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Comparatively profiling the transcriptome of human, Porcine and mouse oocytes undergoing meiotic maturation

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Abstract

Background Oocyte maturation is a critical process responsible for supporting preimplantation embryo development and full development to term. Understanding oocyte gene expression is relevant given the unique molecular mechanism present in this gamete. Comparative transcriptome analysis across species offers a powerful approach to uncover conserved and species-specific genes involved in the molecular regulation of oocyte maturation throughout evolution.

Results Transcriptome analysis identified 4,625, 3,824, 4,972 differentially expressed genes (DEGs) between the germinal vesicle (GV) and metaphase II (MII) stage in human, porcine and mouse oocytes respectively. These DEGs showed dynamic changes associated with oocyte maturation. Functional enrichment analysis revealed that the DEGs in all three species were mainly involved in DNA replication, cell cycle and redox regulation. Comparative transcriptome analysis identified 551 conserved DEGs in the three species with significant enrichment in mitochondria and mitochondrial intima.

Conclusions This study provides a systematic comparative analysis of oocyte meiotic maturation in humans, pigs and mice identifying both conserved and species-specific patterns during oocyte meiosis. Our findings also implied that the selection of oocyte expressed genes among these three species could form a basis for further exploring their functional roles in human oocyte maturation.

Keywords Transcriptome, Human, Pig, Mouse, Oocyte maturation

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Introduction

In humans, oocytes can be the targets of treatment for reproductive diseases since they might be the cause of infertility [1]. Sexual reproduction requires the fertilization of a female gamete after it has achieved optimal development. Mice have long been the models of choice to elucidate the complicated mechanisms that regulate oogenesis and to identify the major genes and molecular actors involved throughout the process [2, 3]. However, in recent decades, progress in genomics has made it possible to explore molecular mechanisms in a range of species, including pigs. Many studies have reported that some molecular mechanisms regulating oocyte maturation are conserved among species [4]. However, our understanding of the oocyte transcriptome and the identity of key oocyte-expressed genes is far from accomplished. Furthermore, characterization of gene expression in oocytes is necessary and will offer another insight into the regulation of oocyte maturation, fertility, and preimplantation development. Studies that have used mice as models often contribute to our understanding of mammals, however, less is known about the molecular mechanisms involved in oocyte maturation in humans and pigs including their similarities and differences.

Compared with mice, pigs appear to have more advantages as model animals, not only in their physiological and anatomical similarities to humans, but most importantly, porcine oocytes are closely related to human oocytes in terms of morphology and timing of meiotic maturation [5, 6]. It takes an average of 7–13 h for mouse oocytes to reach MII post luteinizing hormone surge, whereas in humans and pigs this occurs after about 40 h [6, 7]. In addition, while some oocyte RNAs are translated to ensure cellular metabolism, others are deadenylated or stored in the cytoplasm.

The occurrence of oocyte meiotic maturation depends on the accumulation of maternal mRNAs and proteins during oocyte growth [8–10], and the transcription of fully-grown oocytes is still maintained at a low level until they complete meiotic maturation [11]. Therefore, transcription from oocytes at GV stage supports oocyte meiotic maturation [12]. The transcripts stored in oocytes at the GV stage exhibit differential fate during oocyte meiotic maturation: some undergo selective degradation, while others undergo translation to generate proteins essential for oocyte meiosis completion. Upon fertilization of oocytes to form zygotes and subsequent transition to embryos, certain transcripts are degraded, contributing to the establishment of a novel regulatory program [13–15]. Maternal mRNA degradation is a common phenomenon during oocyte maturation in humans, pigs, and mice, however, the alterations in oocyte maternal mRNA profiles vary across these three species. An estimate of the RNA content of a fully-grown oocyte in most mammalian species is 0.3–0.5 ng (mouse, human) to 0.7–2 ng (pig).

RNA sequencing (RNA-seq) has marked significant strides in most life sciences and health areas. These advancements provide a nuanced view into complex regulatory networks and cellular dynamics, including cellular heterogeneity, developmental biology and reproductive mechanisms. Furthermore, a substantial number of genes have been identified through RNA-seq analysis in both human and mouse oocyte maturation, and are crucial for the regulation of this process [16–19]. In pigs, prior research has revealed transcriptomic profiles indicating the involvement of numerous genes in the regulation of oocyte maturation [20, 21].

Here, we compared transcriptomic profiles of human, porcine and mouse oocytes by RNA-seq, screened the DEGs of oocytes within and between species, and analyzed the expression characteristics and potential roles of DEGs during oocyte maturation. The comparative analysis revealed species-specific differences and conservation among maternal transcripts expressed in human, porcine and mouse oocytes. Working simultaneously with three distantly related species will be helpful for us to better understand the oocyte transcriptome and the identity of key oocyte expressed genes with functions in important evolutionarily conserved mechanisms. Moreover, transcriptomic profiles of oocyte maturation among these three species enables us a profound understanding of oocyte meiosis.

Materials and methods

In vitro maturation of human oocytes

Oocytes used in this study were obtained from the women who received reproductive assisted technology treatment at the Center for Reproduction and Genetics, the First Affiliated Hospital of the University of Science and Technology of China, Division of Life Sciences and Medicine, Hefei, Anhui, China. Inclusion criteria were that the oocytes came from women within couples with male factor etiology infertility. Ovarian stimulation was performed according to a short GnRH agonist stimulation protocol [22]. Oocyte retrievals were performed 36 h after hCG administration. Oocytes donated by patients were divided in two groups: the GV-stage group was not maturated and was immediately stored for RNA isolation, and the maturation group was cultured for 24 h in maturation medium (G-IVF™ PLUS fertilization medium supplemented with 0.075 IU/mL FSH, 0.1 IU/mL LH) covered with mineral oil at 37 °C in an atmosphere of 6% CO_2 incubator.

In vitro maturation of Porcine oocytes

Porcine ovaries were collected from a local slaughterhouse. Cumulus-oocyte complexes (COCs) were

collected and selected under a stereomicroscope. Oocytes were divided in two groups: the GV-stage group was not maturated and was immediately stored for RNA isolation, and the maturation group was cultured for 44 h in maturation medium (TCM-199 supplemented with 5% FBS, 10% porcine follicular fluid, 10 IU/mL eCG, 5 IU/mL hCG, 100 ng/mL L-Cysteine, 10 ng/mL EGF, 0.23 ng/mL melatonin, 2.03×10^{-5} ng/mL LIF, 2×10^{-5} ng/mL IGF-1, 4×10^{-5} ng/mL FGF_2, 100 U/mL penicillin and 100 mg/mL streptomycin) covered with mineral oil at 38.5 °C in an atmosphere of 5% CO_2 incubator [23]. After maturation, 1 mg/mL hyaluronidase in DPBS without $Ca^{2+}$and Mg^{2+} (Gibco, Grand Isle, NY) was used to remove the cumulus cells surrounding the oocytes.

In vitro maturation of mouse oocyte

Kunming mice from the Laboratory of the Animal Center of Anhui Medical University were used for oocyte collection. All experiments were conducted according to the Institutional Animal Care and Use Committee (IACUC) guidelines under current approved protocols at Anhui Agricultural University.

Young female mice (4–5 weeks old) were sacrificed by cervical dislocation. Oocytes were divided in two groups: the GV-stage group was not maturated and was immediately stored for RNA isolation, and the maturation group was cultured for 16 h in M16 medium covered with mineral oil at 37 °C in an atmosphere of 5% CO_2 incubator [24].

Sample collection and RNA isolation

Sample collection comprised of 9 GV and 9 MII oocytes from humans, 15 GV and 15 MII oocytes from pigs, 24 GV and 24 MII oocytes from mice. The zona pellucida of human and mouse oocytes underwent digestion using acid tyrode's solution, while that of porcine oocytes underwent digestion using 3.3 mg/mL streptomysin. Oocytes lacking the zona pellucida were evaluated for cytoplasmic integrity and subsequently transferred into lysis buffer containing RNase inhibitor.

The oocytes from humans, pigs and mice were collected at GV and MII stage, respectively. Total RNA of oocytes was extracted and isolated using RNeasy Mini Kit (QIAGEN, Germany) according to the manufacturer's instructions [25].

Transcriptome sequencing

SMART preamplification of the sample involves utilizing poly(A) RNA as a template and oligo (dT) sequence with splice as a primer, for carrying out first-strand cDNA synthesis using SMART reverse transcriptase. The SMART pre-amplified products were constructed as follows: the SMART products obtained underwent enzyme digestion to generate segments, and fragments within the range of 150–300 bp were isolated using magnetic beads. These selected fragments were modified by adding "A" at the 3' end, followed by ligation with Y-shaped sequencing adapters. The resulting constructs were utilized as templates for PCR amplification to produce libraries. Upon completion, the constructed libraries underwent additional quality assessment measures to ensure high quality. Subsequently, the libraries were sequenced using the Illumina NovaseqTM6000 platform with a paired-end read length of 2*150 bp (PE150). The entire process of RNA amplification and sequencing was carried out by Lianchuan Company (Hangzhou, China).

Real-time quantitative PCR (RT-qPCR)

Reverse transcription was performed using a reverse transcription kit (TRANSGEN, China) according to the manufacturer's instructions. Quantitative PCR was conducted using the Quanti Nova SYBR Green PCR Kit (QIAGEN, Germany) on a Step One Plus Real-Time PCR System (Applied Biosystems) [25]. The primers used in this study were listed in supplementary Table S1. The universal reverse primer was provided by the PCR kit. The quantification cycle samples of each target gene were normalized to the abundance of *EF1α1* mRNA transcript in pigs, *GAPDH* mRNA transcript in humans and mice. Three independent biological replicates were performed for all experiments.

Biological analysis of transcriptome sequencing

Raw data required preprocessing to generate valid data through the utilization of the Cutadapt software. This process entailed the elimination of reads containing adaptors and those of poor quality (defined as reads with a mass value $Q \leq 10$ accounting for over 20% of the total read). Trimmed reads were aligned to the reference genome using Hisat software with default parameters, and transcripts were assembled based on the alignment results. StringTie software was employed to assemble complex datasets into transcripts and enhance the prediction of gene expression levels. Subsequently, EdgeR from the R package was utilized for differential expression analysis with significance defined as a log2 fold change of at least 1 and FDR p-value less than 0.05. Graphical representation of the differential expression results included a heat map of DEGs, scatter plot, volcano plot, and principal component analysis plot of DEGs. The functional analysis of DEGs involved conducting Gene Ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) signaling pathway enrichment analysis. The results of the enrichment analyses were presented graphically, while the disparities in gene expression abundance related to GO or KEGG functions and pathways were depicted in a tabulated format.

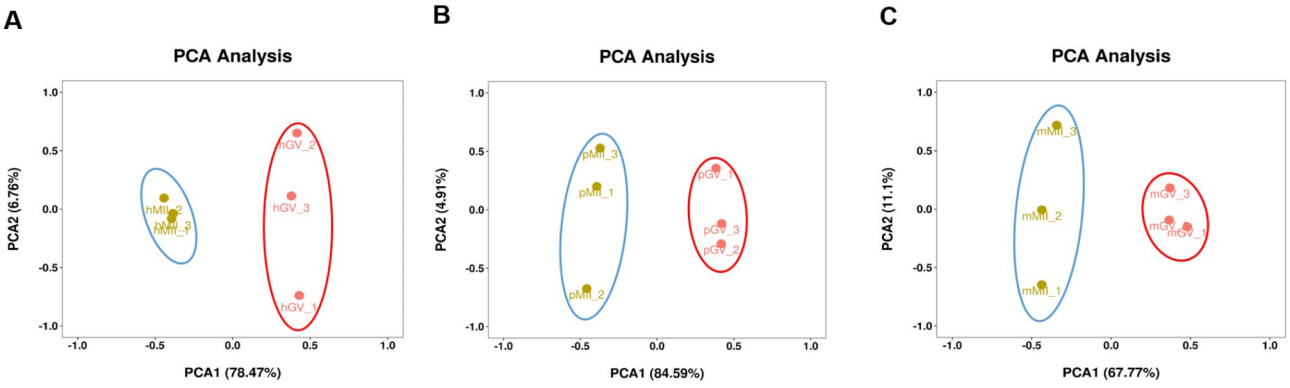

Fig. 1 Principal component analysis (PCA) of oocytes from humans, pigs and mice. **A** PCA of all samples from humans. **B** PCA of all samples from pigs. **C** PCA of all samples from mice. Ellipses show the 95% confidence interval in each stage. PCA, principal component analysis; hGV, human germinal vesicle; hMII, human metaphase II; pGV, pig germinal vesicle; pMII, pig metaphase II; mGV, mouse germinal vesicle; mMII, mouse metaphase II

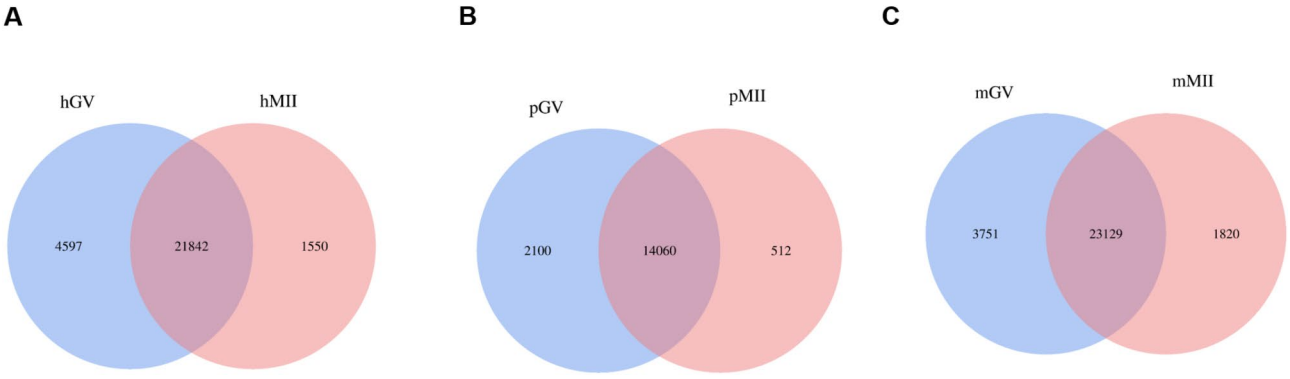

Fig. 2 Gene expression of human porcine and mouse between GV and MII oocytes. **A** Overlap of genes expression identified between GV and MII oocytes from humans. **B** Overlap of genes expression identified between GV and MII oocytes from pigs. **C** Overlap of genes expression identified between GV and MII oocytes from mice. hGV, human germinal vesicle; hMII, human metaphase II; pGV, pig germinal vesicle; pMII, pig metaphase II; mGV, mouse germinal vesicle; mMII, mouse metaphase II

Statistical analysis

The RT-qPCR data were analyzed by student's *t*-test or one-way ANOVA (SPSS 19.0) and were presented as mean ± standard error of mean (mean ± S.E.M). $P < 0.05$ was considered to be statistically significant.

Results

Principal component analysis of human, Porcine and mouse oocytes

The gene expression profile matrix was constructed using samples from oocytes at GV and MII stages in humans, pigs, and mice, respectively by using Fragments Per Kilobase of exon model per Million mapped fragments (FPKM). Principal component analysis (PCA) of samples was performed. In humans, the variance contribution rate of first principal component (PC1) in the expression profile was high at 78.47% while the contribution rate of second principal component (PC2) was relatively low at 6.76% (Fig. 1A). In pigs, the variance contribution rate of PC1 in the expression profile was high at 84.59% while the contribution rate of PC2 was relatively low at 4.91% (Fig. 1B). In mice, the variance contribution rate of PC1 in the expression profile was high at 67.77% while the contribution rate of PC2 was relatively low at 11.1% (Fig. 1C). The above results showed the biological samples of humans, pigs and mice showed well clustering.

Identification of differentially expressed genes during oocyte maturation in humans, pigs and mice

The results showed that there were 26,439 genes in GV oocytes and 23,392 genes in MII oocytes, and 4,597 and 1,550 genes were specifically expressed in GV and MII oocytes, respectively, and 21,842 genes were co-expressed in humans (Fig. 2A). In pigs, there were a total of 16,160 genes in GV oocytes and 14,572 genes in MII oocytes, and 2,100 and 512 genes were specifically expressed in GV and MII oocytes, respectively, and 14,060 genes were co-expressed (Fig. 2B). In mice, there were 26,880 genes in GV oocytes and 24,949 genes in MII oocytes, 3,751 and 1,820 genes were specifically expressed in GV and MII oocytes, respectively, and 23,129 genes were co-expressed (Fig. 2C).

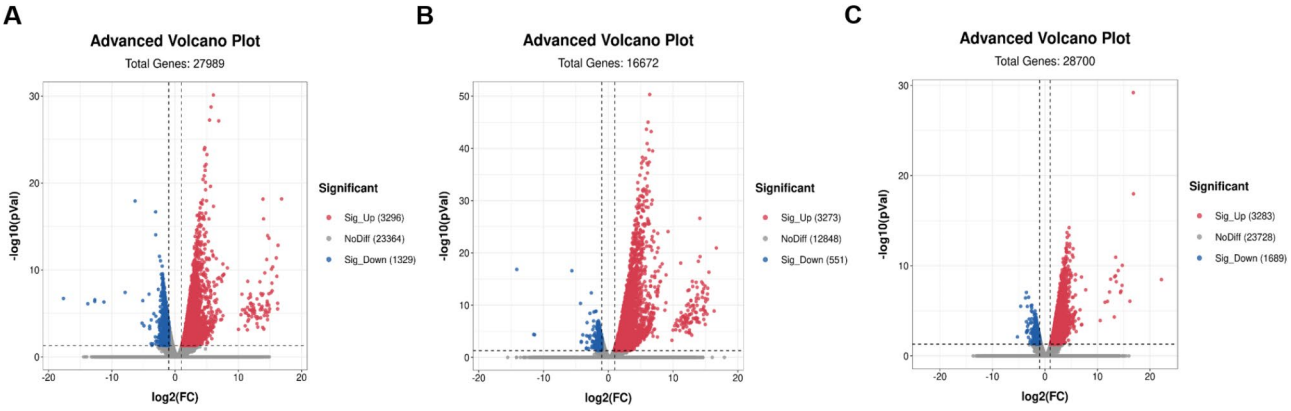

Fig. 3 Volcano plots showing differentially expressed genes identified between GV and MII oocytes in humans, pigs and mice. **A** DEGs identified between GV and MII oocytes in humans. **B** DEGs identified between GV and MII oocytes in pigs. **C** DEGs identified between GV and MII oocytes in mice. DEGs, Differentially Expressed Genes

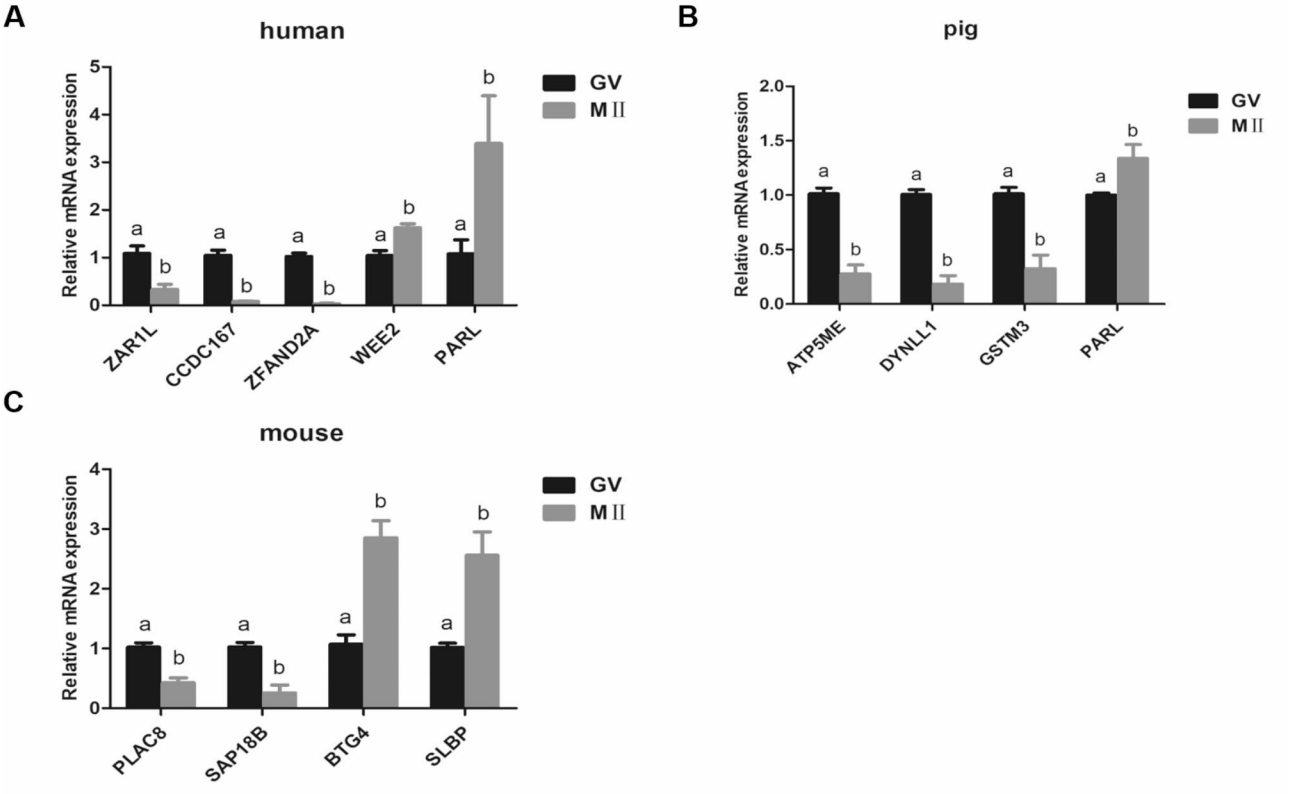

Fig. 4 Validation of differentially expressed genes during oocyte maturation in humans, pigs and mice. **A** Validation of DEGs between GV stage and MII stage in human oocytes. **B** Validation of DEGs between GV stage and MII stage in porcine oocytes. **C** Validation of DEGs between GV stage and MII stage in mouse oocytes. DEGs, Differentially Expressed Genes

Further, DEGs during oocyte maturation in humans, pigs and mice respectively were obtained according to screening conditions $|\log2(FC)| > 1$ and $p < 0.05$. The findings revealed a total of 4,625 DEGs in humans, with 1,329 genes significantly up-regulated and 3,296 genes significantly down-regulated during oocyte maturation (Fig. 3A). In pigs, a total of 3,824 DEGs were identified, with 551 genes showing significant up-regulation and 3,273 genes displaying significant down-regulation during oocyte maturation (Fig. 3B). In mice, the analysis revealed a total of 4,972 DEGs, with 1,689 genes exhibiting significant up-regulation and 3,283 genes showing significant down-regulation during oocyte maturation (Fig. 3C). The transcriptome sequencing data were validated through RT-qPCR, serving as the foundation for the forthcoming experiments (Fig. 4A, B and C).

The hierarchical clustering analysis of DEGs in human, porcine and mouse oocytes revealed contrasting expression patterns between GV and MII stages. Furthermore, the three biological replicates within each stage exhibited comparable gene expression clustering patterns (Fig. 5A, B and C). Hence, the expression profiles of these genes exhibited dynamic changes across the three species, indicating substantial transcriptional modifications during oocyte meiotic maturation.

GO enrichment analysis of DEGs during oocyte maturation in humans, pigs and mice respectively

To uncover the putative functions of DEGs, GO enrichment analysis was leveraged to scrutinize the DEGs (Fig. 6A, B and C). The outcomes indicated that 9,577 GO functional annotations were enriched by 4,625 DEGs originating from human samples, of which 764 GO function terms showed significant enrichment ($p < 0.05$), encompassing 417 biological process terms, 180 cell component terms, and 167 molecular function terms (Fig. 6A). Among the three categories of enrichment, biological processes exhibited the highest level of enrichment among the DEGs. For instance, 227 DEGs (e.g., *KLF1*, *ZNF671*, *PDCD6-AHRR* and *ZNF491*) were implicated in transcriptional regulation utilizing DNA as a template. Additionally, 220 DEGs (e.g., *CCL25*, *ENHO*, *OPN1LW*, *RASLlB*) participated in signal transduction, while 190 DEGs (e.g., *CDKN2D*, *ZNRD2*, *E4F1*, *MAPK12*) were involved in cell cycle. Furthermore, 177 DEGs (e.g., *SERTAD1*, *MESP2*, *KLF1*, *PDCD6-AHRR*) were associated with the positive regulation of RNA polymerase II-mediated transcription, and 174 DEGs (e.g., *AL360181*, *ACADS*, *PDIA2*, *SCO2*) were linked to the reoxidation process.

The results revealed that a total of 8,171 GO function terms were enriched in 3,824 DEGs in pigs (Fig. 6B). Among these, 345 GO function terms exhibited significant enrichment ($p < 0.05$), comprising 171 biological process terms, 95 cell component terms, and 79 molecular function terms. Notably, of the three types of enrichment, biological processes demonstrated the highest enrichment among the DEGs. For instance, 174 DEGs (e.g., *MSRB1*, *CYP4F22*, *DUOX1*, and *TM7SF2*) were found to be involved in redox processes, 172 DEGs (e.g., *MAPK8IP1*, *ZNF628*, *NOTCH4*, and *KLF1*) were associated with DNA-template transcriptional regulation, 143 DEGs (e.g., *HRAS*, *REXO4*, *SERTAD1*, and *ZNF628*) participated in the positive regulation of RNA polymerase II on transcription, 131 DEGs (e.g., *GGT7*, *VARS*, *EIF3F*, *MRPS11*) were linked to translation, and 113 DEGs (e.g., *HRAS*, *MAPK8IP1*, *NPR2*, *PTH1R*) played a role in signal transduction.

The results indicated that a total of 10,081 GO function terms were enriched by 4,972 DEGs in mice, with 508 GO function terms demonstrating significant enrichment ($p < 0.05$) (Fig. 6C). These included 249 biological process terms, 125 cell component terms, and 134 molecular function terms. Among the three types of enrichment, biological processes exhibited the highest level of enrichment among the DEGs. For instance, 243 DEGs (e.g., *ARID3C*, *OVOL3*, *ZFP593*, *NR1H3*) were implicated in the positive regulation of RNA polymerase II on transcription, 241 DEGs (e.g., *GM14418*, *PPIE*, *GM14403*, *ZSCAN4-PS2*) were involved in DNA-template

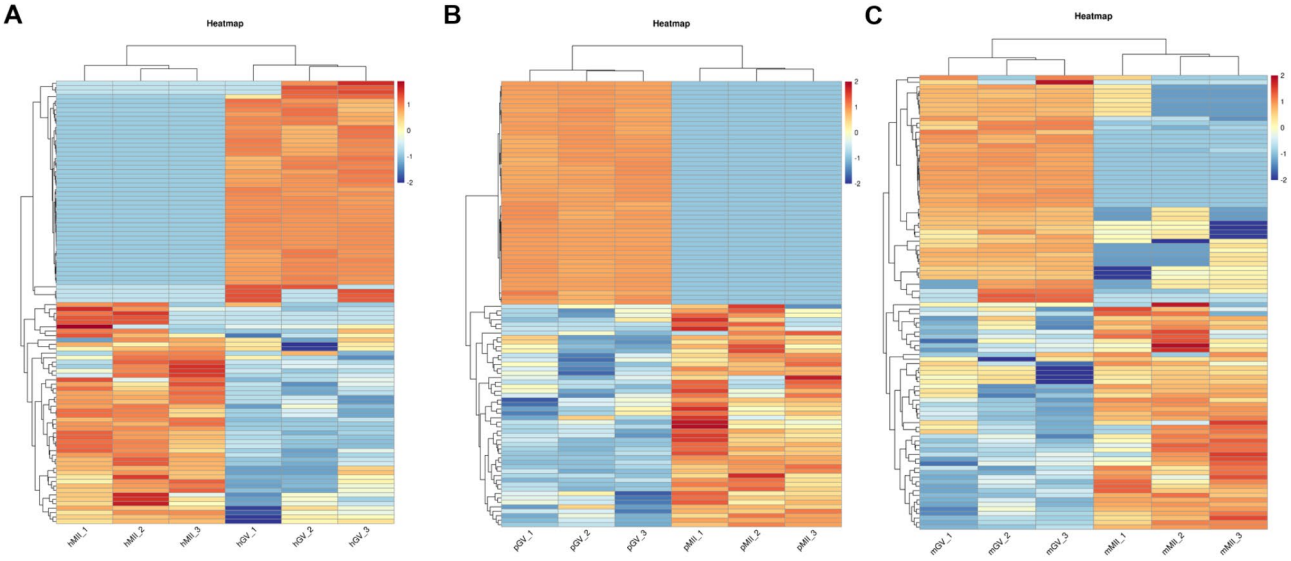

Fig. 5 Hierarchical clustering analysis of differentially expressed genes during oocyte maturation in humans. pigs and mice. **A** Hierarchical clustering analysis of DEGs between GV stage and MII stage in human oocytes. **B** Hierarchical clustering analysis of DEGs between GV stage and MII stage in porcine oocytes. **C** Hierarchical clustering analysis of DEGs between GV stage and MII stage in mouse oocytes. DEGs, Differentially Expressed Genes. hGV, human germinal vesicle; hMII, human metaphase II; pGV, pig germinal vesicle; pMII, pig metaphase II; mGV, mouse germinal vesicle; mMII, mouse metaphase II

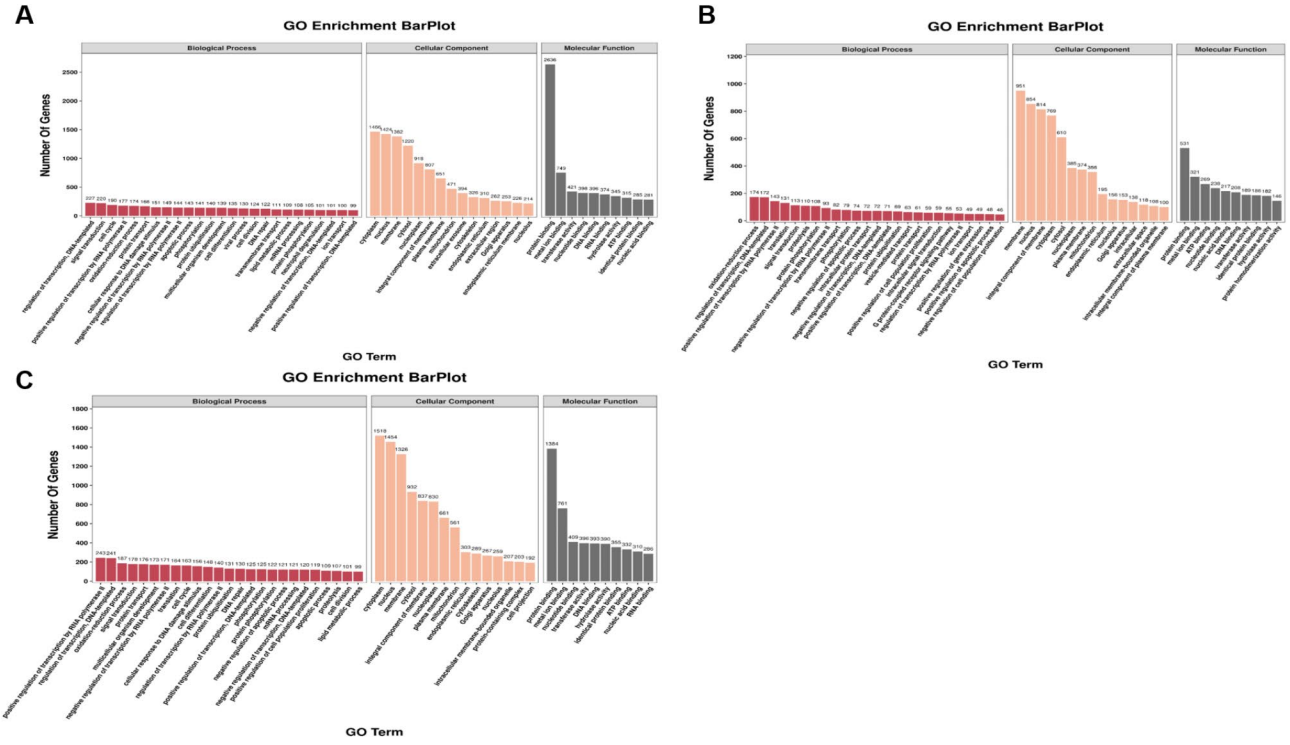

Fig. 6 The enriched GO categories of differentially expressed genes between GV stage and MII stage in human, porcine and mouse oocytes, respectively. **A** The enriched GO categories of DEGs in human oocytes. **B** The enriched GO categories of DEGs in porcine oocytes. **C** The enriched GO categories of DEGs in mouse oocytes. DEGs, Differentially Expressed Genes

transcriptional regulation, 187 DEGs (e.g., *DOHH*, *NDUFB7*, *NDUFB10*) were associated with redox process. Additionally, 178 DEGs (e.g., *RETN*, *SECTM1A*, *TNFSF13*, *TNFRSF17*) were found to be involved in signal transduction.

KEGG enrichment analysis of DEGs during oocyte maturation in humans, pigs and mice respectively

The DEGs of human oocytes were analyzed by KEGG (Fig. 7A). The results revealed that KEGG enrichment analysis of DEGs in human oocytes was involved in 318 signaling pathways enriched with 4,625 DEGs, with 40 signaling pathways showing significant enrichment ($p < 0.05$). These pathways included RNA transport, oxidative phosphorylation, RNA degradation, cell cycle, spliceosome, pyrimidine metabolism, N-Glycan biosynthesis, basal transcription factors, pentose phosphate pathway, progesterone-mediated oocyte maturation, ribosome biogenesis in eukaryotes, cellular senescence, TCA cycle, apoptosis, cysteine and methionine metabolism, oocyte meiosis and DNA replication and so forth.

KEGG enrichment analysis of DEGs in porcine oocytes was shown in Fig. 7B.The results demonstrated that 319 signaling pathways were enriched with 3,824 DEGs, out of which 23 signaling pathways exhibited significant enrichment ($p < 0.05$).These pathways included ribosome, oxidative phosphorylation, pentose phosphate pathway, fructose and mannose metabolism, DNA replication, RNA polymerase, mismatch repair, protein processing in endoplasmic reticulum, proteasome, glycolysis/gluconeogenesis, lysosome, glutathione metabolism, folate biosynthesis, glycerophospholipid metabolism and so forth.

The DEGs of mouse oocytes were also analyzed using KEGG (Fig. 7C). The results indicated that a total of 315 signaling pathways were enriched by 4,972 DEGs, with 41 signaling pathways demonstrating significant enrichment ($p < 0.05$). The pathways included ribosome, oxidative phosphorylation, DNA replication, RNA transport, nucleotide excision repair, proteasome, insulin signaling pathway, RNA polymerase, cell cycle, basal transcription factors, TCA cycle, pyrimidine metabolism and so forth.

Comparative analysis of DEGs during oocyte maturation among humans, pigs and mice

Cross-species comparison of oocyte transcripts among humans, pigs and mice can provide valuable insights into the genetic characteristics of each species. Differential gene analysis was performed based on homologous gene comparison (Fig. 8A). The analysis revealed 1,146 DEGs during oocyte meiotic maturation between humans and pigs, 1,147 DEGs between pigs and mice, and 1,223 DEGs between humans and mice. Additionally, 551 DEGs were identified as being differentially expressed across all

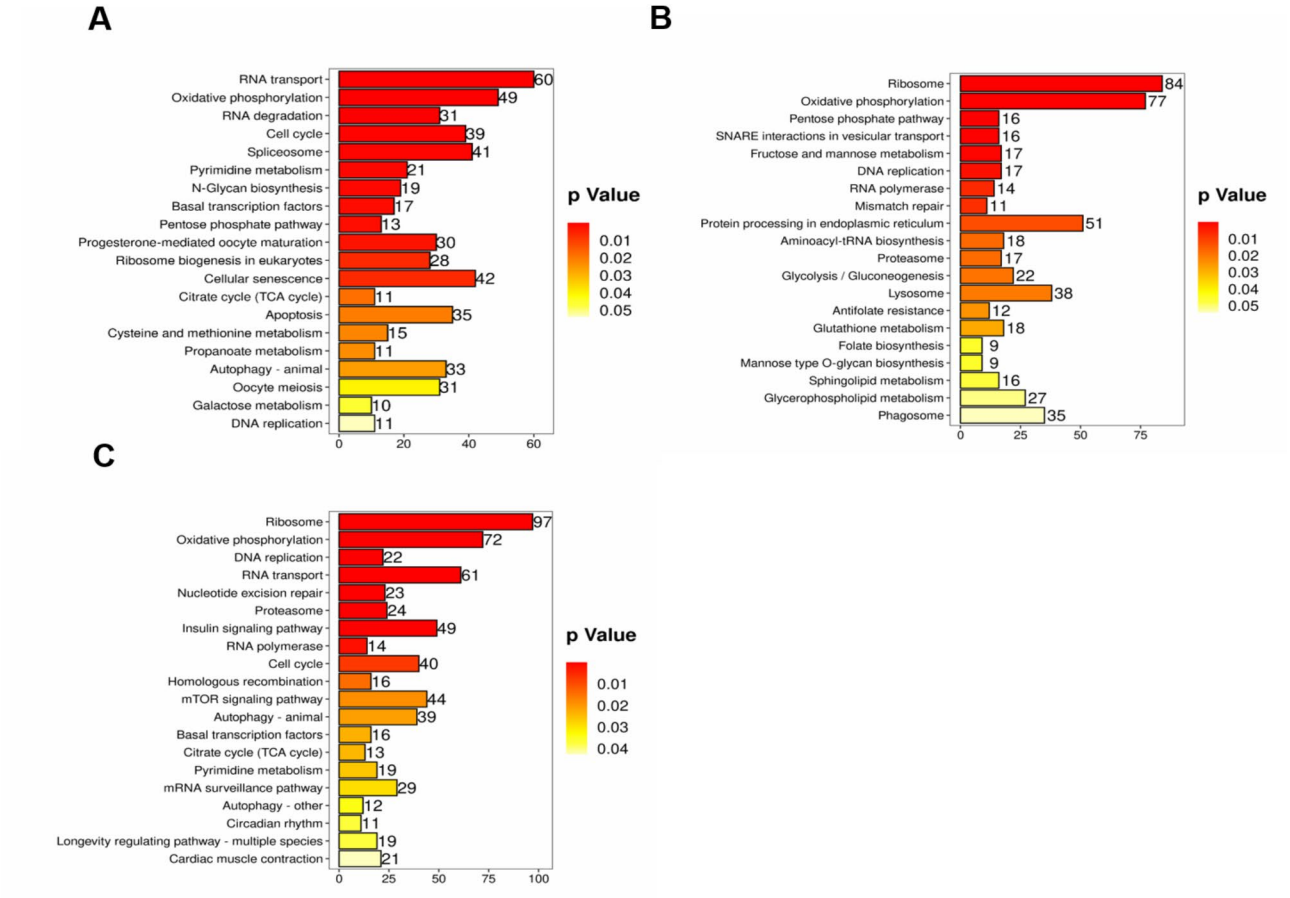

Fig. 7 The enriched KEGG categories of differentially expressed genes between GV stage and MII stage in human, porcine and mouse oocytes. **A** The enriched KEGG categories of DEGs in human oocytes. **B** The enriched KEGG categories of DEGs in porcine oocytes. **C** The enriched KEGG categories of DEGs in mouse oocytes. DEGs, Differentially Expressed Genes

three species during oocyte maturation. In order to further elucidate the characteristics and functions of these DEGs, GO enrichment analysis was conducted (Fig. 8B, C and D). The results showed that the 551 DEGs shared by humans, pigs and mice were enriched in a total of 16 function terms, of which 5 function terms were common across all three species, including mitochondria, mitochondrial inner membrane, mitochondrial large ribosome subunit, mitochondrial respiratory chain complex I assembly and ribosome structure. It indicated that a significant portion of the DEGs shared by the three species were related to mitochondrial functions and structure.

Discussion

The exploration of human reproductive diseases is largely based on studies of experimental animal models. It is considered that oogenesis is an extremely complex species-specific cell differentiation process, and oocyte meiosis is an indispensable biological process during oogenesis [26, 27]. Therefore, meiotic maturation enables the oocyte to achieve fertilization, embryo development and the generation of healthy offspring [28].The acquisition of oocyte developmental ability is regulated by complicated and sophisticated signaling pathways [29, 30].To date, the mechanisms governing human oocyte maturation have remained inadequately elucidated. Ethical and logistical constraints frequently limit the utilization of human oocytes, underscoring the necessity to employ animal oocytes as materials to investigate the molecular mechanisms of human oocyte maturation. Historically, mice, serving as a classic model organism, and their oocytes have been commonly employed to dissect the molecular mechanisms supporting oocyte maturation. More recently, pigs have emerged as an appealing alternative for modeling human diseases. Given the similarities between pigs and humans in terms of embryonic development, anatomy, and physiology, pigs have gained prominence in this field [31]. In this study, we conducted transcriptomic profiles of human, porcine, and mouse oocytes at GV and MII stages. Additionally, comparative analysis of the transcriptomic data yielded crucial insights into the disparities among human, porcine, and mouse oocytes.

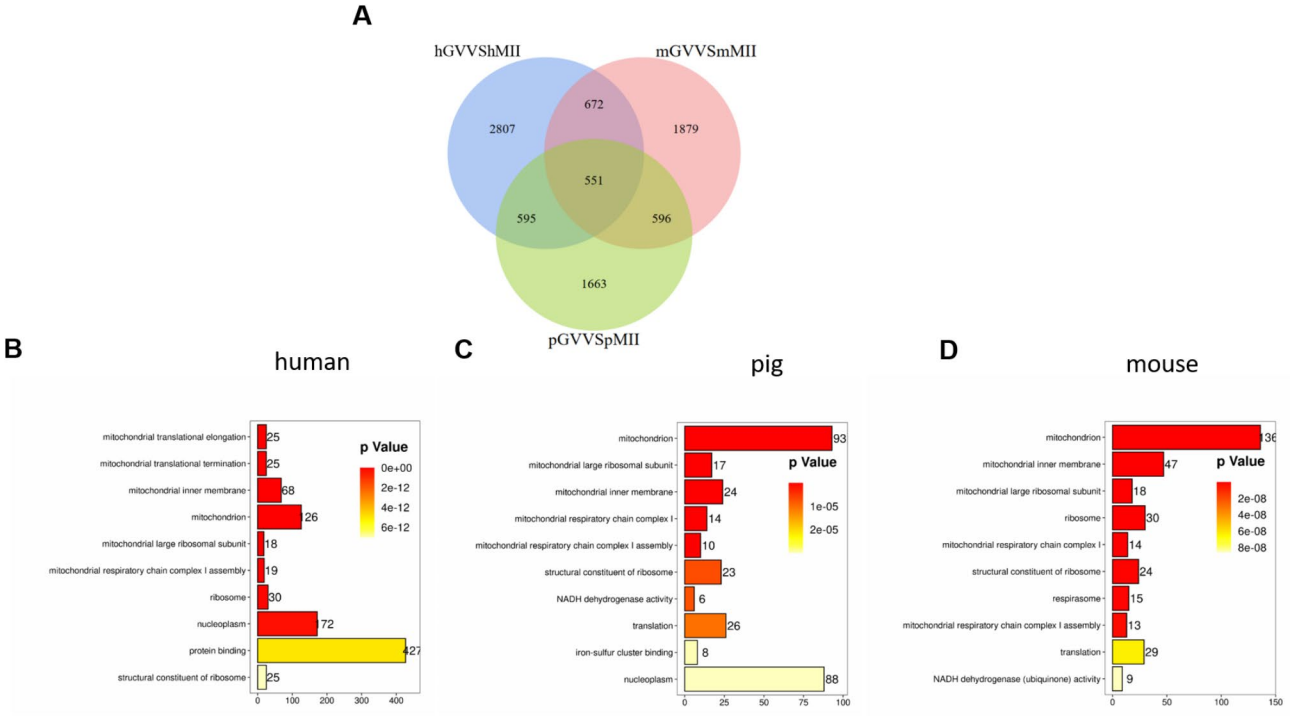

Fig. 8 Comparative analysis of differentially expressed genes during maturation of human, porcine and mouse oocytes. **A** DEGs during oocyte maturation among humans, pigs and mice. **B** The enriched GO categories in humans of DEGs among human, porcine and mouse oocytes. **C** The enriched GO categories in pigs of DEGs among human, porcine and mouse oocytes. **D** The enriched GO categories in mice of DEGs among human, porcine and mouse oocytes. DEGs, Differentially Expressed Genes

Previous studies have shown a higher level of gene conservation revealed by comparative transcriptomic analysis [4], however, significant differences in gene expression exist among species [32]. The maintenance of meiotic arrest and regulation of meiotic maturation in mammalian oocytes are crucial for acquisition of oocyte developmental capacity [33, 34]. Transcripts associated with oocyte maturation in humans, pigs and mice have also been documented [17, 35, 36]. Nonetheless, the molecular mechanisms governing oocyte maturation across these species remain obscure. Therefore, we conducted a transcriptomic analysis of human, porcine and mouse oocytes at both GV and MII stages to investigate the differences in oocyte maturation. Our findings suggested distinct regulatory characteristics were present in oocyte maturation among humans, pigs and mice. Specifically, we identified that 4,597, 2,100 and 3,751 transcripts were completely degraded during oocyte maturation in humans, pigs and mice, respectively. Furthermore, when comparing GV oocytes, the specific gene expression in MII oocytes was found to be 1,550, 512 and 1,820 in humans, pigs and mice, respectively. These results imply that a substantial number of transcripts undergo degradation or inhibition during oocyte maturation, while a few transcripts are translated and activated to promote maturation process. The observed variation in transcript numbers among these three species leads us to speculate that these discrepancies may be due to interspecies differences [3, 37]. Previous studies have provided evidence that oocytes possess significant substantial mRNA reserves, and the control of temporal and spatial translation plays a crucial role in governing the process of oocyte meiotic maturation. These findings agree with the results obtained in our study [12, 38]. Furthermore, our findings revealed the presence of 4,625, 3,824 and 4,972 DEGs in human, porcine and mouse oocytes, respectively. The DEGs are primarily implicated in various biological processes, including cell cycle, oxidative phosphorylation and DNA replication. Existing research has emphasized that certain DEGs in mouse oocytes are predominantly associated with ribosome synthesis, translation and regulation of mitochondrial protein complex at GV stage while other DEGs are primarily involved in the regulation of cell cycle, DNA recombination and chromosome separation at MII stage [19]. Moreover, a comparative transcriptome analysis of MII oocytes derived from younger and older females revealed that the DEGs were closely associated with mitochondrial structure and function, encompassing biological processes such as mitochondrial respiratory chain complex I assembly and mitochondrial translation termination [22]. Oocyte maturation involves a multitude of signaling pathways, each of which plays a critical role in the regulation of oocyte meiosis [39, 40].

During oocyte maturation, a substantial number of transcripts undergo degradation, which is essential for the transition from oocyte development to embryo formation. Previous studies have demonstrated that oocytes initiate the processes of de-adenylation or degradation of numerous mRNAs during meiotic maturation [41, 42]. Specific transcripts, such as *BTG4*, *CNOT6L*, and *ZAR1/2*, have been identified as targets for degradation during this process [34, 43, 44]. Additionally, researches have highlighted the roles of *cyclin B1*, *mos*, *emi2* and *weel* in activating translation during oocyte maturation [45–48], thereby facilitating the progression of meiosis. Our findings indicate that the majority of maternal mRNAs degradation occurs during human oocyte maturation. According to Zhao [3], while the quantity of degraded maternal mRNAs was comparable, only 120 transcripts were found to overlap in degradation between humans and mice during oocyte maturation. Furthermore, analysis revealed differences in the GO function of these shared transcripts in humans and mice, suggesting distinct molecular mechanisms regulate oocyte meiotic maturation across different species.

Our findings revealed distinct gene expression patterns. 1,146, 1,147, and 1,223 DEGs were identified between humans and pigs, pigs and mice, and humans and mice, respectively. The presence of common transcripts displayed some conservation across species. Previous studies have reported 9,853 and 10,046 genes were detected in bovine and Xenopus oocytes, respectively. Comparative analysis with mouse oocytes identified 7,275 conserved genes among bovine, Xenopus and mouse oocytes, highlighting inter-species conservation [4], which is similar with our findings. Variations in oogenesis across species have been documented [49], emphasizing that mice may not be the ideal models for studying human oocyte maturation [50, 51]. Our analysis also revealed a modest 551 DEGs during oocyte meiotic maturation across humans, pigs and mice. Among the higher enriched cellular components within these 551 DEGs, *MRPL17* and *MRPS28* are involved in mitochondria function, *TIMM50* and *NDUFAF3* function in mitochondrial inner membrane, *NDUFS5* and *NDUFA10* play roles in mitochondrial respiratory chain complex I assembly. In consequence, these findings suggest distinct molecular mechanisms governing oocyte meiotic maturation among humans, pigs, and mice.

Transcriptome analysis displayed dynamic changes during oocyte maturation. Comparative analysis of transcriptomic profiles revealed species-specific differences and conservation during oocyte maturation [52]. Our findings predict that oocyte expressed genes may have important functions in oogenesis, oocyte maturation, fertilization and/or early embryonic development and should therefore be further characterized.

Conclusion

In conclusion, our transcriptome results indicate that there are a large number of genes which are involved in regulating meiotic maturation among human, porcine and mouse oocytes. Cross-species comparative analysis of transcriptomic profiles revealed the conservation and diversity of oocyte maturation. In addition, it also implied that the selection of oocyte expressed genes among three species can provide a reference for exploring their functional roles in human oocyte maturation.

Finally, deciphering the convergences and divergences in transcriptomic landscapes of human, porcine and mouse oocytes undergoing IVM might facilitate the classification of species-specific molecular criteria for the purposes of either positive selection of high-quality nuclear recipient ova or negative selection of poor-quality nuclear recipient ova intended for a variety of modern assisted reproductive technologies (ARTs). The latter entail conventional in vitro fertilization (IVF) by gamete co-incubation [53–55], microsurgical IVF by intracytoplasmic sperm injection (ICSI) [56–58] and somatic cell cloning [59–62].

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12864-025-11431-1.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

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Author contributions

Y.Z. and N.Z. conceived the study. Z.C. designed the experiments. X. W., Y. X., L. L., R. J., X. T.,Z.S., Z. W. and H. S.performed the experiments. Z. L., X. W. and Y. X. analyzed the results.Y. M. and Y.Li. provided scientific suggestions. X. W. and Y. X. prepared Figs. 1, 2, 3, 4, 5, 6, 7 and 8. N.Z. wrote the main manuscript. Z. C. and Y.Z. revised the manuscript. All authors reviewed the manuscript.

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Data availability

Data is provided within the manuscript or supplementary information files.

Declarations

Ethics approval and consent to participate

All experiments involving samples from human patients were conducted according to the ethical policies and procedures approved by the ethics committee of the First Affiliated Hospital of University of Science and Technology of China (Approval no.2021-KY-210). All participants signed the informed consent form prior to entering the study. All experiments were conducted according to the Institutional Animal Care and Use Committee guidelines under current approved protocols at Anhui Agricultural University.

Consent for publication
Not applicable to this study.

Competing interests
The authors declare no competing interests.

Generative AI and AI-assisted technologies in the writing process
During the preparation of this work, we used ChatGPT as an AI-assisted tool in order to polish our English article. After using this tool, we reviewed and edited the content as needed and take full responsibility for the content of the published article.

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