The role of KIR, their ligands HLA-C and HLA-G and endoplasmic reticulum aminopeptidases in recurrent implantation failure following *in vitro* fertilization

Nowadays, infertility is considered as a common disease worldwide as well as a social problem that affects more and more couples in reproductive age. Despite significant advances in assisted reproductive techniques, such as *in vitro* fertilization (IVF), women undergoing these procedures increasingly experience recurrent implantation failure. Recurrent implantation failure (RIF) is defined as the absence of pregnancy after at least three IVF procedures in which good quality embryos were transferred in women under the age of 40. The etiology of RIF is ambiguous. Maternal factors as well as factors related to the embryo and reproductive cells of the parents (egg quality, sperm quality, and chromosomal aberrations in these cells) influence implantation failures after IVF. However, the cause of more than 50% of RIF cases remains unclear.

Proper implantation depends on the tolerance of the maternal immune system to the fetus expressing the foreign paternal antigens without losing the mother's ability to fight infection. A unique feature of the human trophoblast (the cells that form the placenta), is the expression of human leukocyte antigens HLA-C and non-classical HLA-G. Killer immunoglobulin-like receptors (KIR) on natural killer (NK) cells or T cells are involved in the recognition of HLA-C and HLA-G molecules. During implantation and early pregnancy, the dominant population of leukocytes in the uterus is the population of uterine NK cells. In normal pregnancy, NK cells lack cytolytic activity. Due to their structure and interaction with ligands, KIR receptors can activate or inhibit the NK cell to produce cytokines and growth factors necessary for the proper implantation of the embryo. The polymorphism of *KIR* genes influences the activity status of NK cells and therefore may play a role in shaping interactions with HLA-C and HLA-G molecules expressed by the embryo. The antigenic peptides presented by HLA are formed by their cleavage to the appropriate length by endoplasmic reticulum aminopeptidases ERAP1 and ERAP2. The polymorphism of the *ERAP1* and *ERAP2* genes may affect the correct presentation of antigens to the respective receptors.

The main aim of the study was to assess the association between the polymorphism of *KIR* genes and their *HLA-C* and *HLA-G* ligands, as well as *ERAP1* and *ERAP2* polymorphisms with recurrent implantation failure after *in vitro* fertilization. The combinations of *ERAP, KIR, HLA-C* polymorphisms among couples suffering from RIF were also analyzed. Additionally, the concentration of aminopeptidases in the blood plasma of women with RIF before and after

the IVF procedure was tested. Moreover, the role of the soluble form of HLA-G (sHLA-G) in female and male infertility was examined.

A predisposition to RIF was observed in women with the combination of *KIR* genes AA telomeric region and *HLA-C2C2* genotype. In turn, the genes *KIR2DS2, KIR2DS3, KIR2DL2* and *KIR2DL5* gr. 2 from the centromeric region AB and BB in men were associated with an increased risk of RIF and infertility. Seven polymorphic sites in the genes encoding the endoplasmic reticulum aminopeptidases were investigated. *ERAP1* and *ERAP2* polymorphisms, both in women and men, may be involved in the pathogenesis of RIF. The *ERAP1* rs26653 G>C and rs26618 T>C polymorphisms with the *HLA-C2* allotype in women, have the greatest influence on infertility and RIF. In the combination of female *ERAP1/HLA-C* partner, the rs30187 C>T and rs27044 C>G polymorphisms play an important role in the implantation failure. In the combination of partner's *ERAP* and female's *HLA-C*, the rs30187 C>T has an impact on RIF. Moreover, the secreted ERAP1 and ERAP2 has been associated with female infertility. The concentration of ERAP2 associated with the miscarriage in patient was determined. For the first time in the world, the effect of the soluble form of HLA-G before and after embryo transfer on recurrent implantation failure has been shown. Women who did not become pregnant or experienced a miscarriage had significantly lower plasma levels of sHLA-G compared to women who became pregnant after IVF and fertile women. The analyses took into account clinical conditions such as the ovarian stimulation protocol or the cycle of embryo transferred (fresh or frozen/thawed). Some *HLA-G* rs1632947G>A-rs1233334G>C/T-rs371194629ins/del haplotypes and diplotypes were associated with infertility, while others showed a protective effect. The results also showed that sHLA-G concentrations were correlated with the haplotype and diplotype of *HLA-G*. In men, the low concentration of sHLA-G in semen was associated with the most unfavorable haplotype G-C-ins and diplotype G-C-ins/G-C-ins. Low sHLA-G level was also associated with the risk of teratozoospermia.

The results of this study show the important role of factors that may influence the susceptibility to recurrent implantation failure after IVF, depending on the individual (genetic and immunological) predispositions of couples with the infertility problem. The research may be helpful in the diagnosis of recurrent implantation failure and prognosis of therapy outcome, and explain the pathomechanism of this disease.