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"Cell cycle process", "cell division" and "cell proliferation" belong to ontology groups highly regulated during long–term culture of porcine oviductal epithelial cells

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Abstract

Morphological and biochemical changes in the cells surrounding the oocyte seem to be extremely important in an effective fertilization process. Thanks to advanced cell culture techniques, as well as biochemical and bioinformatics analyses, we can partly imitate the phenomena occurring in the living organism. Previous studies showed a possibility of short – and long – term OEC in vitro cultivation, during which these cells have shown to have significant proliferation and expression of genes responsible for differentiation. Our research was aimed at maintaining a culture of porcine oviduct epithelial cells and analyzing their gene expression profile. The study employed cross-bred gilts at the age of about 9 months, obtained from commercial herds. With the use of Affymetrix® Porcine Gene 1.1 ST Array Strip, we have examined the expression of 12257 transcripts. Genes with fold change higher than abs (2) and with corrected p-value lower than 0.05 were considered as differentially expressed. We chose 20 genes with the most marked expression (10 up – regulated, 10 down – regulated) for further investigation in the context of literature sources. These genes belonged to three ontological groups: "cell cycle process", "cell division" and "cell proliferation". The results obtained from these studies may be the basis for further molecular analyses.

Running title: Regulation of metabolic enzymes receptor signaling pathway

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

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Introduction

The processes taking place in the female reproductive system cells are very complicated, regulated in many ways and have not yet been fully understood. However, morphological and biochemical changes affecting the cells lining the pathways used to transport oocytes and fertilized zygotes seem to be extremely important in successful fertilization process [1]. It is believed that these changes are regulated by processes such as the ovulation and interaction of maternal tissues with the embryo, including implantation. These processes are stimulated by the hormones of hypothalamus and pituitary gland, as well as through cellular interactions between the mucous membrane of the fallopian tubes and oocytes [2]. Recently, attempts have been made to propagate porcine epithelial cell *in vitro* cultures, which brings a wide range of opportunities to learn about the pathways governing the physiological and *in vitro* processes associated with this cell type [3]. Morphological and biochemical changes in *in vitro* cultured (IVC) oviductal epithelial cells (OECs) have been previously documented. These are reflected in changes in levels of gene expression. Previous studies (unpublished data) showed that, during short – and long – term *in vitro* culture, OECs exhibit significant proliferation and expression of genes responsible for differentiation. However, the exact molecular basis of differentiation and other important life processes of OECs are not yet fully understood. Therefore, our research is focused on the early life stages of cultured cells, their gene expression profiles and morphological changes. An important aspect of this work is also the search for new cellular markers, which may be responsible for the processes of cell division, cell cycle and proliferation.

The aim of the study was to analyse the genetic profile of porcine oviduct epithelial cell *in vitro* cultures, which may bring us closer to understanding the molecular basis of such important processes as cell cycle, cell division and cell proliferation.

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 oestrus 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 in Dulbecco'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 resuspention 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, Poland). 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) [4], where genes belonging to "cell cycle process", "cell division" and "cell proliferation" 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 the GOplot package [5]. 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) [6]. 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. 32/2012.

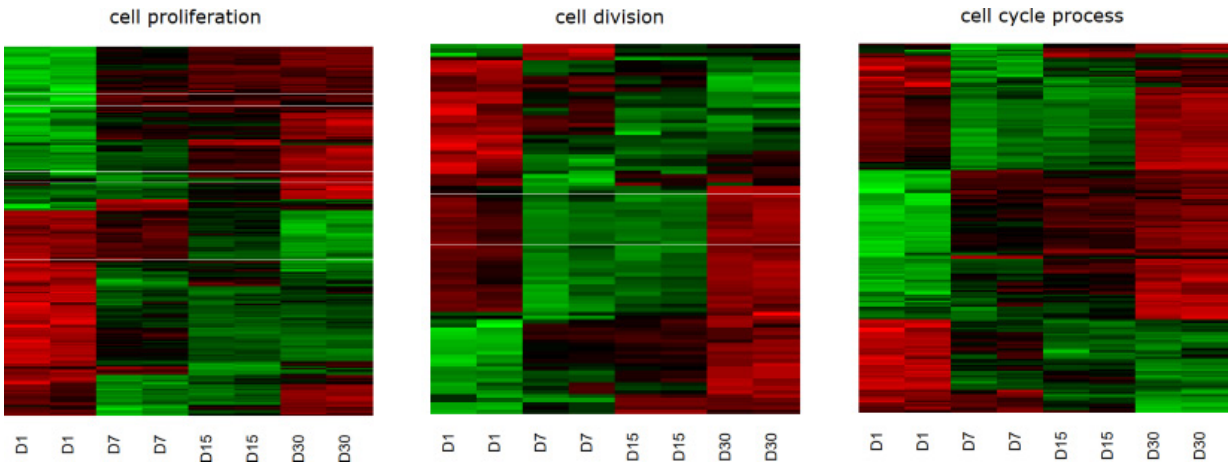

FIGURE 1 Heat map representation of differentially expressed genes belonging to the "cell cycle process", "cell division" and "cell proliferation" GO BP terms. Arbitrary signal intensity acquired from microarray analysis is represented by colors (green, higher; red, lower expression). Log2 signal intensity 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
CD274	0,04424	0,04443	0,041834	2,50E-06	7,45E-07	2,31E-07
TXNIP	0,082179	0,64453	0,63523	1,11E-05	0,024019	0,017645
IL24	0,083371	0,062192	0,071399	9,50E-06	3,21E-06	1,76E-06
IDO1	0,134544	0,12111	0,123331	7,22E-05	3,53E-05	2,36E-05
IFIT3	0,172101	0,195012	0,31317	1,87E-05	1,28E-05	4,60E-05
SERPINB5	0,176908	0,080613	0,009833	4,09E-05	4,16E-06	2,31E-07
SNAI2	0,183766	0,134521	0,130079	0,000411	0,000129	7,85E-05
SYCE2	0,201141	0,201846	0,180612	1,03E-05	4,70E-06	1,84E-06
KLF4	0,205489	0,089904	0,057459	4,71E-05	3,93E-06	1,01E-06
SLFN11	0,227149	0,375498	0,252502	0,000154	0,000817	0,000101
ARNT2	7,767447	13,56793	17,62109	7,45E-06	1,63E-06	4,44E-07
FN1	8,29918	15,9846	14,15968	4,67E-05	7,40E-06	5,68E-06
UBE2C	8,523298	4,717957	0,372658	0,000914	0,00366	0,025648
PPARD	9,200973	6,826522	5,602991	2,25E-05	2,24E-05	2,49E-05
FSCN1	9,234483	9,891081	5,56772	5,23E-06	1,84E-06	2,86E-06
HHEX	9,447447	11,82395	11,30739	2,69E-05	9,59E-06	6,25E-06
CENPF	9,506242	6,960497	0,773239	0,000484	0,000742	0,52224
NOX4	11,05623	21,12763	32,07053	4,90E-06	9,73E-07	2,31E-07
ITGB3	12,67365	13,15962	10,9347	4,27E-06	1,55E-06	8,03E-07
LIPG	36,5469	19,67815	39,01801	2,50E-06	1,39E-06	2,57E-07

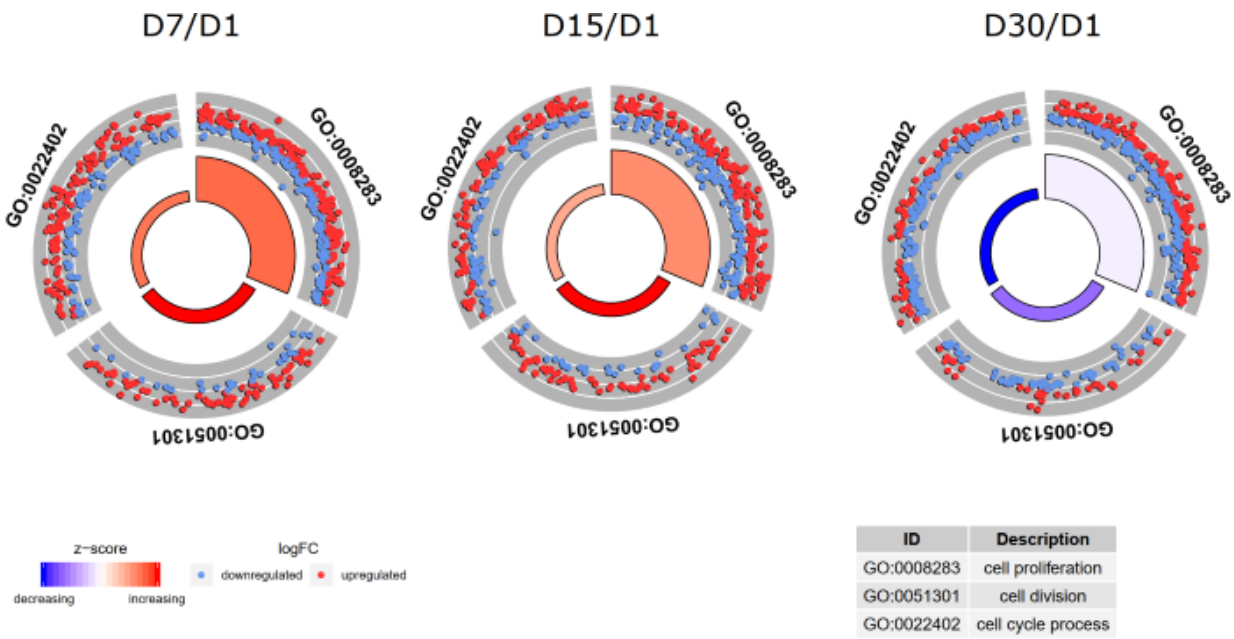

FIGURE 2 The circle plot showing the differently expressed genes and z-score of the "cell cycle process", "cell division" and "cell proliferation" GO BP terms. The outer circle shows a scatter plot for each term of the fold change of the assigned genes. Red circles display up- regulation and blue ones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

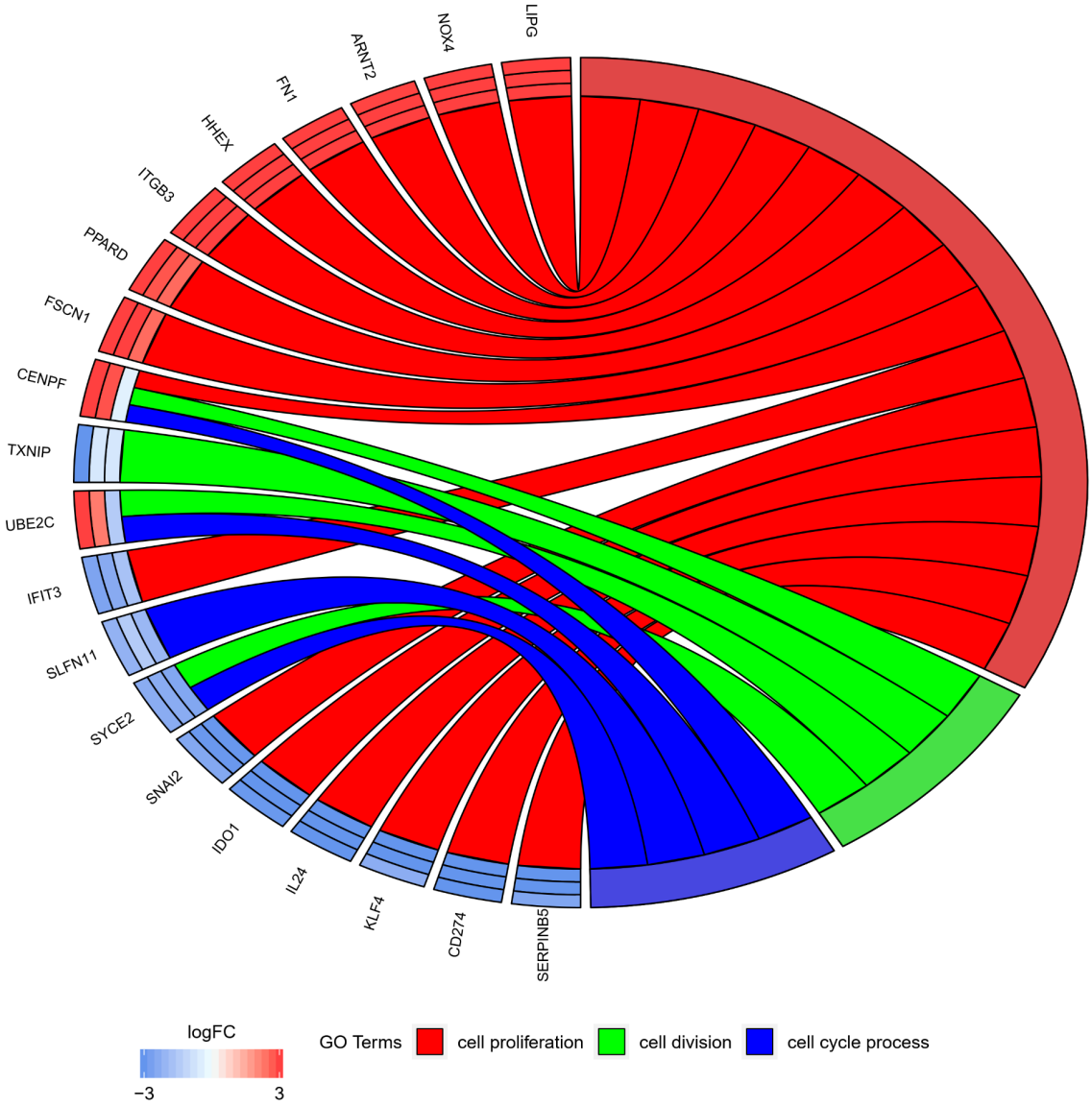

FIGURE 3 The representation of the mutual relationship 10 most upregulated and 10 most downregulated genes that belongs to the "cell cycle process", "cell division" and "cell proliferation" GO BP terms. The ribbons indicate which gene belongs to which categories. The middle circle represents logarithm 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

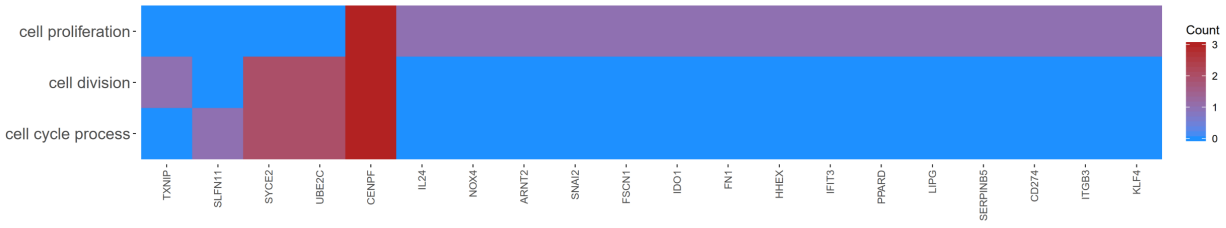

FIGURE 4 Heatmap showing the gene occurrence between 10 most upregulated and 10 most downregulated genes that belongs to "cell cycle process", "cell division" and "cell proliferation" GO BP terms. The red color is associated with gene occurrence in the GO Term. The intensity of the color is corresponding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

Results

Whole transcriptome profiling using Affymetrix microarrays allowed us 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 terms. In this paper, we focused on 378 genes (160 downregulated and 218 upregulated) belonging to "cell cycle process", "cell division" and "cell proliferation" 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 expression, Entrez gene IDs and corrected p-values of these genes were shown in **table 1**.

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**).

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. The results were shown in (**Fig. 6**). REACTOME database found any interactions only between FN1 and ITGB3 genes.

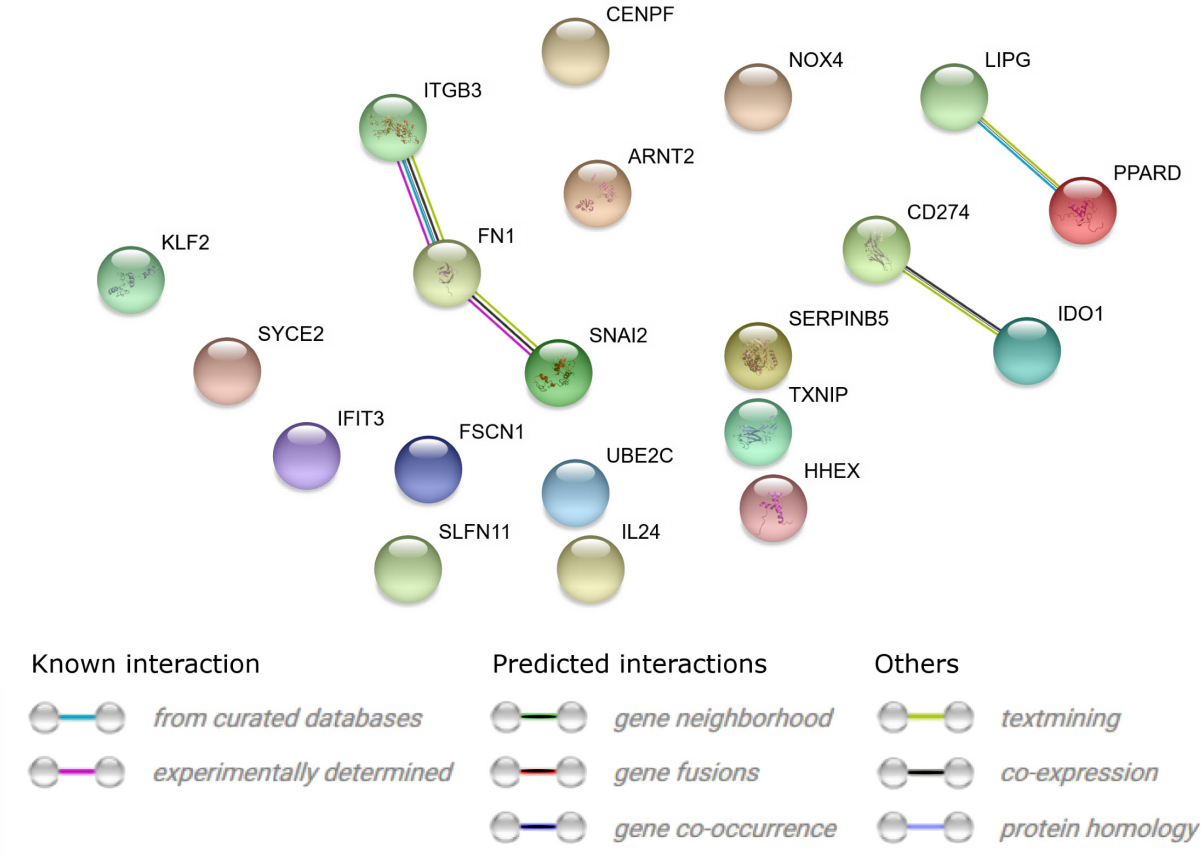

FIGURE 5 STRING-generated interaction occurrence between 10 most upregulated and 10 most downregulated genes that belongs to "cell cycle process", "cell division" and "cell proliferation" GO BP terms. The intensity of the edges reflects the strength of interaction score

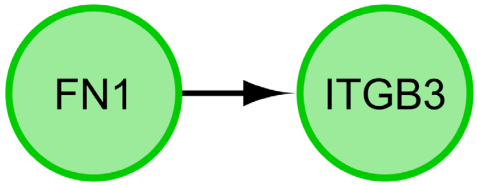

FIGURE 6 Functional interaction (FI) between 10 most upregulated and 10 most downregulated genes that belongs to "cell cycle process", "cell division" and "cell proliferation" GO BP terms. In following figure "->" stands for activating/catalyzing, "-|" for inhibition, "-" for FIs extracted from complexes or inputs, and "---" for predicted FIs

Discussion

According to common knowledge, changes of the oviductal epithelium are important for the process of successful fertilization. Relations between the transported oocyte and the oviductal tissue play an especially important role. The basis for these changes is the secretion of gonadal hormones, which affect the tissues of the oviduct, facilitating their interaction with oocytes. However, the molecular background of these biochemical changes is not yet fully understood. Nowadays, very advanced cell culture techniques enable in – depth cellular research, analyzing their morphological and biochemical changes, reflected in gene expression profiles. Bioinformatic analyses allow us to identify different groups of genes and their interrelationships. Therefore, in our work, we decided to present genes that belong to 3 groups describing genes involved in "cell cycle process", "cell division" and "cell proliferation". This work may serve as a basis for future studies to identify new cellular markers of these processes.

In the course of our research, we managed to maintain a 30 – day *in vitro* culture (IVC) of porcine oviductal epithelial cells (OECs). During this culture, we analyzed their transcriptomic profile at different time intervals: 1, 7, 15 and 30 days. In all the ontological groups of interest, we observed similar gene expression trends. Day 1 is characterized by an abundance of down – regulated genes. In turn, the next days of cultivation showed an upwards trend. Only day 30 exhibited many down – regulated genes in the "cell division" and "cell cycle process" groups, and about half of such genes were members of the "cell proliferation" group. We can see that as many as 17 of the 20 described genes presented in our study belong to only one group – "cell proliferation". The remaining genes belong to two (two genes: SYCE2 and UBE2C) and all three (1 gene: CENPF) of the discussed groups. Such a distribution of affiliation can be an argument for the usefulness of these study in the search for markers associated with cell proliferation processes. The "cell proliferation" group consists of 16 differentially expressed genes, one of which (CENPF) is also related to the other two groups. The "cell division" group is represented by 4 genes, of which only one is exclusive (TXNIP). Similarly, the "cell cycle process" group is composed of 4 genes, of which only one is exclusive (SLFN11). Among the "cell proliferation" group, 9 of the genes were up – regulated and 7 down – regulated. In the "cell cycle process" and "cell division" groups, there were 2 down – regulated and 2 up – regulated genes respectively.

Genes belonging to the "cell proliferation" group and showing up – regulation in our in vitro cultured OECs research are involved in metabolism but also related to cell differentiation and development. The first, *NADPH oxidase 4*, *NOX4*, is involved in the reactive oxygen species (ROS) production, also participating differentiation of fibroblasts into myofibroblasts in the heart [7] and the differentiation of renal fibroblasts [8]. *NOX4* also supports survival of cerebral microvascular endothelial cells (CMVEC) during apoptosis induced by TNF-α in newborn piglets [9,10]. The *integrin subunit β-3* gene (*ITGB3,* also known as *CD61*), also belonging to the "cell proliferation" ontological group, showed up – regulation in our studies. B-3 integrins form transmembrane glycoproteins, essential for many physiological processes, and the presence of *CD61* transcripts is recorded mainly in blood platelets and hematopoietic tissues [11]. Another gene from the "cell proliferation" group is *LIPG* (*lipase G, endothelial type*, also known as *EL*), which is linked with the formation of new cell membranes due to its participation in hydrolysis of extracellular lipoproteins, which in turn can be absorbed and used to build membrane phospholipids [12]. The *FN1* gene (*fibronectin 1*), belonging to the cell proliferation group, showed a significant increase in expression during *in vitro* culture. This gene relates to differentiation, but also to stemness of cells. Its up – regu-

lation was demonstrated during research on the *in vitro* cultured porcine buccal tissue mucous membrane cells, which ir was connected with the proliferation processes [13]. In our research, we have shown up – regulation of the *ARNT2* gene (*aryl hydrocarbon receptor nuclear translocator 2*). This gene has been identified as one of the key factors in the development of the mammary gland in pigs during late pregnancy, where its down – regulation has been demonstrated [14]. Subsequent gene, *PPARD* (*peroxisome proliferator activated receptor delta*) significantly contributes to fat metabolism, differentiation of adipocytes or preadipocytes, as well as myoblasts towards adipocytes [15]. However, its expression is widespread in tissues such as adipose tissue, intestines, muscles, heart or brain. It was also shown that female hormones (especially FSH) have a significant influence on *PPARD* expression in cultured *in vitro* ovarian follicles in pigs [16]. Further, the *FSCN1* gene (*fascin actin-bundling protein 1*) shows a role in cell movement, adhesion and intercellular interactions. The expression of this gene in skeletal muscles of different breeds of pigs, which showed significant racial differences, was studied [17]. This gene is closely related to muscle tissue and, consequently, to meat production. The last gene that showed up – regulation in this ontological group is *HHEX*. The *HHEX* gene (*hematopoietically expressed homeobox*) is a transcription factor that regulates the self – renewal of hematopoietic stem cells (HSC). Genetic control of HSC is essential to maintain them throughout life for blood production processes [18]. *HHEX* shows expression in the dorsal aorta (DA) endothelium, in which HSC is formed in the development [19].

On the other hand, genes belonging to the cell proliferation group but showing down-regulation in our study were related to the immune response, antiviral action, anti-inflammatory processes or apoptosis. The *CD274* gene encodes the programmed death ligand -1 (PD-L1), which is strongly expressed in the placenta, as an immunomodulator associated with the defense of the embryo against the mother's immune system, with its expression associated with high oxygen concentrations [20]. *CD274* expression in macrophages co-cultured with pMSC during the change of phenotype from inflammatory M1 to anti-inflammatory M2 has also been shown [21]. The next gene, *IDO1* (*2,3-dioxygenase 1 indolamine*), also significantly contributes to the immune response by participating in the metabolism of tryptophan. Increased enzymatic activity of IDO was demonstrated after stimulation of LPS in domestic swine *in vivo* and *ex vivo*, which was found to be the basis for research on IDO immunomodulatory functions [22]. A recently published study describes the expression of genes in the process of atresia of pig ovarian follicles, *including interleukin 24* (*IL24*), as one of the main regulators of their atresia [23]. This interleukin induces apoptotic processes, which has been implicated in cancer research. In our study, *IL24* exhibited down – regulation and belonged to the "cell proliferation" gene ontology. Down – regulation of the *SNAI2* gene (*snail family transcriptional repressor 2*) has been demonstrated in our *in vitro* OEC study. This gene, as a transcriptional factor, proves to be of significant importance in the regulation of stem cell niches, as demonstrated by research on porcine skin – derived progenitor (pSKP) cells [24]. This gene is active during the regulation of cell movement, epithelial – mesenchymal transformation and anti – apoptotic processes within the neural crest [25]. We also noted down – regulation of the *IFIT3* gene. Research on *IFIT3* (*interferon induced protein with tetratricopeptide repeats 3*) shown that it has a strong ability to eliminate the swine influenza virus (SIV) through high expression, which inhibits virus replication [26]. The expression of this gene is observed in multiple porcine tissues [27]. The next gene from this group is *KLF4*. *Kruppel like factor 4* (*KLF4*) is one of the factors that regulate gene networks in the anti – inflammatory, antioxidant and anticoagulant profiles, which has been proven to give endothelium atherosclerosis protective properties [28]. As a marker of embryonic stem cells, *KLF4* is one of the important genes for stem cell function, and its expression has been demonstrated in the culture of porcine spermatogonial stem cells (PSSCs) [29]. The last example of the down – regulated genes belonging to this group is the *SERPINB5* (*serpin family B member 5*). This gene belongs to the Serpin superfamily, whose main function is to regulate the distribution of proteins by inhibiting the catalytic activity of proteases, with their expression detected various tissues [27].

The only gene exclusive to the "cell division" GO is TXNIP. *Thioredoxin – interacting protein* has been shown to be of key importance for cellular glucose metabolism [30]. The down – regulation of *TXNIP* in *in vitro* cultured porcine oocytes was observed after estrogen stimulation [31]. Down - regulation of *TXNIP* expression can therefore be a potential marker of cell growth, as a manifestation of advanced metabolic processes. The second gene in this group, showing up – regulation, is *UBE2C* which also belongs to the "cell cycle process" group. Apparent up – regulation of the *UBE2C* gene (*ubiquitin conjugating enzyme E2 C*) in our studies may indicate an important role of this gene in cell cycle and cell division processes, as it is an important mitotic factor It has also been proved that this gene has a specific meaning in pig oocyte maturation [32]. The *SYCE2* gene also belongs to two groups: "cell division" and "cell cycle process", but exhibits reduced expression. The *SYCE2* gene (*synaptonal complex central element of proteins 2*) plays a role in the construction of the synaptonemal complex, which maintains paired chromosomes during meiosis prophase and promo-

tes genetic recombination [33]. It shows ubiquitous expression in different tissues [27].

SLFN11 belongs only to the "cell cycle process" group and showed down-regulation in our study. *SLFN11* (*schlafen family member 11*) gene expression in humans sensitizes cancer cells to factors damaging DNA [34,35], which makes it an interesting candidate for anti-cancer treatment. Additionally, there are reports that this gene influences selective blocking of viral protein expression. SLFN family proteins have a role in the development, immune response and proliferation of cells [34].

The last of the described genes belongs to all 3 ontological groups. In this study, we observed its up – regulation. The *CENPF* gene (*centromere protein F*) is primarily responsible for the formation of centromere proteins under the attachment of microtubules to chromosomes. This process is of key importance in cell division processes and its expression was demonstrated during all stages of pig oocyte meiosis [36].

In our analyses we also evaluated the mutual relations between genes, which is expressed by STRING – generated interaction network. Co – expression was demonstrated in three cases: *ITGB3* and *FN1, FN1* and *SNAI2* genes and *IDO1* and *CD274* genes. 13 of the analyzed genes did not show direct interactions with other genes. However, indirect impacts are not excluded, either through genes not described in this study or through interactions with genes that have not shown significant changes in this study.

This paper presents genes that belong to different ontological groups related to cell cycle, cell division and cell proliferation. Most of the described genes belonged to only one ontological group, so they may be potential markers of OEC cell development in *in vitro* cultures. Some of them were specific to the reproductive system, but most corresponded to the physiological processes in all cells. This work provides basic information on the *in vitro* culture of porcine oviduct epithelial cells, as well as gene expression profile, and may serve as a basis for further research into the molecular processes involved in this culture type.

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Conflict of interest statement

The authors declare they have no conflict of interest.

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