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Abnormal amino acid synthesis and glutathione metabolism may affect PCOS blastocyst development: an examination of in vitro mouse blastocysts model utilizing RNA-sequencing

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Abstract

Background Extensive research has been conducted on embryonic developmental disorders linked to Polycystic Ovary Syndrome (PCOS), a pathological condition that affects 5–10% of women and is characterized by irregularities in the menstrual cycle and infertility. By employing RNA sequencing (RNA-seq), we performed an in-depth investigation of PCOS-related changes in gene expression patterns at the mouse blastocyst stage.

Methods The zygotes of female B6D2 mice were obtained and then differentiated into blastocysts in K + Simplex Optimised Medium (KSOM) cultures containing exo-NC (negative control for exosomes) or exo-LIPE-AS1 (a novel exosomal marker of PCOS). Subsequently, blastocysts were collected for RNA-seq. The bioinformatics was performed to analyze and compare the differences of gene expression profile between blastocysts of control and PCOS group.

Results There were 1150 differentially expressed genes (DEGs) between the two groups of mouse blastocysts; 243 genes were upregulated and 907 downregulated in the blastocysts of the exo-LIPE-AS1 group compared to those of the exo-NC group. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis revealed that the genes involved in amino acid synthesis and glutathione metabolic pathways were down-regulated in exo-LIPE-AS1 group.

Conclusion This study has revealed that blastocyst developmental retardation may be associated with the downregulation of amino acid synthesis and glutathione metabolism, which may affect energy metabolism, biosynthesis, cellular osmotic pressure, antioxidant synthesis, ROS clearance or mitochondrial function, and ultimately

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cause blastocyst cell development abnormalities. Our research offers encouraging data on the mechanisms underlying aberrant embryonic development in patients with PCOS as well as potential treatment strategies.

Keywords PCOS, RNA-seq, Blastocyst, Exosome, Embryonic development

Background

PCOS is a medical condition that is identified by irregular menstrual bleeding, chronic anovulation, excessive androgen production, and distinctive ovarian ultrasound findings [1]. Infertility, caused by this syndrome, affects an estimated 5−10% of women in their reproductive years [2]. The etiology of this syndrome remains unknown. Many women with PCOS require assisted reproductive procedures for a successful pregnancy. The number of eggs obtained in patients with PCOS has been found to be higher than that in individuals without PCOS, but the rate of high-quality embryos was lower than that in individuals without PCOS [3]. The process of IVF in women with PCOS can present various challenges, including a potential for either a suboptimal or an exaggerated response, an imbalance between the number of eggs and follicles, difficulties in fertilization, limited success in blastocyst development, and the risk of ovarian hyperstimulation syndrome [4]. Embryo abnormalities resulting from the excessive level of androgen and Luteinizing hormone in PCOS women may induce a higher miscarriage rate [5, 6]. Therefore, assisted reproductive techniques currently focus on obtaining high-quality oocytes rather than large numbers of oocytes and embryos [7]. The challenge of accurately assessing oocyte quality remains a significant barrier that must be overcome to enhance pregnancy rates.

It is essential to establish a standardized protocol for evaluating the quality of embryo viability used in morphological and morphometric grading systems, as it will ensure consistency and accuracy in the assessment and comparison of different grading techniques [8]. Additionally, the appearance of an embryo does not indicate its quality, considering that around 30–50% of morphologically normal embryos may have chromosomal abnormalities [9]. The assessment of metabolic indicators in embryo culture media, such as glucose, lactate, pyruvate, amino acids, and oxygen consumption, may provide valuable insights. The mtDNA/gDNA ratio in the culture media of embryos from patients with PCOS may be a promising marker for predicting the quality of morphologically good embryos [10, 11]. When mitochondrial antioxidant activity is compromised, there is an increase in ROS production, causing oxidative damage to cellular components, including DNA. Therefore, a decreased mtDNA / gDNA ratio may serve as a marker for poor mitochondrial antioxidant activity and potential cell apoptosis, which are important for ATP production and overall cell function. However, disruptions in mitochondrial structure, dynamics, biogenesis, and mitochondrial membrane potential (MMP) are observed in individuals diagnosed with PCOS [12].

Long non-coding RNA (lncRNA) LIPE-AS1 is located on the plus strand of chromosome 19 in humans and functions as an antisense transcript to the hormone-sensitive lipase (HSL) gene, *LIPE*. LIPE-AS1 was reportedly crucial in the development of PCOS [13]. Previous research has demonstrated that the increased expression of LIPE-AS1, facilitated by follicular fluid exosomes, has a significant and negative impact on both oocyte maturation and embryonic development [13]. During embryonic development, the group with increased LIPE-AS1 expression exhibited blocked embryonic development, exhibiting abnormal morphology and eventually leading to cellular apoptosis. Additionally, this group displayed a reduced proportion of 4–8 cell stage embryos, morulae, and blastocyst formation compared to the control group with normal levels of LIPE-AS1 expression [13]. Therefore, we believe that in vitro culture of fertilized eggs with overexpressed exosomes can well simulate in vitro embryonic development in PCOS patients.

The presence of PCOS may result in alterations in gene expression within embryos or oocytes. A study have identified a significant correlation between PCOS and two diagnostic markers (*GLIPR1* and *MAMLD1*), both of which were involved in metabolic pathways such as glycine, serine, threonine metabolism, and propionic acid metabolism. It has been observed that the pathogenesis of PCOS may be associated with an elevated immune response level resulting from abnormal TCA cycle metabolism [14]. Additionally, changes in mitochondrial gene expression (*NDRG4*, *UCP2*, and *MRPS26*) were linked to mitochondrial dysfunction in oocytes and cumulus cells, resulting in reduced fertilization capacity and impaired embryonic development [15]. Moreover, transcriptomic changes observed in PCOS embryos indicate that mitochondrial dysfunction contributes to the arrest of PCOS embryos [16]. Oxidative phosphorylation genes were significantly down-regulated, while lipopolysaccharide biosynthesis and glutamate breakdown genes were up-regulated [16]. This suggested that elevated ROS production in PCOS oocytes may lead to decreased ATP production and embryo arrest [16]. Therefore, it is imperative to investigate the potential alterations in gene expression of embryos or oocytes induced by PCOS.

Currently, there is a lack of relevant literature regarding the utilization of exosomes for inducing in vitro differentiation of mouse oocytes to establish a cellular model

of mouse oocyte developmental disorders, and subsequently investigate the impact of PCOS-related target genes on mouse oocyte differentiation. Until the recent advent of RNA-seq, transcriptomic analyses of oocytes and early embryos were limited. By employing oligo (dT)-based reverse transcription, RNA-seq was utilized to identify disrupted biological processes and transcripts during development.

To explore the influence of PCOS-related gene LIPE-AS1 on embryonic development, we treated embryos with exosomes overexpressing LIPE-AS1. Our findings revealed that supplementation with exo-LIPE-AS1 significantly impeded embryonic development outcomes when added to KSOM culture medium. Additionally, through RNA-seq analysis comparing exo-NC and exo-LIPE-AS1 embryos, we discovered potential associations between abnormal embryonic development and down-regulation of amino acid synthesis as well as glutamine metabolism pathway. This study provides a deeper understanding into the molecular mechanisms underlying abnormal embryonic development caused by exo-LIPE-AS1 in relation to PCOS.

Methods

Experimental mice and study design

All research procedures were approved by the Animal Science Ethics Committee at the Fudan University (Approval number: 202105002 S). B6D2F1 (C57BL/6 × DBA/2) mice were obtained from the Research Centre for Laboratory Animal Science of Fudan University and raised in a special pathogen-free animal facility under the conditions of a 12:12-h light: dark cycle. Mice had access to food and water during the experiments. We collected exosomes from overexpression empty vector and overexpression LIPE-AS1 cell culture medium and prepared KSOM (#M1450, AiBei Biotechnology) culture medium containing two groups of exosomes with a protein concentration of 100 μg/ml [13]. Empty vector exosomes were included in the KSOM culture medium as a control group (exo-NC group), and exosomes overexpressing LIPE-AS1 were added to the experimental group (exo-LIPE-AS1 group). The development of zygotes between the two groups was observed.

Blastocyst collection

For in vivo zygote collection, 10 female mice were superovulated by injection 10 IU of PMSG, followed by an injection of 10IU of human chorionic gonadotropin (hCG, Ningbo Sansheng) after 48 h. After a period of 16–18 h, adult male mice (12–14 weeks old) were used for fertilization. The vaginal plug was checked the next morning to confirm successful mating. Zygotes were retrieved from the ampulla of the fallopian tube after cervical dislocation without anesthesia in female mice. The collected zygotes were then incubated in KSOM culture medium supplemented with two groups of exosomes. Embryo development was assessed at 24, 48, 72, and 96 h to determine the percentage of embryos at the 2-cell, 4-cell, morula, and blastocyst stages respectively.

Approximately 25–30 blastocysts were collected from each group, and three biologically repeated tests were conducted. Cell lysis was performed using the SMARTer® Ultra™ Low RNA Kit for Illumina® Sequencing (Clontech). Each group of blastocysts was aspirated into 4 μL lysate using a microsuction tube, and the total sample volume was less than 5 μL. The sample was immediately placed on dry ice. All experimental processes were performed in a sterilized cell room without external contamination. All samples were stored at a temperature of -80°C.

RNA sample assessment

Total RNA was extracted and isolated from the blastocysts using Picopure RNA isolation kit (Thermo Fisher Scientific, USA) according to the manufacturer's protocol. The concentration and purity assessment of the RNA samples were performed using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The Agilent 2100 Bioanalyzer (Agilent Technologies Inc., Santa Clara, CA, USA), along with the 2100 RNA Nano 6000 assay kit (Agilent Technologies Inc.), was employed for assessing the quality of the RNA samples. For optimal cDNA synthesis, it was recommended to use a total RNA amount ranging from 10pg to 10ng, ensuring an OD260/280 ratio between 1.8 and 2.1, as well as a RNA integrity number (RIN) value greater than 7.

Transcriptome sequencing library preparation, quantification, and qualification.

After the quality control, a total amount of 3 μg RNA per sample was enriched with the TIANSeq mRNA Capture Kit (TIANGEN, Beijing, China), specifically targeting RNA with polyA. Thereafter, we used the obtained RNA and the Illumina TIANSeq Fast RNA Library Kit (TIANGEN) for the construction of transcriptome sequencing libraries. Briefly, a transcriptome sequencing library was constructed using random RNA fragmentation, cDNA strand 1 and strand 2 synthesis, end repair, A-tailing, ligation of sequencing adapters, size selection, and library PCR enrichment. The concentration of the library was initially measured using a Qubit 2.0 fluorometer (Thermo Fisher Scientific). After being diluted to a concentration of 1 ng/μl, the insert size was evaluated using an Agilent 2100 instrument (Agilent Technologies Inc.).

Clustering and sequencing

The index-coded samples underwent clustering on a cBot Cluster Generation System using the TruSeq PE Cluster

Kit v3-cBot-HS (Illumina, San Diego, CA, USA), following the manufacturer's guidelines. Subsequently, the Illumina sequencing platform was utilized to sequence the library preparations, resulting in the generation of 150 bp paired-end reads.

Data analysis

The initial step involved the utilization of internally developed Perl scripts to process the raw data. Clean data (clean reads) were obtained in this stage using of Trimmomatic to remove reads containing adapters and trimming the low-quality bases. Simultaneously, the metrics for Q20, Q30, and GC contents were derived for the clean data. All downstream analyses were based on clean, high-quality data. Reference genome and gene model annotation files were downloaded from genome website directly (http://asia.ensembl.org/Mus_musculus/Location/Genome?ftype=DnaAlignFeature;id=Mm.10). The Hisat2 (version 2.1.0) was used to create the reference genome index, and clean paired-end reads were aligned with the reference genome using Hisat2. Our choice of using Hisat2 as the mapping tool was driven by its ability to create a comprehensive database of splice junctions using the gene model annotation file, resulting in superior mapping outcomes compared to alternative non-splice mapping tools.

The number of reads mapped to each gene was counted using HtSeq count (version 0.6.0). After determining the gene length and the corresponding read count, the Fragments Per Kilobase of transcript sequence per Million base pairs sequenced (FPKM) value was calculated. The FPKM metric incorporates both sequencing depth and gene length to accurately measure gene expression levels, making it the prevailing approach in this area.

Differential gene expression analysis

Differential expression analyses were conducted using the DESeq2 R package (version 1.30.0) to compare gene expression between the two groups. DESeq2 is a statistical tool specifically designed for analyzing digital gene expression data and identifying differences in expression levels. The resulting p-values were adjusted using Benjamini and Hochberg's approach to control for the false discovery rate. The DEGs were screened as the absolute value of Fold change (FC) for $|\text{Log2(FC)}| \geq 2$, and $p < 0.05$ was considered statistically significant.

The topGO R package (version 2.36.0) was used to perform Gene Ontology (GO) (http://www.geneontology.org/) enrichment analysis on DEGs, with the incorporation of gene length bias correction. The GO terms with corrected p-values below 0.05 were considered significantly enriched by DEGs. The KEGG is a collection of databases and tools that integrate and analyze large-scale molecular datasets to provide comprehensive information on various biological processes, pathways, and interactions. KEGG contains information on genes, proteins, small molecules, diseases, and drugs, allowing researchers to explore and study complex biological systems (http://www.genome.jp/kegg/). The statistical enrichment of in KEGG pathways was tested using the clusterProfiler R package (version 3.12.0).

Results

Effect of LIPEAS1 on embryonic development in vitro

During the embryonic development, the exo-LIPE-AS1 group exhibited a retardation in embryo development (Fig. 1a) and a lower proportion of formation of 4- to 8-cell, morula, and blastocyst compared to the exo-NC group (Fig. 1b) [13]. The findings of this study suggested that the upregulation of LIPE-AS1, facilitated by follicular fluid exosomes, significantly impacted embryo development.

Transcriptome of mouse blastocysts

The analysis of blastocysts treated with exo-NC and exo-LIPE-AS1 was carried out using RNA-seq. In the case of every sample, 35 million Illumina reads were produced, 80% of which were mapped to the reference genome. The FPKM was used to calculate the expression value for every gene. A total of 63,470 expressed genes within mouse blastocysts were identified based on a cut-off FPKM value of >0.

Boxplots were used to analyze the log10-transformed FPKM values for each sample, which demonstrated the samples' consistent distribution and range of FPKM values (Fig. 2a), indicating that the RNA-seq data of this study were reliable, reproducible, and of excellent quality.

Global gene expression characteristics of mouse blastocysts

Cluster analysis of FPKM in each sample revealed differences in gene expression profiles between blastocysts treated with exo-NC and exo-LIPE-AS1, revealing that the RNA-seq data in this study fulfilled the necessary conditions for conducting differential expression analysis (Fig. 2b). There were 1150 DEGs between the two groups of mouse blastocysts; 243 genes were upregulated and 907 downregulated in the blastocysts of the exo-LIPE-AS1 group compared to those of the exo-NC group (Fig. 2c, d). According to the P value, we have marked the genes with the most significant differences in the volcano plots. The up-regulated genes included *Polr2k*, *Khdc3*, *Atp5k*, *Rpl5* and *Cox7c*, while the down-regulated genes consist of *Tmsb10*, *Rpl10-ps6*, *Rps10-ps2*, *Psmd13*, and *Gm9843*.

We utilized GO enrichment analysis to detect the distribution of differential gene expression related to PCOS in biological functions (Fig. 3a). The most notable

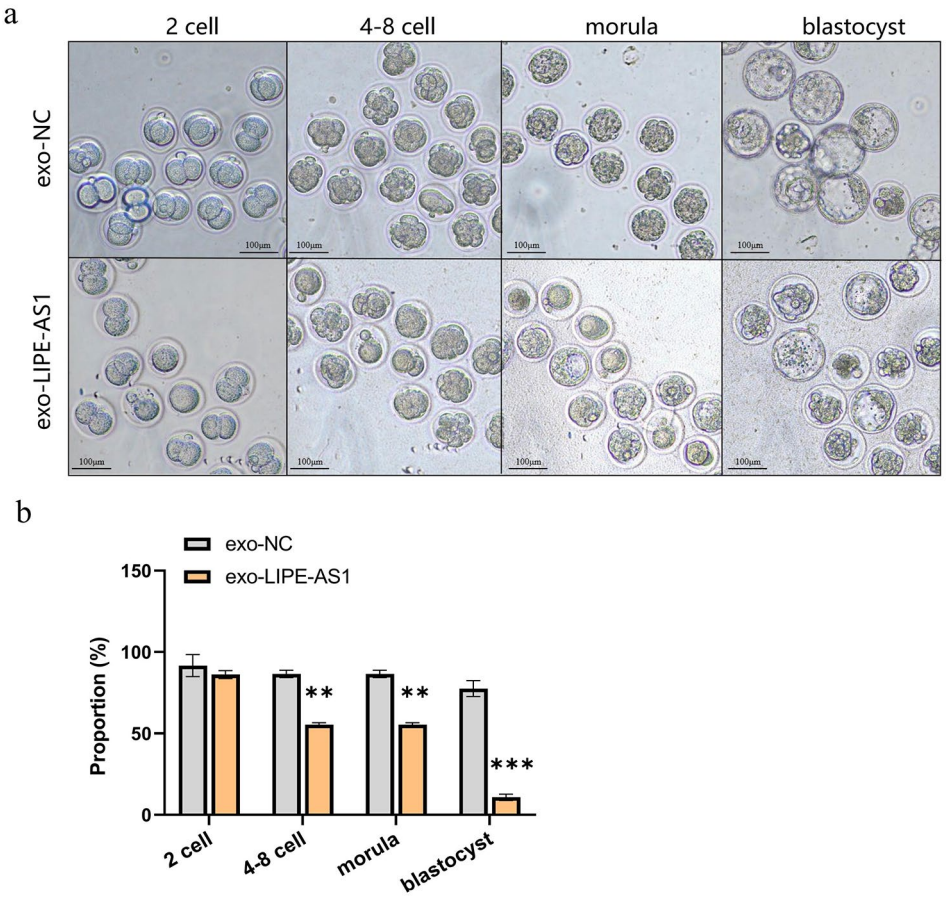

Fig. 1 Effect of exo-LIPE-AS1 on embryonic development in vitro [13]. (**a**) Analysis of embryonic development between two groups. (**b**) The proportion of 2-cell, 4- to 8-cell, morula, and blastocyst between two groups during embryonic development in vitro (scale bars, 100 μm)

enrichment terms in the biological process category were "Cellular metabolic process" and "primary metabolic process", with 373 and 355 DEGs, respectively. "Intracellular membrane-bounded organelle" and "cytoplasm" were the most significant enrichment terms in the cellular component category, including 395 and 379 DEGs, respectively. "Enzyme binding" and "DNA binding" were highlighted as the most significant enrichment terms, with 101 and 90 DEGs, respectively. Additionally, we conducted KEGG enrichment analysis to identify the distribution of PCOS-related differential gene expression in biological pathways (Fig. 3b). The most remarkable enrichment terms were "Systemic lupus erythematosus", "alcoholism", and "Endocrine and other factor-regulated calcium reabsorption".

Enrichment of upregulated differential genes in PCOS blastocysts

In the biological process category of the GO enrichment pathway (Fig. 4a), the most notable enrichment terms were "regulation of transcription by RNA polymerase II" and "positive regulation of nucleobase-containing compound metabolic process" with 24 and 14 DEGs respectively. The cellular component category highlighted "nucleus" and "inner mitochondrial membrane protein complex" as the most significant terms, involving 49 and 5 DEGs, respectively. Additionally, the molecular function category showcased "heterocyclic compound binding" and "organic cyclic compound binding" as the most significant terms, associated with 45 and 45 DEGs, respectively. In the KEGG enrichment pathway analysis (Fig. 4b), the three most significant enrichment terms included "prion disease", "Huntington's disease" and "cardiac muscle contraction".

Enrichment of downregulated differential genes in PCOS blastocysts

In the enrichment pathway for GO terms (Fig. 4c), the terms "primary metabolic process" and "organization of organelles" were identified as the most significantly enriched terms within the biological process category, with 299 and 128 DEGs, respectively. Within the cellular component category, the terms "organelle surrounded by membrane" and "cytoplasm" were found to be the most significantly enriched, with 349 and 333 DEGs,

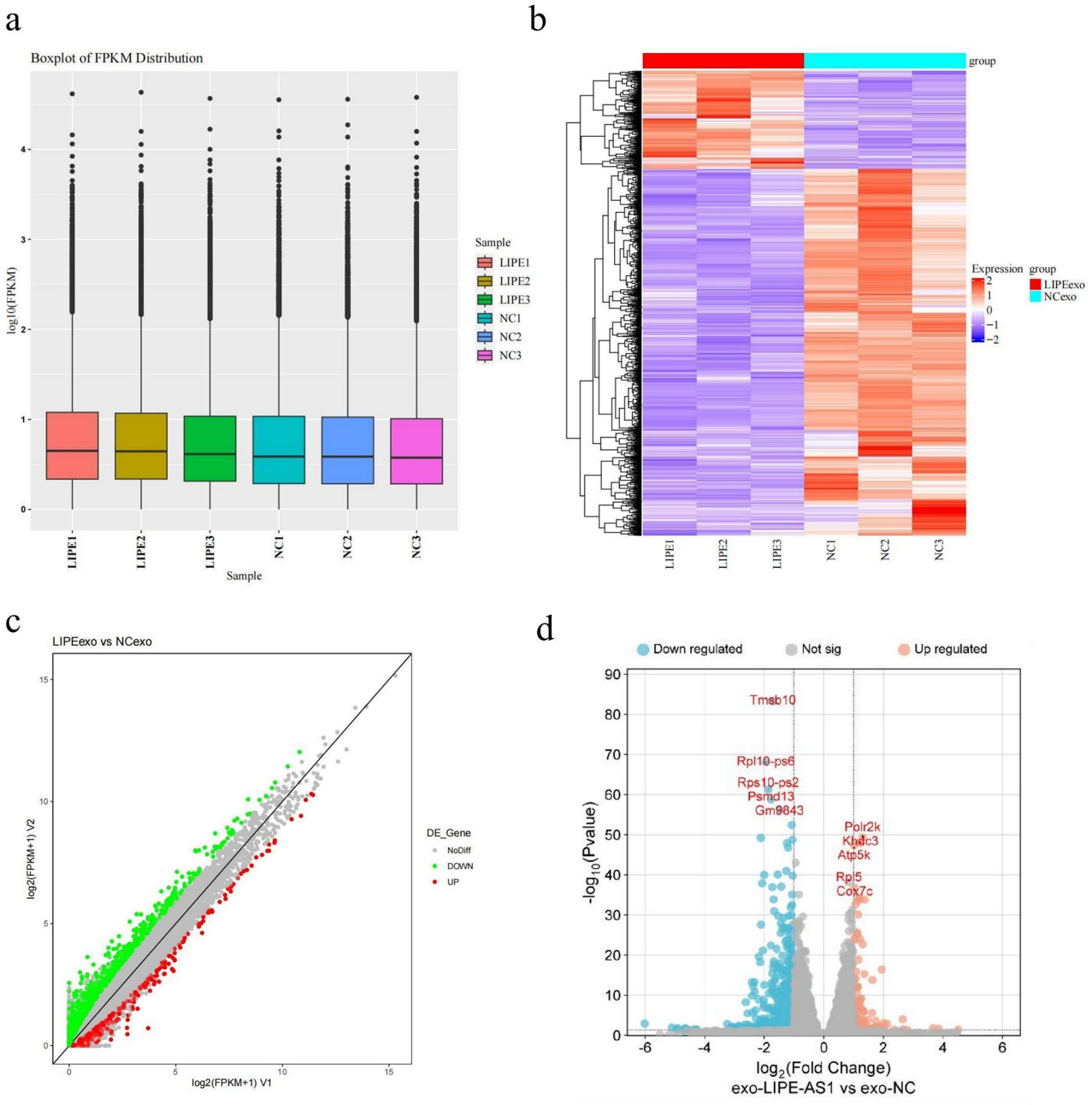

Fig. 2 Gene expression levels of blastocysts from mice with PCOS and the control group. (**a**) The boxplot shows the global gene expression level (log10 FPKM) of each sample. (**b**) The heat map shows the clustering analysis of gene expression levels (FPKM) in each sample. **c**-**d**. The scatter plot and volcano plot show the DEGs between blastocysts from mice with PCOS and the control group, and the genes with the highest significance according to the *P*-value was labeled in the volcano plot

respectively. In the molecular function category, the terms "catalytic activity" and "binding of enzymes" were identified as the most significantly enriched, with 174 and 96 DEGs, respectively. In the KEGG pathway enrichment analysis (Fig. 4d), several terms were observed to be significantly enriched, including "systemic lupus erythematosus", "alcohol abuse disorder", "formation of neutrophil extracellular traps", "biosynthesis of amino acids" and "metabolism of glutathione". The genes related to the biosynthesis of amino acids were significantly downregulated in the blastocysts of the exo-LIPE-AS1 group, including glycolytic enzymes phosphofructokinase, liver type (*PFKL*),

phosphoglycerate mutase 1 (*PGAM1*), enolase 1B (*ENO1B*), enolase 1 (*ENO1*), enolase 3 (*ENO3*), amino acid synthetase aminoacylase 1 (*ACY1*),

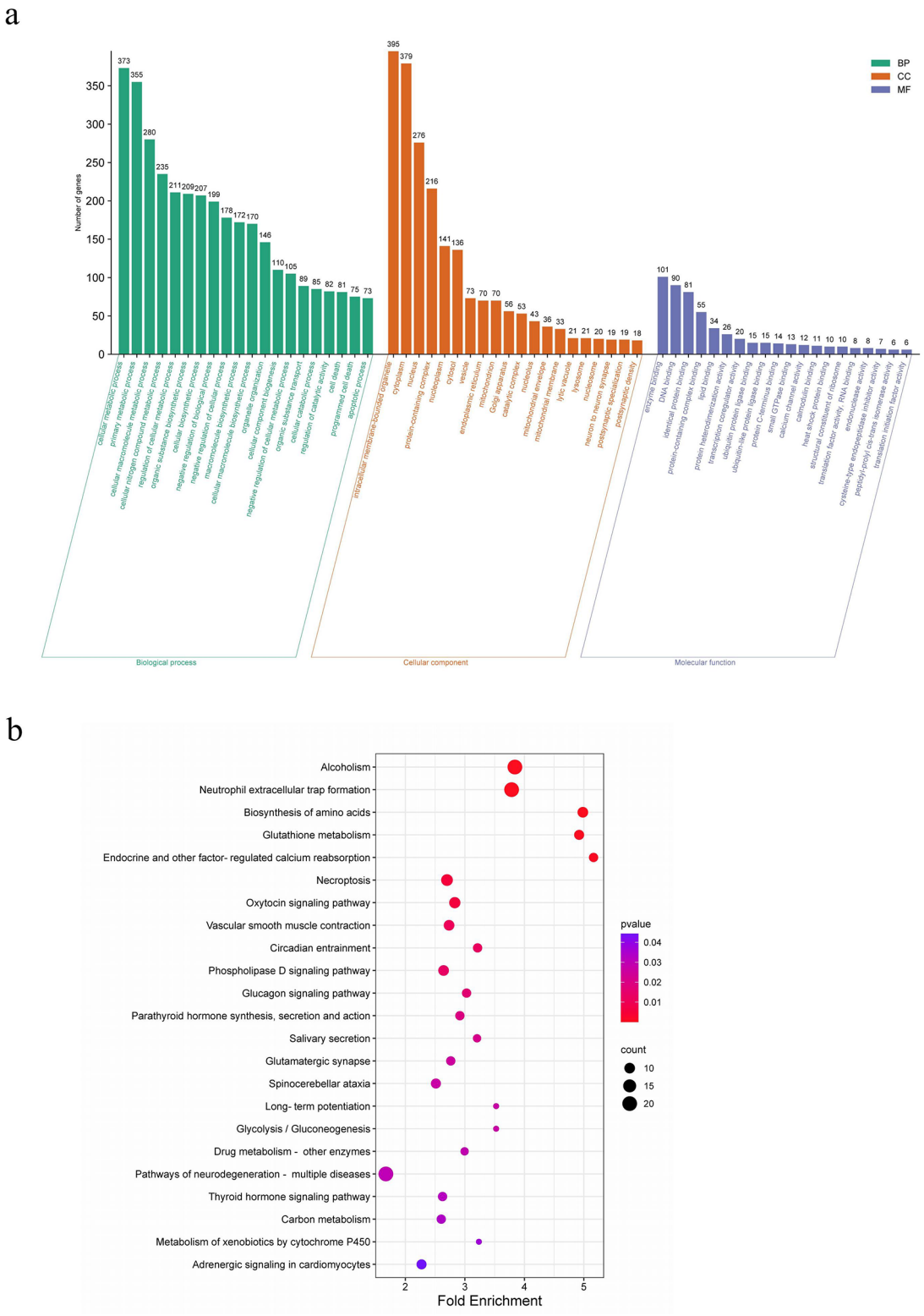

Fig. 3 The analysis of DEGs between mice with PCOS and the control group. (**a**) GO enrichment analysis of DEGs. Green refers to terms relating to biological processes, orange refers to terms relating to cellular components and purple refers to terms relating to molecular function. (**b**) KEGG pathways enriched from DEGs. The dot size denotes the number of DEGs, while colors correspond to the *p*-value range

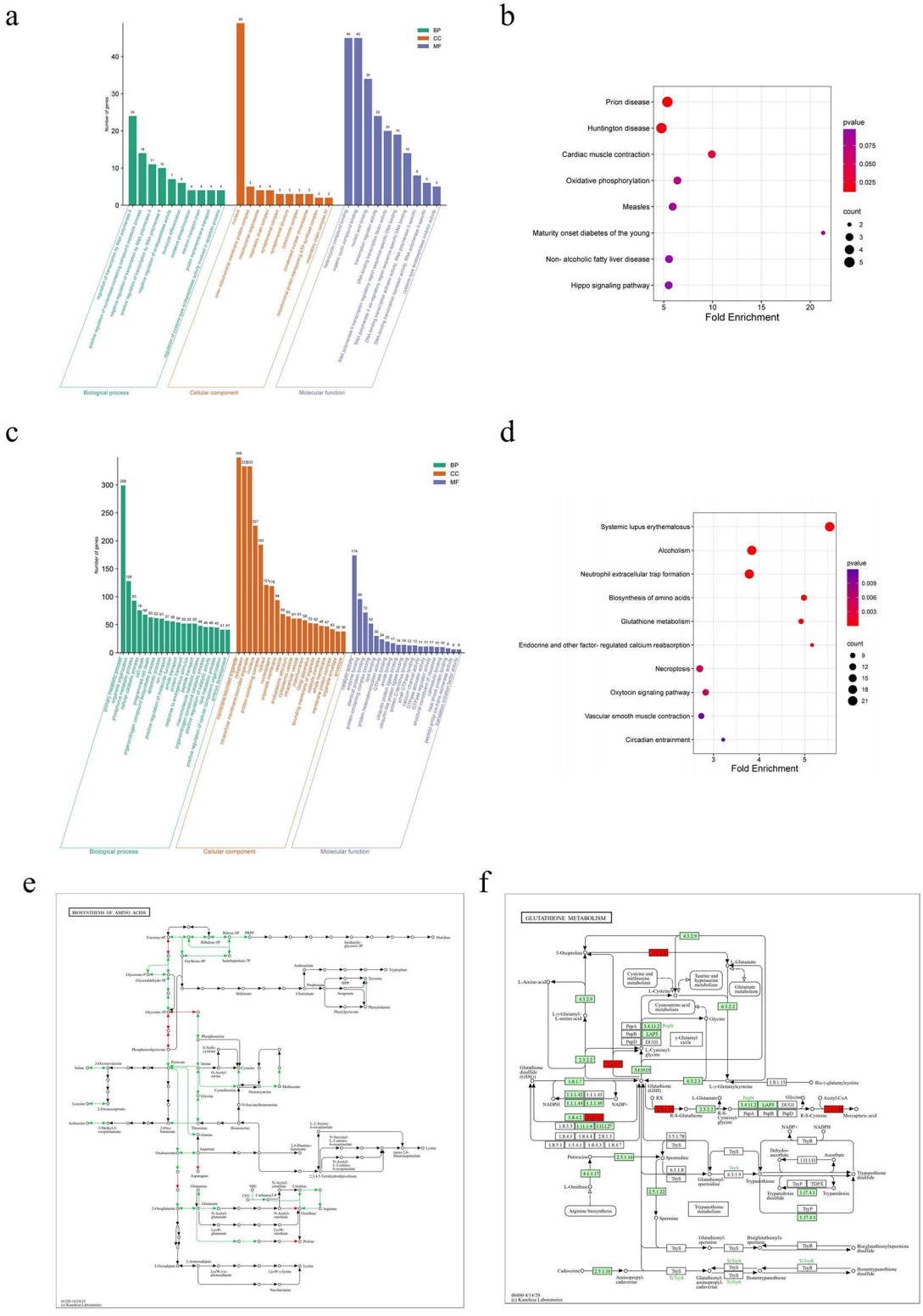

Fig. 4 The analysis of upregulated and downregulated DEGs between mice with PCOS and the control group. **a**-**b**. GO enrichment and KEGG pathway analysis of upregulated DEGs. **c**-**d**. GO enrichment and KEGG pathway analysis of downregulated DEGs. **e**-**f**. Amino acid synthesis and glutathione metabolism in the KEGG pathway. Red dots or lines represent down-regulated genes in the results

pyrroline-5-carboxylate reductase 1 (*PYCR1*), asparagine synthetase (*ASNS*), phosphoglycerate dehydrogenase (*PHGDH*), and tricarboxylic acid (*TCA*) cycle enzyme aconitase 2 (*ACO2*) (Fig. 4e). A group of DEGs participating in the glutathione metabolism were also downregulated in the blastocysts of the exo-LIPE-AS1 group, including glutathione S-transferase mu 1 (*GSTM1*), glutathione S-transferase mu 2 (*GSTM2*), glutathione peroxidase 4 (*GPX4*), glutathione S-transferase P2 (*GSTP2*), glutathione S-transferase P3 (*GSTP3*), N-acetyltransferase 8 family member 2 (*NAT8F2*), microsomal glutathione S-transferase (*MGST3*), ChaC glutathione specific gamma-glutamylcyclotransferase 1 (*CHAC1*), and 5-oxoprolinase, and ATP-hydrolysing (*OPLAH*) (Fig. 4f).

Discussion

The etiology and pathophysiology of PCOS, which is a major reason for anovulatory infertility in women, have not been understood thus far. Patients with PCOS who undergo assisted reproductive techniques may experience various complications, such as a poor to exaggerated response, low oocyte quality, ovarian hyperstimulation syndrome, and alterations in the metabolic composition of the follicular fluid. These irregularities cause a decrease in metaphase II (MII) oocytes; decreased rates of fertilization, cleavage, implantation, and blastocyst conversion; a poor egg-to-follicle ratio; and an increased risk of miscarriage. Currently, there is a significant focus within the medical community on the acquisition of superior-quality embryos for patients diagnosed with PCOS to enhance the chances of successful pregnancies.

The follicular fluid is crucial for the development of follicles and maturation of oocytes within the reproductive system. Follicular fluid exosomes can transport long non-coding RNAs (lncRNAs), microRNAs (miRNAs), messenger RNAs (mRNAs), and proteins that play a crucial rule in follicular development and oocyte maturation [17, 18]. Exosomes transfer genetic information to the target cells, thereby inducing functional changes. This novel mechanism provides insight into the etiology of PCOS [19]. Therefore, follicular fluid exosomes may be used as new diagnostic markers for PCOS. Previous studies have shown that increased expression of LIPE-AS1 facilitated by follicular fluid exosomes, has a significant impact on both oocyte maturation and embryonic development [13]. LIPE-AS1 may have a vital function in the development of PCOS. In this study, the blastocysts were collected for RNA-seq in order to investigate the molecular mechanisms underlying the blocked development of PCOS blastocysts.

According to our data, it was found that blastocysts from both the exo-NC and exo-LIPE-AS1 groups displayed comparable gene expression profiles at the transcriptomic level. Only 1150 genes (1.8%) exhibited statistically significant expression differences, among which 243 were upregulated and 907 were downregulated in the blastocysts of the exo-LIPE-AS1 group. However, gene expression analysis revealed that many DEGs were intricately linked to crucial cellular processes. After enrichment analysis, the most notable enrichment terms in the biological process category were "Cellular metabolic process" and "primary metabolic process", with 373 and 355 DEGs, respectively. Therefore, these DEGs were involved in cellular growth-related biological functions and metabolic pathways.

The genes related to the biosynthesis of amino acids were significantly downregulated in the blastocysts of the exo-LIPE-AS1 group. The DEGs included *PFKL*, *PGAM1*, *ENO1B*, *ENO1*, *ENO3*, *ACY1*, *PYCR1*, *ASNS*, *PHGDH*, *TCA*, *ACO2*. These DEGs were found to be involved in the biosynthesis of proline, ornithine, asparagine, phosphoenolpyruvate, glycerate-3p and other related metabolites. Amino acids serve as the fundamental components of proteins and fulfill various other roles, such as energy substrates, osmolytes, antioxidants, and electron carriers [20]. The amino acid profiles of early embryos obtained in this manner are predictive of embryo sex [21], aneuploidy [22], oxidative stress, as well as developmental ability [23, 24]. Moreover, amino acid production of human oocytes reflects several key features, including developmental competence and hormone regimen during superovulation [25]. Besides, amino acids promote embryonic development in the in vitro culture environment [26]. Therefore, we speculated that the downregulation of thses genes involved in amino acid synthesis may potentially hinder blastocyst development and maturation by impacting energy metabolism, biosynthesis, antioxidant activity or cellular osmotic pressure.

Our study also indicated that the glutathione metabolism might play essential roles in the deterioration of blastocyst quality in PCOS group. These DEGs included *GSTM1*, *GSTM2*, *GPX4*, *GSTP2*, *GSTP3*, *NAT8F2*, *MGST3*, *CHAC1*, *OPLAH*. The downregulation of these DEGs may be associated with the decline in glutathione function, such as oxidative damage mediated by ROS.

Glutathione, a potent antioxidant and universal free radical scavenger, plays a crucial role in neutralizing harmful free radicals during the fertilization process. After fertilization, glutathione is immediately mobilized through two ATP-dependent steps: γ-glutamylcysteine synthetase and glutathione synthetase, both associated with cysteine availability. Glutathione is involved in antioxidant defense and is closely related to gene expression regulation, cell proliferation, apoptosis, signal transduction, and immune response [27]. Glutathione mobilization has an immediate impact on subsequent embryonic development, resulting in an increased rate of blastocyst formation and an increased cell number per blastocyst

[28]. ROS perform crucial functions within cells, including defense against microorganisms and in signaling pathways involved in cell survival, such as the induction of apoptosis, inflammation, and immune responses. However, they also damage DNA, proteins, and lipids, and cells have evolved protective mechanisms, such as the presence of glutathione, a tripeptide of glutamine, glycine, and cysteine, in response to ROS [20]. Oxidative stress caused by elevated ROS levels during in vitro culture can lead to reduced oocyte developmental capacity and increased apoptosis, which are the main reasons for the low efficiency of in vitro maturation and in vitro embryo development.

The germinal vesicle and metaphase I-stage oocytes obtained from females diagnosed with PCOS demonstrate elevated levels of ROS in the mitochondria and a reduction in the mitochondrial membrane potential [29]. The usage of antioxidants for mitochondria during in vitro embryo cultivation can reduce the oxidative stress level in the embryo, inhibit the decoupling of mitochondria in the ovarian mitochondria, reduce the level of blastocyst apoptosis, and improve the quality of blastocysts, thus increasing the success rate of embryo transfer [30].

A limitation of this study was the number of embryos analyzed. In addition, there are still differences between the mouse transcriptome and the human transcriptome. To further test our hypothesis, a well-designed survey with large embryo sample sizes are needed. And functional or protein expression assays should also be devised to validate alterations in the blastocyst transcriptome.

Conclusions

The aim of this study was to use RNA-seq analysis to uncover the molecular mechanism behind abnormal blastocyst development in patients with PCOS. And the application of RNA-seq in this study has revealed that blastocyst developmental retardation may be associated with the downregulation of amino acid synthesis and glutathione metabolism, which may affect energy metabolism, biosynthesis, cellular osmotic pressure, antioxidant synthesis, ROS clearance or mitochondrial function, and ultimately cause blastocyst cell development abnormalities. Thus, our findings offer novel insights into the underlying pathogenic mechanisms governing embryonic developmental arrest in PCOS.

Abbreviations

FC	Fold change
GO	Gene Ontology
KEGG	Kyoto Encyclopedia of Genes and Genomes
MMP	Mitochondrial membrane potential
PCOS	Polycystic Ovary Syndrome
RNA-seq	RNA sequencing
ROS	Reactive oxygen species

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Author contributions

CW, LY collected samples; ML, QC, JT, XW, XD performed mouse blastocyst differentiation experiment; CW, LY, WC and TL designed and finished the RNAseq; BP, BW, SL and WG designed the study; CW analyzed the data and wrote the paper. The authors read and approved the final manuscript.

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Data availability

The data and materials supporting the study are available to the corresponding author upon reasonable request. The accession number for the raw sequence of mouse blastocysts in the SRA data is PRJNA1121980.

Declarations

Ethical approval and consent to participate

All animal experiments were approved by the Animal Science Ethics Committee at the Fudan University (Approval number: 202105002S). This study was conducted in accordance with the Guide for the Care and Use of Laboratory Animals (NIH Publication NO.85–23, revised 1996). All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. There are no human subjects in this article and informed consent is not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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