67  | sp|Q9GZT4|SSR_HUMAN | Serine racemase (SRR) | 0.03 | 0.81 |
| 68  | sp|Q9NYQ3|HAOX2_HUMAN | Hydroxycacid oxidase 2 (HAO2) | 0.00 | 0.72 |
| 69  | sp|A8RTX5|YTYC2_HUMAN | Probable theonine-riRNA ligase 2, cytoplasmic (TARSL2) | 0.00 | 0.77 |
| 70  | sp|Q30453|I3A4_HUMAN | HLA class I histocompatibility antigen, A-34 alpha chain (HLA-A) | 0.02 | 0.74 |
| 71  | sp|P73332|RB6_HUMAN | RNA-binding protein 6 (RBM6) | 0.02 | 0.83 |
| 72  | sp|P01743|H3V16_HUMAN | Immunoglobulin heavy variable 1-46 (IGHV1-46) | 0.00 | 0.8 |
| 73  | sp|Q9NG1|TNS14_HUMAN | Tetraspanin-14 (TSPAN14) | 0.01 | 0.82 |
| 74  | sp|Q8BTPS|F174A_HUMAN | Membrane protein FAM174A (FAM174A) | 0.01 | 0.6 |
| 75  | sp|Q6SO5|JNMT2_HUMAN | Glycylpeptide N-tetradecanoyltransferase 2 (NMT2) | 0.01 | 0.81 |
| 76  | sp|Q99ES9|CPNE1_HUMAN | Copine-1 (CPNE1) | 0.00 | 0.83 |
| 77  | sp|Q9Y644|CF20_HUMAN | Cilia- and flagella-associated protein 20 (CFAP20) | 0.00 | 0.79 |
| 78  | sp|Q88BF9|GLIS1_HUMAN | Zinc finger protein GLIS1 (GLIS1) | 0.05 | 0.72 |
| 79  | sp|Q9BQ75|CM51_HUMAN | Protein CM51 (CM51) | 0.00 | 0.65 |
| 80  | sp|Q15055|PER2_HUMAN | Period circadian protein homolog 2 (PER2) | 0.00 | 0.69 |
| 81  | sp|Q96Q2|TEFM_HUMAN | Transcription elongation factor, mitochondrial (TEFM) | 0.01 | 0.61 |
| 82  | sp|P0114A|ARCP_HUMAN | Apolipoprotein B-100 (APOP) | 0.05 | 0.83 |
| 83  | sp|Q8IY8|H01_HUMAN | Interactor of HORMAD1 protein 1 (CCDC36) | 0.02 | 0.7 |
| 84  | sp|P08571|CD14_HUMAN | Monocyte differentiation antigen CD14 (CD14) | 0.00 | 0.82 |
| 85  | sp|Q96V8|DTB01_HUMAN | Dysbindin (DTNB1) | 0.02 | 0.76 |
| 86  | sp|Q15166|PON3_HUMAN | Serum paraoxonase/lactonase 3 (PON3) | 0.01 | 0.82 |
| 87  | sp|Q81V63|VRK3_HUMAN | Inactive serine/threonine-protein kinase VRK3 (VRK3) | 0.04 | 0.83 |
| 88  | sp|P01099|AT1_HUMAN | Alpha-1-antitrypsin (SERPINA1) | 0.02 | 0.82 |
| 89  | sp|Q15022|SU12_HUMAN | Polycomb protein SUZ12 (SUZ12) | 0.00 | 0.7 |
| 90  | sp|P30711|GSTT1_HUMAN | Glutathione S-transferase theta-1 (GSTT1) | 0.01 | 0.69 |
| 91  | sp|Q99C4|SCRN3_HUMAN | Secernin-3 (SCRN3) | 0.00 | 0.75 |
| 92  | sp|P35443|TS4_HUMAN | Thrombospondin-4 (THBS4) | 0.00 | 0.68 |
| 93  | sp|P14680|MELK_HUMAN | Maternal embryonic leucine zipper kinase (MELK) | 0.00 | 0.62 |
| 94  | sp|Q66D8|ZNF787_HUMAN | Zinc finger protein 787 (ZNF787) | 0.00 | 0.83 |
| 95  | sp|P00488|F13A_HUMAN | Coagulation factor XIII A chain (F13A1) | 0.03 | 0.81 |
| 96  | sp|P01766|HV313_HUMAN | Immunoglobulin heavy variable 3-13 (IGHV3-13) | 0.01 | 0.78 |
| No. | Protein_ID | Description                                                                 | P   | Mean_Ratio_treated-VS-control |
|-----|------------|------------------------------------------------------------------------------|-----|------------------------------|
| 97  | sp|Q9Y3D7|TIM16_HUMAN | Mitochondrial import inner membrane translocase subunit TIM16 (PAM16) | 0.02 | 0.83 |
| 98  | sp|Q1S843|NEDD8_HUMAN | NEDD8 (NEDD8) | 0.02 | 0.73 |
| 99  | sp|P02533|K1C14_HUMAN | Keratin, type I cytoskeletal 14 (KRT14) | 0.01 | 0.79 |
| 100 | sp|Q5UCC4|EMC10_HUMAN | ER membrane protein complex subunit 10 (EMC10) | 0.00 | 0.8 |
| 101 | sp|Q9S2588|UCPS_HUMAN | Brain mitochondrial carrier protein 1 (SLC25A14) | 0.03 | 0.8 |
| 102 | sp|Q9Q615|SMG1_HUMAN | Serine/threonine-protein kinase SMG1 (SMG1) | 0.01 | 0.78 |
| 103 | sp|Q8N59|CL045_HUMAN | Uncharacterized protein C12orf45 (C12orf45) | 0.00 | 0.75 |
| 104 | sp|P51157|RAB28_HUMAN | Ras-related protein Rab-28 (RAB28) | 0.02 | 0.83 |
| 105 | sp|P27037|AVR2A_HUMAN | Activin receptor type-2A (ACVR2A) | 0.05 | 0.78 |
| 106 | sp|Q9BT9|TCHP_HUMAN | Trichoplein keratin filament-binding protein (TCHP) | 0.04 | 0.8 |
| 107 | sp|P15427|SF3B4_HUMAN | Splicing factor 3B subunit 4 (SF3B4) | 0.04 | 0.82 |
| 108 | sp|P24593|IBPS_HUMAN | Insulin-like growth factor-binding protein 5 (IGFBP5) | 0.05 | 0.78 |
| 109 | sp|Q9Y229|COQ6_HUMAN | Ubiquinone biosynthesis monoxygenase COQ6, mitochondrial (COQ6) | 0.02 | 0.8 |
| 110 | sp|P14136|GFAP_HUMAN | Gial fibrillary acidic protein (GFAP) | 0.03 | 0.75 |
| 111 | sp|Q8NC5|CHST4_HUMAN | Carbohydrate sulfotransferase 4 (CHST4) | 0.00 | 0.8 |
| 112 | sp|P01834|IGKC_HUMAN | Immunoglobulin kappa constant (IGKC) | 0.05 | 0.83 |
| 113 | sp|Q4ZH4|FND1C_HUMAN | Fibronectin type III domain-containing protein 1 (FND1C) | 0.00 | 0.77 |
| 114 | sp|Q15615|IKBB_HUMAN | NF-kappa-B inhibitor beta (NFKBIB) | 0.04 | 0.82 |
| 115 | sp|P49184|DNLS1_HUMAN | Zinc finger protein S87B (ZNF587B) | 0.05 | 0.83 |
| 116 | sp|P49184|DNLS1_HUMAN | Deoxyribonuclease-1-like 1 (DNASE1L1) | 0.00 | 0.66 |
| 117 | sp|Q96H9|FM1_HUMAN | Protein FM1 homolog (FM1) | 0.04 | 0.82 |
| 118 | sp|Q9B8N8|OTUL_HUMAN | Ubiquitin thioesterase otulin (OTULIN) | 0.00 | 0.82 |
| 119 | sp|Q9BH15|C014_HUMAN | Uncharacterized protein C3orf14 (C3orf14) | 0.01 | 0.8 |
| 120 | sp|Q9S80|TTC4_HUMAN | Tetratricopeptide repeat protein 4 (TTC4) | 0.02 | 0.83 |
| 121 | sp|Q9B592|NP53_B_HUMAN | Protein NipScan homolog 3B (NIPSAP3B) | 0.01 | 0.81 |
| 122 | sp|A0A07566|KVD29_HUMAN | Immunoglobulin kappa variable 2D-29 (IGKV2D-29) | 0.02 | 0.82 |
| 123 | sp|A0A07566|KVD29_HUMAN | Immunoglobulin heavy variable 1-18 (IGHV1-18) | 0.00 | 0.71 |
| 124 | sp|Q96X5|UPT4_HUMAN | U3 small nucleolar RNA-associated protein 4 homolog (UPT4) | 0.02 | 0.81 |
| 125 | sp|Q9MV5|SDA1_HUMAN | Protein SDA1 homolog (SDADI) | 0.02 | 1.22 |
| 126 | sp|Q9BL7|UPLS_HUMAN | Ubiquitin-like protein 5 (UBL5) | 0.02 | 1.35 |
| 127 | sp|Q96K80|ZC3HA_HUMAN | Zinc finger CCCH domain-containing protein 10 (ZC3H10) | 0.00 | 1.24 |
| 128 | sp|O0479|HMGN4_HUMAN | High mobility group nucleosome-binding domain-containing protein 4 (HMGN4) | 0.04 | 1.31 |
| 129 | sp|P3553|PKGC_HUMAN | Phosphoenolpyruvate carboxykinase, cytosolic [GTP] (PCK1) | 0.04 | 1.21 |
| 130 | sp|Q3Mrf|CSO8_HUMAN | Cell cycle control protein 50B (TMEM30B) | 0.01 | 2.09 |
| 131 | sp|Q5SRD9|FBLN7_HUMAN | Fibulin-7 (FBLN7) | 0.02 | 1.34 |
| 132 | sp|P0969|GMFB_HUMAN | Glia maturation factor beta (GMFB) | 0.00 | 1.22 |
| 133 | sp|Q1S041|AR6P1_HUMAN | ADP-ribosylation factor-like protein 6-interacting protein 1 (ARL6IP1) | 0.00 | 1.52 |
| 134 | sp|Q9BY7|ACTBM_HUMAN | Putative beta-actin-like protein 3 (POTECP) | 0.00 | 1.21 |
| 135 | sp|Q9H07|TM165_HUMAN | Transmembrane protein 165 (TMEM165) | 0.01 | 1.27 |
| 136 | sp|Q9H06|RPA2_HUMAN | DNA-directed RNA polymerase I subunit RPA2 (POLR1B) | 0.05 | 1.27 |
| 137 | sp|Q8UIF8|ROX2_HUMAN | Ribosomal oxygenase 2 (ROX2) | 0.00 | 1.73 |
| 138 | sp|Q92833|JARD2_HUMAN | Protein Jumonji (JARID2) | 0.04 | 1.25 |
| 139 | sp|Q5279|SFT2C_HUMAN | Vesicle transport protein SFT2C (SFT2D3) | 0.01 | 1.25 |
| 140 | sp|P1341|CY24A_HUMAN | Cytochrome b-245 light chain (CYBA) | 0.01 | 1.28 |
| 141 | sp|Q5BE2|SDK2_HUMAN | Protein sidekick-2 (SDK2) | 0.00 | 1.3 |
| 142 | sp|P5282|STC1_HUMAN | Stanniocalcin-1 (STC1) | 0.02 | 2 |
| 143 | sp|Q9687|TBBI1_HUMAN | Tubulin beta-1 chain (TUBB1) | 0.04 | 1.25 |
| 144 | sp|Q9HA82|CER54_HUMAN | Ceramide synthase 4 (CER54) | 0.02 | 1.88 |
### Table 2 (continued)

| No. | Protein_ID | Description | P | Mean_Ratio_{treated-VS-control} |
|-----|------------|-------------|---|-------------------------------|
| 146 | sp|Q128666|MERTK_HUMAN | Tyrosine-protein kinase Mer (MERTK) | 0.01 | 1.28 |
| 147 | sp|Q812V59|RDH10_HUMAN | Retinol dehydrogenase 10 (RDH10) | 0.01 | 1.76 |
| 148 | sp|O150144|ZN609_HUMAN | Zinc finger protein 609 (ZNF609) | 0.00 | 1.26 |
| 149 | sp|P60468|SC61B_HUMAN | Protein transport protein Sec61 subunit beta (SEC61B) | 0.02 | 1.22 |
| 150 | sp|Q96AA31|RFT1_HUMAN | Protein RFT1 homolog (RFT1) | 0.01 | 1.31 |
| 151 | sp|Q6PHR2|ULK3_HUMAN | Serine/threonine-protein kinase ULK3 (ULK3) | 0.01 | 3.02 |
| 152 | sp|Q5BJF2|SGMR2_HUMAN | Sigma intracellular receptor 2 (TMEM97) | 0.01 | 1.4 |
| 153 | sp|P63267|ACTH_HUMAN | Actin, gamma-enteric smooth muscle (ACTG2) | 0.00 | 1.37 |
| 154 | sp|P78563|RED1_HUMAN | Double-stranded RNA-specific editase 1 (ADARB1) | 0.03 | 1.28 |
| 155 | sp|P16422|EPCAM_HUMAN | Epithelial cell adhesion molecule (EPCAM) | 0.01 | 1.24 |
| 156 | sp|P31371|FGF9_HUMAN | Fibroblast growth factor 9 (FGF9) | 0.01 | 1.23 |
| 157 | sp|P57053|H2BFS_HUMAN | Histone H2B type F-S (H2BFS) | 0.00 | 1.27 |
| 158 | sp|Q9HBR06|S38AA_HUMAN | Putative sodium-coupled neutral amino acid transporter 10 (SLC38A10) | 0.04 | 1.56 |
| 159 | sp|Q5W0Z9|ZDH20_HUMAN | Palmitoyltransferase ZDHHC20 (ZDHHC20) | 0.00 | 1.29 |
| 160 | sp|P161122|PGCA_HUMAN | Aggrecan core protein (ACAN) | 0.04 | 1.22 |
| 161 | sp|Q9H954|CB39L_HUMAN | Calcium-binding protein 39-like (CAB39L) | 0.00 | 1.25 |
| 162 | sp|Q9BBW0|REPI1_HUMAN | Replication initiator 1 (REPIN1) | 0.01 | 1.28 |
| 163 | sp|Q9GG2U6|CTDS1_HUMAN | Carboxy-terminal domain RNA polymerase II polypeptide A small phosphatase 1 | 0.00 | 1.26 |
| 164 | sp|P42680|TEC_HUMAN | Tyrosine-protein kinase TEC (TEC) | 0.05 | 1.24 |
| 165 | sp|Q9BR32|ZN2_HUMAN | Zinc transporter 2 (SLC30A2) | 0.04 | 1.36 |
| 166 | sp|O124632|GOSR2_HUMAN | Golgi SNAP receptor complex member 2 (GOSR2) | 0.02 | 1.25 |
| 167 | sp|Q75665|TOF1_HUMAN | Oral-facial-digital syndrome 1 protein (OFT1) | 0.04 | 1.52 |
| 168 | sp|Q14687|GSE1_HUMAN | Genetic suppressor element 1 (GSE1) | 0.00 | 1.21 |
| 169 | sp|Q9BQX8|N3H23_HUMAN | Condensin complex subunit 3 (NCAPG) | 0.04 | 1.36 |
| 170 | sp|Q96N9Y8|NECT4_HUMAN | Nectin-4 (NECT4) | 0.00 | 1.21 |
| 171 | sp|Q7P750|DERM_HUMAN | Dermatopontin (DPT0) | 0.01 | 1.5 |
| 172 | sp|P619562|SUMO2_HUMAN | Small ubiquitin-related modifier 2 (SUMO2) | 0.02 | 1.22 |
| 173 | sp|Q9BZ678|FRMD8_HUMAN | FERM domain-containing protein 8 (FRMD8) | 0.00 | 1.22 |
| 174 | sp|Q9Y6241|JAM1_HUMAN | Junctional adhesion molecule A (JAM1) | 0.00 | 1.26 |
| 175 | sp|P30486|B48_HUMAN | HLA class I histocompatibility antigen, B-48 alpha chain (HLA-B) | 0.01 | 2.04 |
| 176 | sp|Q13601|KRR1_HUMAN | KRR1 small subunit processome component homolog (KRR1) | 0.00 | 1.21 |
| 177 | sp|P27987|IPK8_HUMAN | Insoluble-triphosphatase 3 kinase B (ITPKB) | 0.00 | 1.22 |
| 178 | sp|P151515|PVR_HUMAN | Poliovirus receptor (PVR) | 0.00 | 1.21 |
| 179 | sp|Q14925|TIM23_HUMAN | Mitochondrial import inner membrane translocase subunit Tim23 (TIMM23) | 0.00 | 1.34 |
| 180 | sp|Q8N5567|AFAP1_HUMAN | Actin filament-associated protein 1 (AFAP1) | 0.02 | 1.3 |
| 181 | sp|Q9Y3C11|NOP16_HUMAN | Nucleolar protein 16 (NOP16) | 0.00 | 1.33 |
| 182 | sp|P52520|CAD13_HUMAN | Cadherin-13 (CDH13) | 0.00 | 1.32 |
| 183 | sp|Q9KW60|SENP5_HUMAN | Sentrin-specific protease 5 (SENP5) | 0.00 | 1.4 |
| 184 | sp|Q9UJI30|ZBTB21_HUMAN | Zinc finger and BTB domain-containing protein 21 (ZBTB21) | 0.01 | 2.55 |
| 185 | sp|P27847|DPP4_HUMAN | Dipetidyl peptidase 4 (DPP4) | 0.03 | 1.49 |
| 186 | sp|Q8NH190|O1AG_HUMAN | Olfactory receptor 10A1 (OR10A1) | 0.02 | 2.06 |
| 187 | sp|P15309|PPAP_HUMAN | Prostatic acid phosphatase (ACP5) | 0.00 | 1.34 |
| 188 | sp|Q9UR005|ISY1_HUMAN | Pre-mRNA-splicing factor 5 (ISY1) | 0.02 | 2.08 |
| 189 | sp|Q9M6690|DNHD1_HUMAN | Dynein heavy chain domain-containing protein 1 (DNHD1) | 0.02 | 1.55 |
| 190 | sp|P4C220|LIM5_HUMAN | LIM and senescent cell antigen-like-containing domain protein 4 (LIM5) | 0.01 | 1.27 |
| 191 | sp|O75503|CLN5_HUMAN | Ceroid-lipofuscinosis neuronal protein 5 (CLN5) | 0.03 | 1.67 |
| 192 | sp|Q9HC360|MRRM3_HUMAN | rRNA methyltransferase 3, mitochondrial (MRRM3) | 0.00 | 1.22 |
| 193 | sp|Q9H9Y2|JUPI2_HUMAN | Jupiter microtubule associated homolog 2 (JPT2) | 0.00 | 1.25 |
Functional classification of differentially expressed proteins (DEPs) in the endometrium

To determine the functional differences in the increased and decreased proteins, the quantified proteins were analyzed for the following three types of enrichment-based clustering analyses: gene ontology (GO) enrichment analysis of DEPs, pathway enrichment analysis of DEPs, and eukaryotic orthologous groups (KOOGs) annotation of DEPs.

GO enrichment analysis showed the GO terms in which the DEPs were enriched in all identified proteins. It represented the important or typical biological functions in the study. We performed pathway enrichment analysis of DEPs based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. KOOGs were delineated by comparing protein sequences encoded in complete genomes, which represented major phylogenetic lineages.

Through the GO enrichment analysis of biological processes, we found that these different proteins were closely associated with cellular processes, metabolic processes, and biological regulation. Based on their molecular functions, these proteins with altered levels were strongly associated with binding, catalytic activity, and molecular function regulators (Fig. 3, Additional file 1: Fig. S2).

The results from KEGG pathway enrichment showed that the DEPs were mainly involved in allograft rejection, cell adhesion molecules (CAMs), type I diabetes mellitus, allograft rejection, phagosomes, and the necrotic factor (NF)—kappa B signaling pathway (Fig. 4, Additional file 1: Fig. S3). Moreover, we constructed a scatter plot for the top 20 KEGG enrichment results as shown in Fig. 5.

For DEPs, their KOOG terms were also extracted and showed that the DEPs were mainly associated with inorganic ion transport and metabolism, lipid transport and metabolism, and energy production and conversion. We plotted bar plots accordingly (Fig. 6). Thus, we could easily obtain their functional categories.

Predicted protein–protein interactions (PPI) of DEPs and subcellular localization prediction of DEPs

Proteins usually interact with each other to participate in certain biological functions. STRING is a database of known PPI. Based on Fig. 7, we determined the interaction between proteins (Fig. 7). Proteins can be targeted in the inner space of an organelle, different intracellular membranes, the plasma membrane, or to the exterior of...
the cell through secretion. This delivery process is performed on the basis of the information present in the protein. Correct sorting is important for the cell; errors can lead to the development of diseases. We predicted protein subcellular localization using bioinformatics tools (WoLF PSORT). The bar plot of subcellular localization prediction showed that different proteins are mainly present in the nucleus, extracellular space, cytosol, plasma membrane, and mitochondria (Fig. 8).

Taken together, these results showed that these DEPs mainly play a role in metabolic processes, cell adhesion molecules, and immunity.

Discussion
Embryo implantation is a key process in pregnancy. For successful embryo implantation, the process must be sequential, which means that the three phases, namely apposition, adhesion, and invasion, should occur...
sequentially [16]. For pregnancy, endometrium transition to the pregnancy state is the key to embryo implantation, and a change in several proteins in the endometrium during this process is a prerequisite [17, 18]. The DEPs discovered in the present study were mainly involved in energy metabolism, inflammation, and cell–cell adhesion functions, as well as the cell and cell parts in cellular components and catalytic activity. Energy metabolism may affect embryo implantation, whereas inflammation and CAMs may affect both endometrial conversion and receptivity.

Impairment of embryo implantation because of energy metabolism deficit

The exact mechanism of embryo implantation is not clear, and probably energy metabolism is a crucial factor in implantation [19]. PCOS is an endocrine disorder characterized by hyperinsulinemia and obesity [20]. These characteristics can cause an insulin-resistant state and metabolic disorder in organs such as the endometrium [21, 22]. As insulin resistance in the endometrium leads to no response or sensitivity to the metabolic effects of insulin, the endometrium needs more insulin for normal metabolism [23]. The gene for insulin-like growth factor-binding protein 5 (IGFBP5) is downregulated in patients with PCOS than in healthy people, and IGFBP5 is an important member of the IGFBP family. IGFBP5 may affect cell metabolism. A decrease in IGFBP5 level may be associated with the pathogenesis of type 2 diabetes [24, 25], and decreased GLUT4 expression may be one of the mechanisms by which IGFBP causes insulin resistance [26]. Moreover, the results of our subcellular localization analysis show that many different proteins are located in mitochondria. Importantly, mitochondria play a key role in energy production by converting nutrients into energy, and altered proteins
may negatively affect energy metabolism. For example, mitochondrial pyruvate carrier 1 (MPC1) and transcription elongation factor mitochondrial (TEFM) levels were significantly decreased in patients with PCOS. Pyruvate, carried by MPC1 into the mitochondrion, is essential to mitochondrial energy metabolism. The lack of MPC1 can impair pyruvate transport and then can damage mitochondrial energy metabolism [27].
glucose metabolism is in mitochondria, in which TEFM regulates the formation of mitochondrial RNA primers. As RNA primers are necessary for the initiation of mitochondrial DNA replication, the lack of TEFM reduces mitochondrial DNA replication [28]. Therefore, abnormalities in MPC1 and TEFM must affect mitochondrial oxidation, thus leading to a bioenergetic crisis. Therefore, we hypothesized that energy metabolism deficits may cause embryo implantation failure, and treatment including energy supplements may improve the endometrial microenvironment.

**CAM deficiency causes miscarriage**
Apart from energy metabolism deficits, embryo implantation also requires adhesion molecules. Increasing or decreasing adhesion molecules can lead to embryo implantation failure. In our proteomics analysis results, we observed the differential expression of adhesion molecules in the PCOS group including CAMs,
receptor–ligand activity, and cell adhesion. Among these, epithelial CAM (EpCAM) level was increased in endometrial samples of women with PCOS. EpCAM regulates many important cellular functions such as cell migration, metastasis, proliferation, and cell differentiation [29, 30]; however, the main role of EpCAM is intercellular adhesion [31]. A specific EpCAM is necessary for embryo implantation, and the amount of EpCAM during the implantation window should be reduced [32]. EpCAMs are maintained mainly at the basal cell surface to maintain a polarized epithelial surface, and then uterine epithelial cells connect with the underlying stroma to prevent premature detachment before implantation [33]. However, higher concentrations of EpCAM can impair adhesion or promote deadhesion by competitively binding to extracellular matrix proteins and blocking cell attachment. Proteomics analysis results show that T-lymphoma invasion and metastasis-inducing protein 1 (TIAM1) were decreased in the PCOS group, which regulates cell migration, motility, and cell adhesion in some cells [34, 35]. TIAM1 is decreased by estradiol and increased by progesterone in a dose-dependent manner [36]. Patients with PCOS lack a complete menstrual cycle as a result of oligo- or anovulation; thus, the endometrium is exposed to estradiol for an extended period and lacks progesterone [37]. The reduction in TIAM1 level is consistent with the characteristics of patients with PCOS. TIAM1 is essential in embryo implantation in mice by increasing the implantation site of the endometrium [38]. Studies have shown that increased levels of TIAM1 during the implantation window facilitates embryo implantation, and decreased TIAM1 levels might be associated with the failure of embryo implantation in patients with repeated implantation failure [35]. More studies need to be established to explore the details of adhesion mechanisms underlying the endometrium of PCOS.

Immune disorders lead to miscarriage
The embryo is a natural semi-allograft, and tolerance mechanisms for successful embryo implantation involve the acceptance of allografts [39]. A recent study highlighted that immune imbalance plays a key role in recurrent miscarriage [40]. Our pathway analysis reports that allograft rejection, natural killer (NK)-cell-mediated cytotoxicity, and primary immunodeficiency in patients with PCOS were significantly abnormal compared with those in healthy women. For instance, human leukocyte antigen C (HLA-C), a marker of recurrent miscarriage, was significantly increased in the PCOS group [41]. In the fetal–maternal interface, NK cells recognize and eliminate exogenous cells mainly resulting from HLA expressed on the foreign cell surface [42]. Thus, the increased HLA-C levels may negatively affect the process by which NK cells recognize embryo antigens, resulting in immune tolerance disorders. Hemeoxynase 1 (HMOX1) was significantly decreased in patients with PCOS. HMOX1 is a central player in anti-inflammatory, antioxidant, and cytoprotective activities, and HMOX1 can inhibit the cytotoxicity of other immune cells, cytokine release, and proliferation [43, 44]. HMOX1 is necessary for protecting fetuses from rejection [45, 46]. Therefore, HMOX1 deficiency may affect fetal and allograft rejection, thereby leading to embryo implantation failure. Thus, curing immune disorders in the endometrium will improve the probability of embryo implantation success.

Strengths and limitations of the study
Our results show that endometrial receptive damage in patients with PCOS is not only associated with a single factor but also multiple proteins, pathways, systems, and other abnormalities; these factors also interact with each other. Due to difficulty in obtaining the desired endometrial tissues repeatedly at the same time, we only compared endometrial proteomics in the luteal phase between the experimental group and the control group, rather than comparing the endometrial proteomics in different phases in one group. Moreover, animal validation model tests are in preparation.

Conclusion
Our results show that endometrial receptive damage in patients with PCOS is not a single factor event but occurs because of multiple proteins, pathways, systems, and other abnormalities, and they also interact with each other, thereby greatly increasing the difficulty of endometrial receptive research. More studies are needed to support the hypothesis of this study and to establish a better understanding of the molecular mechanistic details underlying impaired endometrial implantation in patients with PCOS.

Supplementary Information
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Additional file 1. Fig. S1. CV distribution in replicate. Fig. S2. Gene Ontology Analysis of Differentially Expressed Proteins. Fig. S3. Pathway analysis of Differentially Expressed Proteins.

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Author contributions
JL and XJ designed research. JL and CL performed the research. JL, XJ and LL analyzed the data and drafted the final version of the manuscript. CL and ZW supervised the study, and provided financial support, editing and final approval of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE [47] partner repository with the dataset identifier PXD024735.

Declarations

Competing interests
No conflicts of interest, financial or otherwise, are declared by the authors.

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