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# Transcriptome profiling of in vitro-matured oocytes from a korean native cow (hanwoo) after cysteamine supplementation

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**ABSTRACT**

This study elucidated the molecular markers that decrease oocyte quality during *in vitro* culture, restricting optimal developmental potential. Here, we evaluated the transcriptomic differences between cysteamine-treated and non-treated bovine cumulus oocyte complexes (COCs) after 22 h of co-culture in the maturation media using RNA sequencing. In total, 39,014 transcripts were sequenced between cysteamine-treated and non-treated mature COCs. We evaluated the relative expression of 21,472 genes, with 59 genes showing differential expression between the two COC groups. The cysteamine-treated group had 36 up-regulated gene transcripts and 23 down-regulated gene transcripts. Moreover, gene ontology (GO) enrichment analysis revealed that multiple biological processes were significantly enriched after cysteamine supplementation. Differentially expressed genes appeared to maintain normal oocyte physiology, regulation of apoptosis, differentiation, ossification or bone formation, cardiac and muscle physiology, hormonal secretion, and membrane construction for further embryonic development. In conclusion, cysteamine affects the mRNA level of COCs during oocyte maturation by upregulating potential molecular markers and downregulating genes that affect further embryonic development.

**KEYWORDS**

Cysteamine; oocyte maturation; transcriptome; bovine embryo

## Introduction

*In vitro* maturation (IVM) and *in vitro* fertilization (IVF) of oocytes have been extensively used to produce embryos in bovine industry assisted reproductive technology (ART). However, blastocysts productivity and quality are still lower *in vitro* than *in vivo*.[1] A healthy oocyte is the key factor for successful fertilization, embryonic development, and subsequent implantation after transfer.[1] It is very important to understand the basic physiology of the maturation process in mammalian oocytes because oocyte quality usually determines the developmental competence of fertilized eggs. Inadequate *in vitro* maturation conditions could affect the efficiency of *in vitro*-produced embryos and reduce the calving rate after embryo transfer.[2–4]

Cysteamine belongs to a group of antioxidants that increase glutathione level during oocyte maturation, and it has been used for *in vitro* embryo development to increase intracellular glutathione (GSH) content in mice, cows, pigs, and other mammalian species.[5,6] Thiol compounds are known for general antioxidant properties such as free radical quenching, and they play a crucial role in protecting cells from oxidative damage.[7] Cysteamine supplementation during *in vitro* maturation results in increased GSH content in bovine oocytes,[8] maintaining the meiotic spindle morphology of the oocytes.[9] This enhances pronuclear formation due to the increased GSH concentration in the ooplasm, thus protecting the oocyte in later fertilization stages.[10,11] Cysteine is an obligatory external substrate for GSH synthesis in maturing bovine oocytes.

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Outside the cell cysteine is an unstable compound that is usually auto-oxidized to cystine.[12,13] During oocyte maturation, decreased GSH concentration within the oocytes had adverse effects on cleavage and embryonic development.[6] Cysteamine supplementation generates oocytes with large GSH stores to protect the embryo until the blastocyst stage.[14]

Whole-transcriptome or RNA sequencing (RNA-seq) analysis during oocyte maturation is a powerful and versatile approach for functional analysis of different RNA molecules. Embryonic development is mostly dependent on maternal transcripts that are synthesized within the oocytes during gametogenesis.[14] A comparison study of mRNA during oocyte maturation *in vitro* would explain oocyte physiology and further developmental ability. Therefore, this study was designed to clarify gene expression regulation by cysteamine supplementation.

## Materials and methods

### Experimental design

Cysteamine was added to the maturation media at a concentration of 100 µM, which is the most commonly used concentration with IVM media in cattle.[14–19] The COCs were retrieved from slaughterhouse ovaries and transferred to the laboratory within 4–6 h. The experiment was performed with three biological replicates, and 450 oocytes were cultured *in vitro* in the maturation media for 22 h. After this period, matured COCs were processed for RNA extraction for gene expression analysis. In total, 39,014 transcripts were determined by sequencing between cysteamine-treated and non-treated COCs, respectively.

### Collection of oocytes

Ovaries from Korean native cows (Hanwoo) were collected after slaughter at the local slaughterhouse, placed in physiological saline (0.9% NaCl) at approximately 37.5 °C, and transported to the laboratory within 4 h of slaughter. After washing the ovaries with fresh Dulbecco's phosphate-buffered saline (D-PBS), COCs were retrieved as described by Mesalam.[20] The ovaries were pooled regardless of donor estrous cycle stage. COCs were aspirated from antral follicles (2–8mm) using an 18-G needle. COCs were examined under a stereo-microscope (Olympus Co., Japan), and only cumulus-intact oocytes with multiple layers of compact cumulus cells and evenly granulated cytoplasm were selected for further experiments.

### In vitro maturation (IVM)

The oocytes were washed twice in TCM-199 buffered with 25 mM HEPES and containing 5% (v/v) FBS before washing twice in IVM medium. The basic medium used for IVM was 25 mM HEPES-buffered TCM199 supplemented with 2 mM sodium pyruvate, penicillin (50 IU/mL), streptomycin (50 µg/mL), 5% FBS, FSH (0.1 U/mL), and 100 µM cysteamine [+Cys group] or without cysteamine [−Cys group]. Groups of 15 COCs were transferred into 50 µL of IVM medium under mineral oil and matured for 22 h at 38.5 °C in an atmosphere of 5% CO2 in humidified air. For transcriptome analysis, matured COCs ($n = 150$ per group) were transferred to a 1.5 mL cryotube, directly plunged into liquid nitrogen, and transferred to the genome sequencing laboratory for RNA extraction and sequence analysis.

### RNA extraction and sequence analysis

RNA was extracted from cultured COCs using the RNeasy Micro kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. A portion of total RNA from cultured COCs treated with or without cysteamine ($n = 150$ per group) was pooled. Total RNA integrity was checked using an Agilent Technologies 2100 Bioanalyzer and RNA 6000 Nano LabChip Kit (Agilent, Palo Alto, CA, USA) with the RNA integrity number >8.0. Approximately 10 µg of total RNA of each sample was used to isolate poly (A) messenger RNA (mRNA) with poly-T oligo-attached magnetic beads (Invitrogen). Following purification, the mRNA was fragmented into small pieces using divalent cations at an elevated temperature. The cleaved RNA fragments were reverse transcribed to create the final complementary DNA (cDNA). cDNA amplification and library preparation were performed for transcriptome sequencing. First-strand cDNA (from total RNA) was synthesized according to the SMART-Seq™ protocols, and sequenced reads containing polyA tails, low-quality regions, and adapters were prefiltered before mapping. cDNA was sheared into 100- to 150-bp short fragments according to the manufacturer's instructions. Libraries were pooled and sequenced on Illumina HiSeq2000 sequencers. cDNA of control and treated samples were run on an Illumina HiSeq2000 sequencer located at the Animal Genetic Resources Research Center, Korea, and sequenced in parallel to ensure the data generated for each run were accurately calibrated during data analysis. HTSeq v0.6.1 was used to count the read numbers mapped to quantify gene expression levels.

The FPKM of each gene was calculated based on the length of the gene and read counts mapped to this gene.[21] Data normalization was carried out by transforming mapped transcript reads to fragments per kilobase of transcript per million mapped reads (FPKM). Genes with fragments per kilobase of transcript per million mapped reads > 0.5 were retained for analysis. Moreover, the transcripts were removed when they did not have a significant FDR ($q < 0.05$) in any of the treatment and control groups. The log2 fold change (FC) values were calculated to estimate the effect of cysteamine treatment. After generating sequencing images, the pixel-level raw data collection, image analysis, and base calling were performed using the Real-Time Analysis Software (Illumina).

### GO enrichment analyses for DEGs using bioinformatics

The Gene Ontology database (GO: http://www.geneontology.org/) is a structured, standard biological annotation system built in 2000 by an organization (Gene Ontology Consortium). GO aims to establish a standard vocabulary of systematic knowledge of genes and their products. The probable enrichment of Gene Ontology (GO) terms and InterPro entries with genes differentially expressed between the control and cysteamine-treated oocyte were analyzed using a test of proportions based on the cumulative hypergeometric distribution.[22] Furthermore, The Database for Annotation, Visualization and Integrated Discovery (DAVID, http://david.abcc.ncifcrf.gov)[23] was used to ascertain major biochemical and functional pathways. The differentially expressed genes (DEGs) were categorized into three main GO categories: biological process, cellular component, and molecular function. The full list of differentially expressed transcripts and the list of the up- and down-regulated genes were separately analyzed and compared.

### Statistical analysis

The gene expression data were projected according to fragments per kilobase of transcript per million fragments mapped (FPKM).[24] Gene ontology was analyzed via hypergeometric distribution. Heatmap was constructed by using R software. Differential expression analysis between the control and treated groups was performed using the DESeq R package (1.18.0).[21] DESeq provides statistical routines for determining differential expression in digital gene expression data using a model based on the negative binomial distribution. The genes with an adjusted $p$-value $\leq 0.05$ and a fold change $\geq 2$ found by DESeq were considered differentially expressed. Fold change represents the ratio of the expression between the two groups. The resulting $P$-values were adjusted to control the false discovery rate.

## Results

### Overall gene expression analysis between cysteamine-treated and non-treated bovine COCs

The genes that were differentially expressed in the *in vitro* matured COCs and could be genetic markers for predicting oocyte maturation. We performed RNA-seq to analyze the transcriptomes of matured oocytes and therefore greatly facilitates this study to know the influence of cysteamine on the *in vitro* matured oocyte gene expression profiles. We detected 59 DEGs among non-treated (control) and cysteamine-treated groups. Cluster analysis also showed that differences occurred among the control and treated samples, confirming that our RNA-seq data encountered the conditions for differential gene expression analysis (Fig. 1a). The expression of each transcript in the individual sample was evaluated by the expected number of fragments per kilobase of transcript sequence per million base pairs sequenced (FPKM). We defined genes with values of FPKM >1 as expressed genes. Boxplots of log10-transformed FPKM values for each replicate showed that the overall range and distribution of the FPKM values were reliable and consistent among the control and treated samples (Fig. 1b). Additionally, DEGs seem to be evenly distributed in the range between low and moderate abundance levels (Fig. 1c–d). Our data detected a total of 21,472 expressed genes and 59 known transcripts (36 up-regulated vs. 23 down-regulated as shown in pie chart- Fig. 2a, Tables 1 and 2) exhibited a significant change in expression (FDR, $P < 0.05$) between the non-cysteamine and cysteamine-treated groups COCs (Fig. 2a–b). Fig. 2c shows the log2 fold change (i.e., the log2 of the ratio of expression levels for each gene between the two experimental groups) against the average log2 expression (i.e., the overall average expression level for each gene across the two experimental groups). As expected, most of the genes showed a fold difference very close to zero (0). Tables 1 and 2 include a list of selected up-regulated and down-regulated transcripts.

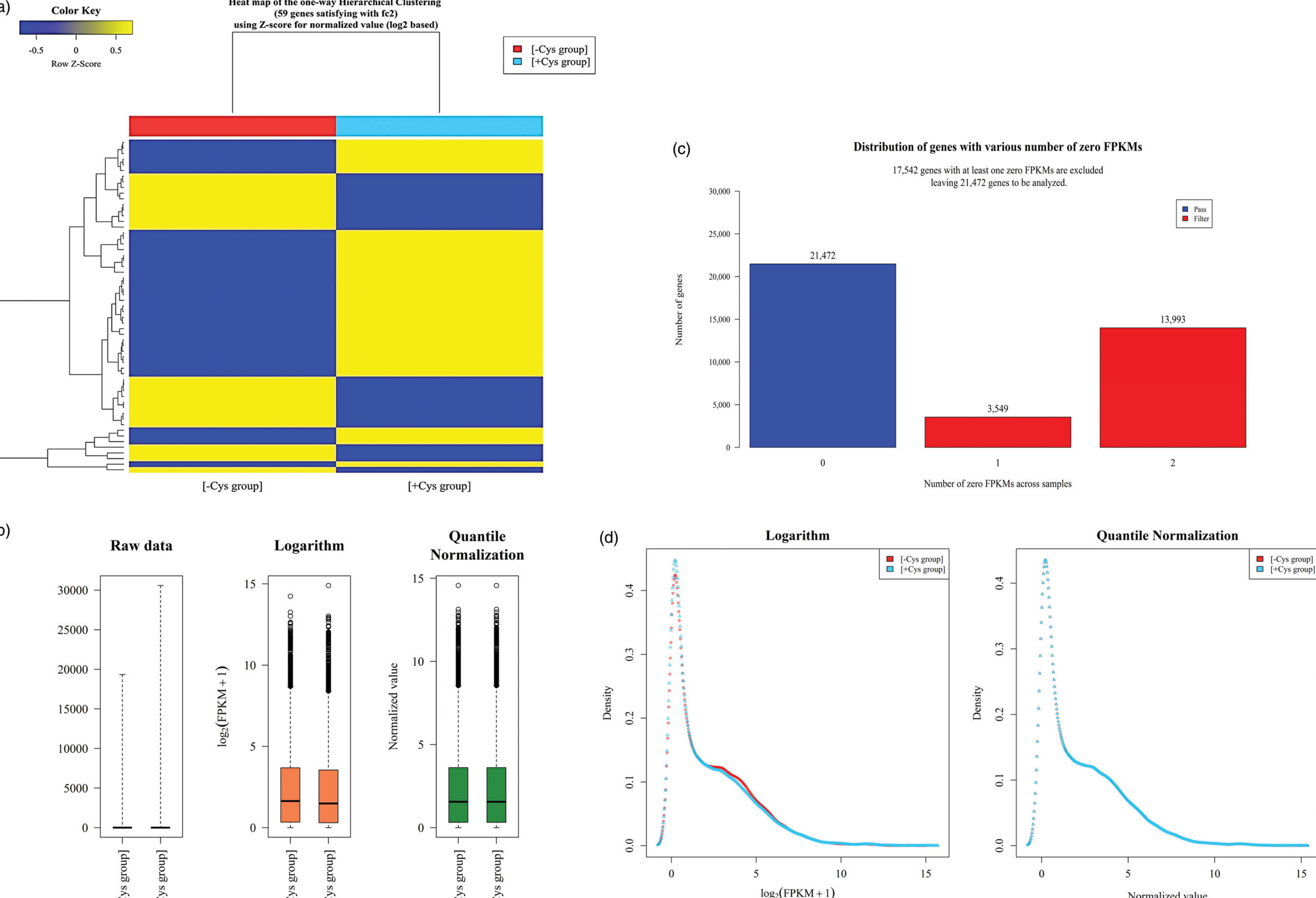


**Figure 1.** Differential gene expression in bovine COCs treated with cysteamine and non-treated COCs. Heatmap (a) shows different gene cluster image of DEG levels of two samples [−Cys group] and [+Cys group] in bovine COCs and demonstrated the results of a clustering analysis of gene expression levels (FPKM). Each column represents an experimental sample, and each row represents a gene. Differences in expression are shown in different colors. The boxplot (b) shows the gene expression level (log10 FPKM) in each sample. The figures (c–d) show the distribution of gene expression levels with a various number of zero FPKMs in each sample.

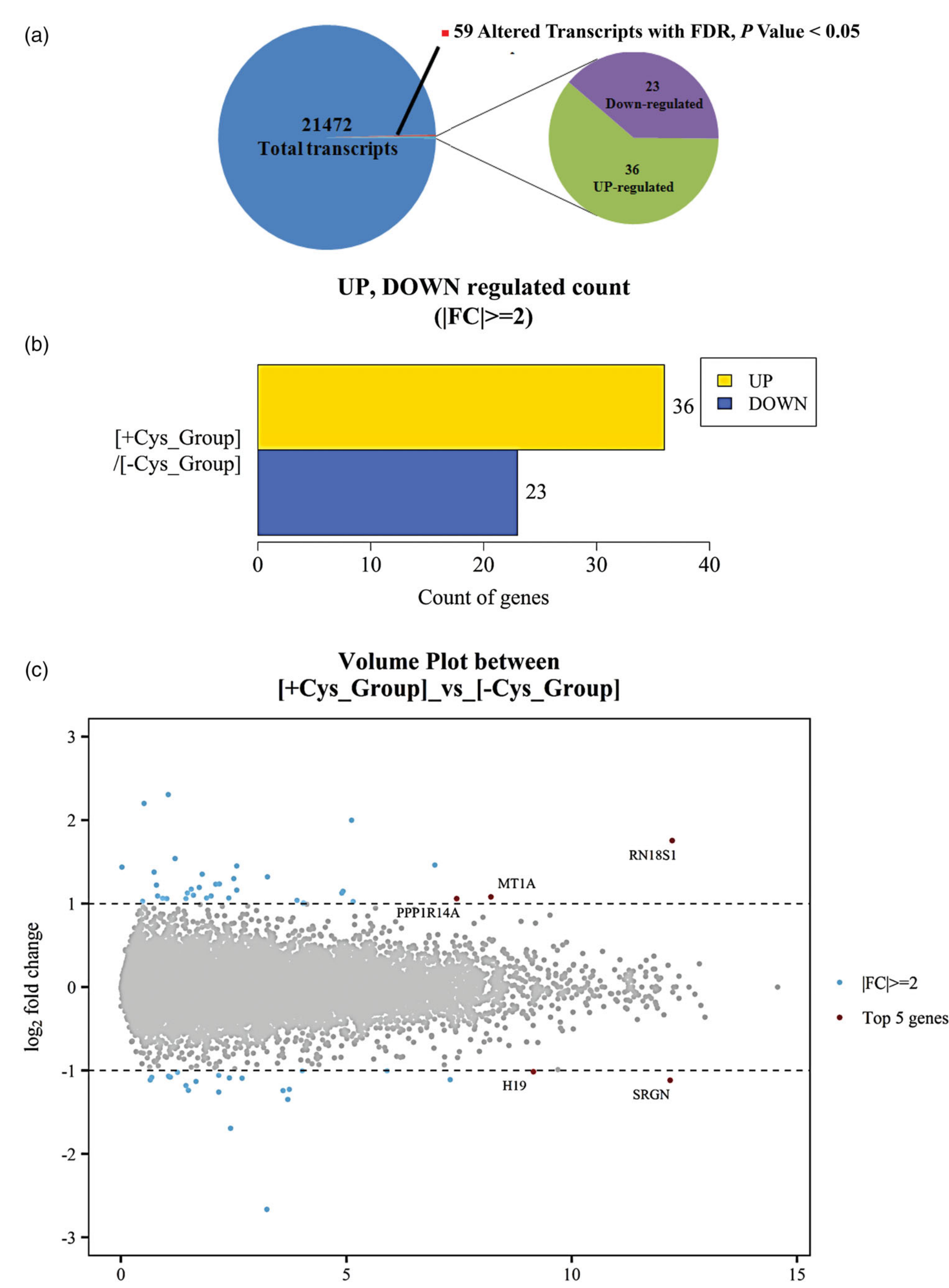


**Figure 2.** RNA-sequencing was performed on COCs of control [−Cys group] and cysteamine-treated group [+Cys group] under differentiating conditions. (a) Pie chart showing the number of transcripts with altered expression levels in COCs expressed in control and cysteamine supplementation group. (b) Analysis of transcripts affected by control and cysteamine-treated group in bovine COCs. (c) Volcano plot demonstrated the difference in gene expression level between control group and cysteamine treated group. The X-axis corresponds to the mean expression value of log10 $P$ values, and the Y-axis shows displays the log2 fold changes values. The blue dots point having a fold-change more than 2 (log2 = 1) represent the up regulated expressed transcripts ($p < 0.05$), false discovery rate (FDR), and the blue dots points having a fold-change less than -1 (log2 = 1) represent transcripts with down-regulated expression ($p < 0.05$), (FDR). The top 5 ranking genes are showed as the red dots. Points having a fold-change less than 2 (log2 = 1) are shown as gray dots.

### *Specific transcribed genes enriched by non-cysteamine and cysteamine treatment groups in COCs*

Here, we demonstrated the differential expression of transcripts either up-regulated or down-regulated between the control and cysteamine-treated group at mRNA levels. This is a useful and frequently used visual tools in gene sequencing analysis and represented in enrichment map test (Fig. 3a). Moreover, the biological processes including practical investigation with

**Table 1.** Relative expression of up-regulated genes between cysteamine treated [+cys group] and non-treated [−cys group] COCs determined using gene sequencing analysis.

| Gene Symbol | Type | Function | Cys(−)/Cys(+) |
|---|---|---|---|
| LOC104974594 | lncRNA | | 4.9 |
| LOC104970089 | lncRNA | | 4.6 |
| RN7SL1 | SRP_RNA | RNA, 7SL, cytoplasmic 1 | 4.0 |
| RN18S1 | rRNA | 18S ribosomal RNA | 3.4 |
| LOC101906344 | lncRNA | | 2.9 |
| SEPW1 | protein_coding | selenoprotein W, 1 | 2.8 |
| LOC101904948 | lncRNA | | 2.7 |
| LOC101907463 | V_segment | | 2.7 |
| LOC519274 | pseudogene | | 2.6 |
| LOC104970007 | lncRNA | | 2.6 |
| LOC513842 | misc_RNA | ferritin, heavy polypeptide 1 pseudogene | 2.5 |
| GSTA3 | protein_coding | glutathione S-transferase, alpha 3 | 2.5 |
| CATHL5 | protein_coding | cathelicidin 5 | 2.4 |
| LOC101906273 | pseudogene | | 2.3 |
| B3GAT2 | protein_coding | beta-1,3-glucuronyltransferase 2 (glucuronosyltransferase S) | 2.3 |
| LOC781250 | protein_coding | insulin-like growth factor-binding protein-like 1 | 2.3 |
| LOC104973472 | lncRNA | | 2.3 |
| LOC104970304 | lncRNA | | 2.2 |
| LOC530932 | misc_RNA | | 2.2 |
| LOC787803 | protein_coding | 40S ribosomal protein S23 | 2.2 |
| CT | protein_coding | calcitonin | 2.2 |
| LOC101907575 | lncRNA | | 2.1 |
| SCGB2A2 | protein_coding | secretoglobin, family 2A, member 2 | 2.1 |
| LOC104973007 | lncRNA | | 2.1 |
| MT1A | protein_coding | metallothionein-1A | 2.1 |
| CRYAB | protein_coding | crystallin, alpha B | 2.1 |
| CCK | protein_coding | cholecystokinin | 2.1 |
| WNK3 | protein_coding | WNK lysine deficient protein kinase 3 | 2.1 |
| LOC104972680 | lncRNA | | 2.1 |
| PPP1R14A | protein_coding | protein phosphatase 1, regulatory (inhibitor) subunit 14A | 2.1 |
| MYOCD | protein_coding | Transcriptional co-activator of serum response factor | 2.1 |
| LOC783726 | misc_RNA | ubiquitin-like protein FUBI | 2.1 |
| LOC104971090 | lncRNA | | 2.0 |
| LOC104976794 | lncRNA | | 2.0 |
| LOC100336109 | pseudogene | | 2.0 |
| LOC787803 | protein_coding | 40S ribosomal protein S23 | 2.0 |

**Table 2.** Relative expression of down-regulated genes between cysteamine treated [+cys group] and non-treated [−cys group] chikso cow COCs determined using gene sequencing analysis.

| Gene symbol | Type | Function | Cys(−)/Cys(+) |
|---|---|---|---|
| LOC101909775 | lncRNA | | −6.3 |
| NUP54 | protein_coding | nucleoporin 54 kDa | −3.2 |
| INHBB | protein_coding | inhibin, beta B | −2.5 |
| LOC104968914 | lncRNA | | −2.4 |
| FATE1 | protein_coding | fetal and adult testis expressed 1 | −2.4 |
| SCAMP4 | protein_coding | secretory carrier membrane protein 4 | −2.4 |
| LOC614091 | pseudogene | | −2.3 |
| OLR1 | protein_coding | oxidized low density lipoprotein (lectin-like) receptor 1 | −2.3 |
| PTAR1 | protein_coding | protein prenyltransferase alpha subunit repeat containing 1 | −2.2 |
| LOC104969943 | lncRNA | | −2.2 |
| SRGN | protein_coding | serglycin | −2.2 |
| VCAN | protein_coding | versican | −2.2 |
| LHFPL3 | protein_coding | lipoma HMGIC fusion partner-like 3 | −2.1 |
| LOC101904768 | pseudogene | | −2.1 |
| LOC104969287 | lncRNA | | −2.1 |
| HAND2 | protein_coding | | −2.1 |
| CPSF1 | protein_coding | cleavage and polyadenylation specific factor 1, 160 kDa | −2.1 |
| LOC104969395 | lncRNA | | −2.1 |
| LOC104973132 | lncRNA | | −2.1 |
| LOC104976825 | lncRNA | | −2.0 |
| H19 | lncRNA | H19, imprinted maternally expressed transcript (non-protein coding) | −2.0 |
| LOC101908842 | protein_coding | | −2.0 |
| LEO1 | protein_coding | Leo1, Paf1/RNA polymerase II complex component, homolog (S. cerevisiae) | −2.0 |

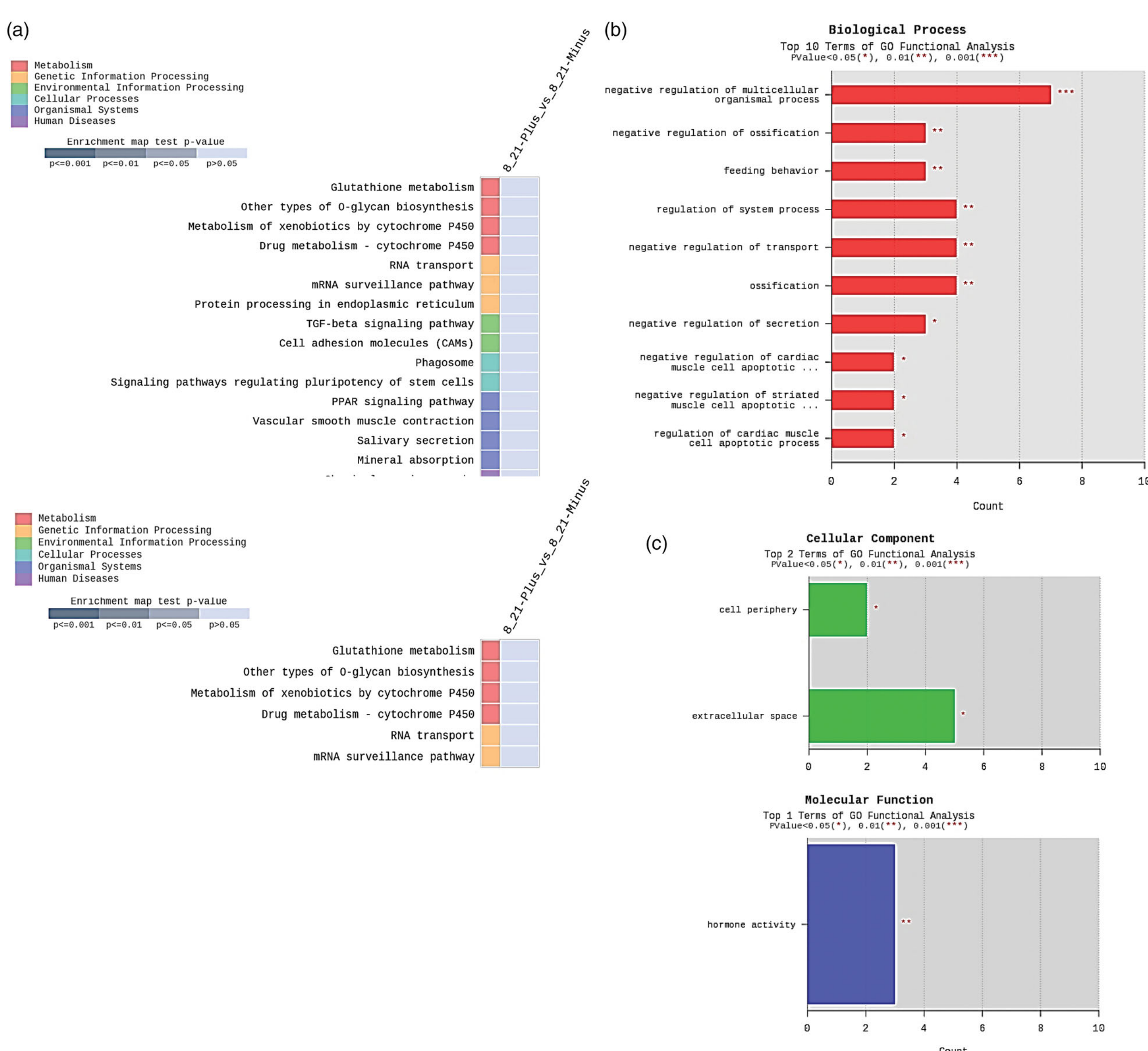


Figure 3. The gene ontology analysis was performed using DAVID Bioinformatic Resources. The DEGs were categorized into three main GO categories: biological process, cellular component, and molecular function. The mRNAs were analyzed to identify the biological process with molecular and cellular functions associated with genes overexpressed in cysteamine-treated and non-treated COCs. (a) Illustration of the functional classification and numbers of genes over and under expressed in control and cysteamine supplementation group. (b) The biological processes included in the top ten GO terms functional analysis significantly changed with the proportion of transcripts involved in over expression between cysteamine-treated and non-treated COCs in bovine. (c). The proportion of transcripts associated with the GO term was calculated using the number of genes overexpressed in one sample divided by the total number of genes involved in that given GO term multiplied by 100. Enrichment map test $P$-value, $^{*}p < 0.05$; $^{**}p < 0.01$ or $^{***}p < 0.001$ was considered statistically significant compared to the non-cysteamine-treated COCs.

GO analysis enabled us to query the gene functions using large-scale experimental data with a number of statistical analyses using DAVID Bioinformatic Resources between the control and cysteamine treated COCs at the transcriptome level (Fig. 3b). Our data highlighted that the GO classifications of genes with top ten terms relative to each other were the most significant or overexpressed transcripts involved in processes like physiological system or processes, regulation of striated or cardiac muscle physiology, hormonal regulation of secretion, ossification of bone, feed intake and transport, and negative regulation of various processes. These data are represented in Fig. 3b. Furthermore, these were related to cellular physiology like cell component, cell development, cellular morphogenesis, cell physiology at the molecular level, developmental processes such as embryonic development, embryonic morphogenesis, membrane structure, anatomical structure development, and hormonal response (Fig. 3c).

## Discussion

This study compared the transcriptome profiles of cysteamine-treated and non-treated COCs, and gene expression profiling was acknowledged as a marker of oocyte quality.[8] Cysteamine-treated COCs showed 36 gene transcripts that were up-regulated, 18 of which are known and 18 genes have an unknown function and still remain to be explored. However, among the 23 gene transcripts down-regulated in the cysteamine-treated COCs, 12 gene functions still need to be explored, and 11 known genes were depicted as below. Among the upregulated transcripts, α-crystallins or *CRYAB* are the principal members of small HSP family that inhibit apoptosis.[11] Gene sequencing data demonstrated that *CRYAB* expression is upregulated after cysteamine supplementation during oocyte maturation. This might play a crucial role in maintaining the normal oocyte physiology. These data correlated with the results of Xiongjie et al.[25] who mentioned that cells lacking the *CRYAB* gene accumulate p53 protein.[25] *CRYAB* is a negative regulator of inflammation with anti-apoptotic properties, and it can bind with already produced cytochrome-c and caspase3.[25,26] This protein can also prevent apoptosis caused by heat shock, oxidative stress, ischemia, and apoptosis effectors. *CRYAB* can stop the pro-apoptotic gene translocation of *Bax* and *Bcl-2* from the cytoplasm to mitochondria.[26] Therefore, these findings suggest that the *CRYAB* gene is very important for oocyte maturation and preimplantation embryo development.

Cysteamine supplementation within the maturation medium stimulates the *LOC781250* gene or insulin-like growth factor 1 (*IGF-1*) within matured oocytes. *IGF-1* is a hormone similar to insulin with regard to molecular structure. It promotes amino acid and glucose uptake, and it may stimulate mitosis via its mitogenic effects during cell division and proliferation of the preimplantation embryos.[20,27] *IGF-1* is one of the most potent natural activators of the AKT signaling pathway, a stimulator of cell growth and proliferation and a potent inhibitor of programed cell death.[28] Our data highlighted that *LOC781250* upregulation might be due to increased GSH content in oocytes, which acts as a reservoir to protect zygotes and promotes embryonic development. Our data support the results of the study by Guangmang et al.[29] who established that *IGF-I* mRNA levels were upregulated after dietary supplementation with cysteamine on growth hormone receptor and insulin-like growth factor in finishing pigs.[29] It also increases the proportion of preimplantation embryos developing into blastocysts, alters blastocyst gene expression, and improves embryo resistance to various stresses.[14]

Cysteamine supplementation within the maturation medium upregulates calcitonin (CT, also known as thyrocalcitonin), providing intracellular calcium homeostasis and regulating normal calcium homeostasis during the oocyte maturation process.[30] Gene sequencing data showed higher expression of *CT* gene transcript compared to control. As an intracellular $Ca^{2+}$ mobilizing agent, CT may effectively circumvent $Ca^{2+}$ overload that effects the oocyte maturation process.[30] This study will provide an important bridge regarding the relationship between upregulated calcitonin during oocyte maturation and further embryonic development after pregnancy. The *SCGB2A2* gene seems to be involved in cell signaling, immune response, chemotaxis, and it may also serve as a transporter for steroid hormones.[31] The *SCGB2A2* gene was significantly upregulated in the treatment group compared to control, but further studies are needed to elucidate its effects on the oocyte maturation process because there are no reports about this molecular marker's effects on oocyte maturation. The *B3GAT2* gene is a trans-membrane protein involved in the synthesis of a carbohydrate epitope, a sulfated trisaccharide involved in cellular migration.[32] Although gene sequencing data showed higher expression of the transcript than control, there is scant evidence of *B3GAT2*'s involvement in oocyte maturation. Hence further studies are warranted. *SCAMP4* is also known as secretory carrier membrane protein. Secretory carrier membrane proteins (SCAMPs) are integral membrane proteins with four transmembrane spans that reside mainly in functioning cell membranes.[33] Accordingly, downregulation of this gene was observed in gene sequencing data of cysteamine-treated MII oocytes. *SCAMP4* levels were remarkably increased on the surface of proliferating cells, and the suppressive activity usually associated with aging conditions of the senescent cells might be an indication of the oocyte maturation status.[33] There is no previous report that *SCAMP4* has any effect on the oocyte maturation process. Therefore, more studies are needed.

Other transcripts stimulated by cysteamine are *LOC513842* or *ferritin* genes, which regulate iron transport.[20] *Ferritin* is associated with bio-mineralization of the embryo, and it acts as a detoxifying protein by removing metals. It promotes embryonic development by overcoming the embryo two-cell stage block via hydroxy radical formation inhibition and lipid peroxidation.[34] It protects cells from oxidative

injury by reducing free radical production.[20] In fact, it can scavenge free oxygen radicals and compete with lipid components of the membrane in chelating the iron ions that accelerate the lipid peroxidation rate.[28] Cysteamine supplementation also upregulates selenoprotein (*SEPW1*), which might provide antioxidant action. Similar data were demonstrated by Whanger[35] who found that selenoprotein acts as an antioxidant involved in cell immunity and has thioredoxin-like action. The high antioxidant activity of cysteamine should be a target of further investigations with focus on detailed molecular mechanisms. *GSTA3* is a membrane-bound form of glutathione S-transferase and catalase reduced GSH to foreign substrates for detoxification.[36] During oocyte maturation, *GSTA3* mRNA might be accumulated in oocytes for further embryonic development.[36] Our data support Cho et al.[37] who revealed that *GSTA3* was transcriptionally motivated in response to oxidative stress. This response might be related to changes in the redox state of cellular response on oxidative stress.[37]

One of the important outputs of cysteamine-supplemented oocytes compared to control oocyte is the transcriptional downregulation of *OLR1* (oxidized low-density lipoprotein, LDL receptor 1), also known as lectin-type oxidized LDL receptor 1 (LOX-1). These data demonstrate that LDL oxidation can cause oxidative damage during the maturation process counteracting or blocking oocyte contact. Cysteamine inhibits LDL oxidative reactions facilitated by free radicals and ameliorates lysosomal stresses and protects from proteolysis.[38] The oxidized LDL is associated with NF-kB activation.[39] Besides, LOX-1 may be involved in cyclic AMP (cAMP) signaling pathway regulation in oocyte maturation.[40] The meiotic resumption of COCs must involve a decrease in oocyte cAMP levels to escape from meiotic arrest. Cumulus cell-synthesized cAMPs can pass through the gap junctions between oocytes and cumulus cells and maintain oocyte meiotic arrest *in vivo*.[40] The drastic change in cAMP levels is known to be an important stimulus for meiosis re-initiation. *OLR1* downregulation by the cysteamine supplementation might be an important molecular marker for the oocyte meiotic resumption status. Moreover, cysteamine may protect oocytes from premature aging before the MII stage in *in vitro* culture and mitochondrial redox stress within oocytes.[41,42] It is also essential that cysteamine is stored during the maturation phases to contribute to the initial stages of embryonic development by regulating genes that concluded zygotic genome activation.[42]

Inhibin, beta B (*INHBB*) produces a subunit of activin and inhibin, which are two closely related glycoproteins with opposing biological effects. *INHBB* is a pituitary FSH secretion inhibitor that negatively regulates gonadal stromal cell proliferation and acts as both growth differentiation factor.[43] Downregulated *INHBB* gene prepare the matured COCs for further biological processes and molecular activities like fertilization. Therefore, downregulation of inhibin beta B unit might be due to the action of FSH hormone in *in vitro* matured COCs.[32] Matured COCs do not require FSH action, providing negative signals to stop the secretion of FSH.[43] *CPSF1* is involved in the cleavage of the 3’ signaling region from a newly synthesized pre-messenger RNA (pre-mRNA) molecule in the process of gene transcription.[44] There are no studies of oocyte maturation effects focusing on the transcripts after cysteamine supplementation, and such studies could find new clues for the maturation mechanism. Downregulation of *CPSF1* and *LHFPL3* in matured COCs might be due to the MII stage or matured oocytes that do not require estrogen activity.[45,46] Downregulation of *MYOCD* and *PPP1R14A* gene expression in COCs after cysteamine supplementation might influence oocyte maturation; however, further studies are needed.

Among the other downregulated transcripts, *H19* is an uncommon gene that blocks mRNA from being translated.[47] Suppression of *H19* expression after cysteamine supplementation will open a new window in assisted reproduction technologies or in cloning research since this lethal gene causes genomic imprinting effects.[48] These data supported[48,49] that *H19* expression regulates DNA methylation and genomic imprinting during oocyte maturation. However, *H19* gene function remains elusive and hence more detailed studies are required. The *SRGN* is well known for its role in granule-mediated apoptosis through binding to the CD44 receptor in cumulus cells, and it can modify sperm activation.[50] *Versican* is also a proteoglycan that mediates many cellular processes such as cell adhesion, migration, and signaling.[51] Moreover, it acts as a selective barrier for sperm with high fertilization potential[51]. MII oocytes must bind to sperm, and thus down-regulation of *SRGN* and *Versican (VCAN)* is essential for successful fertilization and subsequent embryonic development.[41] *SRGN* and *VCAN* mRNAs were down-regulated in cysteamine-treated COCs. These data are in accordance with those of previous studies[50,51] and suggest that the complicated regulation of mRNA accumulation in COCs should be studied further.

The analysis of the transcriptomes discovered in cysteamine-treated COCs shows extensive changes in the expression of genes involved in a wide range of physiological mechanisms associated with developmental processes, apoptosis regulation, cellular differentiation, calcium and mineral regulation, cell structure physiology, hormonal secretion, and membrane construction for further embryonic development. A large number of abnormally expressed transcripts are associated with membrane structure formation and functions. Furthermore, all these functions might allow the response to internal and external stimuli, contributing to COC homeostasis maintenance and cell-to-cell communications that are essential for normal early embryogenesis. We hope that these molecular markers will offer clues regarding oocyte metabolism, which is a prerequisite for further embryonic development.

In conclusion, our data highlighted that cysteamine supplementation overcomes the difficulties encountered during *in vitro* maturation of oocytes by upregulating potential molecular markers and downregulating genes that affect oocyte maturation. Another important feature of cysteamine is that even a minute dose (0.1 mM) can induce *in vitro* oocyte maturation. Furthermore, this study will provide a foundation for future research on regulatory factors and molecular markers for oocyte maturation, and it will also overcome the constraints that lead to infertility.

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