

Investigation of the ability to differentiate and transdifferentiate porcine ovarian follicular cells under *in vitro* culture conditions.

In a mature ovarian follicle there are several cell populations. Theca cells (TCs), among which we distinguish the interna and externa layers, are the most outer layer of the follicle wall. The largest population of ovarian follicular cells are mural granulosa cells (GCs) - filling the interior of the follicle and constituting the layer surrounding the oocyte - cumulus cells (CCs) building the cumulus oophorus and corona radiata. Each of these cell populations performs a specific role in the follicle. Follicular granulosa cells are becoming more and more popular among scientists because of their reported stem-like potential and differentiation abilities.

Studies of the last few years on the analysis of stem properties of human ovarian granulosa cells have shown, among other things, that these cells have the characteristics/potential of stem cells. Numerous literature data emphasize that granulosa cells, originating from women referred for *in vitro* fertilization, filling the interior of the follicle, show expression of molecular markers characteristic for mesenchymal stem cells (MSCs), such as; CD29, CD44, CD105, CD90, as well as pluripotent stem cells (OCT-4, NANOG, SOX2, TERT). The stem potential of human granulosa cells is an issue quite well recognized in the literature. Numerous publications indicate that these cells can differentiate to osteoblasts, chondrocytes, which is a confirmation of mesenchymal properties of these cells.

However, less known are stem properties of porcine GCs or TCs cells. The main goal of this project is to study secretion properties and identify markers of stem cells properties of GCs, and TCs isolated from porcine follicles during primary *in vitro* culture, as well as to test their ability to differentiate towards cells from 3 germ layers: ectoderm (neural cells), mesoderm (osteoblasts, chondroblasts, adipocytes, cardiomyocytes, skeletal muscle cells, vascular endothelial cells) and endoderm (hepatocytes, pancreatic islet – like cells). In addition, the ability to form spheroids and interactions between specific cell types will be explored using 3D culture cell culture model. The assumed goal will be achieved by growing individual types of follicular cells in a basic medium and a medium containing differentiation factors. The assessment of the secretory properties of the above cells will be conducted during proteomic / metabolomic assays (mass spectrometry) of the cells.

So far, the question of whether TCs cells and GCs cells have the stem-like potential has not been answered. Additionally, it has not been studied whether GCs, and TCs, showing different properties in the follicle, may differentiate towards cells from the above mentioned germ layers.

Thus, the identified differentiation inducing factors can be used as supplements during the procedures of regenerative medicine. At the same time, the presented research will provide completely new information (primarily molecular markers as well as created data libraries), thanks to which it will be possible to identify the process of GCs, and TCs differentiation towards cells from the above mentioned germ layers. A wide field for the use of porcine stem cells may become the basis of new forms of the therapy of human and animal neurodegenerative diseases, but also the broadly understood civilization's diseases. Positive results of cellular (flow cytometry), molecular (RNAseq) and proteomic and metabolic (LCMS/MS) analyses confirming the presence of different cell populations of different types may indicate completely new properties of follicular cells as a therapeutic tool in regenerative medicine.