DESCRIPTION FOR THE GENERAL PUBLIC

Progesterone (P4) is the main regulator of the estrous cycle duration and creates suitable conditions for the embryo implantation and maintenance of pregnancy in many animals. The physiological effects of P4 on the cells is evoked by nuclear P4 receptor (PGR) and also through a nongenomic mechanism via membrane receptors: PGRMC (*progesterone receptor membrane component*) and mPR (*membrane progestin receptor*). While the impact of steroids on the genomic way is fairly well-understood, the role and the mechanism of nongenomic P4 effect on the female reproductive tract is not yet fully elucidated. Therefore, **the aim of this project is:**

(a) to determine the function of membrane P4 receptors: PGRMC and mPR in the regulation of uterine function,

(b) to identify of signal pathways activated by these receptors and the interaction between them, and

(c) to define cellular processes regulated by these receptors in the endometrial tissues.

The experimental material will be the endometrial cells of (i) the luminal epithelium, (ii) the endothelium of blood vessels (isolated from cow uteri; 15-17 days of the estrous cycle, (iii) EnCL-1 cell line (bovine luteal endothelial cell lines) and/or (iv) slices of endometrium. In the studies, particular attention will be focused on intracellular mechanisms of P4 action through membrane receptors, their role in modulation of signaling pathways, influence on selected gene expression and regulation of secretory and proliferation processes in endometrial cells and endothelium of the blood vessels of the uterus.

In the planned tasks we will want to define the PGRMC and mPR functions in endometrial cells and/ or EnCL line cells by knockdown gene for these receptors by siRNAs transfection and pharmacological modification of PGRMC and mPR action using an available mPRα agonist and PGRMC1 antagonist) and/ or the P4-BSA conjugate. Next, using new generation sequencing analysis (NGS), we will identify genes regulated by PGRMC and mPR in control and knockdown cells. In the next stage, we will determine the participation of these receptors in the secretory and proliferative function of endometrium and/ or EnCL cells and we will identify intracellular signaling pathways modulated by P4 in the studied cells. Molecular biology techniques, such as: Real Time PCR, Western blot, NGS, reporter gene assays, microscopic and time-laps technique for analysis of proliferation, migration, apoptosis, angiogenesis, immunohistochemical and inmmunoenzymatic analysis will be used in this project.

The results obtained from such comprehensive studies will contain innovative elements, and therefore we expect that they will extend our knowledge on uterine function. Moreover, these studies extend our earlier studies on the nongenomic influence of P4 on the function of uterine and ovarian cells using cow tissue/cells as a model. We assume these studies will help to understand better the changes in the reproductive system during the estrous cycle and pregnancy. Especially that, dysfunction of corpus luteum (CL) and a reduction of P4 level in the blood may affect the so-called “early embryos mortality”, that in farm animals is up to 40%. From this point of view, better understanding the mechanisms regulating the function of these organs at the level of cellular and sub-cellular regulation may also have some practical significance in medical and veterinary practice.