

1. PHD PROJECT DESCRIPTION (4000 characters max., including the aims and work plan)

Project title:

Investigation of the differentiation and trans-differentiation ability of porcine theca cells during in vitro primary culture

- 1.1. **Project goals:** The main goal of this project is to study secretion properties and to identify markers for stem cell (SC) properties of theca cells (TCs) isolated from porcine follicles during primary in vitro culture. Moreover, the project's aim is to test the ability of TCs to differentiate towards cells from three 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 model.
- 1.2. **Outline:** In a mature ovarian follicle there are several cell populations. Theca cells, among which we distinguish the interna and externa layers, are the most outer layer of the follicle wall. The granulosa cells (GCs) form the very inner layer of a follicle since they are surrounding the oocyte. GCs became more and more popular among scientists due to their reported stem-like potential and differentiation abilities. Studies of the last few years on the analysis of stem properties of human ovarian GCs have shown their characteristics/potential of SC. Numerous data provided evidence that human GCs undergoing in vitro fertilization showed an expression of molecular markers for mesenchymal SC and pluripotent SC. These cells can differentiate to osteoblasts, chondrocytes, which is a confirmation of mesenchymal properties of these cells. So far, the questions of whether TCs cells have the stem-like potential and if they may differentiate towards cells from the earlier mentioned germ layers have not been answered.
- 1.3. **Work plan:** The proposed 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. Thus, the identified differentiation inducing factors can be used as supplements during the procedures of regenerative medicine. This research will provide completely new information (primarily molecular markers and created data libraries), thanks to which it will be possible to identify the process of TCs differentiation towards the cells from germ layers. A wide field for the use of porcine SC may become the basis of new therapeutic strategies for human and animal neurodegenerative and other diseases. Positive results of cellular (flow cytometry), molecular (RNAseq) and proteomic/metabolic (LCMS/MS) analyses confirming the presence of different cell populations of different types may indicate completely new properties of porcine TCs as a therapeutic tool in regenerative medicine.
- 1.4. **Literature:** 1. Chachuła, A.; et al. The differentiation of mammalian ovarian granulosa

cells living in the shadow of cellular developmental capacity. *J. Biol. Regul. Homeost. Agents* 30, 627–634. 2. W. Chen, L.; et al. Potential Application of Induced Pluripotent Stem Cells in Cell Replacement Therapy for Parkinsons Disease. *CNS Neurol. Disord. - Drug Targets* 2012, 10, 449–458. 3. Yin, K.; et al. Exosomes from mesenchymal stem/stromal cells: a new therapeutic paradigm. *Biomark. Res.* 2019, 7, 8. 4. Li, J.; Dong, S. The Signaling Pathways Involved in Chondrocyte Differentiation and Hypertrophic Differentiation. *Stem Cells Int.* 2016, 2016, 1–12. 5. Bi, S.; et al. Human Umbilical Cord Mesenchymal Stem Cells Therapy for Insulin Resistance: A Novel Strategy in Clinical Implication. *Curr. Stem Cell Res. Ther.* 2018, 13, 658–664. 6. Dzafic, E.; et al. Plasticity of granulosa cells: On the crossroad of stemness and transdifferentiation potential. *J. Assist. Reprod. Genet.* 2013, 30, 1255–1261. 7. Dzafic, E.; Set al. Expression of mesenchymal stem cells-related genes and plasticity of aspirated follicular cells obtained from infertile women. *Biomed Res. Int.* 2014, 2014, 508216. 8. Merkwitz, C.; et al. Progenitor cells harvested from bovine follicles become endothelial cells. *Differentiation* 2010, 79, 203–210. 9. Varras, M.; et al. Markers of stem cells in human ovarian granulosa cells: is there a clinical significance in ART? *J. Ovarian Res.* 2012, 5, 36. 10. Chermuła, B.; et al. New Gene Markers of Angiogenesis and Blood Vessels Development in Porcine Ovarian Granulosa Cells during ShortTerm Primary Culture In Vitro. *Biomed Res. Int.* 2019, 2019, 1–12. 11. Koos, R.D. Stimulation of endothelial cell proliferation by rat granulosa cell-conditioned medium. *Endocrinology* 1986, 119, 481–489. 12. Basini, G.; et al. Swine Granulosa Cells Show Typical Endothelial Cell Characteristics. *Reprod. Sci.* 2016, 23, 630– 7. 13. Koga, K.; et al. Evidence for the Presence of Angiogenin in Human Follicular Fluid and the UpRegulation of Its Production by Human Chorionic Gonadotropin and Hypoxia 1 . *J. Clin. Endocrinol. Metab.* 2000, 85, 3352–3355. 14. Skowronski, M.T.; et al. Pituitary hormones (FSH, LH, PRL, and GH) differentially regulate AQP5 expression in porcine ovarian follicular cells. *Int. J. Mol. Sci.* 2019, 20. 15. Skowronski, M.T. et al. Pituitary gonadotropins, prolactin and growth hormone differentially regulate AQP1 expression in the porcine ovarian follicular cells. *Int. J. Mol. Sci.* 2018, 19

1.5. Required initial knowledge and skills of the PhD candidate

The requirements for the PhD candidates contain a MSc degree in biology, cell biology, molecular biology, biochemistry, or related fields, and the candidate should have a well-structured knowledge in cell biology and molecular biology. Further, the candidate should have had Previous hands-on training in molecular biology, cell biology, or in a related topic lab. A good command of English is essential, and the candidate should have good communication skills. Previous exposure to mammalian cell culture techniques would be advantageous.

1.6. Expected development of the PhD candidate's knowledge and skills

It is expected that the PhD candidate will focus on developing skills and harnessing opportunities that will help the candidate to proceed with the project adequately in the proposed time frame. Due to hands on trainings, practical and theoretical seminars the candidate will be provided with tools and resources to finish successfully the PhD programme and to develop a career beyond the PhD. The attendance in the Journal Club will ensure the gain of new knowledge of the candidate in a very wide field of biological and medical topics. The candidate's development of practical skills will be ensured due to the guidance by other experienced lab-members and co-supervisor.