

## **Involvement of the arachidonic acid in shaping the quality of oocytes of the domestic pig**

Due to several physiological and anatomical similarities to humans, the domestic pig is a well-recognized model organism in the field of reproductive biotechnology. A dynamic development of advanced reproductive bio techniques (eg transgenesis) requires a big number of oocytes and in vitro produced porcine embryos. The yield of the complex IVF procedure is limited to 30-50% of blastocysts in relation to fertilized oocytes and is mainly attributed to the developmental potential (quality) of oocytes. Lipid metabolism is one of the factors influencing the oocyte quality. High concentration of lipids in the porcine oocytes is of special importance to research on lipid metabolism in female gametes. It has been demonstrated that lipids (e.g. fatty acids) present in oocytes and the follicular fluid regulate reproductive processes due to involvement in hormonal metabolism. The porcine oocytes are characterised by a high content of arachidonic acid (AA), the precursor of bioactive components like prostaglandins and leukotrienes. Thus AA may play a crucial role in maturation of porcine oocytes as previously shown in cattle. Bovine oocytes showed distinct AA content before and after in vitro maturation (IVM). Despite a significant involvement of lipids in the regulation of oocyte growth and maturation, the current knowledge on this phenomenon is limited. Therefore the motivation to undertake the proposed research is to analyse possible functions of the arachidonic acid in shaping the developmental potential of porcine oocytes by modifying selected pathways of lipid metabolism during in vitro maturation.

**Working hypothesis:** Oocytes of the domestic pig are characterized by high content of arachidonic acid, prostaglandin precursor. Experimental modification of the selected pathways of the AA metabolism during in vitro maturation of porcine oocytes will help to elucidate the involvement of this fatty acid in shaping the developmental potential of oocytes.

**The aim of the project:** evaluation of the developmental potential of porcine oocytes matured in vitro under culture conditions modified with respect to selected pathways of AA metabolism by applying a broad panel of parameters related to oocyte quality

**M&M** Cumulus-oocyte complexes (COC) obtained from ovaries of slaughtered cyclic gilts subjected to IVM in the presence of factors modifying selected pathways of AA metabolism. The experiment will include 5 groups of COCs: 1) before IVM, 2) after IVM in standard conditions (control), 3) after IVM with phospholipase A2 inhibitor, 4) after IVM with inhibition of cyclooxygenase activity and 5) after IVM with media supplementation with exogenous arachidonic acid. The following biological material will be analysed: 1) before IVM - follicular fluid (FF), oocytes, follicular cells (FC), 2) after IVM - oocytes, FC and 3) seven days after chemical activation of the matured oocytes – embryos at blastocyst stage.

The applied panel of procedures will include: real-time PCR (mRNA expression of a panel of genes regulating lipid metabolism and markers of oocyte quality in FC and oocytes), gas chromatography (FA content and profile in the FF), ELISA (hormone concentration in the FF), fluorescent staining Bodipy 488/50 (the number and size of lipid droplets, oocyte diameter, stadium of meiosis).

The pioneering aspect of this project concerns getting a new insight into involvement of lipids, especially the arachidonic acid, in shaping the development potential of porcine oocytes during IVM. The expansion of the existing knowledge will help to improve the condition of oocyte maturation and embryo development in vitro and will result in production of better quality embryos.