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'Cell cycle' and 'cell death'- related genes are differentially expressed during long – term in vitro real-time cultivation of porcine oviductal epithelial cells

Magdalena Kulus[1], Jakub Kulus[1], Małgorzata Popis[2], Blanka Borowiec[2,3], Katarzyna Stefańska[3], Piotr Celichowski[3], Mariusz J. Nawrocki[2], Klaus P. Brüssow[1], Bartosz Kempisty[2,3,4], Michal Jeseta[4], Paweł Antosik[1], Dorota Bukowska[1]

Abstract

Alterations in cells depend on their genetic material, its activation and translation of the products. The genes responsible for the cell cycle processes and apoptosis of porcine oviductal cells have been presented in our study. The processes occurring in the reproductive system of females are extremely complex and require in-depth knowledge. Thanks to *in vitro* studies on the fallopian tube epithelium cells, we can get closer to understanding the biochemical and morphological changes occurring in mammalian organisms. Our research was conducted on fallopian tubes obtained from commercially bred pigs and its aim was to assess the expression profile of genes responsible for the most important processes of cellular life. Cell cultures were carried out for 30 days, with the obtained cells subjected to molecular analysis. We have shown significant regulation of "cell death" and "cell cycle" genes, some of which are related to the reproductive system. The alterations in transcriptomic profile and mutual relations between the genes were analyzed and related to the literature findings. The knowledge gained could help in identifying new potential markers of the *in vitro* occurrence of processes described by the ontology groups of interest.

Running title: pig, oocytes, microarray assays, *in vitro* maturation (IVM)

Keywords: Regulation of metabolic enzymes receptor signaling pathway

[1]Veterinary Centre, Nicolaus Copernicus University in Torun, Torun, Poland
[2]Department of Anatomy, Poznan University of Medical Sciences, Poznan, Poland
[3]Department of Histology and Embryology, Poznan University of Medical Sciences, Poznan, Poland
[4]Department of Obstetrics and Gynecology, University Hospital and Masaryk University, Brno, Czech Republic
*** Correspondence:** bkempisty@ump.edu.pl
Full list of author information is available at the end of article

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Introduction

Due to their utmost importance, the undisturbed processes of cell life are subject to continuous genetic control, including segregation of material, correctness of subsequent events and cell division. The cell cycle is therefore an extremely complex process, with its mechanisms focused on biochemical and morphological changes of replicating cells [1]. In contrast, the permanent retention of the vital functions of cells causes their irreversible death. It can take place due to accidental damage or a physiological mechanism called apoptosis [2].

The processes associated with the femalereproductive system are very complicateddue to a vastness of biochemical changes. Therefore, they have not yet been fully understood. Proper functioning of the reproductive systems requires many hormonal changes in the body, especially the effective feedback of the hypothalamus, pituitary gland and gonads [3]. The transport of the oocyte through the fallopian tube and the interaction between the embryo and maternal tissues are of great importance in the context of effective fertilization. Morphological and biochemical changes in the oviductal cells are mainlycaused by processes occurring after ovulation, related to the influence of both oocyte and embryo on oviductal cells [4]. General changes occurring at this stage are relatively well described, but the changes on the molecular ground are not fully known. Therefore, in our study, we described the changes in expressionof genes associated with "cell cycle" and "cell death"gene ontology processes.

Recent studies indicate the possibility of short- and long-term*in vitro* culture of oviductal cells, including epithelial cells. This gives an opportunity to observe many morphological and biochemical changes during in vitro culture (IVC) processes of oviductal epithelial cells (OECs). These changes are related to the modification of genes and protein expression [5], including the mechanisms of oocyte influence on fallopian tube epithelial cells in relation to both cell cycle and cell death. The occurrence of the above changes *in vivo* enables effective fertilization and later development of the embryo through pathways that are not fully discovered. Therefore, the analysis of these *in vitro* changes can contribute a great deal to the understanding of the interactions between the mother and embryo.

Current studies conducted by our team (not published data) indicate the possibility of effective *in vitro* culture of epithelial cells of the fallopian tube and even changes in their proliferation. Changes in cell morphology, cell growth and differentiation may be related to cell cycle and cell death processes. Therefore, the aim of our work was to find new cellular markers associatedwith cell cycle and cell death processes in *in vitro* OEC cultures.

Material and methods

Animals

The animals in our study- crossbred gilts (n=45) at the age of around 9 months, came from a commercial breeding herd. The selected individuals expressed two regular estrus cycles. All the animals were checked daily for estrus behavior and were slaughtered after reaching the anestrus phase of the estrus cycle. The uteri were then transported to the laboratory within 30 min at 38°C.

Oviductal epithelial cell (OEC) selection and culture

Oviducts were washed twice in Dulbecco's phosphate buffered saline (PBS) (137 mM NaCl, 27 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, pH 7.4). Epithelial cells were surgically removed using sterile blades. Then, the epithelium was incubated with collagenase I (Sigma Aldrich, Madison, USA), 1mg/mL inDulbecco's modified Eagle's medium (DMEM; Sigma Aldrich, Madison, USA) for 1 h at 37°C. The cell suspension obtained from this digestion was filtered through 40 µm pore size strainer to remove blood and single cells. The residue was collected by rinsing the strainer with DMEM. The cell samples were then centrifuged (200 x g, 10 min.). Next, they were washed in PBS and centrifuged again. Later, they were incubated with 0.5% Trypsin/EDTA (Sigma Aldrich, Madison, USA) at 37°C for 10 min. The reaction was stopped with fetal calf serum (FCS; Sigma Aldrich, Madison, USA). After incubation, cells where filtered and centrifuged for the last time. The final cell pellet was suspended in DMEM supplemented with 10% FCS, 100U/mL penicillin, 100 µg/mL streptomycin and 1µg/mL amphotericin B. The cells were cultured at 37°C in a humidified atmosphere of 5% CO_2. Once the OEC cultures attained 70–80% confluency, they were passaged by washing with PBS, digestion with 0.025% Trypsin/EDTA, neutralization by a 0.0125% trypsin inhibitor (Cascade Biologics, Portland, USA), centrifugation, and resuspension at a seeding density of $2x10^4$ cells/cm^2. The culture medium was changed every three days. The culture lasted 30 days.

RNA extraction from porcine oviductal epithelial cells (OECs)

Oviductal epithelial cells from specific time periods were pooled into three independent samples for each experimental group. Total RNA was extracted from samples using TRI Reagent (Sigma, St Louis, MO, USA) and RNeasy MinElute cleanup Kit (Qiagen, Hilden, Germany). The amount of total mRNA was determined from the optical density at 260 nm, and the RNA purity was estimated using the 260/280 nm absorption ratio (higher than 1.8) (NanoDrop spectrophotometer, Thermo Scientific, ALAB, Po-

land). The RNA integrity and quality were checked on a Bioanalyzer 2100 (Agilent Technologies, Inc., Santa Clara, CA, USA). The resulting RNA integrity numbers (RINs) were between 8.5 and 10, with an average of 9.2 (Agilent Technologies, Inc., Santa Clara, CA, USA). The RNA in each sample was diluted to a concentration of 100 ng/µl with an OD260/OD280 ratio of 1.8/2.0. From each RNA sample, 500 ng of RNA was taken for microarray expression assays.

Microarray expression analysis and statistics

Total RNA (100 ng) from each pooled sample was subjected to two rounds of sense cDNA amplification (Ambion® WT Expression Kit). The obtained cDNA was used for biotin labeling and fragmentation by Affymetrix GeneChip® WT Terminal Labeling and Hybridization (Affymetrix). Biotin-labeled fragments of cDNA (5.5 µg) were hybridized to the Affymetrix® Porcine Gene 1.1 ST Array Strip (48°C/20 h). Microarrays were then washed and stained according to the technical protocol using the Affymetrix GeneAtlas Fluidics Station. The array strips were scanned with the use of Imaging Station of the GeneAtlas System. Preliminary analysis of the scanned chips was performed using Affymetrix GeneAtlas™ Operating Software. The quality of gene expression data was confirmed according to the quality control criteria provided by the software. The obtained CEL files were imported into downstream data analysis software.

All of the presented analyses and graphs were performed using Bioconductor and R programming languages. Each CEL file was merged with a description file. To correct background, normalize, and summarize the results, we used the Robust Multi-array Averaging (RMA) algorithm. To determine the statistical significance of the analyzed genes, moderated t-statistics from the empirical Bayes method were performed. The obtained p-value was corrected for multiple comparisons using Benjamini and Hochberg's false discovery rate. The selection of significantly altered genes was based on a p-value beneath 0.05 and expression higher than two-fold.

Differentially expressed genes were subjected to selection by examination of their involvement in the gene ontologies of interest. The differentially expressed gene lists (separate for up- and down-regulated genes) were uploaded to the DAVID software (Database for Annotation, Visualization and Integrated Discovery) [6], where genes belonging to "cell cycle" and "cell death" GO BP terms were extracted. Expression data of these genes was also subjected to a hierarchical clusterization procedure, with results presented as a heat map.

Subsequently, we analyzed the relation between the genes belonging to chosen GO terms with theGOplot package [7]. The GoPlot package calculated the z-score: the number of up- regulated genes minus the number of down- regulated genes divided by the square root of the count. This information allowed estimating the change course of each gene-ontology term.

Interactions between differentially expressed genes/proteins belonging to the studied gene ontology group were investigated using the STRING10 software (Search Tool for the Retrieval of Interacting Genes) [8]. The list of gene names was used as a query for an interaction prediction. The search criteria were based on co-occurrences of genes/proteins in scientific texts (text mining), co-expression, and experimentally observed interactions. The results of such analyses generated a gene/protein interaction network where the intensity of the edges reflected the strength of the interaction score.

Finally, the functional interactions between genes belonging to the chosen GO BP terms were investigated using the REACTOME FIViz application to the Cytoscape 3.6.0 software. The ReactomeFIViz app is designed to find pathways and network patterns related to cancer and other types of diseases. This app accesses the pathways stored in the Reactome database, allowing to perform pathway enrichment analysis for a set of genes, visualize hit pathways using manually laid-out pathway diagrams directly in Cytoscape and investigate functional relationships among the genes in hit pathways. The app can also access the Reactome Functional Interaction (FI) network, a highly reliable, manually curated pathway-based protein functional interaction network covering over 60% of human proteins.

Ethical approval

The research related to animal use has been complied with all the relevant national regulations and instructional policies for the care and use of animals. Bioethical Committee approval no. 83/2012/DNT.

Results

Whole transcriptome profiling using Affymetrix microarrays allowedus to analyze the gene expression changes between 7, 15 and 30 days of porcine oviductal epithelial cells culture. Using the Affymetrix® Porcine Gene 1.1 ST Array Strip, we examined the expression of 12257 transcripts. Genes with fold change higher than abs (2) and with a corrected p-value lower than 0.05 were considered as differentially expressed. This set of genes consists of 2533 different transcripts.

DAVID (Database for Annotation, Visualization and Integrated Discovery) software was used for extraction of gene ontology biological process terms (GO BP) that contains differently expressed transcripts. Up and down regulated gene sets were subjected to the DAVID search separately,with only the sets of adj. p value lower than 0.05 selected. The DAVID software analysis showed that the differently expressed genes belonged to 657 Gene ontology

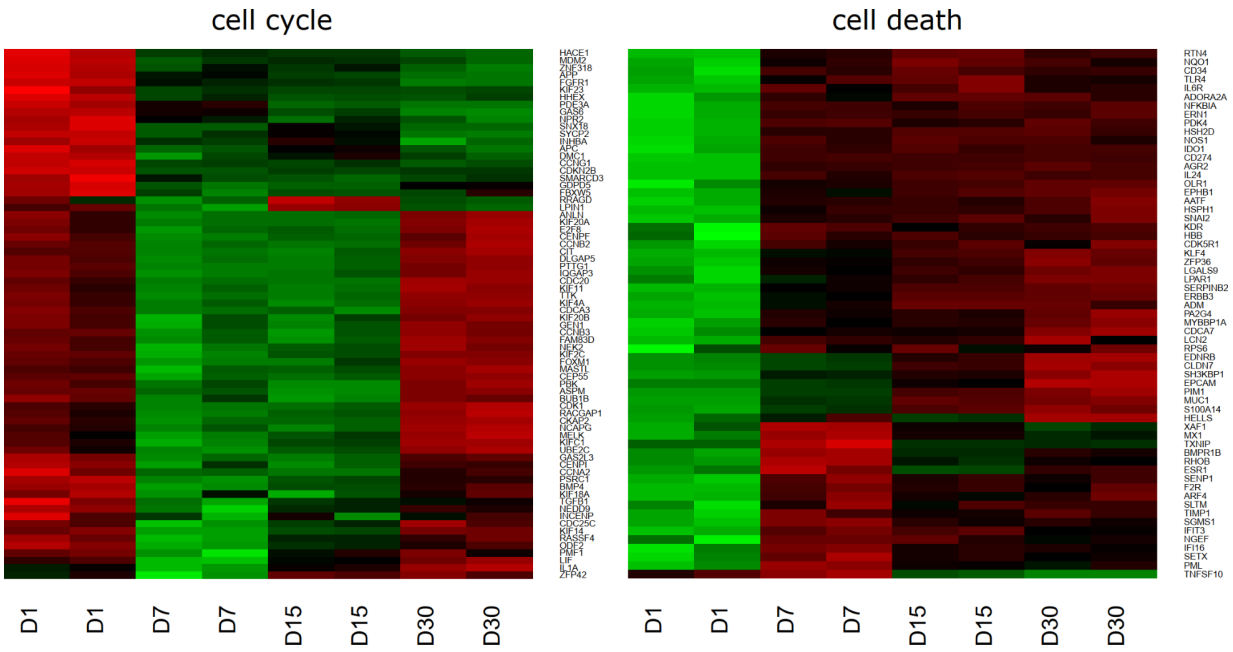

FIGURE 1 Heat map representation of differentially expressed genes belonging to the to "cell cycle" and "cell death" GO BP terms. Arbitrary signal intensity acquired from microarray analysis is represented by colors (green, higher; red, lower expression). Log2 signal intensity values for any single gene were resized to Row Z-Score scale (from -2, the lowest expression to +2, the highest expression for single gene)

TABLE 1 Gene symbols, fold changes in expression, Entrez gene IDs and corrected p values of studied genes

GENE SYMBOL	FOLD. CHANGE D15/D7	FOLD. CHANGE D30/D7	FOLD. CHANGE D30/D15	ADJUSTED P.VALUE D15/D7	ADJUSTED P.VALUE D30/D7	ADJUSTED P.VALUE D30/D15	GENEID
SERPINB2	0,041199	0,012677	0,008239	4,60E-06	7,45E-07	2,31E-07	1,01E+08
CD274	0,04424	0,04443	0,041834	2,50E-06	7,45E-07	2,31E-07	574058
TXNIP	0,082179	0,64453	0,63523	1,11E-05	0,024019	0,017645	733688
IL24	0,083371	0,062192	0,071399	9,50E-06	3,21E-06	1,76E-06	1,01E+08
HSH2D	0,123575	0,083464	0,094409	2,25E-05	5,06E-06	3,79E-06	1,01E+08
IDO1	0,134544	0,12111	0,123331	7,22E-05	3,53E-05	2,36E-05	1,01E+08
MX1	0,147313	0,355975	0,489162	1,87E-05	0,000197	0,00096	397128
IFIT3	0,172101	0,195012	0,31317	1,87E-05	1,28E-05	4,60E-05	1E+08
SNAI2	0,183766	0,134521	0,130079	0,000411	0,000129	7,85E-05	641345
XAF1	0,196155	0,456355	0,66362	0,000162	0,004319	0,06338	---
PTTG1	5,573407	4,839927	0,818891	5,27E-05	4,98E-05	0,269018	397015
CDKN2B	5,906076	5,279248	5,031711	6,22E-06	3,39E-06	1,81E-06	397227
KIF20A	6,182894	6,001182	0,588195	0,000319	0,000249	0,063892	1,01E+08
KIF23	6,49686	7,105965	7,104752	0,000647	0,000382	0,000271	1,01E+08
TTK	6,595833	6,064142	0,629174	0,000194	0,000163	0,078507	---
DLGAP5	7,403844	6,551599	0,699776	9,17E-05	8,22E-05	0,131054	1,01E+08
GAS2L3	7,739168	7,035903	1,559417	8,27E-05	6,71E-05	0,064706	1E+08
UBE2C	8,523298	4,717957	0,372658	0,000914	0,00366	0,025648	1E+08
HHEX	9,447447	11,82395	11,30739	2,69E-05	9,59E-06	6,25E-06	397232
CENPF	9,506242	6,960497	0,773239	0,000484	0,000742	0,52224	1,01E+08

terms. In this paper, we focused on 133 genes (61 downregulated and 72 upregulated) belonging to "cell cycle" and "cell death" GO BP terms.

These sets of genes were subjected to hierarchical clusterization procedure and presented as heatmaps (**Fig. 1**). The gene symbols, fold changes in

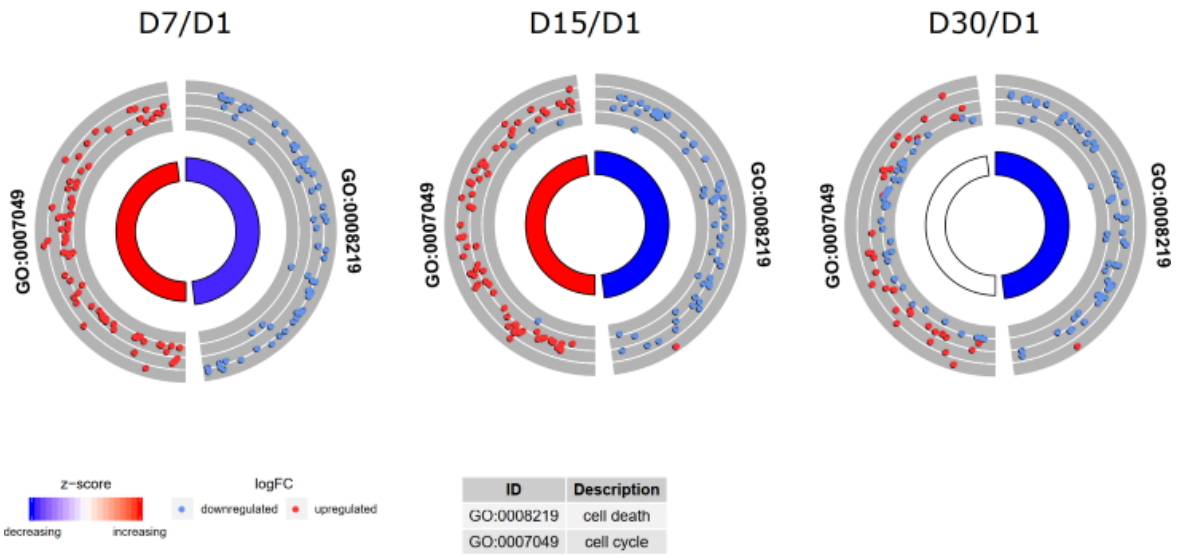

FIGURE 2 The circle plot showing the differently expressed genes and z-score of "cell cycle" and "cell death" GO BP terms. The outer circle shows a scatter plot for each term of the fold change of the assigned genes. Red circles display upregulation and blue ones downregulation. The inner circle shows the z-score of each GO BP term. The width of the each bar corresponds to the number of genes within GO BP term and the color corresponds to the z-score

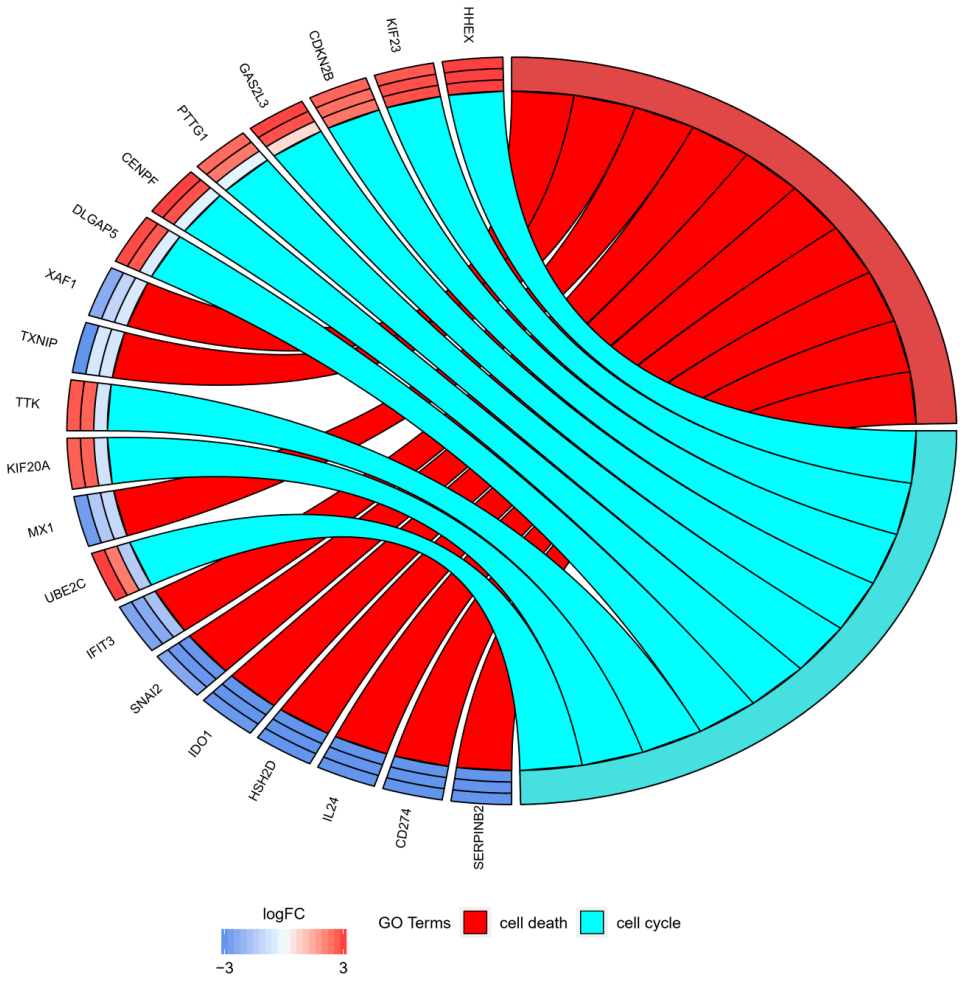

FIGURE 3 The representation of the mutual relationship 10 most upregulated and 10 most downregulated genes that belongs to the to "cell cycle" and "cell death" GO BP terms. The ribbons indicate which gene belongs to which categories. The middle circle represents logarithm from fold change (LogFC) between D7/D1, D15/D1 and D30/D1 respectively. The genes were sorted by logFC from most to least changed gene

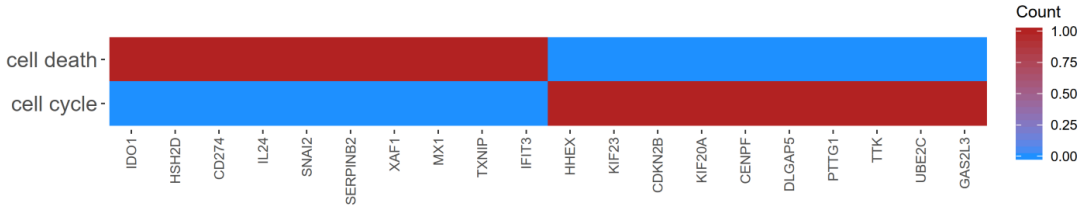

FIGURE 4 Heatmap showing the gene occurrence between 10 most upregulated and 10 most downregulated genes that belongs "cell cycle" and "cell death" GO BP terms. The red color is associated with gene occurrence in the GO Term. The intensity of the color is corresponding to amount of GO BP terms that each gene belongs to

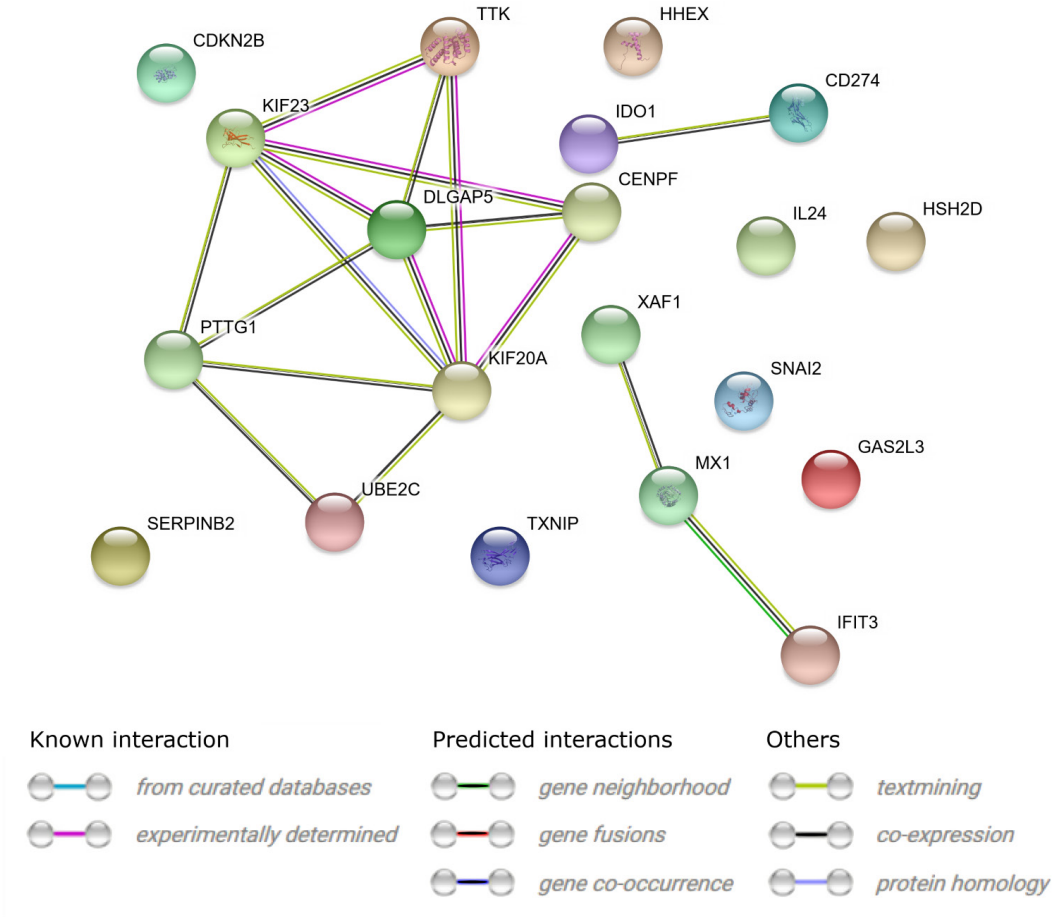

FIGURE 5 STRING-generated interaction occurrence between 10 most upregulated and 10 most dowregulated genes that belongs to "cell cycle" and "cell death" GO BP terms. The intensity of the edges reflects the strength of interaction score

expression, Entrez gene IDs and corrected p-values of these genes were shown in **table1**.

The enrichment of each GO BP term was calculated as a z-score and shown on the circle diagram (**Fig. 2**).

From the differently expressed genes belonging to the studied GO BP terms, we chose 10 most downregulated and 10 most upregulated genes for further analysis. In Gene Ontology database, genes that formed one particular GO group can also belong to other different GO term categories. For this reason, we explored the gene intersections between selected GO BP terms. The relation between those GO BP terms was presented as a circle plot (**Fig. 3**) as well as a heatmap (**Fig. 4**). However none of differently expressed genes were common for these GO BP terms.

STRING interaction network was generated among differentially expressed genes belonging to each of the selected GO BP terms. Using such prediction method provided us with a molecular interaction network formed between protein products of studied genes (**Fig. 5**). Finally, we investigated the functional interactions between chosen genes with REACTOME FIViz app to Cytoscape 3.6.0 software. REACTOME database only found any interactions between PTTG1 and UBE2C genes.

Discussion

The complicated cellular processes that make up the cell cycle are focused on the progress of biochemical and morphological changes during subsequent replications. Genetic control at the level of material segregation and correctness of subsequent events, including cell division, is essential for the life of the cell and the whole organism [1]. In contrast, the cell death process is defined as the permanent cessation of all cell functions, e.g. during the loss of cell membrane integrity or the complete fragmentation of a cell including its nuclei. The cell death can be either accidental or programmed [2].

Thanks to our research on *in vitro*culturedporcine oviductal epithelial cells (OECs), it was possible to recognize the genes belonging to"cell cycle" and "cell death" ontological groups, as they showed high values of expression changes.We analyzed these genes, comparing their trends, mutual relations and level of expression. It is possible that these studies could serve as potential data for establishing new cellular markers of*in vitro* cultures. So far, the molecular mechanisms controlling changes in the mucous membrane of the fallopian tube have been little known. Hence, our research may bring a new value in a more detailed description.

During the in vitro culture (IVC) of OECs, we analyzed their transcriptomic profile at different time intervals: 1, 7, 15 and 30 days of cultivation. For both ontological groups, we compiled heat maps, which illustrate the distribution of expression in particular days of cultivation. As can be seen in **figure 1**, genes from the "cell death" group showed significant up – regulation on the first day of cultivation and then went into the down – regulation mode. This may be due to acute cell death on the first day, caused by mechanical damage during culture preparation, with lack of relation to cell death on the subsequent days. However, the ontological group associated with the "cell cycle"was characterized by the opposite expression in relation to "cell death" (mainly down – regulation)on the first day,which changed into up – regulation in subsequent culture days. Only at the end of cultivation, more precisely at day 30, downregulation was observed again. Of the 20 genes we have selected, 10 belonged to the "cell cycle" group and 10 to "cell death". Each gene belongs to only one ontological group (**Fig. 4**), with expression patterns corresponding to that the group (**Fig. 3**).

Within the "cell death" group, we distinguished genes typically associated with apoptotic processes (4 genes), immune response (2 genes), antiviral effect (2 genes) and metabolic mechanisms (2 genes).

The first gene, associated with apoptotic processes, is *XAF1*. *XIAP associated factor 1* is a pro – apoptotic protein. Its activation sensitizes cancer cells to apoptotic stimuli [9]. It is an essential factor in inhibiting the growth of cancer cells and research is currently underway on its medical use [10]. In our studies this gene, as a member of the "cell death" group, showed down – regulation. Also belonging to the "cell death" GO, the *IL24* (*interleukin 24*) gene, known for inducing apoptotic processes, was also downregulated in our study. It has recently been shown that *IL24* is one of the key regulators of atresia in the ovarian follicles of pigs [11].

The next two genes are related to apoptotic processes but also contribute to cell metabolism. *HSH2D* gene exhibits functions as an adapter protein in tyrosine kinase signaling, cytokine signaling, as well as cytoskeleton reorganization, mainly in hematopoietic cells [12]. It is worth emphasizing that it can be a modulator of apoptotic response through its ability to influence mitochondrial stability [13], which coincides with our results as it belongs to the "cell death" group. *SNAI2* (*snail family transcriptional repressor 2*), as a transcription factor, occurs quite widely in various tissues, especially in subcutaneous adipose tissue in pigs. Downregulation of this gene has also been reported in studies on porcine skin – derived progenitor (pSKP) cultures, and the level of its mRNA has changed significantly in response to mitogen or growth factor stimulation by pSKP cells [14]. *SNAI2* exhibits regulatory action over cell movement, promotes epithelial – mesenchymal transformations and participates in anti – apoptotic processes [15]. The results suggest that it may be one of the main regulators of the pSKPs stem cell niche *in vitro* [14].

IDO1 and *CD274* genes exhibit immunomodulatory properties. The *IDO1* gene (*2,3-dioxygenase 1 indolamine*) is characterized by immunomodulatory actionthrough participation in tryptophan metabolism. It has been shown that increased *IDO* activity after LPS stimulation of porcine cells may be the basis for immune function of *IDO* [16]. Another gene belonging to this group is *CD274*, which also has immunomodulating properties. As a gene, it codes programmed death ligand 1 (PD-L1), with its expression depending on the concentration of oxygen [17]. Expression of this gene was demonstrated in the study of macrophages cultivated in co–culture with mesenchymal stem cells, where the phenotype of the former was changed from inflammatory to anti – inflammatory [18].

Another gene from the cell death group, exhibiting antiviral properties, is *IFIT3* (*interferon induced protein with tetratricopeptide repeats 3*), the expression of which is shown in different tissues of the swine [19]. Studies have been carried out using porcine alveolar macrophages that have been infected with swine influenza virus. Significant up – regulation of *IFIT3* was demonstrated in these cells, which correlated with the inhibition of virus proliferation [20]. Like the *IFIT3* gene, the *MX1* gene (*MX dynamin like GTPase 1*) is associated with antiviral activity. It is widespread in tissues and its action consists of inhibiting virus replication and blocking the endo-

cytic transport of viral particles [21]. Studies on cell lines show significant inhibition of classical swine fever virus through porcine *MX1*, suggesting its potential use in classical swine influenza therapy [22].

The genes associated with molecular mechanisms of cells in our study are *SERPINB2* and *TXNIP*. *SERPINB2*, belonging to the cell death group, also known as the *plasminogen activator inhibitor type 2* (*PAI-2*), as it inhibits serine protease plasminogen activators [23]. Its main cytoplasmic localization indicates intracellular function. It was shown that it exhibits cytoprotective propertiesin neurons. It is one of the most regulated proteins after cellular stress [24]. The *TXNIP* gene encodes thioredoxin - interacting proteins, which are important for glucose metabolism [25]. The relationship between the expression of this gene and hormonal activity of estrogens was demonstrated in Ożegowska's work [26], porcine oocytes stimulated with this hormone exhibited down-regulation of TXNIP. This gene may be a good candidate for describing *in vitro* processes of epithelial cells of the fallopian tube.

The genes that make up the "cell cycle" group in our research showed stronger or weaker links with mitosis, meiosis or intracellular organization processes.

The first, *PTTG1* (*pituitary tumor – transforming 1*) plays an important role in mitosis, cellular transformation, DNA repair and transcription regulation [27]. In studies on porcine oocytes and early stages of porcine embryos, it has been shown that this gene may have an important connection with the process of maternal-to-embryonic transition (MET) [28]. During this process, the germ genome is activated,beginning the transcription. This gene can play an important role in the early development of mammalian embryogenesis.

The *CDKN2B* gene (*cyclin dependent kinase inhibitor 2B*) is a strong cell cycle inhibitorprotein, acting jointly with *CDK4* and *CDK6*. It may cause the cell cycle retention effect of TGF-beta [29,30].

The next gene from this GO is*kinesin family member 20A* (*KIF20A*). This gene is a mammalian mitotic kinesin – like motor protein of the kinesin superfamily proteins (KIFs). It plays a role in the dynamics of the Golgi Apparatus and in the regulation of cell cycle [31]. Recently, it has also been proven to take an active part in the maturation of pig oocytes and embryos [32]. Similarly, *KIF23* (*kinesin family member 23*) is involved in organelle processes, their transport, as well as the movement of microtubules and chromosomes during the divisions [33]. It exhibits expression in ovary and others tissues in pigs [19].

Another gene belonging to this group is *TTK* (*TTK protein kinase*), involved in aligning chromosomes during mitosis and duplicationof centrosomes. It is also involved in cell proliferation. Its expression in ovary, spleen and other organs in pigs has been shown [19,34]. The *DLGAP5* gene (*DLG associated protein 5*) encodes a building material of kinetochore, as well as stabilizes the spindle apparatus and microtubules [34]. It belongs to the "cell cycle" ontological group and was upregulated during *in vitro* culture of porcine oviduct epithelial cells. In pigs, its expression is identified in several tissues, including the ovaries [19]. Another gene in this group, *GAS2L3* (*growth arrest specific 2 like 3*), is also associated with chromosome segregation and alignment during cell division, particularly biding of microtubules [35,36]. In our study, we found that its expression increased during *in vitro* culture.

Thenext gene, *UBE2C* (ubiquitin conjugating enzyme E2 C), which in our *in vitro* cultures of OECs showed upregulation. This gene functions as a factor involved in mitotic processes butits significant role in the course of meiosis of maturing pig oocytes has also been found [37]. The maintenance of hematopoietic stem cell (HSC) populations throughout life is important for the hematopoietic processes. Genetic regulation of their self – renewal is subject to the *HHEX* (*hematopoietically expressed homeobox*) transcription factor [38], the expression of which wasdescribed in the endothelium of the dorsal aorta (DA) during development [39]. Linked to the cell cycle, the *CENPF* gene (*centromere protein F*) has shown up – regulationin our studies. This gene is responsible for the formation of centromere proteins during attachment of microtubes to chromosomes. Their expression was demonstrated during all stages of porcine oocytes meiosis [40].

The network of STRING generated interactions (**Fig. 5**) presents mutual relations between genes. In our study, genes participating in the immune response (*IDO1* and *CD274*) showed relations, including co-expression. In addition, similar relationships were possible in the case of *IFIT3* and *MX1*genes, which have antiviral properties. It is also worth paying attention to the interrelationship of 7 genes closely related to cell division processes.

Thus, our research shows groups of genes that participate in opposing cellular processes. Some of them were closely related to the reproductive system, while others were responsible for normal physiological changes of various tissues. Significant changes in the expression of the selected genes may therefore indicate their potential as markers of physiologychanges in the *in vitro* culturesof OECs. However, in order to preciselyunderstand all of these mechanisms that control cells in cultures and in living organisms, further research will be necessary.

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Corresponding author
Bartosz Kempisty PhD, Department of Histology and Embryology, Department of Anatomy, Poznań University of Medical Sciences, 6 Święcickiego St., 60-781 Poznań, Poland Tel./Fax: +48 61 8546418 / +48 61 8546440, e-mail: bkempisty@ump.edu.pl.

Conflict of interest statement
The authors declare they have no conflict of interest.

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