The investigation of mechanisms responsible for the foliculo- and oogenesis regulation as well as preliminary stages of embryo development and implantation aim at more accurate familiarization with the process of acquiring the developmental competence by female gametes, the proper embryo growth and development as well as obtaining healthy offspring. The application of research methods used in molecular genetics and cell biology may significantly influence the development of assisted reproductive technology. Research hypothesis assumes that aquaporins and conexins participate in the regulation of folliccle-oocyte interaction and have impact on oocyte quality. One of the main aim of this project is to study relation between aquaporins and connexins on the gene and protein levels in maturing oocytes and cells surrounding the oocyte (cumulus, granulosa and theca).

The detailed aims of the research will be:

1. To define the relation between the ovarian follicle size and pig's oocyte morphology as well as the expression of mRNA, aquaporin (1, 5, 9) and conexin proteins (37,43 and 45);
2. To define the relation between the ovarian follicle size and pig's oocyte morphology as well as subcellular protein distribution AQP1, 5, 9 and conexin 37, 43, 45 using confocal microscopy before and after in vitro maturation;
3. To study biological connections between aquaporins and conexins during oocyte development using AQPs and Cxs inhibitors;
4. To define the relation between granulosa, theca, corona radiata cells proliferation and the size of ovarian follicles as well as the pig's oocyte quality.

Ovaries (300 pieces) collected after slaughter from puberal gilts (White Polish Landrace) aged from 140 to 180 days (mean age 160 days) and weighing 95-120 kg (mean weight 100 kg) will be used in this study. The animals will be fed with standard feed. Only clinically healthy specimens will be classified for investigations. The animals will be slaughtered in one of the local slaughterhouses.

To determine the proliferation potential of cumulus cells and granulosa cells (RTCA), both populations of cells (cumulus cells and granulosa cells) will be cultured for 168 hours. Cells for further analyses will be collected every 24 hours. At every stage of primary culture of the cell population the proliferation index and the normalized proliferation index will be defined. Cells will be cultured and used for further investigations, i.e. RTq-PCR, western-blotting, confocal microscopy.