Changes in Proteomics of Endometrial Tissue During Secretion of Polycystic Ovary Syndrome May Affect Endometrial Receptivity

Jun Li

Second Affiliated Hospital of Anhui Medical University

Xiaohua Jiang

First Affiliated Hospital of Anhui Medical University

Caihua Li

First Affiliated Hospital of Anhui Medical University

Huihui Che

Anhui Medical College: Anhui Medical University

Lin Ling

Second Affiliated Hospital of Anhui Medical University

Zhaolian Wei (✉ weizhaolian_1@126.com)

First Affiliated Hospital of Anhui Medical University

Research Article

Keywords: endometrial receptivity, endometrium, PCOS, Proteomics.

Posted Date: July 29th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-726665/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Embryo implantation is a complex developmental process that requires coordinated interactions of embryo, endometrium and microenvironment of endometrium factors. Although impaired endometrial receptivity of patients of PCOS have been identified, our understanding of endometrial receptivity is limited. To understand impaired endometrial receptivity of patients of PCOS during luteal phases, a proteomics study in three PCOS patients and 3 fertility women was performed. Isobaric tags for relative and absolute quantitation (iTRAQ) analyses identified 232 unique proteins involved in metabolism, inflammation and cell adhesion molecule. Finally, novel clues suggest that energy metabolism may affect embryo implantation, while inflammation and cell adhesion molecule may affect both endometrial conversion and receptivity. Taken together, our results show that the endometrial receptive damage in PCOS patients is not a single factor, caused by multiple proteins, pathways, systems and other abnormalities, and they also interact with each other, which greatly increases the difficulty of endometrial receptive research.

Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women of reproductive age that perplex researchers and doctors worldwide[1]. Although many researchers focus on the pathophysiology of PCOS, the etiology of PCOS is still unknown. Nowadays many researchers mainly focused on improving clinical symptoms such as insulin resistance, obesity, metabolic derangements, androgen excess, to achieve a successful pregnancy, reduce pregnancy complications and have a good pregnancy outcome[2-3]. In the past, ovulation disorders were considered to be the main cause of infertility in patients with PCOS. However, the pregnancy rates are still low and have a high risk of biochemical abortion after ovulation disorders were corrected. Many factors may cause this situation, and impaired endometrial receptivity could be responsible for adverse pregnancy outcomes in PCOS. Unfortunately, there were still few researches about the molecular mechanisms regarding impaired endometrial receptivity. We just know some basis essential protein of embryo implantation such as fork head 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 abnormal when compared with healthy people[4]. In addition, considering that single protein changes do not mirror function of endometrium microenvironment due to protein-protein interactions, ongoing program has increasingly concentrated on proteomic analyses. The use of proteomics, which is not constricted by previous knowledge on the problem, can help discover the potential advantage of revealing novel associations with unexpected molecules that might lead to new mechanistic explanations for the impaired endometrial implantation.

In recent years, proteomics approaches have been applied to explore the potential mechanistic about the molecules of adverse pregnancy outcomes of PCOS. To date, there was not any research executing on the secretory endometrial proteome in PCOS patients. With the aim of exploring the molecular basis of infertility related to endometrium implantation in PCOS, this study compared the secretory endometrial

proteomic profile of PCOS patients with that of healthy fertile women using Isobaric tags for relative and absolute quantitation (iTRAQ).

Materials And Methods

The endometrial tissue was obtained from 3 patients of PCOS and 3 healthy volunteers who already have children. Patients with PCOS took letrozole on the third day of menstruation, then ovulation has been continuously monitored since the 10th day of menstruation, and the endometrium was obtained on the fifth day after ovulation.

They were also screened for glucose metabolism and endocrine normality with serum determinations of follicle-stimulating hormone (FSH), luteinizing hormone (LH), Estradiol, glucose and insulin on day 3 of the menstrual cycle. No participants showed any evidence of chromosomal abnormality, pathological uterine disorder or endometrial hyperplasia. None had used oral contraception or hormonal therapy during the previous 3 months. The diagnosis of PCOS was according to the 2003 Rotterdam criteria which included any two or all three of the following features: oligo-/anovulation; clinical or biochemical signs of hyperandrogenism; polycystic ovary morphology on ultrasound[5]. The main demographic characteristics of the patient and control groups are summarized in Table 1; the PCOS and control groups did not differ regarding age, body mass index (BMI), FSH, LH and testosterone but did differ regarding 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 gave informed consent prior to entering the study. Figure 1 shows the basic principle of iTRAQ quantitative proteomics and the main steps of quantitative techniques.

1.1 Protein Extraction

We used lysis buffer 3 (8 M Urea, TEAB or 40 mM Tris-HCl with 1mM PMSF, 2mM EDTA and 10mM DTT, pH 8.5) and two magnetic beads to extract proteins. Then we removed the mixtures into a tissue lyser for 2 minutes at 50Hz to release proteins. After that, the supernatant was transferred into a new tube after centrifugation with 25000g at 4°C for 20 minutes, and reduced with 10 mM dithiothreitol (DTT) at 56°C for 1 hour and alkylated by 55 mM iodoacetamide (IAM) in the dark at room temperature for 45 minutes. Following centrifugation, the supernatant containing proteins was quantified by Bradford.

1.2 QC of Protein Extraction

(1) Protein Quantitation with Bradford assay

First of all, we added 0, 2, 4, 6, 8, 10, 12, 14, 16 and 18 μl BSA solution separately into a 96-well plate, then separately added 20, 18, 16, 14, 12, 10, 8, 6, 4 and 2 μl pure water into the corresponding wells. At the same time, we also made serial dilutions (20 μl each well) of the unknown sample to be measured. After

that, we added 180µl of coomassie blue into each well and mixed. Read the absorbance of each standard and sample well at 595 nm. Each sample had at least two duplicates. Then the absorbance of the standards vs. their concentration would be plotted. Then we calculated the extinction coefficient and the concentrations of the unknown samples.

(2) SDS-PAGE

15–30µg proteins were mixed with loading buffer in the centrifuge tube and heated at 95°C for 5 minutes. Then, the mixture would be centrifuged at 25000g for 5 minutes, then the supernatant would be loaded into sample holes in 12% polyacrylamide gel. Run SDS-PAGE in constant voltage at 120V for 120 minutes. Once it finished, stain gel with coomassie blue for 2 hours, then add destaining solution (40% ethanol and 10% acetic acid) and put it on a shaker (exchange destaining solution for 3 ~ 5 times, 30 minutes a time).

1.3 Protein Digestion

The protein solution (100ug) with 8M urea was diluted 4 times with 100mM TEAB. We applied the trypsin gold (Promega, Madison, WI, USA) to digest the proteins (protein: trypsin = 40:1) at 37°C overnight. After that, we used a Strata X C18 column (Phenomenex) and vacuum-dried to desalt peptides according to the manufacturer's protocol.

1.4 Peptide Labeling

We dissolved the peptides in 30 ul 0.5 M TEAB with vortexing. After the iTRAQ labeling reagents were recovered to ambient temperature, they were transferred and combined with proper samples. We performed the peptide labeling by iTRAQ Reagent 8-plex Kit on the basis of the manufacturer's protocol. Then we combined and desalted the labeled peptides with a Strata X C18 column and vacuum-dried based on the manufacturer's protocol.

1.5 Peptide Fractionation

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

1.6 HPLC

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

1.7 Mass Spectrometer Detection

We subjected the peptides separated from nanoHPLC into the tandem mass spectrometry Q EXACTIVE HF X (Thermo Fisher Scientific, San Jose, CA) for DDA (data-dependent acquisition) detection by nano-electrospray ionization. The relevant parameters of the MS analysis are presented as following: precursor scan range: 350–1500 m/z at a resolution of 60000 in Orbitrap; electrospray voltage: 2.0 kV; MS/MS fragment scan range: >100 m/z at a resolution of 15000 in HCD mode; 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 10000.

2. Bioinformatics Pipeline

After getting raw data, we will do each bioinformatics analysis as the client appoints on contract. Figure 2 demonstrates a complete pipeline for iTRAQ (Quantification) project.

3. Protein Quantification

We applied an automated software called IQuant for quantitatively analyzing the labeled peptides with isobaric tags[6]. It integrates Mascot Percolator[7] to provide reliable significance measures. In order to assess the confidence of peptides, the PSMs were pre-filtered at a PSM-level FDR of 1%. Then based on the "simple principle" (The parsimony principle), identified peptide sequences were assembled into a set of confident proteins. In order to control the rate of false-positive at protein level, a protein FDR at 1%, which is based on picked protein FDR strategy[8], would also be estimated after protein inference (Protein-level FDR < = 0.01). The process of the protein quantification comprised the following steps: Protein identification, Tag impurity correction, Data normalization, Missing value imputation, Protein ratio calculation, Statistical analysis, Results presentation.

Results

The mean CV (0.12) presents that the proteins in this study has quite good reproducibility. (Fig. 3). A total of 6524 proteins were quantitatively identified in samples from the PCOS group and control group. Compared with the control group, 232 proteins displayed statistical changes in their expression levels in

the group of PCOS. Of these, 108 proteins were up-regulated and 124 proteins were down-regulated. The differentially expressed proteins of p -value < 0.05 is detailed in Fig. 4.

Functional classification of differentially expressed proteins upon

Endometrium

To elucidate the functional differences of up-regulated and down-regulated proteins, the quantified proteins were analyzed for three types of enrichment-based clustering analyses: gene ontology (GO) annotation, eukaryotic orthologous groups (KOGs) annotation and pathway annotation.

According to GO enrichment analysis, these different proteins were closely related to the cellular process, metabolic process in biological process, cell and cell part in cellular component, binding and catalytic activity in molecular function (Fig. 5). Pathway enrichment results show that the differentially expressed proteins were mainly involved in allograft rejection, cell adhesion molecules (CAMs), Type I diabetes mellitus, allograft rejection, phagosome and NF – kappa B signaling pathway (Fig. 6). Bar plot of subcellular localization prediction shows different proteins are mainly located at nucleus, extracellular, cytosol, plasma membrane and mitochondria (Fig. 8).

Discussion

Embryo implantation is a key process in pregnancy. In order for an embryo to be successfully implanted, the implantation process must be sequential, which means the three phases: apposition, adhesion and invasion, should take place in sequence[9]. For pregnancy, the transition of endometrium to pregnancy state is the key to embryo implantation, and the change of a large number of proteins in the endometrium during this procession is prerequisite[10, 11]. The differentially expressed proteins discovered in the study were mainly involved in energy metabolism, inflammation and cell-cell adhesion functions, as well as cell and cell part in cellular component, and catalytic activity. Energy metabolism may affect embryo implantation, while inflammation and cell adhesion molecule may affect both endometrial conversion and receptivity.

1.Impairment of embryo implantation due to energy metabolism deficit?

The accurate mechanism of embryo implantation is not clear, it seems that energy is a crucial event of implantation[12]. PCOS is an endocrine disorder characterized by hyperinsulinemia and obesity[13], these characteristics could cause an insulin-resistant state and metabolism disorder in organ such as endometrium[14, 15]. Since insulin resistance in the endometrium result in no response or sensitivity to the metabolic effects of insulin, the endometrium needs more insulin to sustain normal metabolism[16]. Insulin-like growth factor-binding protein 5 (IGFBP5) in PCOS patients is a down-regulated protein

compared with healthy people, IGFBP5 is an important member of the IGFBP family. IGFBP5 may have effect on cell metabolism. It has been reported that a decrease in IGFBP5 may be the pathogenesis of type 2 diabetes [17, 18], and the decreased GLUT4 expression may be one of the mechanisms by which IGFBP causes insulin resistance [19]. Moreover, our subcellular localization analysis shows that many different proteins located at mitochondria. Importantly, mitochondria plays a key role in energy production that change nutrients into available energy, the altered proteins may have negative effect on energy metabolism. Such as mitochondrial pyruvate carrier 1 (MPC1) and transcription elongation factor mitochondrial (TEFM) were significantly decreased in PCOS patients. The pyruvate, carried by MPC1 into the mitochondrion, is essential to the energy metabolism of mitochondrion. The lack of MPC1 can lead to pyruvate transport impairing, then cause damage to the mitochondrial energy metabolism[20]. The final place of glucose metabolism is in mitochondrion, in which the TEFM regulates the formation of the mitochondrial RNA primers. Since RNA primer is needed for the initiation of mitochondrial DNA replication, the lack of TEFM reduces mitochondria DNA expression[21]. So, the abnormality of MPC1 and TEFM must have effect on mitochondrial oxidative resulting in a bioenergetics crisis. Therefore, we hypothesized that energy metabolism deficit may cause embryo implant failure, and energy supplement treatment may improve endometrium microenvironment.

2. cell adhesion molecule deficiency causes miscarriage?

Apart from energy metabolism deficit, The process of embryo implantation also need adhesion molecule. It is proved that increasing or decreasing adhesion molecule can cause embryo implantation failure. In our proteomics result, we also observed the different expression of adhesion molecules in PCOS group including cell adhesion molecules (CAMs), receptor ligand activity and cell adhesion. Among these, epithelial cell adhesion molecule (EpCAM) was increased in endometrial samples of PCOS women. EpCAM regulates many important cellular functions such as cell migration, metastasis, proliferation and cell differentiation[22–24], however, the main role of EpCAM is intercellular adhesion[25]. Appropriate EpCAM is necessary for embryo implantation, and it has been proved that the amount of EpCAM during the window of implantation should be reduced[26]. EpCAM are maintained mainly at the basal cell surface to maintenance of polarised epithelial surface, then, UECs contact with the underlying stroma to prevent their premature detachment prior to implantation[27]. However, higher concentrations of EpCAM can impair adhesion, or promote de-adhesion by competitively binding to the extracellular matrix proteins and blocking cell attachment. Proteomics outcomes show that T-lymphoma invasion and metastasis-inducing protein 1(TIAM1) were decreased in group of PCOS patients, which has been demonstrated to regulate cell migration, motility and cell adhesion in some cells[28, 29]. There is evidence that TIAM1 is down-regulated by oestradiol, and dose-dependent increase by progesterone[30]. The patients with PCOS lack a complete menstrual cycle as a result of oligo-or anovulation, thus, endometrium is exposed to estradiol for a long time and lack of progesterone[31]. The reduction of TIAM1 is consistent with the characteristics of patients with PCOS. TIAM1 plays an important role in embryo implantation in mice by increasing the implantation site of the endometrium [32]. The studies showed the increased expression of TIAM1 during the implantation window facilitated embryo implantation, decreased TIAM1 might be involved in the failure of embryo implantation in patients with repeated implantation failure[29]. More

studies need to be established to explore the adhesion mechanistic details underlying in endometrium of PCOS.

3. Immune disorders lead to miscarriage?

The embryo is considered as a natural semi-allograft, the tolerance mechanisms for successful embryo implantation involves in the acceptance of allografts[33]. It was recently highlighted that immune imbalance plays key roles in recurrent miscarriage[34]. Our pathway analysis reports that allograft rejection, natural killer cell mediated cytotoxicity, and primary immunodeficiency in the PCOS patients were significantly abnormal compared to healthy women. For instance, it has been observed that human leukocyte antigen C (HLA-C) significantly elevated in PCOS group, which is known as a marker of recurrent miscarriage[35]. In the fetal–maternal interface, the NK cell recognizes and eliminates exogenous cells mainly resulting from the HLA expressed on the surface of foreign cells[36]. Thus, the increased HLA-C may have negative influence on the process that the NK cells recognize embryo antigens, resulting in immune tolerance disorder. Hemeoxygenase1(HMOX1) was significantly down-regulated in the PCOS patients. HMOX1 has been widely described as a central player in anti-inflammatory, antioxidant, and cytoprotective, it proposes that HMOX1 can inhibit cytotoxicity of other immune cells, cytokine release and proliferation[37, 38]. It has been proved that the HMOX1 is necessary for protected fetuses from rejection[39]. Therefore, HMOX1 deficiency may affect fetal and allograft rejection causing embryo implantation failure. Thus, correcting immune disorders in the endometrium will improve probability of embryo implantation success.

Strengths and limitations

Our results show that the endometrial receptive damage in PCOS patients is not a single factor, caused by multiple proteins, pathways, systems and other abnormalities, and they also interact with each other.

Because of the difficulty in getting the endometrial tissue again at the same time, we only did a comparison of the 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. Besides, the animal validation model tests are in preparation.

Conclusion

our results show the endometrial receptive damage in PCOS patients is not a single factor, caused by multiple proteins, pathways, systems and other abnormalities, and they also interact with each other, which greatly increases the difficulty of endometrial receptive research. More studies are needed to support the hypothesis of this study and to establish better understanding of the molecular mechanistic details underlying impaired endometrial implantation of PCOS patients.

Declarations

Acknowledgements

The authors acknowledge support from professor Haijian Cai in Center for Scientific Research of Anhui Medical University.

Funding

This research was funded by national natural science foundation of china youth science fund development project of the first affiliated hospital of anhui medical university [grant number 2017kj02]

Ethics approval and consent to participate

Yes

Consent for publication

Yes

Availability of data and materials

The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE[40] partner repository with the dataset identifier PXD024735".

Authors' contribution

Jun Li. and Xiaohua Jiang designed research. Jun Li and Caihua Li performed the research. Jun Li, Xiaohua Jiang and Lin Ling analyzed the data and drafted the final version of the manuscript. Caihua Li and Zhaolian Wei. supervised the study, provided financial support, editing and final approval of the manuscript.

Conflict of interest statement

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

Author details

¹ Department of Gynecology and Obstetrics, The Second Affiliated Hospital of Anhui Medical University, Hefei, People's Republic of China. ²Reproductive Medicine Center, Department of Obstetrics and Gynecology, the First Affiliated Hospital of Anhui Medical University, Hefei 230022, China; ³ Anhui Province Key Laboratory of Reproductive Health and Genetics, Anhui Medical University, Hefei 230022, China; ⁴ Anhui Provincial Engineering Technology Research Center for Biopreservation and Artificial Organs, Hefei 230022, China;

References

1. Al WB, Teede H, Garad R, Franks S, Balen A, Bhide P, et al. Harmonising research outcomes for polycystic ovary syndrome: an international multi-stakeholder core outcome set. *Hum Reprod.* 2020;35:404–12.
2. Gonzalez F, Considine RV, Abdelhadi OA, Acton AJ. Inflammation triggered by saturated fat ingestion is linked to insulin resistance and hyperandrogenism in PCOS. *J Clin Endocrinol Metab.* 2020.
3. Zhu X, Fu Y. Randomized. Controlled Pilot Study of Low-Dose Human Chorionic Gonadotropin Administration Beginning From the Early Follicular Phase for Women With Polycystic Ovarian Syndrome Undergoing Ovarian Stimulation Using the Progesterone Protocol. *Front Endocrinol (Lausanne).* 2019;10:875.
4. Piltonen TT. Polycystic ovary syndrome: Endometrial markers. *Best Pract Res Clin Obstet Gynaecol.* 2016;37:66–79.
5. Revised 2003 consensus. on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). *Hum Reprod.* 2004;19:41–7.
6. Wen B, Zhou R, Feng Q, Wang Q, Wang J, Liu S. IQuant: an automated pipeline for quantitative proteomics based upon isobaric tags. *Proteomics.* 2014;14:2280–5.
7. Brosch M, Yu L, Hubbard T, Choudhary J. Accurate and sensitive peptide identification with Mascot Percolator. *J Proteome Res.* 2009;8:3176–81.
8. Brosch M, Yu L, Hubbard T, Choudhary J. Accurate and sensitive peptide identification with Mascot Percolator. *J Proteome Res.* 2009;8:3176–81.
9. Lopes IM, Baracat MC, Simoes MJ, Simoes RS, Baracat EC, Soares JJ. Endometrium in women with polycystic ovary syndrome during the window of implantation. *Rev Assoc Med Bras (1992).* 2011;57:702–9.
10. Kasvandik S, Saarma M, Kaart T, Rooda I, Velthut-Meikas A, Ehrenberg A, et al. Uterine Fluid Proteins for Minimally Invasive Assessment of Endometrial Receptivity. *J Clin Endocrinol Metab.* 2020;105.
11. Sadigh AR, Mihanfar A, Fattahi A, Latifi Z, Akbarzadeh M, Hajipour H, et al. S100 protein family and embryo implantation. *J Cell Biochem.* 2019;120:19229–44.
12. Perakakis N, Upadhyay J, Ghaly W, Chen J, Chrysafi P, Anastasilakis AD, et al. Regulation of the activins-follistatins-inhibins axis by energy status: Impact on reproductive function. *Metabolism.* 2018;85:240–9.
13. Teede HJ, Misso ML, Costello MF, Dokras A, Laven J, Moran L, et al. Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome. *Fertil Steril.* 2018;110:364–79.
14. Cabrera-Cruz H, Orostica L, Plaza-Parrochia F, Torres-Pinto I, Romero C, Vega M. The insulin-sensitizing mechanism of myo-inositol is associated with AMPK activation and GLUT-4 expression in human endometrial cells exposed to a PCOS environment. *Am J Physiol Endocrinol Metab.* 2020;318:E237–48.

15. Shafiee MN, Seedhouse C, Mongan N, Chapman C, Deen S, Abu J, et al. Up-regulation of genes involved in the insulin signalling pathway (IGF1, PTEN and IGFBP1) in the endometrium may link polycystic ovarian syndrome and endometrial cancer. *Mol Cell Endocrinol.* 2016;424:94–101.
16. Diamanti-Kandarakis E, Dunaif A. Insulin resistance and the polycystic ovary syndrome revisited: an update on mechanisms and implications. *Endocr Rev.* 2012;33:981–1030.
17. Gleason CE, Ning Y, Cominski TP, Gupta R, Kaestner KH, Pintar JE, et al. Role of insulin-like growth factor-binding protein 5 (IGFBP5) in organismal and pancreatic beta-cell growth. *Mol Endocrinol.* 2010;24:178–92.
18. Rojas-Rodriguez R, Lifshitz LM, Bellve KD, Min SY, Pires J, Leung K, et al. Human adipose tissue expansion in pregnancy is impaired in gestational diabetes mellitus. *Diabetologia.* 2015;58:2106–14.
19. Xiang A, Chu G, Zhu Y, Ma G, Yang G, Sun S. IGFBP5 suppresses oleate-induced intramyocellular lipids deposition and enhances insulin signaling. *J Cell Physiol.* 2019;234:15288–98.
20. Li X, Zheng H, Shi L, Liu Z, He L, Gao J. Stress-seventy subfamily A 4, A member of HSP70, confers yeast cadmium tolerance in the loss of mitochondria pyruvate carrier 1. *Plant Signal Behav.* 2020;15:1719312.
21. Jiang S, Koolmeister C, Misic J, Siira S, Kühl I, Silva Ramos E, et al. TEFM regulates both transcription elongation and RNA processing in mitochondria. *Embo Rep.* 2019;20:n/a-n/a.
22. Munz M, Zeidler R, Gires O. The tumour-associated antigen EpCAM upregulates the fatty acid binding protein E-FABP. *Cancer Lett.* 2005;225:151–7.
23. Osta WA, Chen Y, Mikhitarian K, Mitas M, Salem M, Hannun YA, et al. EpCAM is overexpressed in breast cancer and is a potential target for breast cancer gene therapy. *Cancer Res.* 2004;64:5818–24.
24. Schmidt DS, Klingbeil P, Schnolzer M, Zoller M. CD44 variant isoforms associate with tetraspanins and EpCAM. *Exp Cell Res.* 2004;297:329–47.
25. Lei Z, Maeda T, Tamura A, Nakamura T, Yamazaki Y, Shiratori H, et al. EpCAM contributes to formation of functional tight junction in the intestinal epithelium by recruiting claudin proteins. *Dev Biol.* 2012;371:136–45.
26. Poon CE, Madawala RJ, Day ML, Murphy CR. EpCAM is decreased but is still present in uterine epithelial cells during early pregnancy in the rat: potential mechanism for maintenance of mucosal integrity during implantation. *Cell Tissue Res.* 2015;359:655–64.
27. Murphy CR. Uterine receptivity and the plasma membrane transformation. *Cell Res.* 2004;14:259–67.
28. Diaz J, Mendoza P, Silva P, Quest AF, Torres VA. A novel caveolin-1/p85alpha/Rab5/Tiam1/Rac1 signaling axis in tumor cell migration and invasion. *Commun Integr Biol.* 2014;7.
29. Miyamoto Y, Yamauchi J, Tanoue A, Wu C, Mobley WC. TrkB binds and tyrosine-phosphorylates Tiam1, leading to activation of Rac1 and induction of changes in cellular morphology. *Proc Natl Acad Sci U S A.* 2006;103:10444–9.
30. Ma HL, Gong F, Tang Y, Li X, Li X, Yang X, et al. Inhibition of Endometrial Tiam1/Rac1 Signals Induced by miR-22 Up-Regulation Leads to the Failure of Embryo Implantation During the

- Implantation Window in Pregnant Mice. *Biol Reprod.* 2015;92:152.
31. Li X, Feng Y, Lin JF, Billig H, Shao R. Endometrial progesterone resistance and PCOS. *J Biomed Sci.* 2014;21:2.
32. Ma HL, Zhang T, Meng J, Qin ZY, Du F, Wang QY, et al. The role of T-lymphoma invasion and metastasis inducing protein 1 in early pregnancy in mice. *Mol Hum Reprod.* 2008;14:589–94.
33. Sollwedel A, Bertoja AZ, Zenclussen ML, Gerlof K, Lisewski U, Wafula P, et al. Protection from abortion by heme oxygenase-1 up-regulation is associated with increased levels of Bag-1 and neuropilin-1 at the fetal-maternal interface. *J Immunol.* 2005;175:4875–85.
34. Franasiak JM, Scott RT. Contribution of immunology to implantation failure of euploid embryos. *Fertil Steril.* 2017;107:1279–83.
35. Meuleman T, van Beelen E, Kaaja RJ, van Lith JM, Claas FH, Bloemenkamp KW. HLA-C antibodies in women with recurrent miscarriage suggests that antibody mediated rejection is one of the mechanisms leading to recurrent miscarriage. *J Reprod Immunol.* 2016;116:28–34.
36. Kofod L, Lindhard A, Hviid T. Implications of uterine NK cells and regulatory T cells in the endometrium of infertile women. *Hum Immunol.* 2018;79:693–701.
37. Chauveau C, Remy S, Royer PJ, Hill M, Tanguy-Royer S, Hubert FX, et al. Heme oxygenase-1 expression inhibits dendritic cell maturation and proinflammatory function but conserves IL-10 expression. *Blood.* 2005;106:1694–702.
38. Sun L, Shi T, Qiao H, Jiang X, Jiang H, Krissansen GW, et al. Hepatic overexpression of heme oxygenase-1 improves liver allograft survival by expanding T regulatory cells. *J Surg Res.* 2011;166:e187–94.
39. Ozen M, Zhao H, Lewis DB, Wong RJ, Stevenson DK. Heme oxygenase and the immune system in normal and pathological pregnancies. *Front Pharmacol.* 2015;6:84.
40. Deutsch EW, Bandeira N, Sharma V, Perez-Riverol Y, Carver JJ, Kundu DJ, et al. The ProteomeXchange consortium in 2020: enabling 'big data' approaches in proteomics. *Nucleic Acids Res.* 2020;48:D1145–52.

Tables

Table 1 Demographic characteristics of PCOS and control subjects.

	PCOS (n=3)	Control (n=3)	P
Age	29.67±0.58	30.67±1.15	0.25
BMI	24.96±2.15	21.10±1.47	0.06
FSH	5.24±1.30	6.44±1.28	0.32
LH	6.43±2.62	3.41±2.23	0.20
Testosterone	1.41±0.26	1.11±0.20	0.35
insulin	12.14±2.01	6.24±0.92	0.01
glucose	5.80±0.31	5.23±0.19	0.05

Figures

Figure 1

Bioinformatics Analysis Pipeline.

Figure 2

This plot depicts volcano plot of log2 fold-change (x-axis) versus -log10 Qvalue (y-axis, representing the probability that the protein is differentially expressed). Qvalue<0.05 and Foldchange > 1.2 are set as the significant threshold for differentially expression. The red and green dots indicate points-of-interest that display both large-magnitude fold-changes as well as high statistical significance. Dots in red mean significant up-regulated proteins which passed screening threshold. Dots in green mean significant down-regulated proteins which passed screening threshold. And gray dots are non-significant differentially expressed protein.

Figure 3

Gene Ontology Analysis of Differentially Expressed Proteins. x-axis displays protein count y-axis displays GO term

Figure 4

Statistics of pathway enrichment of differentially expressed proteins in each pairwise.

Figure 5

Subcellular localization prediction of DEPs

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplyTable.docx](#)