

Description for the general public

Fertilization and the earliest stages of development of the embryo/s take place in the oviduct. However, the conditions of these initial periods of pregnancy, are not fully understood. Despite the fact that prenatal medicine and techniques for pregnancy abnormalities diagnosis still are improved, only 30% of women is reported to carry of successful pregnancy. The vast majority of embryos die, in particular, during the first stages of pregnancy. Similarly, in the case of farm animals e.g. pig embryos, mortality is still high and fluctuates within 30-40%. In spite of rapid progress of assisted reproductive techniques, their effectiveness has to be improved. It turns out that even 90% of blastocysts obtained by *in vitro* fertilization is not able to properly hatch in the endometrium of a woman's uterus. Also in the case of farm animals, where up to 43% bovine embryos, obtained by *in vitro* fertilization, dies before their transport to the uterus. However, in the pig, the effectiveness of *in vitro* fertilization is even 100%, but up to 92% of the oocytes are fertilized by more than one sperm (polyspermy), which cause their death soon after fertilization. Thus, it has become clear that there is a need to fully understanding the conditions in which occur maturation of the gametes, fertilization and development newly formed embryos, which remain in constant contact with the reproductive tract of female.

The most optimal conditions for the final gamete development and formation of the embryos, occur within the oviduct. This complex organ is not, as it was thought, a simple connection between the ovary and the uterus. On the contrary, it is a site of the very specific and dynamically changing microenvironment. The endocrine, and locally synthesized molecules, factors from immune and central nervous systems as well as cell-to-cell contacts between the epithelium of the oviduct and oocytes, sperm or embryos, affect the microenvironment of this organ. Dysfunction of any of those factors result in interference in the course of early pregnancy and let to death of the embryos. In addition, different compartments of the oviduct perform different functions. The utero-isthmic junction and isthmus are the site of sperm selection, their reservoir and hyperactivation. In ampullar-isthmic junction of the oviduct processes of recognition of the gametes and fertilization occur, followed by the transport of the embryos, which undergo constant meiotic cell divisions. Ampulla, in the first place, is involved in oocytes maturation and then, their transport to the site of the fertilization.

Taking under consideration complexity of the processes occurring in the oviduct, the aim of this project is to analyze the global gene expression in this organ, on the model of domestic pig. It is planned to use transcriptomic microarray analysis, which allows to determine the expression of thousands of genes simultaneously. Thus, the main aim of the study is to compare the gene expression in two compartments of the oviduct (ampulla and isthmus) between pregnant and not pregnant pigs. Moreover, it will be possible to determine one of the mechanisms that may regulate the expression of genes – DNA methylation. During this process methyl groups binds cytosine which is one of the four nucleotides of DNA. As a result of this binding the expression of the gene is diminished. Analysis of the gene expression with the possibility of simultaneous detection of methylation in the sequences of the studied genes (methylation specific analysis of gene expression), will let to answer the question of whether DNA methylation is also involved in the genes expression regulation of ampullar and isthmic epithelial cells in the oviduct.

In order to determine differentially expressed genes, the oviducts will be harvested from pigs, during 2-3 days of pregnancy. Embryos at 2-/4-cells stage are present at this time inside this organ. Another oviducts will be collected from non-pregnant pigs, during 2-3 days of the estrous cycle, when there are only nonfertilized oocytes in this organ.

The planned experiment will let to determine the genes, which may be involved in succesfull development of the embryo from the zygote to two-/four-cells-stage embryo and which may be characteristic for early pregnancy. Moreover, determination of the involvement of DNA methylation in regulation of genes expression will be possible. In the future, determination of concentration of proteins encoded by the genes, which shown increased or decreased expression, may also be interesting. These findings will therefore allow to include the results in the procedures for optimization of *in vitro* fertilization and oocytes/embryos cultivation. Changes in the protocols would increase the efficiency of the methods and may reduce e.g. incidence of polyspermy in pigs, in the future.