Infertility and reproductive disorders are a growing problem in today's world. Currently, women decide first to achieve a higher level of education and economic stabilization, and only then (about the age 30 years) on motherhood, and yet the woman's fertility is conditioned by her age. Therefore, more and more of them require assisted reproductive technology (ART). Techniques for assisted reproduction, which include *in vitro* fertilization (IVF), are often the last chance to have an own child. Despite significant advances in these techniques, approximately 10% of women undergoing *in vitro* procedure experience recurrent implant failure (RIF). RIF is considered when a woman under the age of 40 has undergone at least three IVF procedures in which four good quality embryos were transferred and, despite this, did not get pregnant. Over 50% of such cases remains unexplained. So, why do not all embryos succeed in implantation into uterus? In searching for causes of this frustrating failure, it is important to understand better the immune system, especially the mechanisms of mother's immune tolerance to semi-allogeneic embryo.

During implantation and early pregnancy, uterine NK cells are the largest population in uterus. On the surface of uterus there are receptors, including killer immunoglobulin-like receptors (KIR). These receptors can recognize specific ligands that are found on the surface of nesting embryo. Such ligands are HLA class I leukocyte antigens (HLA-C, HLA-G), which are inherited in a half from mother and father. In studies carried out in our laboratory, we analyze the diversity (polymorphism) of genes that encode KIR receptors and their HLA ligands in couples with recurrent implantation failure after *in vitro* fertilization. From the proper interaction of HLA antigens on cells of invading trophoblast (cells, which give placenta in further stages of pregnancy) with KIR receptors on NK cells in uterus, may depend the response of NK cells to the developing embryo. Therefore, the polymorphism of both maternal and paternal genes may determine successful implantation. During the KIR-HLA interaction, the antigenic peptide is also presented in the context of HLA. It turns out that most of the antigenic peptides presented by HLA are formed by their cleavage to the appropriate length by endoplasmic reticulum aminopeptidases ERAP1 and ERAP2. If these aminopeptidases and their genetic differentiation affect the formation of suitable antigenic peptide complex - HLA-C and/or HLA-G on the embryo surface, it can impact on the implantation failure. It seems that our hypothesis is justified. Our preliminary studies showed significant differences in the polymorphism of ERAP genes between women experiencing recurrent spontaneous abortion, women suffering from RIF and healthy fertile women.

The main aim of this project is to investigate the role of aminopeptidases of the endoplasmic reticulum ERAP1 and ERAP2 in patients with RIF, which is in a way a continuation of research on the role of KIR and HLA and may be a missing element in understanding of reproductive disorders pathomechanism. Women with successful conceive after *in vitro* treatment and fertile women with at least two healthy-born children will be constitute control groups. The use of Real-Time PCR will allow to assess the polymorphism of *ERAP* genes in women with RIF and their partners. In addition, the amount of secreted form of aminopeptidases in women with RIF before and after the IVF-embryo transfer procedure in plasma will be examined by the ELISA test. It allow us to combine the genetics of ERAP with its secretion into the peripheral blood and will explain the role of both aminopeptidases in RIF. What's more, the combinations of *ERAP*, *KIR*, *HLA-C* and *HLA-G* polymorphisms will be analyzed in couples suffered from RIF. Perhaps different genetic variants of *HLA* in combination with certain sets of *KIR* and *ERAP* genes have an impact on recurrent implantation failure.

In this project, we also want to study the immune profile (the level of pro- and anti-inflammatory cytokines and growth factors) of women before and after *in vitro* treatment in patients' plasma. This is to check whether the different ovarian stimulation protocols that patients undergo to collect as many eggs as possible to IVF, and also the IVF-embryo transfer procedure itself, can affect the induction of inflammatory reactions. Because it has been reported that ERAP1 can shed receptors for cytokines, including for TNFR-α and ILR-6, its effect on the level of cytokines and their receptors during implantation and subsequent pregnancy may be significant. An important fact is also that in polish clinical practice, patients are not routinely tested for the level of pro- and anti-inflammatory cytokines as well as growth factors.

We believe that the research carried out in this project will expand knowledge on factors that may affect the susceptibility of recurrent implantation failure after *in vitro* procedure, depending on individual (genetic and immunological) predispositions of couples with infertility problem. Our research may be helpful in the diagnosis of RIF and prognosis of therapy results and may contribute to the explanation of the pathogenesis of this disease.