

The main objective of this project is to evaluate the association between the expression of interleukin 35 and its polymorphisms and the risk of miscarriage. The research hypothesis assumes that in women who have had a miscarriage, there is a change in the expression of interleukin 35, which may be crucial for maintaining pregnancy. Because cytokine expression may be influenced by polymorphism in cytokine genes, polymorphisms localized in genes encoding cytokines may be potential factors associated with the risk of miscarriage. The research hypothesis assumes that interleukin 35 administration in abortion-prone female mice, with impaired ability to induce immunological tolerance to developing pregnancy, will increase the proportion of regulatory cells and decrease Th17 cell population, thereby limiting the fetal resorption rate. In the proposed project, we also want to broaden the knowledge about different populations of regulatory cells (Breg and Treg) during pregnancy and indicate subpopulations responsible for its maintenance.

The majority of the work is focused on the role of interleukin 35 mainly during the course of autoimmune diseases and cancers. The role of this cytokine in maintaining pregnancy remains unclear. However, it is known that women with recurrent miscarriages show a decreased level of interleukin 35 in serum compared with the control group. The research conducted by our team demonstrated a decrease in the percentage of regulatory B cells expressing interleukin 35 on day 3 of pregnancy in the uterus, uterus draining lymph nodes and peripheral blood in mice from the abortion group compared to the normal pregnancy group. Literature data and preliminary studies prompted us to analyze interleukin 35 expression in regulatory cells in humans during normal pregnancy and in the case of spontaneous abortion (SA) and recurrent pregnancy loss (RPL). Additionally, we would like to determine whether the level of expression of this protein and polymorphisms in the gene correlate with the risk of miscarriage. Until now, interleukin 35 levels have been studied only in the serum, trophoblast, peritoneal fluid or endometrial tissue of patients. There are no data on the expression of this protein in individual populations of regulatory cells (Breg and Treg). The detailed phenotype of B lymphocytes that are involved in the maintenance of pregnancy is also unknown, hence the application of the proposed tests is reasonable. More detailed knowledge on this subject seems to be highly desirable, due to the fact that the problem of miscarriage and infertility has become one of the greatest challenges of modern medicine. The problem of miscarriages affects approximately 1% of all women. However, it is believed that the incidence of this phenomenon is significantly higher than clinically recognized. About 30-40% cases of pregnancy failures occurs in the preimplantation period of pregnancy, and the majority of causes remains unknown. It is most probable that the immunological and endocrine dysregulation play a major part in the loss of pregnancy, therefore, conducting this research is justified.

Indication of interleukin 35 and comprehensive characterization of regulatory cells as potential therapeutic tools in pregnancies at high risk of miscarriage is the innovation of this project. These studies will allow us to answer the question whether interleukin 35 determines the success of pregnancy. The proposed experiments are designed to broaden and improve knowledge about determinants of successful pregnancy, especially those that determine the tolerance of fetal antigens. In addition, linking *interleukin 35* polymorphisms with the risk of miscarriage may be helpful in the diagnosis and possible therapy of future mothers. The results of this research may be helpful in developing programs to improve animal reproduction performance, and to indicate the directions of therapeutic intervention at the systemic level in the event of pregnancy failure in humans.