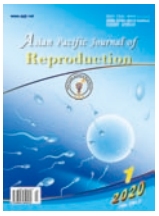

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Comparative proteomic analysis of mature and immature oocytes in domestic cats

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ABSTRACT

Objective: To evaluate changes of feline (*Felis catus*) oocytes proteins during *in vitro* maturation by using the proteomic approach.

Methods: Immature oocytes (germinal vesicle) isolated from female cats were cultured and collected at 0 h and 24 h. After collection, oocytes were investigated into immature (germinal vesicle) and mature (metaphase Ⅱ) stages. The qualitative profiles of the proteins at the immature and mature stages were determined by one-dimensional electrophoresis and liquid chromatography-mass spectrometry.

Results: Our data revealed that following 24 h *in vitro* maturation the maturation rate (metaphase Ⅱ stage) was 58.7%. Eighty-one of the 260 proteins analyzed were differentially expressed between the germinal vesicle stage and the metaphase Ⅱ-arrest stage. Proteomic analysis of germinal vesicle and metaphase Ⅱ oocytes showed abundant expression of proteins involved in transportation (10%), indicating that this was a major characteristic of germinal vesicle oocytes. Similarly, analysis of the proteome of metaphase Ⅱ oocytes indicated that cell cycle proteins were overexpressed. Interestingly, proteins involved in DNA repair and apoptosis were only expressed in germinal vesicle oocytes and proteins involved in fertilization were only expressed in metaphase Ⅱ oocytes.

Conclusions: The overexpression of certain proteins in germinal vesicle and metaphase Ⅱ is necessary for oocyte development and maturation. Our findings provide a valuable resource for further investigations into protein expression in oocytes at different developmental stages.

KEYWORDS: Domestic cats; Immature oocytes; Mature oocytes; Proteomics

1. Introduction

Most wild members of the felidae family are vulnerable, threatened, or in danger of extinction in nature. The domestic cat (*Felis catus*) serves as an animal model in the reproductive studies of endangered or nondomestic species. *In vitro* maturation (IVM) is a technique that allows saving the genetic materials from endangered species. The oocyte maturation process has been described by the changes in chromosomal morphology in the meiosis stage[1]. Oocytes are arrested at the germinal vesicle (GV) stage which is the first meiotic prophase. Upon the surge of gonadotropin secreted by the anterior pituitary gland, the immature oocytes have been stimulated to resume the first meiosis, ovulate and thereby arrest at metaphase Ⅱ (MⅡ)[2]. Approximately 80% or less cultured cat oocytes achieve nuclear maturation and only 60% of the mature oocytes are fertilized by *in vitro* fertilization (IVF)[3]. To understand the molecular mechanisms of oocyte biology, it is important to recognize the processes that regulate meiotic maturation of oocytes. Oocyte maturation is a complex process that involves the regulation of protein synthesis, degradation, and phosphorylation[4]. The processes of cellular differentiation and

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maturation are characterized by specific protein expression[5]. Many proteins with well-defined functions have been identified during oocyte maturation. For example, high levels of glucose-6-phosphate dehydrogenase are essential for viable oocytes and for the generation of triphosphopyridine nucleotide, which is required in the process of fertilization[6]. Nucleoplasmin 2 and peptidylarginine deiminase 6 are proven maternal-effect proteins, which play crucial roles in early embryonic development[7,8]. Peroxiredoxin 2, glutathione-S-transferase, and myomegalin 1 are involved in redox regulation and the cAMP-dependent signaling pathway. Both of them have been found to be correlated with oocyte maturation[9,10]. The activation of some protein kinases plays a key role in the meiotic maturation of oocytes. Several studies have investigated mammalian oocyte proteomics, including the exploration of bovine[11], pig[12], and mouse[13] oocyte proteins. The processes of IVM and IVF of oocytes collected from excised ovarian tissue have reached a level of consistency in certain species, to allow replacement of the costly and labor-intensive processes of *in vivo* embryo production and recovery[14]. The ability to grow and fertilize immature oocytes is beneficial to produce large numbers of embryos for developmental biology, cryopreservation, and genetic studies, as well as for live animal production. The domestic cat is an important model to study human genetic diseases and to develop the assisted reproduction in taxonomically-related endangered species. Proteomic analysis is a valuable technique that can be applied to differentiate protein expression between different stages of nuclear maturation of oocytes[5]. This information is helpful for further understanding the mechanism of oocyte maturation, which will improve the quality of oocytes after IVM. However, no previous reports have revealed proteomics-based investigations of fresh immature and mature oocytes in the felid family. Therefore, this study was to employ proteomic analyses to identify the proteins necessary for IVM of feline oocytes.

2. Materials and methods

2.1. Chemicals

Chemicals used in this study were purchased from Sigma (Sigma, St. Louis, MO, USA), unless indicated otherwise. Media were prepared once a week, filtered, and then kept in sterile bottles.

2.2. Oocyte collection

Prior to ovarian tissue collection, animal care and ethics approval was informed to Mahidol University Ethic Committee on Animal research. However, no ethical approval was required due to ovaries were collected after ovariohysterectomy for the purpose of permanent contraception from the veterinary clinic of the Veterinary Public Health Division, Bangkok Metropolitan Administration (total number of cats = 150). To collect cumulus-oocyte complexes (COCs), ovaries from normal females of various breeds (> 6 months old) were repeatedly sliced in Petri dishes which contained TCM 199 (Invitrogen, Carlsbad, CA, USA) supplemented with 25 mM 2-hydroxyethyl, 0.1% polyvinylalcohol, 0.1 mM glutamine, 2.5 mM sodium pyruvate, and 1% penicillin-streptomycin. COCs with more than three layers of cumulus cells with a darkly pigmented oocyte cytoplasm (grade 1 and grade 2) were selected for the experiments. Grade 1 COCs with more than 5 layers of compact cumulus cells and grade 2 COCs with 3 to 5 layers of compact cumulus cells[15].

2.3. IVM

The COCs were cultured in Dulbecco's modified eagle medium supplemented with follicle-stimulating hormone, luteinizing hormone, and estradiol. Ten COCs were cultured in Petri dishes containing 100 μL culture medium under mineral oil in each drop for 24 h at 38.5 ℃ in 5% CO_2.

2.4. Assessment of nuclear maturation

After 24 h of culture, COCs were denuded by exposure to 0.5% hyaluronidase for 5 min and gently pressed the pipette to remove cumulus cells. To analyze the stages of oocytes, the denuded oocytes were characterized as being in MⅡ-arrest stage according to the presence of the first polar body within the perivitelline space under a stereomicroscope (200×, Nikon SMZ1500, Japan).

2.5. Protein extraction and one-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

A total of 900 GV and MⅡ denuded oocytes were lysed by adding 0.5% SDS supplemented with protease cocktail inhibitor. Lysates were centrifuged at 17 530×*g*, at 4 ℃, for 20 min. The supernatant was collected and the protein concentration was determined by Lowry method. The protein lysates were mixed with 5× sample buffer and heated at 95 ℃ for 10 min before loading onto a 12.5% gel for SDS-PAGE. Electrophoresis was performed at 70 V in electrophoresis buffer and the gel was then silver-stained[15].

2.6. Gel slicing and tryptic in-gel digestion

To perform in-gel digestion of proteins, 20 μL of trypsin solution (10 ng/μL trypsin in 50% acetonitrile/10 mM ammonium bicarbonate) was added to the gels, followed by incubation at room temperature for 20 min. To keep the gels immersed throughout digestion, 30 μL of 30% acetonitrile was added and incubated overnight. To extract the peptide digestion products, 30 μL of 50% acetonitrile in 0.1% formic acid was added to the

gels and incubated with shaking for 10 min, and this was repeated three times. Extracted peptides were collected, dried by vacuum centrifugation, and stored at −80 ℃ for further mass spectrometric analysis. Prior to liquid chromatography–tandem mass spectrometry (LC-MS/MS) analysis, the peptides were dissolved in 20 μL 0.1% formic acid[15].

2.7. LC–MS/MS analysis

Peptide solutions were analyzed by using the HCTultra PTM Discovery System (BrukerDaltonics Ltd., UK) coupled to the UltiMate 3000 LC System (Dionex Ltd., UK). Peptides were separated on a nanocolumn (PepSwift monolithic column 100 μm i.d. 50 mm). Eluent A contained 0.1% formic acid and eluent B contained 80% acetonitrile/water with 0.1% formic acid. Peptide separation was carried out with a linear gradient from 10% to 70% eluent B at a flow rate of 300 nL/min for 13 min, including a regeneration step at 90% eluent B and an equilibration step at 10% eluent B, which took 20 min. Peptide fragment mass spectra were achieved in data-dependent AutoMS mode with a scan range of 300–1 500 *m/z*, three averages, and up to five precursor ions selected from the MS scan 50–3 000 *m/z*[15,16].

2.8. Protein quantitation and identification

DeCyder MS Differential Analysis software (DeCyderMS, GE Healthcare) was used to quantify the protein. The analyzed MS/MS data from DeCyderMS were submitted for database searches by using the Mascot software (Matrix Science, London, UK). The data were searched in the NCBI database for protein identification. Data normalization and quantification of the changes in protein abundance between the GV and MⅡ stages were performed and visualized by using MultiExperiment Viewer (Mev) software version 4.6.1. Data were normalized and quantified the changes of protein abundance between the GV and MⅡ, subsequently visualized by using MultiExperiment Viewer (Mev) software version 4.6.1. Gene ontology annotation including molecular function and biological process was assigned to the proteins identified according to the Uni-Prot database. The identified proteins were then submitted to the search tool STITCH (V4.0) to gain insight into their cellular functions and to annotate all of the functional interactions among proteins in the cell[15,16].

2.9. Statistical analysis

Statistical tests of the variance (ANOVA) tests of differences for these data sets were performed for statistically significant proteins. $P<0.05$ was considered statistically significant.

3. Results

3.1. IVM of feline oocytes

To verify the changes of protein during IVM, a total of 1 533 oocytes were cultured *in vitro* to induce oocyte maturation. The different stages of oocytes during IVM were referred to the GV, GV breakdown, MⅠ, and MⅡ stages, which were classified by morphological observations. Following 24 h of IVM, cat oocytes were divided into the GV and MⅡ stages by morphological analysis (Figure 1A and B). In Dulbecco's modified eagle medium supplemented with follicle-stimulating hormone, luteinizing hormone, and estradiol, the maturation rate was 58.7%. Based on morphological analysis, the GV and MⅡ stages of IVM were collected and samples were prepared for protein fractionation by SDS-PAGE and analysis by LC-MS/MS.

3.2. Quantitative proteome profile of feline GV and MII –arrested oocytes

A representative SDS-PAGE image from 900 GV and MⅡ-arrested oocytes were shown in Figure 2A. Gels were sliced into 15 pieces and assigned to in-gel tryptic digestion. Peptides were analyzed by LC-MS/MS. A total of 1 702 protein identifications were made from 20 μg of protein. Of these 1 702 proteins, 1 442 proteins were found in both the GV and MⅡ stages and 260 proteins were differentially expressed between GV and MⅡ stages (Figure 2B).

All 1 702 proteins were analyzed to determine the gene ontology annotations for biological processes and cellular compartments. The majority of proteins were found to involve in the metabolism (4.11%), transportation (4.00%), transcription (3.06%), and cell cycle (2.76%) (Figure 3A). Classification based on the subcellular localization (Figure 3B) indicated that 7.68% of proteins were from the plasma membrane and 7.31% were from the nucleus.

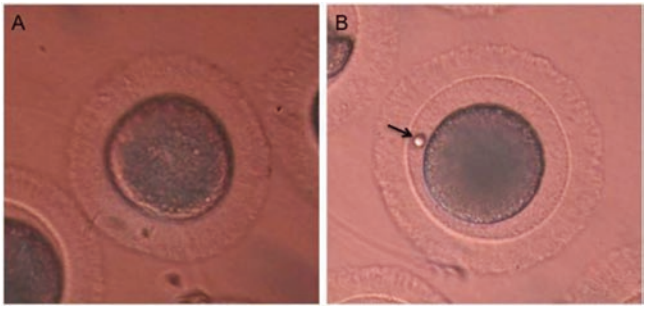

Figure 1. Morphological characterization of feline oocytes during *in vitro* maturation. (A) An intact germinal vesicle; (B) Metaphase Ⅱ stage with extrusion of the first polar body (arrow).

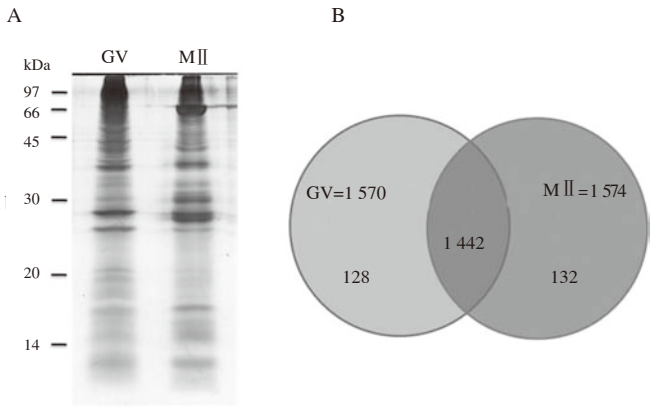

Figure 2. SDS-PAGE fractionation of denuded germinal vesicle (GV) and metaphase Ⅱ (MⅡ) oocyte proteins. Pooled protein (20 μg of denuded GV or MⅡ oocyte protein) is run on a 12.5% acrylamide gel and then subjected to silver staining. A: Lane 1: total protein of GV oocytes; Lane 2: total protein of MⅡ oocytes. Each gel lane is excised and then subjected to in-gel digestion prior to LC/MS-MS analysis. B: 1 442 proteins are found in both the GV and MⅡ stages and 260 proteins were differentially expressed between GV and MⅡ stages.

A total of 260 proteins showed expression differences between the GV and MⅡ stages during IVM. Of these, 81 proteins showed at least two unique tryptic peptides with protein ID scores >10. Changes in protein expression over time in GV stage compared with MⅡ stage were evident using Multi-Experiment Viewer (MeV, version 4.9.0) software. In the MⅡ stage of feline oocytes during IVM, 41 proteins were overexpressed and 40 proteins were downregulated, the details and biological functions of which were presented in Table 1 and 2, respectively. Proteins participating in transportation, signal transduction, and cell cycle events were found to predominate among overexpressed proteins in GV stage. Compared with MⅡ oocytes, GV oocytes contained higher levels of proteins involved in transportation including inward rectifier potassium channel 9, stonin-2, lysosomal-associated transmembrane protein 5-like protein, and Shaw type potassium channel (Table 1 and 2). These results suggested that the feline IVM process may be dependent upon 81 specific proteins that were differentially expressed. These proteins might play an important role in the molecular events involved in feline oocyte development.

3.3. Classification of proteins identified in GV and MII stage oocytes

The 81 proteins that were differentially expressed during oocyte IVM were subjected to classify according to their associated biological processes and molecular functions using information based on the Gene Ontology and Uni-Prot databases.

The proteins that were overexpressed in MⅡ during IVM were clasified into the following biological functions: cell cycle 13%, metabolic process 10%, cellular organization 10%, development 8%, transcription 8%, signal transduction 8%, immune response 5%, transportation 5%, stress response 3%, translation 3%, fertilization 2%, differentiation 2%, and unknown function 23%. The upregulated proteins included the following: activin receptor type-1C, KIAA0445 (Rootletin), centromere protein T, claspin, DNA topoisomerase 2-alpha, rotatin, growth/differentiation factor 7, and titin all involved in the cell cycle and development; C-1-tetrahydrofolate synthase and cytoplasmic involved in biosynthesis; delta-like protein 1 precursor and zonapellucida sperm-binding protein 4 precursor involved in fertilization; complement factor H-like protein, tumor necrosis factor receptor superfamily member 10B-like protein, and interleukin-34 precursor involved in the immune response and apoptosis; ethanolamine-phosphate cytidylyltransferase-like protein, beta-1,4-*N*-acetyl-galactosaminyl transferase 3, galactocerebrosidase, UDP-GalNAc:beta-1,3-*N*-acetylgalactosaminyl-transferase 2-like protein, and adenosine triphosphate-dependent RNA helicase DDX47-like protein involved in metabolic processes; high mobility group B2-like protein, ninein isoform 2, and espin-like protein involved in cell organization; cryptic-like protein, 52 kDa repressor of the inhibitor of protein kinase, and torsin-1A-interacting 2-like protein involved in signal transduction; histone-lysine *N*-methyltransferase SETD1A isoform 2, snRNA-activating protein complex subunit 3, RNA-binding protein 14, and 40S ribosomal protein S3-like isoform 2 involved in transcription and translation; solute carrier family 25 member 39 and nuclear pore membrane glycoprotein 210 precursor involved in transportation.

The downregulated proteins in MⅡ during IVM were classified into the following biological functions: cell cycle 10%, signal transduction 10%, transportation 10%, transcription 8%, metabolic processes 8%, cellular organization 5%, apoptosis 5%, DNA repair 3%, tumor suppressor 3%, translation 3%, biosynthesis 2%, development 2%, and unknown function 31%. The downregulated proteins included the following: cyclin-dependent kinase 5 and ABL1 enzyme substrate 1 (CABLES1), synaptonemal complex protein 1, and sarcoma antigen NY-SAR-48, part involved in the cell cycle; alkyl dihydroxyacetone phosphate synthase and carnitine *O*-palmitoyl-transferase 1 involved in metabolic processes; hypothetical protein LOC100017349 and protein yippee-like 3 involved in apoptosis; Rho GTPase activating protein 17, signal-induced proliferation-associated 1-like protein 2, and serine/threonine-protein phosphatase 4 regulatory subunit 4 involved in signal transduction; zinc finger protein castor homolog 1-like protein (CASZ1), zinc finger protein 226-like protein, and sex-determining region Y protein involved in transcription; 60S ribosomal L13a-like protein and diphthamide biosynthesis 1-like protein involved in translation; lysosomal-associated transmembrane 5-like protein, inward rectifier potassium channel 9, and Shaw type potassium channel involved in transportation; vascular endothelial growth factor receptor-2 involved in angiogenesis; and transmembrane 187-like protein and KIAA1110 protein involved in cellular organization.

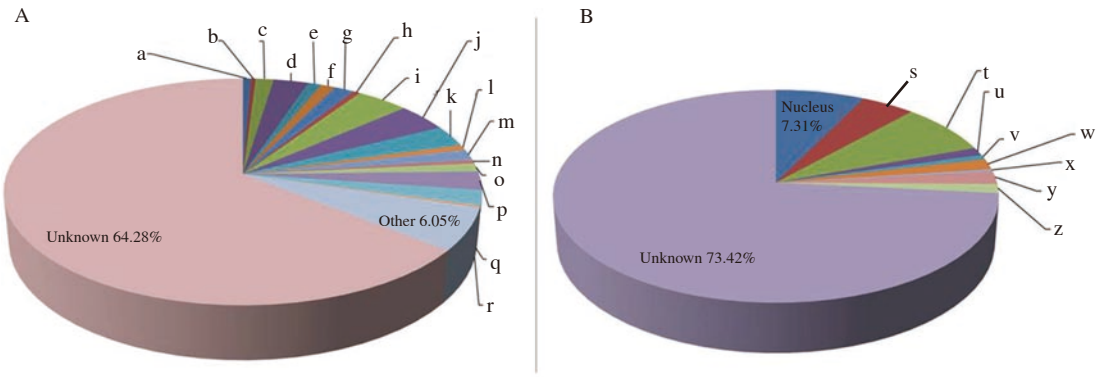

Figure 3. Gene ontology analyses of proteins identified in both germinal vesicle and metaphase Ⅱ stages. Proteins are classified according to A) biological processes, and B) cellular compartment. Results are displayed as percentage of genes classified into a category over total number of class hits. a: apoptosis 0.65%; b: angiogenesis 0.35%; c: biosynthesis 1.41%; d: cell cycle 2.76%; e: cell adhesion 1.06%; f: DNA repair 1.12%; g: development 1.59%; h: differentiation 0.53%; i: metabolism 4.11%; j: transportation 4.00%; k: transcription 3.06%; l: translation 0.71%; m: immune response 1.59%; n: Oxidative stress response 0.53%; o: proteolysis 1.18%; p: signal transduction 2.64%; q: cellular organization 2.17%; r: fertilization 0.24%; s: cytoplasm 4.40%; t: plasma membrane 7.68%; u: endoplasmic reticulum 1.18%; v: Golgi apparatus 0.74%; w: mitochondria 1.61%; x: lysosome 0.31%; y: extracellular space 1.98%; z: cytoskeleton 1.36%.

Table 1. Overexpressed proteins in metaphase Ⅱ of *in vitro* maturation.

Protein name	Biological process	MOWSE score	Database ID No.	Species	Peptide sequence
Activin receptor type-1C (ACVR1C)	Cell cycle	14	gil126326424	*Monodelphis domestica*	RSDIYSLGLVYWEIAR
KIAA0445 (Rootletin)	Cell cycle	10	gil29421172	*Homo sapiens*	AQREEAAAAHAQEVR
Centromere protein T (CENPT)	Cell cycle	12	gil28893125	*Mus musculus*	TQTAGPR
Claspin isoform 1 (CLSPN)	Cell cycle	13	gil296207468	*Callithrix jacchus*	SLLSDSTLLLFKDSSSK
DNA topoisomerase 2-alpha (TOP2A)	Cell cycle	14	gil47523094	*Sus scrofa*	KPNPPKPK
Rotatin (RTTN)	Development	16	gil291394477	*Oryctolagus cuniculus*	GAPSSSLMLCILK
Titin (TTN)	Development	10	gil334329989	*Monodelphis domestica*	ELTPGPKYK
Delta-like protein 1 precursor (DLL1)	Differentiation	31	gil14091746	*Rattus norvegicus*	GGEVPDRK
Zonapellucida sperm-binding protein 4 precursor (ZP4)	Fertilization	34	gil57163799	*Felis catus*	NSQSLSQWPILVK
Growth/differentiation factor 7 (GDF7)	Cell growth	26	gil50400625	*Homo sapiens*	SPGGGGGGGR
	Cellular organization	10	gil125346156	*Mus musculus*	SSPIPAGDCGEHWK
High mobility group protein B2-like (HMG2)	Cellular organization	12	gil297297620	*Macaca mulatta*	FEGMAK
Espin-like (ESPNL)	Cellular organization	11	gil194208107	*Equus caballus*	GVTSNRK
C-1-tetrahydrofolate synthase, cytoplasmic (MTHFD1)	Biosynthesis	19	gil222136639	*Homo sapiens*	CTHWAEGGK
Complement factor H-like (CFH)	Immune response	19	gil334321844	*Monodelphis domestica*	CINGR
Interleukin-34 precursor (IL34)	Immune response	13	gil302564283	*Macaca mulatta*	PRGFAWLR
UDP-GalNAc:beta-1,3-N-acetylgalactosaminyl transferase 2-like (B3Gal-T3)	Metabolic process	19	gil297281799	*Macaca mulatta*	TGVLVLR
Probable ATP-dependent RNA helicase DDX47-like (DDX47)	Metabolic process	19	gil334348319	*Monodelphis domestica*	IQIEAIPMALDGR
Ethanolamine-phosphate cytidylyltransferase (PCYT2)	Metabolic process	16	gil301754189	*Ailuropoda melanoleuca*	ETLCSEGSSQCPGGR
B4galnt3 protein (beta-1,4-N-acetyl-galactosaminyl transferase 3)	Metabolic process	12	gil141795858	*Mus musculus*	GGDHPGR
Galactocerebrosidase (GALC)	Metabolic process	22	gil329664800	*Bos taurus*	DARLTK
Solute carrier family 25 member 39	Transportation	15	gil115495355	*Bos taurus*	NFFQRLNR
Nuclear pore membrane glycoprotein 210 precursor (NUP210)	Transportation	17	gil27477134	*Homo sapiens*	TVKAYVR
Histone-lysine N-methyltransferase SETD1A isoform 2 (hSET1A)	Transcription	10	gil114662089	*Pan troglodytes*	APRGVER
snRNA-activating protein complex subunit 3 (SNAPC3)	Transcription	17	gil89242152	*Homo sapiens*	GAGDLSLR
RNA-binding protein 14 (RBM14)	Transcription	10	gil86262142	*Mus musculus*	TQPMAAQAASYR
40S ribosomal protein S3 (RPS3)	Translation	14	gil332220518	*Nomascus leucogenys*	GGKPEPPVMPQPVPTA
Cryptic protein-like (CFC1)	Signal transduction	16	gil297668342	*Pongo abelii*	EKPNGGR
Torsin-1A-interacting protein 2-like	Signal transduction	11	gil301770861	*Ailuropoda melanoleuca*	DPAEASQK
52 kDa repressor of the inhibitor of the protein kinase (PRKRIR)	Stress response	14	gil332210861	*Nomascus leucogenys*	FNTSEGHHADMYR
Pecanex-like protein 1-like	Unknown function	12	gil301782760	*Ailuropoda melanoleuca*	SSCTSDKR
hCG1654079, isoform CRA_b	Unknown function	13	gil119586908	*Homo sapiens*	KSQIPVR
Peptide ABC transporter substrate-binding protein	Unknown function	12	gil488747097	*Treponema denticola*	MMESGAFNTLLNK
Unknown (protein for MGC:176400)	Unknown function	11	gil223461298	*Homo sapiens*	AGVVAR
Putative PRAME family member 24-like	Unknown function	10	gil94374974	*Mus musculus*	CSQLTK
Hypothetical protein LOC100428998	Unknown function	13	gil297296253	*Macaca mulatta*	LPLLVPGST
Protein FAM71F1-like	Unknown function	11	gil194209870	*Equus caballus*	LHPDHPR
Von Willebrand factor	Unknown function	21	gil254728529	*Tupaia longipes*	LLDLVFLLDGSXR
Uncharacterized protein LOC100296067	Unknown function	12	gil297490716	*Bos taurus*	HGLQPSSAFVPMSDHK

Table 2. Downregulated proteins in metaphase Ⅱ-arrest stage of *in vitro* maturation.

Protein name	Biological process	MOWSE score	Database	Species	Peptide sequence
CDK5 and ABL1 enzyme substrate 1 (CABLES1)	Cell cycle	15	gil296222383	*Callithrix jacchus*	DSTQAGDLK
Synaptonemal complex protein 1 (SYCP1)	Cell cycle	20	gil334324527	*Monodelphis domestica*	DELDAVK
Sarcoma antigen NY-SAR-48 (HAUS8)	Cell cycle	11	gil29164881	*Homo sapiens*	GKMADSSGR
Thrombopoietin receptor (MPL)	Development	16	gil126305853	*Monodelphis domestica*	NKPPP
Aspartate dehydrogenase domain containing (ASPDH)	Biosynthesis	19	gil42490927	*Homo sapiens*	RPDLVVEVAHPK
Dynein heavy chain domain-containing protein 1 isoform 1 (CCDC35)	Cellular organization	25	gil222144249	*Homo sapiens*	GGPIK
KIAA1110 protein	Cellular organization	12	gil5689557	*Homo sapiens*	GVDDGADIPR
Transmembrane protein 187 (TMEM187)	Cellular organization	15	gil301786893	*Ailuropoda melanoleuca*	LSPEGKVH
Protein Shroom 3 (SHROOM3)	Cellular organization	18	gil297292672	*Macaca mulatta*	SSSMDNTSAR
Zinc finger protein Castor homolog 1-like (CASZ1)	Transcription	10	gil301784136	*Ailuropoda melanoleuca*	DDSGPGAK
Zinc finger protein 226-like (ZNF226)	Transcription	15	gil301788644	*Ailuropoda melanoleuca*	NVGGNNQSELR
Zinc finger and BTB domain-containing protein 44 (ZBTB44)	Transcription	24	gil74760158	*Homo sapiens*	VQDKIFR
Sex-determining region Y protein (SRY)	Transcription, sex differentiation	21	gil323530757	*Colobus guereza*	KMELDNR
Stonin-2 (STON2)	Transportation	16	gil126282180	*Monodelphis domestica*	DEFSGVLR
Lysosomal-associated transmembrane protein 5-like (LAPTM5)	Transportation	12	gil297282785	*Macaca mulatta*	GGDSSTMDPR
Inward rectifier potassuimchannel9 (KCNJ9)	Transportation	12	gil609672	*Rattus rattus*	TSPAPR
Shaw type potassium channel	Transportation	12	gil2143521	Mouse	HGGGGGDSGK
Alkyldihydroxyacetonephosphate synthase (AGPS)	Lipid metabolism	16	gil4501993	*Homo sapiens*	RYPLSGMGLPTFK
Carnitine O-palmitoyl-transferase 1, brain isoform-like (CPT1)	Metabolism	11	gil334329080	*Monodelphis domestica*	AGNAVYAMMR
DNA repair protein complementing XP-C cells isoform 2	DNA repair	15	gil297670012	*Pongo abelii*	SADGPAK
Hypothetical protein LOC100017349	Apoptosis	15	gil334333518	*Monodelphis domestica*	KMSVEIQSSLR
Protein yippee-like 3 (YPEL3)	Apoptosis	15	gil332265954	*Nomascus leucogenys*	ALGVPR
PRP8 pre-mRNA processing factor 8 homolog (PRPF8)	Protein interaction	10	gil119610996	*Homo sapiens*	MMSTLYR
Rho GTPase activating protein 17 (RICH-1)	Signal transduciton	15	gil34531434	*Homo sapiens*	LLLETGMK
Signal-induced proliferation-associated 1-like protein 2 (SIPA1-L2)	Signal transduction	12	gil329664354	*Bos taurus*	SLVHGR
Serine/threonine-protein phosphatase 4 regulatory subunit 4 (PPP4R4)	Signal transduction	12	gil126282106	*Monodelphis domestica*	TDETILLSLSLHLGK
60S ribosomal protein L13a-like (RPL13A)	Translation	21	gil126330006	*Monodelphis domestica*	QAEKNVEGK
Diphthamide biosynthesis protein 1-like (DPH1)	Translation, proliferation	10	gil335298294	*Sus scrofa*	ERPLQAAGR
Glioma tumor suppressor candidate region gene 2, isoform CRA_d	Tumor supressor	10	gil119577914	*Homo sapiens*	SSSGGGRR
Vascular endothelial growth factor receptor-2 (VEGFR2)	Angiogenesis	13	gil21359789	*Ovis aries*	LILNCTAR
Collagen, type XIV, alpha 1 (undulin), isoform CRA_a	Unknown function	15	gil119612404	*Homo sapiens*	AMNASANITSDGVEVLGK
Uncharacterized protein C6orf130 homolog isoform 2	Unknown function	10	gil335307525	*Sus scrofa*	KSGEVAGITK
hCG1801336	Unknown function	15	gil119612676	*Homo sapiens*	MLVSNLKR
T-cell surface glycoprotein CD5, partial	Unknown function	14	gil114637848	*Pan troglodytes*	VLDAGDPTSR
Wdr78 protein, partial	Unknown function	18	gil53734209	*Rattus norvegicus*	VTEDEVK
Sulfide:quinoneoxidoreductase, mitochondrial	Unknown function	13	gil297696565	*Pongo abelii*	GYWGGPAVLR
Unnamedd protein product	Unknown function	22	gil7020068	*Homo sapiens*	LQSPVFAR
ALYE870	Unknown function	13	gil37182346	*Homo sapiens*	AADAPFVINAIIR
Uncharacterized protein LOC100403426, partial	Unknown function	12	gil296237824	*Callithrix jacchus*	LLENSPGQTVEK
Hypothetical gene supported by AK126539	Unknown function	14	gil119605560	*Homo sapiens*	NREVEATLK
Unnamed protein product	Unknown function	16	gil194388300	*Homo sapiens*	GGRPHQVPGAHILGRV

4. Discussion

The complex process of oocyte nuclear maturation involves dynamic regulation of protein synthesis, degradation, and phosphorylation. Before IVM, oocytes remain at the diplotene stage of prophase Ⅰ and after 24 h of culture, the rate of maturation was found to be 58.7%. To improve the rate of maturation and subsequent fertilization, it is essential to understand the molecular mechanisms and proteins associated with meiotic maturation.

In this study, 81 differentially expressed proteins were identified by LC-MS/MS of GV- and MⅡ-stage oocytes. The results of this study was consistent with that of Wang *et al*[17] who reported that primary transporters and cation channel family members were more abundant in GV oocytes than in MⅡ oocytes.

In addition to proteins involved in the cell cycle, proteins involved in signal transduction and transcription were also identified in GV oocytes. CABLES1, Synaptonemal complex protein 1 (SCP1), and sarcoma antigen NY-SAR-48 were involved in the cell cycle. Cyclin-dependent kinase 5 and CABLES1 were cell cycle regulatory proteins that interact with both p53 and p73[18,19] and modulate the activity of female germline stem cells and oocytes. In mice, knockout of CABLES1 led to an increase in atretic immature oocytes within the ovaries and an increased occurrence of degenerating oocytes[20]. SCP1 is a key component of the protein complex that retains recombining chromosomes in prophase Ⅰ of the first meiotic stage in germ cells[21]. SCP1-deficient ovaries exhibited completion of meiotic prophase Ⅰ that endowed oocytes with the capability to orchestrate follicle assembly in rat ovaries[22].

Rho GTPase activating protein 17, signal-induced proliferation-associated 1-like protein 2 and protein phosphatase 4 were involved in cellular signal transduction. Rho GTPase activating protein acts as a molecular switch regulating the actin cytoskeleton[23]. Rho GTPase was necessary for oocyte polar body emission and spindle rotation during meiosis in mouse oocytes[24]. Protein phosphatase 4 is a member of the serine-threonine phosphatases, which hydrolyze and remove phosphate groups from phosphoproteins and therefore antagonize protein phosphorylation and have been involved in regulating oocyte meiosis in mice[25]. Protein phosphatase 4

regulates the activity of histone deacetylase 3[26] and histone deacetylase 3 is implicated in cell cycle progression, proliferation, and differentiation during oocyte maturation[27].

CASZ1, zinc finger protein 226-like protein, and sex-determining region Y protein were involved in transcription. CASZ1 is a conserved transcription factor required for vascular patterning[28]. The formation of the vascular system is important for embryonic development and homeostasis. In the absence of CASZ1, *Xenopus* embryos failed to develop a branched and lumenized vascular system, and CASZ1-depleted human endothelial cells displayed considerable changes in adhesion, morphology, and sprouting. CASZ1 was found throughout the developing myocardium and was downregulated in cells that re-enter the cell cycle[29].

Mature oocytes contain the complement of maternal proteins essential for fertilization and egg-embryo transition. In this study, the majority of proteins found to be overexpressed in MⅡ stage participated in cell cycle events including: activin receptor type-1C, KIAA0445 (Rootletin), centromere protein T, claspin, and DNA topoisomerase 2-alpha activin receptor type-1C has been detected in several types of cells including granulose cells, cumulus cells, and oocytes[30]. Activin is secreted by granulosa cells, acting on the oocyte and granulosa cells through type Ⅰ and type Ⅱ activin receptors[31]. Activin also promotes IVM and IVF in primate oocytes[32]. Centromere protein-T is a member of the centromere proteins essential for the attachment of microtubules to chromosomes, which occurs in the kinetochore[31]. Kinetochores are key structures in oocytes that control chromosome alignment leading to the completion of meiosis. Alterations of centromere proteins (in kinetochores) relate to the motion of chromosomes during pig oocyte maturation[33]. In human cells, ectopically localizing the N-termini of centromere protein-T and centromere protein-C to chromatin engages sufficient centromere components to drive the formation of pseudokinetochores that can bind microtubules and enhance chromosome segregation when the N-termini of centromere protein-T was mutated contributing to defective kinetochores[34]. Claspin is a checkpoint mediator protein that functions during cell cycle arrest in response to inhibit DNA replication. Its function is to phosphorylate and activate checkpoint kinase 1 for the regulation of DNA replication. In human cells, claspin and checkpoint kinase 1 are essential for the normal rates of replication fork progression[35]. In *Xenopus* egg extracts, Claspin-depleted extracts were not able to arrest the cell cycle in response to DNA replication[36]. DNA topoisomerase Ⅱ is an enzyme that activates DNA replication and chromosome segregation, and becomes localized and functions during oocyte maturation, egg activation, and embryo development by playing an important role in chromosome condensation and separation, and as a decatenation checkpoint during oocyte meiosis in mice[37].

Additionally, proteins involved in cellular organization, proteins involved in cellular development and transcription were also identified in MⅡ stage. High mobility group B2-like protein, ninein isoform 2, and espin-like protein were involved in cell organization. Ninein is associated with the centrosome throughout the cell cycle, where it binds and stabilizes the minus ends of microtubule anchoring them at the centrosome[38]. The espins, microfilament binding proteins, constitute an emerging family of actin-binding and actin-bundling proteins[39]. Rotatin, titin, and Delta-like protein 1 are proteins involved in cellular development. Rotatin is an essential protein for determination of the key left-right specification in vertebrate embryos. In mice, embryos deficient in rotatin showed randomized heart looping, delayed neural tube closure, and failure to undergo the critical step of axial rotation as a result of an embryonic defect[40]. Titin is a calcium-responsive protein and calcium currents are responsible for oocyte meiosis resumption. The expression of titin in human trophoblasts is recognized to participate in the processes of placentation and embryo development[41]. Delta-like protein 1 is a member of the epidermal growth factor-like family. It is widely expressed in embryonic tissues, as well as the ovaries of adult tissues[42]. The function of Delta-like protein 1 is still unknown.

In conclusion, the identification of differentially expressed proteins in GV and MⅡ stages aids our understanding of the processes of meiotic maturation and fertilization. Our current finding provides a valuable resource for further investigations into the functions of proteins specifically expressed in oocytes at different developmental stages.

Conflict of interest statement

We hereby confirm there are no conflicts of interest associated with this publication.

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Authors' contributions

Bongkoch Turathum participated in all aspects of the experiment and writing the manuscript. Sittiruk Roytrakul contributed to the proteomic analysis. Chinnarat Changsangfa, Morakot Sroyraya and Supita Tanasawet were involved in oocyte collection, IVM, protein extraction and analysis. Kulnasan Saikhun and Yindee Kitiyanant designed the experiment and contributed to the analysis and discussion of data. All authors read and approved the final manuscript.

References

[1] Coticchio G, Dal Canto M, Renzini MM, Guglielmo MC, Brambillasca F, Turchi D, et al. Oocyte maturation: Gamete-somatic cells interactions, meiotic resumption, cytoskeletal dynamics and cytoplasmic reorganization. *Hum Reprod Update* 2015; **21**(4): 427-454.

[2] Darbandi S, Darbandi M, Khorram Khorshid HR, Shirazi A, Sadeghi MR, Agarwal A, et al. Reconstruction of mammalian oocytes by germinal vesicle transfer: A systematic review. *Int J Reprod Biomed* 2017; **15**(10): 601-612.

[3] Freistedt P, Stojkovic M, Wolf E. Efficient *in vitro* production of cat embryos in modified synthetic oviduct fluid medium: Effects of season and ovarian status. *Biol Reprod* 2001; **65**(1): 9-13.

[4] Landim AF, Maziero R. Control of oocyte maturation. *Anim Reprod* 2014; **11**(3): 150-158.

[5] Chen L, Zhai L, Qu C, Zhang C, Li S, Wu F, et al. Comparative Proteomic analysis of buffalo oocytes matured *in vitro* using iTRAQ technique. *Sci Rep* 2016; **6**: 31795.

[6] Mohammadi-Sangcheshmeh A, Soleimani M, Deldar H, Salehi M, Soudi S, Hashemi SM, et al. Prediction of oocyte developmental competence in ovine using glucose-6-phosphate dehydrogenase (G6PDH) activity determined at retrieval time. *J Assist Reprod Genet* 2012; **29**(2): 153-158.

[7] Yurttas P, Morency E, Coonrod SA. Use of proteomics to identify highly abundant maternal factors that drive the egg-to-embryo transition. *Reproduction (Cambridge, England)* 2010; **139**(5): 809-823.

[8] Zhang K, Smith GW. Maternal control of early embryogenesis in mammals. *Reprod Fertil Dev* 2015; **27**(6): 880-896.

[9] Kim J, Kim JS, Jeon YJ, Yang TH, Soh Y, Lee HK, et al. Identification of maturation and protein synthesis related proteins from porcine oocytes during *in vitro* maturation. *Prot Sci* 2011; **9**: 28.

[10]Virant-Klun I, Krijgsveld J. Proteomes of animal oocytes: What can we learn for human oocytes in the *in vitro* fertilization programme? *BioMed Res Int* 2014; **2014**: 1-11.

[11]Ríos GL. Combined epidermal growth factor and hyaluronic acid supplementation of *in vitro* maturation medium and its impact on bovine oocyte proteome and competence. *Theriogenology* 2015; **83**(5): 874-880.

[12]Kołakowska J, Franczak A, Saini RKR, Souchelnytskyi S. Progress and challenges in the proteomics of domestic pig in research on the female reproductive system. *J Elem* 2016; **21**(4): 1055-1069.

[13]Wang B, Pfeiffer MJ, Drexler HC, Fuellen G, Boiani M. Proteomic analysis of mouse oocytes identifies PRMT7 as a reprogramming factor that replaces SOX2 in the induction of pluripotent stem cells. *J Proteome Res* 2016; **15**(8): 2407-2421.

[14]Trounson A, Wood C, Kausche A. *In vitro* maturation and the fertilization and developmental competence of oocytes recovered from untreated polycystic ovarian patients. *Fertil Steril* 1994; **62**(2): 353-362.

[15]Turathum B, Roytrakul S, Changsangfa C, Sroyraya M, Tanasawet S, Kitiyanant Y, et al. Missing and overexpressing proteins in domestic cat oocytes following vitrification and *in vitro* maturation as revealed by proteomic analysis. *Biol Res* 2018; **51**(1): 27-37.

[16]Maksup S, Roytrakul S, Supaibulwatana K. Physiological and comparative proteomic analyses of Thai jasmine rice and two check cultivars in response to drought stress. *J Plant Interact* 2014; **9**(1): 43-55.

[17]Wang S, Kou Z, Jing Z, Zhang Y, Guo X, Dong M, et al. Proteome of mouse oocytes at different developmental stages. *Proc Natl Acad Sci* 2010; **107**(41): 17639-17644.

[18]Hernández-Ramírez LC, Gam R, Valdes N, Lodish MB, Pankratz N, Balsalobre A, et al. Loss-of-function mutations in the *CABLES1* gene are a novel cause of Cushing's disease. *Endocr Relat Cancer* 2017; **24**(8): 379-392.

[19]Wang N, Guo L, Rueda BR, Tilly JL. Cables1 protects p63 from proteasomal degradation to ensure deletion of cells after genotoxic stress. *EMBO Rep* 2010; **11**(8): 633-639.

[20]Lee HJ, Sakamoto H, Luo H, Skaznik-Wikiel ME, Friel AM, Niikura T, et al. Loss of CABLES1, a cyclin-dependent kinase-interacting protein that inhibits cell cycle progression, results in germline expansion at the expense of oocyte quality in adult female mice. *Cell Cycle* 2007; **6**(21): 2678-2684.

[21]Panula S, Medrano JV, Kee K, Bergstrom R, Nguyen HN, Byers B, et al. Human germ cell differentiation from fetal- and adult-derived induced pluripotent stem cells. *Hum Mol Genet* 2010; **20**: 752-762.

[22]Paredes A, Garcia-Rudaz C, Kerr B, Tapia V, Dissen GA, Costa ME, et al. Loss of synaptonemal complex protein-1, a synaptonemal complex protein, contributes to the initiation of follicular assembly in the developing rat ovary. *Endocrinology* 2005; **146**(12): 5267-5277.

[23]Hodge RG, Ridley AJ. Regulating Rho GTPases and their regulators. *Nat Rev Mol Cell Biol* 2016; **17**(8): 1-15.

[24]Duan X, Liu J, Dai XX, Liu HL, Cui XS, Kim NH, et al. Rho-GTPase effector ROCK phosphorylates cofilin in actin-meditated cytokinesis during mouse oocyte meiosis. *Biol Reprod* 2014; **90**: 37.

[25]Smith GD, Sadhu A, Mathies S, Wolf DP. Characterization of protein phosphatases in mouse oocytes. *Dev Biol* 1998; **204**(2): 537-549.

[26]Zhang X, Ozawa Y, Lee H, Wen YD, Tan TH, Wadzinski BE, et al. Histone deacetylase 3 (HDAC3) activity is regulated by interaction with protein serine/threonine phosphatase 4. *Genes Dev* 2005; **19**(7): 827-839.

[27]Li X, Liu X, Gao M, Han L, Qiu D, Wang H, et al. HDAC3 promotes meiotic apparatus assembly in mouse oocytes by modulating tubulin acetylation. *Development* 2017; **144**: 3789-3797.

[28]Charpentier MS, Christine KS, Amin NM, Dorr KM, Kushner EJ, Bautch VL, et al. CASZ1 promotes vascular assembly and morphogenesis through the direct regulation of an EGFL7/RhoA-mediated pathway. *Dev Cell* 2013; **25**(2): 132-143.

[29]Amin NM, Gibbs D, Conlon FL. Differential regulation of CASZ1 protein expression during cardiac and skeletal muscle development. *Dev Dyn* 2014; **243**(7): 948-956.

[30]Silva C, Groome N, Knight P. Immunohistochemical localization of inhibin/activin alpha, beta A and beta B subunits and follistatin in bovine oocytes during *in vitro* maturation and fertilization. *Reproduction (Cambridge, England)* 2003; **125**(1): 33-42.

[31]Matzuk MM, Li Q. How the oocyte influences follicular cell function and why. In: Coticchio G, Albertini DF, De Santis L. (eds.) *Oogenesis*. London: Springer; 2013, p. 75-92.

[32]Heikinheimo O, Gibbons WE. The molecular mechanisms of oocyte maturation and early embryonic development are unveiling new insights into reproductive medicine. *Mol Hum Reprod* 1998; **4**(8): 745-756.

[33]Nishino T, Rago F, Hori T, Tomii K, Cheeseman IM, Fukagawa T. CENP-T provides a structural platform for outer kinetochore assembly. *EMBO J* 2013; **32**: 424-436.

[34]Westhorpe FG, Straight AF. Functions of the centromere and kinetochore in chromosome segregation. *Curr Opin Cell Biol* 2013; **25**(3): 334-340.

[35]Scorah J, McGowan CH. Claspin and Chk1 regulate replication fork stability by different mechanisms. *Cell Cycle* 2009; **8**(7): 1036-1043.

[36]Kumagai A, Dunphy WG. Claspin, a novel protein required for the activation of Chk1 during a DNA replication checkpoint response in *Xenopus* egg extracts. *Mol Cell* 2000; **6**(4): 839-849.

[37]Li XM, Yu C, Wang ZW, Zhang YL, Liu XM, Zhou D, et al. DNA topoisomerase][is dispensable for oocyte meiotic resumption but is essential for meiotic chromosome condensation and separation in mice. *Biol Reprod* 2013; **89**(5): 118.

[38]Goto H, Inoko A, Inagaki M. Cell cycle progression by the repression of primary cilia formation in proliferating cells. *Cell Mol Life Sci* 2013; **70**: 3893-3905.

[39]Yanagishita T, Yajima I, Kumasaka M, Kawamoto Y, Tsuzuki T, Matsumoto Y, et al. Actin-binding protein, espin: A novel metastatic regulator for melanoma. *Mol Cancer Res* 2014; **12**(3): 440-446.

[40]Chartier S, Alby C, Boutaud L, Thomas S, Elkhartoufi N, Martinovic J, et al. A neuropathological study of novel *RTTN* gene mutations causing a familial microcephaly with simplified gyral pattern. *Birth Defects Res* 2018; **110**(7): 598-602.

[41]Du MR, Zhou WH, Yan FT, Zhu XY, He YY, Yang JY, et al. Cyclosporine A induces titin expression *via* MAPK/ERK signalling and improves proliferative and invasive potential of human trophoblast cells. *Hum Reprod* 2007; **22**(9): 2528-2537.

[42]Friedrichsen BN, Carlsson C, Moldrup A, Michelsen B, Jensen CH, Teisner B, et al. Expression, biosynthesis and release of preadipocyte factor-1/delta-like protein/fetal antigen-1 in pancreatic beta-cells: Possible physiological implications. *J Endocrinol* 2003; **176**(2): 257-266.