

## Abstract

The aim of this study was to evaluate the presence of receptors (CD16, CD161, NKG2D, KIR2DL4 and CD85j) on the surface of immunocompetent cells and whether their presence is involved in the *in vitro* fertilization (IVF) and following embryotransfer (ET) success.

We observed significant difference in the age of successful versus unsuccessful patients, which is confirmed by former studies on age-dependence of IVF success. The result from cytotoxic assay confirmed, that neither the stimulation protocol, nor the PBMC source have any effect on effector function. Afterwards, we performed a large-scale analysis of surface markers, starting with healthy donors, compared to patients. Both groups of patients have lower counts of HLA-G binding KIR2DL4 or CD85j receptors bearing cells. Next we observed the expression of these two receptors on various subpopulations of cells ( $CD56^{+dim}$ ,  $CD56^{+bright}$ , T helpers and cytotoxic T cells) and their counts. Here we observed differences in CD56 surface expression on  $CD56^{+bright}$  NK cells, the levels of  $CD56^{+dim}$  NK cells in PBMC (CD16 expression on this population), levels of  $CD56^{+dim}$  NK cells in FFMC. Next we observed a shift in T cell distribution strongly favoring the CTL in PBMC and in FFMC.

Next step was to compare the levels of cell populations between the successful and unsuccessful patients. We observed an increased surface expression of KIR2DL4 on lymphocytic PBMC of unsuccessful patients compared to both successful patients and healthy donors. This increased expression was further tracked to CTL surface. Then we observed increased surface expression of CD85j on unsuccessful patients PBMC. Successful patients on the other hand had lower CD85j surface expression on pure Th cells. Moreover, the successful patients had lower levels of partially activated  $CD56^{+bright}$  NK cells and of fully active  $CD56^{+dim}$  NK cells in their PBMC, when compared to unsuccessful patients. In the follicular fluids of patients we only observed a significant difference in the surface expression of CD161 on monocyte/granulocyte morphology of  $CD56^{+dim}$  NK cells. Lastly, we observed increased NKG2D expression on the surface of CD4-/CD8- double negative T cells in the FFMC of successful patients.

In this study, we identified several cell populations involved in the final successfulness of IVF. We confirmed the influence of HLA-G binding receptors KIR2DL4 and CD85j and their involvement in immunological mechanisms, accompanying the IVF successfulness and we pointed out the vast influence of stimulation protocols on the composition and function of the immune system of the patients.

**Key words:** HLA-G, IVF, NK cells, Immunity