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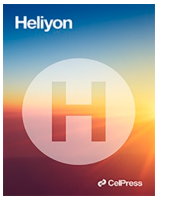

Research article

NCOA3 knockdown delays human embryo development

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ABSTRACT

Embryonic development is a precisely controlled sequential process influenced by complex external and internal factors; therefore, this process holds paramount significance in the context of *in vitro* fertilization and embryo transfer (IVF-ET), with internal oocyte and embryo quality being pivotal in determining its success. Nuclear receptor coactivator 3 (NCOA3), a member of the p160 nuclear receptor coactivators family, has been extensively studied in tumorigenesis and reportedly plays a crucial role in maintaining pluripotency in mouse embryonic stem cells (ESCs). However, its functions in human embryo development remain largely unexplored. In this study, we collected human samples, including oocytes, zygotes, and embryos, from patients at the First Affiliated Hospital of Zhengzhou University to investigate whether NCOA3 regulates human embryonic development. To this end, we employed various assays, including immunofluorescence, quantitative real-time PCR (qPCR), microinjection, and RNA sequencing. Our findings suggested that *NCOA3* expression level was low in inferior embryos (with >50 % fragmentation), and its presence is closely related to the expression of the pluripotency factor NANOG. Deletion of NCOA3 delays human embryonic development. Single-oocyte RNA sequencing revealed that NCOA3 primarily participates in metabolic alterations in oocytes. In sum, these findings elucidate the pivotal roles of NCOA3 in human embryonic development-NCOA3 deletion compromise the developmental potential of embryos. These mechanistic insights into the role of NCOA3 in human embryonic development not only advances our understanding of the intricate molecular mechanisms involved but also holds potential implications for improving assisted reproductive technologies (ART) and addressing developmental disorders in human embryos.

1. Introduction

Embryo quality and developmental potential significantly impact the success of *in vitro* fertilization and embryo transfer (IVF-ET). The internal quality of oocytes and embryos plays a pivotal role in determining fertilization and embryonic development. Despite its complexity, gene profiling based on genome/transcriptome analysis is crucial for comprehending oogenesis and embryogenesis. Notably, studies on metabolism, oxidative stress response, and methylation imprinting in oocytes and embryos have increased; however, the nuclear receptor cofactors, such as nuclear receptor coactivator 3 (NCOA3), have rarely been investigated [1,2].

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The p160 family of steroid receptor coactivators (SRC), including NCOA1, NCOA2, and NCOA3, are the first nuclear receptor coactivators to be identified. They participate in diverse processes, such as somatic cell growth, breast development, tumorigenesis, and immune disorders [3–8]. While previous research has predominantly focused on the role of NCOA3 in tumorigenesis, recent studies on the p160 family have delved into developmental biology, aligning with advancements in assisted reproductive technologies (ART) [9–12]. Knockout mouse models suggest that the absence of NCOA1 and NCOA3 in embryos leads to placental dysfunction and embryo death [9]. Female mice lacking NCOA3 exhibit low ovulation ability, reduced pregnancy rates, small litter sizes, and prolonged estrous cycles [10]. Despite these observations, the specific underlying mechanisms remain unclear.

The interaction between NCOA3 and NANOG could partially explain the role of NCOA3 in embryonic development. Core factors, such as *NANOG* and *OCT4*, together strictly control early embryonic development [13,14]. The absence of NANOG leads to the loss of the epiblast during embryo development [15–17]. NCOA3 was reported to activate *NANOG* expression [18–21]. Based on these findings, we supposed that NCOA3 may participate in early embryonic development.

Therefore, in this study, we aimed to investigate the role of NCOA3 in embryonic development, focusing on its potential influence on developmental processes. We monitored the expression of NCOA3 during embryogenesis, examined its expression levels in poor-quality embryos, assessed the impact of *NCOA3* knockdown on *NANOG* expression and embryonic development, and investigated the effects of *NCOA3* deletion on the transcriptome of oocytes through RNA sequencing (RNA-seq). Collectively, we aimed to contribute to a better understanding of the regulatory role of NCOA3 in embryonic development, and instigate a new research field of cofactors.

2. Materials and methods

2.1. Ethics statement

This study was conducted in accordance with the Declaration of Helsinki, and approved by the Biomedical Ethics Committee of the First Affiliated Hospital of Zhengzhou University on August 7, 2019 (2019-KY-242). Written informed consent was obtained from patients at the Center for Reproductive Medicine in the First Affiliated Hospital of Zhengzhou University to collect human oocytes, zygotes, and embryos.

2.2. Human sample collection and embryo culture

The collected samples included spare immature oocytes at the germinal vesicle (GV) or metaphase I (MI) stage of meiosis from consenting patients undergoing intracytoplasmic sperm injection (ICSI) treatment. Zygotes with three pronuclei (3 PN), representing abnormally fertilized zygotes from consenting patients receiving IVF-ET treatment, were also obtained. Additionally, discarded inferior cleavage-stage embryos and blastocysts were included. The 3 PN zygotes were collected 18 h post-fertilization, and all specimens were cultured in a 37 °C incubator (K-MINC-1000; COOK, Bloomington, IN, USA) with 6 % CO_2 and 5 % O_2. Immature oocytes (GV and MI stages) were cultured for 24 h to the mature metaphase II (MII) stage in the G-IVF Plus medium (Vitrolife, Gothenburg, Sweden). Cleavage-stage embryos were cultured in G-1 Plus medium (Vitrolife, Gothenburg, Sweden), designed for culturing embryos from the pronuclear stage to day 3 embryos. Blastocysts were cultured in G-2 Plus medium (Vitrolife, Gothenburg, Sweden) used for cultivating day 3 embryos until the blastocyst stage. Specimens were vitrified in liquid nitrogen using a KITAZATO Vitrification Kit (Kitazato Biopharma, Shizuoka, Japan) and thawed using a KITAZATO Thawing Kit (Kitazato Biopharma, Shizuoka, Japan). After thawing, specimens were cultured in a 37 °C incubator (K-MINC-1000; COOK, Bloomington, IN, USA) with 6 % CO_2 and 5 % O_2 in G-IVF Plus, G-1 Plus or G-2 Plus medium for 2 h before treatment. The cleavage rate was calculated 48 h post-fertilization. Slow cleavage refers to embryos with five or fewer blastomeres at 72 h post-fertilization, whereas normal cleavage embryos have six or more blastomeres at this time point. Greater than or equal to 50 % fragmentation indicates poor developmental potential [22]. The blastocyst formation rate was calculated on day 6 post-fertilization. Precious samples were cryopreserved in liquid nitrogen until a sufficient quantity was collected for experimentation.

2.3. Immunofluorescence

Oocytes, zygotes, cleavage-stage embryos, and blastocysts were fixed in a 4 % paraformaldehyde (Sangon Biotech, Shanghai, China) solution for 20 min and permeabilized with 0.2 % Triton X-100 (Sangon Biotech, Shanghai, China) for 20 min. Following blocking with 1 % bovine serum albumin (BSA) (Sigma-Aldrich, St. Louis, MO, USA)/phosphate-buffered saline (PBS) for 2 h, the samples were incubated overnight with rabbit anti-NCOA3 (1:400; Cell Signaling Technology, Danvers, MA, USA) and mouse anti-CDX2 (1:250; Biogenex, Fremont, CA, USA) antibodies at 4 °C. Subsequently, samples were washed thrice with PBS and incubated with goat FITC-conjugated secondary antibodies Alexa Fluor 488 anti-rabbit and Alexa Fluor 594 anti-mouse (1:5000; Thermo Fisher, Waltham, MA, USA). Nuclei were stained with Hoechst 33342 (Sigma-Aldrich, St. Louis, MO, USA), and confocal images were captured using a TCS SP5 confocal microscope (Leica, Wetzlar, Germany). Three independent experiments were plotted.

2.4. Quantitative real-time PCR (qPCR)

Cumulus cells were removed using a denuding pipette for the oocyte/embryo (SDP-160; Sunlight Medical, Jacksonville, FL, USA) before qPCR analysis. Ten oocytes/embryos from each group were mixed and lysed. cDNA was directly synthesized from the lysate by reverse transcription (TransScript One-Step gDNA Removal and cDNA Synthesis SuperMix, Transgen, Beijing, China). Quantitative

PCR was performed with TransStart Top Green qPCR SuperMix (Transgen, Beijing, China) on the QuantStudio 12K Flex system (Thermo Fisher, Waltham, MA, USA). The final concentration of the primers is 0.5 μM. The PCR cycling conditions were 94 °C for 30 s, 40 cycles of 94 °C for 5 s, 60 °C for 15 s, and 72 °C for 10 s, followed by the melting curve of the amplified DNA. Data were analyzed using the comparative Ct (ΔΔCt) method [23], and quantification of target genes was normalized with ACTB. Averages and S.D. from three independent experiments were plotted. Primer sequences were as follows: *NCOA3*, forward 5'-TCGAGTGTCTAGTCCTCCCA-3' and reverse 5'- GCCAGCCCTTCATTTCTGAG-3'; *OCT4*, forward 5'-CAGTGCCCGAAACCCACACT-3' and reverse 5'-GTCGCTGCTTGATCGCTTGC-3'; *NANOG*, forward 5'-GCAATGGTGTGACGCAGGGA-3' and reverse 5'-TGGGTCTGGTTGCTCCAGGT-3'; *ACTB*, forward 5'-CAGAAGGAGATTACTGCTCTGGCT-3' and reverse 5'-TACTCCTGCTTGCTGATCCACATC-3'.

2.5. Microinjection

Microinjection was performed on MII oocytes *in vitro* matured from clinically unsuitable immature GV or MI, using an inverted microscope (TE2000-U; Nikon Instruments, Tokyo, Japan) with a micromanipulator (Narishige, Tokyo, Japan) and microinjection controller (Eppendorf, Hamburg, Germany), as described by Homer et al. [24]. Oocytes or zygotes were immobilized using a holding

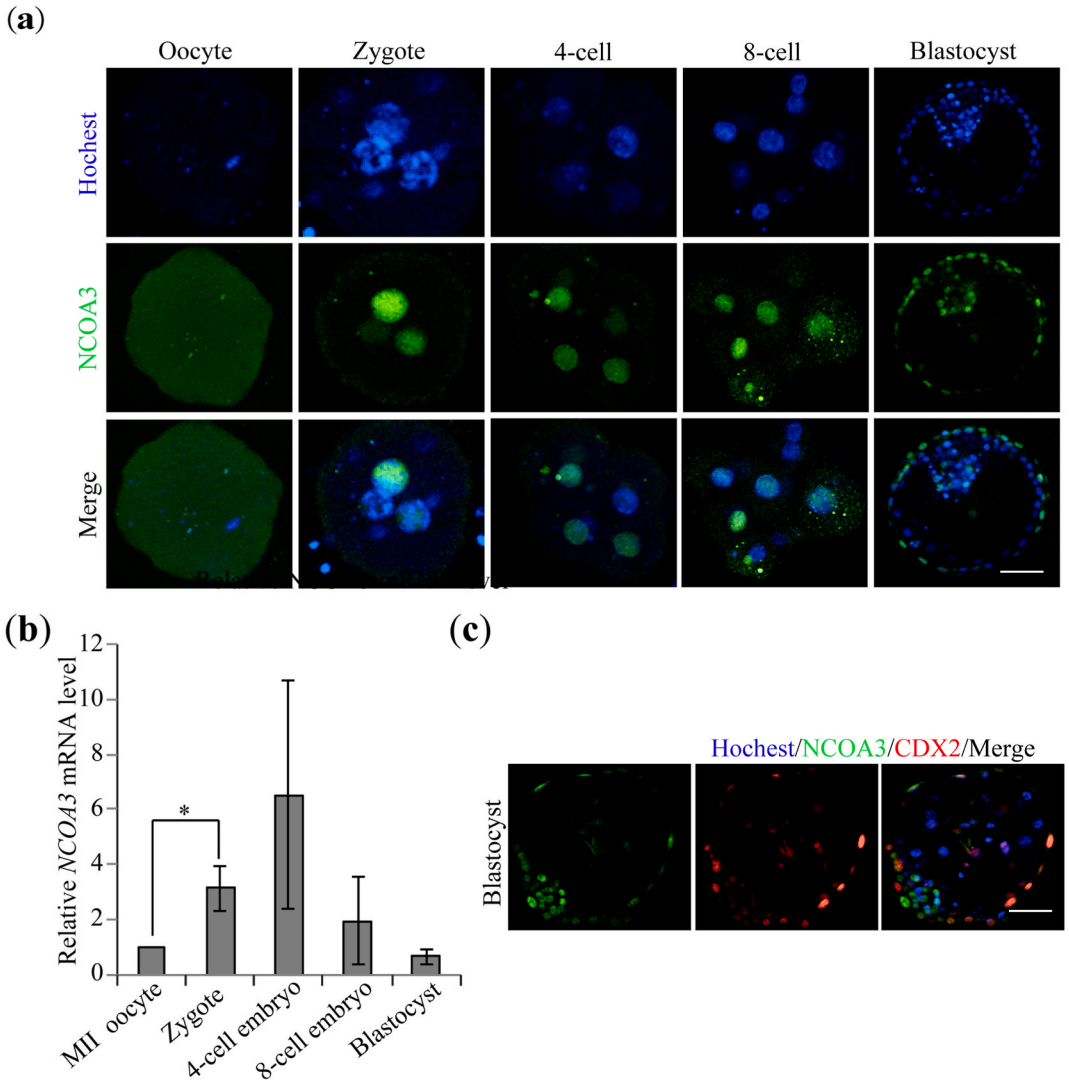

Fig. 1. Expression pattern of NCOA3. **(a)** Confocal images illustrating the dynamic expression of NCOA3 during human embryogenesis. Hoechst33342 staining (blue) denotes the nucleus. Scale bar = 50 μm. **(b)** mRNA levels of endogenous NCOA3 during human embryogenesis. Data were analyzed using the comparative Ct (ΔΔCt) method. Mean and S.D. from three independent experiments are presented. *$p < 0.05$. **(c)** Confocal images of human blastocysts (left: immunostained for NCOA3; middle: immunostained for CDX2, **which is recognized as** a marker for trophectoderm; right: overlap). Hoechst33342 staining (blue) denotes the nucleus. Scale bar = 50 μm.

pipette (Sunlight Medical, Jacksonville, FL, USA) fixed to the micromanipulator, whereas the siRNA solution was loaded into the injection pipette (Sunlight Medical, Jacksonville, FL, USA) controlled by a microinjection controller. The injection pipette tip was introduced into the ooplasm by applying pressure. Approximately 5 % of the zygote/oocyte volume of siRNA targeting NCOA3 (sc-29636; Santa Cruz Biotechnology, Santa Cruz, CA, USA) or scrambled control siRNA (sc-37007; Santa Cruz Biotechnology, Santa Cruz, CA, USA) was injected. SiRNA interference efficiency was verified by qPCR at 24 h post-injection. Embryos were observed using an inverted microscope (TE2000-U; Nikon Instruments, Tokyo, Japan) at 36 h, 48 h, 72 h and 120 h post-fertilization. QPCR results are presented as mean and S.D. from three independent experiments.

2.6. RNA sequencing

Oocytes microinjected with either NCOA3 siRNA or a scrambled control were harvested 24 h after injection for single-oocyte sequencing. Five replicates were obtained for each group from different donors, and sequencing was performed using the DNBSEQ platform at The Beijing Genomics Institute (BGI, Shenzhen, China).

The sequencing data were processed with SOAPnuke (v1.5.2) [25]. Certain reads were removed, including reads that contain the sequencing adapter, have a low-quality base ratio (base quality ≤ 5) over 20 %, or have an unknown base ('N' base) ratio over 5 %. HISAT2 (v2.0.4), Bowtie 2 (v2.2.5) and RSEM (v1.2.12) were used to analyze the clean reads [26–28]. The heatmap was plotted using pheatmap (v1.0.8) (Raivo Kolde, Package 'pheatmap'). Differential expression analysis was performed using DESeq2 (v1.4.5) (Q value ≤ 0.05) [29]. Gene Ontology (GO) (http://www.geneontology.org/) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were performed using Phyper based on Hypergeometric test [30,31]. The significant levels of terms and pathways were corrected using a threshold of Q value ≤ 0.05 by Bonferroni.

2.7. Statistical analysis

Statistical analysis was performed using the IBM Statistical Package for the Social Sciences (SPSS) version 23.0 (SPSS, USA). Proportional data were pooled for statistical analysis and compared using chi-square or Fisher's exact test.

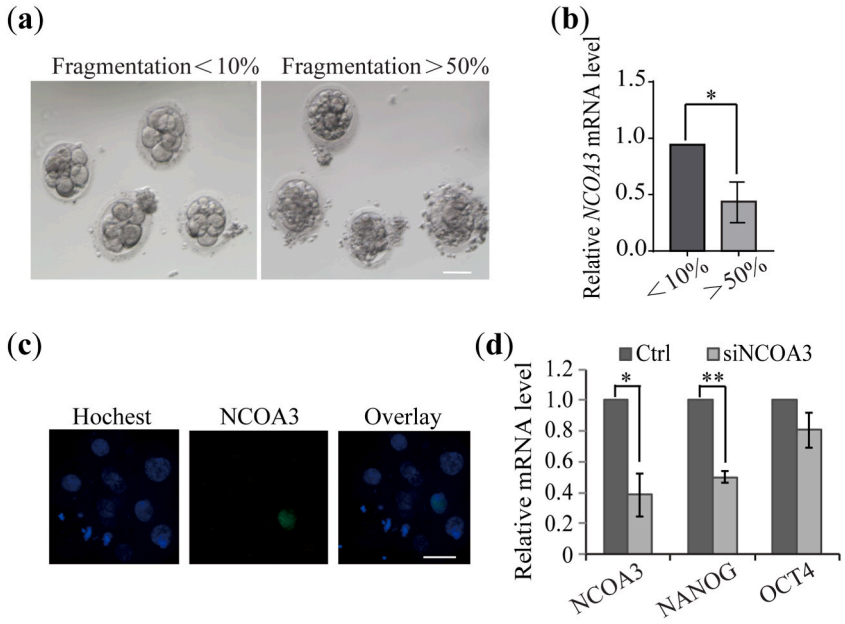

Fig. 2. Low levels of NCOA3 indicate low developmental potential of oocytes and embryos. **(a)** Morphology of day 3 embryos with fragmentation <10 % or >50 %. Scale bar = 100 μm. **(b)** mRNA level of NCOA3 in day 3 embryos with fragmentation <10 % or >50 %. Data were analyzed using the comparative Ct (ΔΔCt) method. Mean and S.D. from three independent experiments are depicted. *, $p < 0.05$. **(c)** Human cleavage-stage embryos developed from 3 PN zygotes were immunostained with an anti-NCOA3 antibody. Hoechst33342 staining (blue) denotes the nucleus. Scale bar = 50 μm. **(d)** mRNA levels of *NCOA3*, *NANOG*, and *OCT4* in oocytes post-injection examined using qPCR and normalized against the levels of *ACTB*. Data were analyzed using the comparative Ct (ΔΔCt) method. Mean and S.D. from three independent experiments are plotted. *, $p < 0.05$; **, $p < 0.01$.

3. Results

3.1. Dynamic changes of NCOA3 during embryonic development

NCOA3 is reportedly required for maintaining pluripotency in mouse ESCs [18]. In mouse embryos, NCOA3 was expressed at the cleavage stage, with significantly higher levels in the ICM of blastocysts compared with those in trophectoderm (TE) cells [18]. To elucidate the dynamics of *NCOA3* expression during human embryogenesis, immature oocytes at the GV or MI stage were cultured to the mature MII stage in G-IVF Plus medium *in vitro*. Cleavage-stage embryos and blastocysts were obtained from 3 PN zygotes cultured in G-1 or G-2 Plus medium until the desired stages.

Simultaneous thawing of MII oocytes, 3 PN zygotes, four- and eight-cell stage embryos, and blastocysts was conducted for immunofluorescence staining. The NCOA3 protein expression, from zygotes to blastocysts, could be detected throughout embryonic development, with specific expression in the nucleus (Fig. 1a). Dynamic changes in *NCOA3* expression during embryonic development, particularly during fertilization, were also observed using qPCR (Fig. 1b). These findings suggest a potential role for NCOA3 in fertilization, warranting further exploration. In human 3 PN blastocysts, *NCOA3* expression was detected in both ICM and TE cells (Fig. 1c). This observation supports our hypothesis regarding the involvement of NCOA3 in embryonic development.

3.2. Reduced NCOA3 expression in poor-quality embryos

Embryo fragmentation serves as an indicator of embryo quality and is closely related to embryonic developmental potential, implantation rate, and clinical pregnancy rate [32]. In the context of IVF-ET, higher degrees of fragmentation, exceeding 35 %, correlate with reduced implantation and pregnancy rates [33]. To further elucidate the correlation between NCOA3 and developmental potential, 3 PN-derived day 3 cleavage-stage embryos with fragment percentages <10 % or >50 % were subjected to qPCR analyses (Fig. 2a). NCOA3 exhibited lower RNA levels in embryos with higher fragmentation, indicative of poor developmental capabilities (Fig. 2b). This suggests a potential role for NCOA3 in embryonic development.

Notably, immunofluorescence staining of cleavage-stage embryos developed from 3 PN revealed varying NCOA3 protein levels among blastomeres within the same embryo (Fig. 2c). Six of the sixteen stained cleavage-stage embryos exhibited inconsistent NCOA3 protein levels among blastomeres, with NCOA3-positive blastomeres constituting 62.7 % of all detected blastomeres. Due to the different developmental potentials of blastomeres and the regulatory role of NCOA3 in NANOG expression, we speculated that NCOA3 might govern the developmental potential of blastomeres through the regulation of NANOG in human embryos. To explore this, we knocked down *NCOA3* expression using siRNA microinjection. GV- or MI-stage oocytes were cultured to the MII stage, and injected with the corresponding NCOA3-targeting siRNA (siNCOA3) or a scrambled control siRNA (Ctrl). The scrambled siRNA was used as a negative control, which consists of a scrambled sequence that will not lead to the specific degradation of any cellular message. The findings demonstrated a significant reduction in *NANOG* levels upon *NCOA3* knockdown, whereas *OCT4* exhibited minimal alteration (Fig. 2d). Therefore, the absence of *NCOA3* implied a diminished *NANOG* level, suggesting a corresponding decrease in the developmental capacity. Blastomeres with lower NCOA3 protein levels may exhibit reduced potential for developing into complete biological individuals, suggesting that NCOA3 may partially indicate the developmental potential of blastomeres.

3.3. NCOA3 deletion compromises the embryonic developmental potential

NCOA3 knockdown in 3 PN zygotes via siRNA microinjection was conducted to investigate its role in embryonic development (Fig. 3a). The efficiency of knockdown was assessed using qPCR (Fig. 3b), and embryos were observed at different time points post-injection.

Thirty-six human 3 PN zygotes were injected for each group. Normal cleavage embryos are expected to contain 6 to 8 blastomeres at 72 h post-fertilization. Whereas NCOA3 deletion resulted in a significant slowing of cleavage (≤5 blastomeres) (11.8 % vs. 32.3 %, $p = 0.045$) at this time point (Table 1). This suggests that the absence of NCOA3 could delay embryonic development.

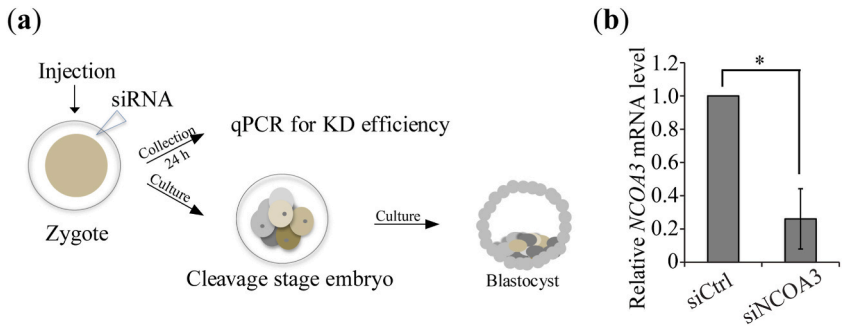

Fig. 3. NCOA3 deletion during embryo development. **(a)** Flow chart. KD, Knockdown. **(b)** Knockdown efficiency evaluated using qPCR. Data were analyzed using the comparative Ct (ΔΔCt) method. Mean and S.D. from three independent experiments are presented. *, $p < 0.05$.

Table 1
Human embryo development following down-regulation of NCOA3 expression in 3 PN zygotes injected with the corresponding siRNA.

	siCtrl	siNCOA3	*p* value
No. oocytes injected	36	36	
Cleavage rate (%)	94.4 % (34/36)	86.1 % (31/36)	0.426
Slow cleavage (%)	11.8 % (4/34)	32.3 % (10/31)	0.045
≥50 % fragmentation (%)	17.6 % (6/34)	29 % (9/31)	0.277
Blastocyst (%)	5.9 % (2/34)	0 % (0/31)	0.493

*Cleavage = embryos with two blastomeres or more at 48 h post-fertilization; slow cleavage = embryos with five blastomeres or fewer at 72 h post-fertilization. Blastocyst formation rate was calculated on day 6 post-fertilization.

3.4. *NCOA3 regulates the metabolic state of oocytes*

While our data highlight the critical role of NCOA3 in embryonic development, the underlying mechanisms remain elusive. Single-oocyte RNA-seq was performed to assess global expression profile changes and understand the mechanisms by which NCOA3 regulates

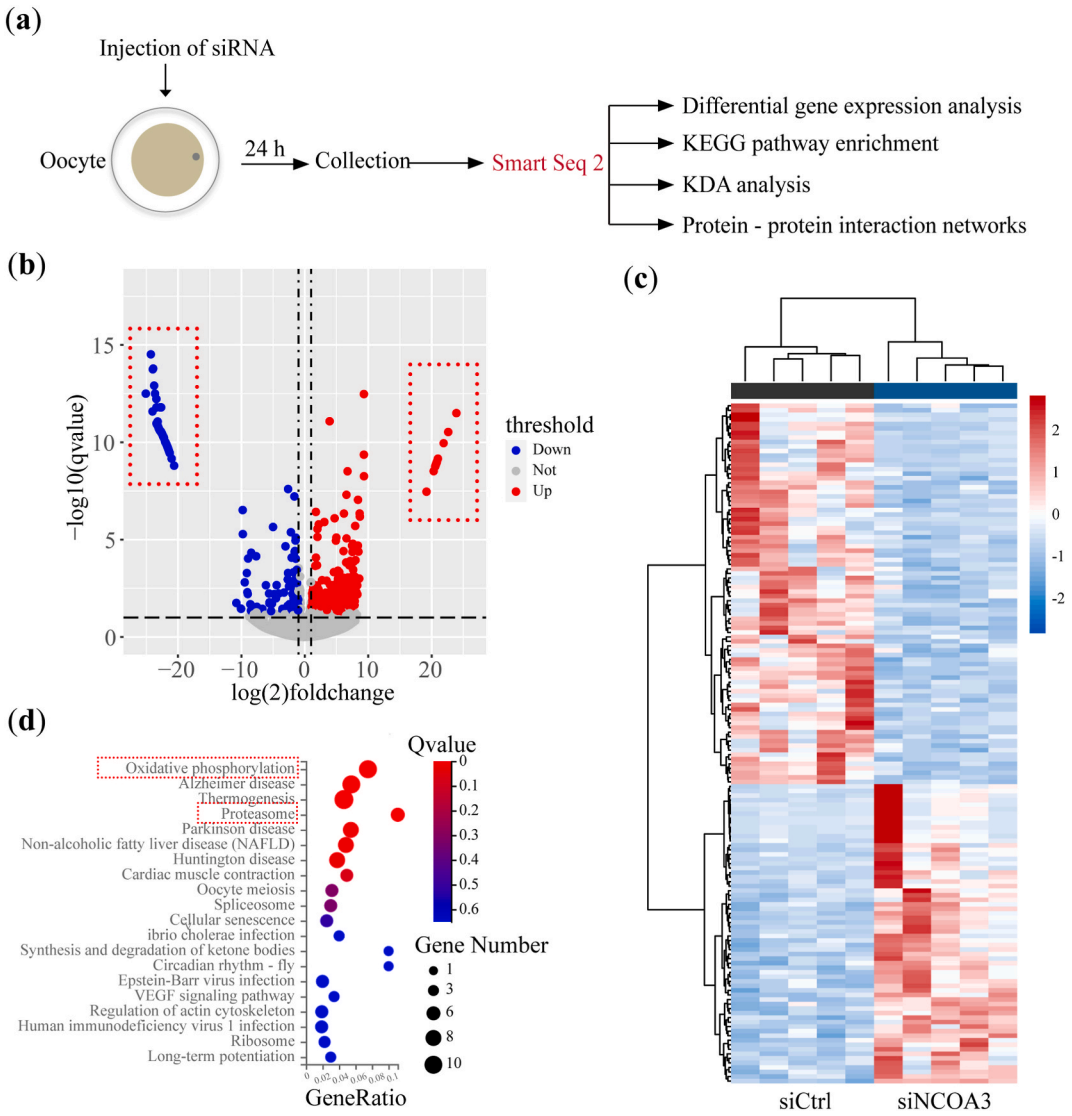

Fig. 4. RNA-seq in human oocytes revealed that NCOA3 knockdown affects the expression of many metabolism-related genes. **(a)** Flow chart of the analysis. **(b)** Volcano plot showing differentially expressed genes as indicated (circled with a red dotted line). **(c)** Heatmap illustrating differential up- and down-regulation gene clusters with significant differences (104 genes). **(d)** KEGG enrichment analysis indicated the enrichment of OxPhos and proteasome pathways.

development (Fig. 4a). Immature oocytes were cultured to maturity and subjected to microinjection with either siNCOA3 or siCtrl (five for each group). Oocytes collected 24 h post-injection underwent RNA-seq analysis. Knockdown efficiency was evaluated, and transcriptome data were subjected to differentially expressed gene (DEGs) analysis, KEGG pathway enrichment analysis, KDA analysis, and protein–protein interaction (PPI) network analysis.

A total of 442 genes with significant differences were identified following NCOA3 knockdown (Supplementary Table S1). The threshold was set as follows:|log(2) fold change|> 1, Q value < 0.05. DEGs with FPKM>1 were screened, and 150 candidates with differentially expressed genes were obtained (Supplementary Table S2). The volcano plot illustrated that 104 genes exhibited significant upregulation or downregulation with NCOA3 knockdown (Fig. 4b, circled with a red dotted line; Supplementary Table S3). The threshold was modified as follows: log(2)fold change >19 or < −20, and Q value < 0.05. A heatmap based on gene clusters with significant changes was generated (Fig. 4c). KEGG pathway analysis indicated the enrichment of pathways, such as oxidative phosphorylation (OxPhos) and proteasome pathways (Fig. 4d; Supplementary Table S4) [34–36]. Although OxPhos and proteasome pathways function differently, their normal operation is an important guarantee for cellular health and function. During autophagy, the degraded components enter lysosomes for further processing, which is beneficial for energy supply, cell renewal and repair. Lysosomes also participate in mitochondrial stress response [37]. NCOA3 might play a role in maintaining cellular functions though participating in both OxPhos and proteasome process. KDA analysis helped identify *COX7A1*, *COX7B*, and *COX14* as key genes (Fig. 5a), mainly involved in OxPhos and heat production-related metabolic pathways. PPI analysis predicted three DEGs—*MED27*, *PSME3*, and *PSMC5*—that directly interact with NCOA3 and were overexpressed in the NCOA3 knockdown group. Functional annotation revealed their direct involvement in pathways related to lysosomal, immune, and hormone signaling events downstream of NCOA3 [38]. PPI networks indicated upregulation of genes in the knockdown group (Fig. 5b, circled with a red dotted line), which was correlated with the energy metabolism of OxPhos, suggesting that NCOA3 downregulation induces metabolic changes in oocytes.

Metabolic alterations play a crucial role in early embryonic fate determination [39]. The number and functional status of

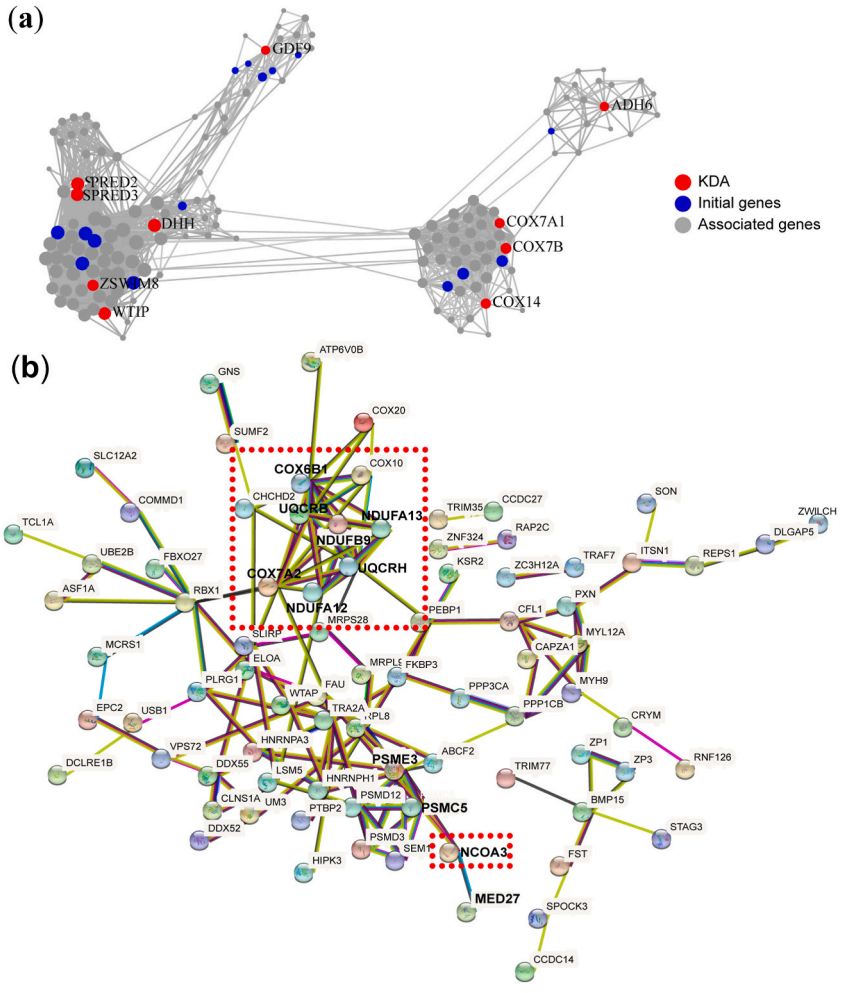

Fig. 5. Key factors involved in NCOA3 regulation pathways in human oocytes. **(a)** KDA analysis of differentially expressed genes identify *COX7A1*, *COX7B*, and *COX14* as key genes. **(b)** PPI network depicting the genes that interact directly with NCOA3.

mitochondria are pivotal in determining oocyte quality and contribute significantly to fertilization and embryonic development [40–43]. Decreased mitochondrial respiratory efficiency in oocytes has been linked to embryonic dysplasia [44,45]. Collectively, we speculate that NCOA3 functions in oocyte and embryo development are primarily mediated through metabolic regulation.

4. Discussion

The functional role of NCOA3 in human reproduction remains elusive. Here, we demonstrate that the nuclear receptor coactivator NCOA3 is a regulator of human embryonic development. We observed diminished NCOA3 RNA levels in poor-quality embryos. And the depletion of *NCOA3* in human oocytes adversely affects the expression of the pluripotency gene *NANOG*, which is pivotal in determining the ICM lineage [46]. Moreover, NCOA3 knockdown delays embryo development. RNA-seq analysis shows that NCOA3 primarily exerts its influence through metabolic regulation. These findings collectively suggest a role for NCOA3 in embryonic developmental potential.

While zygotes and blastomeres in early embryos are considered totipotent, with the capacity to develop into new individuals, not all blastomeres within the same embryo contribute equally to the ICM of blastocysts. Our hypothesis posits that uneven NCOA3 expression may contribute to distinct cell fates among blastomeres. Immunofluorescence results indicate varying NCOA3 protein levels among blastomeres within a single embryo, and *NCOA3* knockdown compromises *NANOG* expression. Development into a whole organism requires a minimum of >4 NANOG-positive cells in preimplantation embryos [47]. Hence, we speculate that disparate NCOA3 expression levels may be related to the developmental potential of blastomeres, serving as a determinant of cell fate. Blastomeres with low NCOA3 levels may exhibit diminished potential for individual development.

Notably, NCOA3 is pivotal for embryo development from zygotes to blastocysts, as *NCOA3* knockdown delayed embryo development to the 6–8 cell stage. However, there is no statistically significant difference in the blastocyst formation rate or the fragmentation rate (Table 1). Owing to the limited sample size and the developmental challenges of 3 PN embryos, which are usually accompanied with chromosomal abnormalities, further clarification is considered necessary.

To further elucidate the role of NCOA3, single-cell RNA-seq profiling of oocytes was performed, revealing its influence on the global metabolic transcriptome. Enrichment of the urine metabolism pathway (Supplementary Table S4) was observed, consistent with upregulated pathways in blastocysts compared with that in two-cell embryos [48]. KEGG enrichment results reveal that the DEGs are primarily associated with the OxPhos pathway. Furthermore, KDA analysis of the DEGs highlights the key function of COX7A1, a vital participant in mitochondrial respiratory chain assembly [49,50]. The suppression of COX7A1 could potentially lead to a premature metabolic shift toward glycolysis. Consequently, we hypothesize that NCOA3 plays a role in the transition from morula to blastocyst through the OxPhos pathway or purine metabolism.

Blastomeres, existing in varied oxygen environments owing to their spherical geometry, exhibit distinct metabolic statuses initiating differentiation [51,52]. The uneven expression of NCOA3 in embryos supports the notion that different NCOA3 levels signify disparate metabolic pathways and energy generation in blastomeres, ultimately determining cell fate and defining embryo developmental potential. But we don't have direct evidence to confirm this speculation. Further exploration still needed.

In this study, we elucidated the regulatory role of NCOA3 in early embryonic development (Fig. 6). As a diverse family of cofactors,

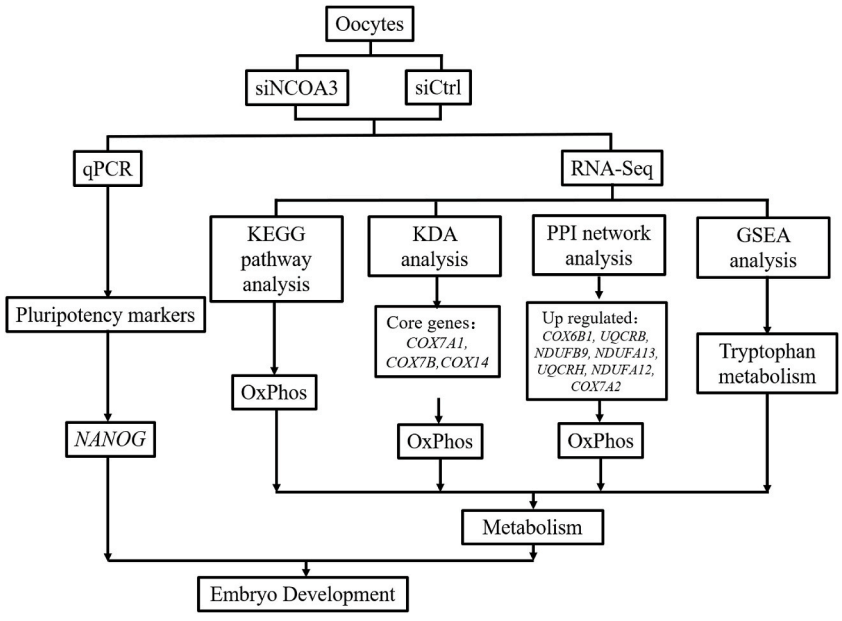

Fig. 6. Summary diagram. GSEA, gene set enrichment analysis; KEGG, Kyoto Encyclopedia of Genes and Genomes; OxPhos, oxidative phosphorylation; PPI, protein–protein interaction.

providing a thorough exploration of their functions will enhance and refine our understanding of biological molecular mechanisms. This elucidation extends the research field of nuclear receptor cofactors, contributing to developmental biology, and opens avenues for investigating reproductive processes. Collectively, our findings elucidate the crucial role of NCOA3 in embryo development, thereby advancing our understanding of embryogenesis. This suggests the potential application of cofactors not only in regenerative medicine but also in ART, providing valuable foundational insights for future research.

Data availability statement

The data presented in this study are available on request from the corresponding author.

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CRediT authorship contribution statement

Zhaoting Wu: Writing – original draft, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Xueshan Ma:** Writing – review & editing, Investigation. **Jingyu Wang:** Writing – review & editing.

Declaration of competing interest

The authors declare no conflicts of interest for our submission titled “NCOA3 Knockdown Delays Human Embryo Development”.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e37639.

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