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Abstract

Title: The influence of sex hormones on immune checkpoint expression in women suffering from miscarriage and women giving birth without complications.

Introduction: In the world around us, difficulties in having children are an increasing challenge for society. One of the elements of this phenomenon are miscarriages, which are the cause of failure in every seventh pregnancy. According to the definition, when there are two or more miscarriages before the 22th week of pregnancy in the same partner relationship, then we are talking about recurrent miscarriages, which affect about 5% of couples trying to conceive, and this problem is becoming more and more noticeable among the public. Increasingly, abnormalities in the functioning of the immune system are recognized as the one of the reasons of miscarriages. In women with recurrent miscarriages, in peripheral blood and decidual tissues, for example, an increased number of NK cells, a decreased number of regulatory T lymphocytes, or an imbalance between the number and functioning of T helper lymphocytes, type 1 and 2, have been noticed. Literature data indicate that a balance between activation and suppression of the immune system is essential for the proper course of pregnancy. The cellular response is regulated by a complex series of mechanisms, ranging from elimination of autoreactive lymphocytes in the thymus to silencing of activated lymphocytes at the site of inflammation. Immunological molecules that control the immune system, called immune checkpoints, can be classified as negative (co-inhibitory) or positive (co-stimulatory). They play an important role in regulating and maintaining immune homeostasis. They are widely studied in the context of restoring immune reactivity to cancer cells, using their potential in the treatment of autoimmune diseases, or in preventing the rejection of transplanted organs. Co-inhibitory receptors, when bound to ligands, can actively provide inhibitory signals to silence T cell activation or to balance stimulating signals. In addition, co-inhibitory receptors on maternal T cells promote an anergic state that ultimately leads to fetal immune tolerance, which is a key element for normal pregnancy development. The controlling molecules can play a significant role in the context of miscarriage. Studies in animal models (mice) have shown that blocking the expression of the gene for Tim-3 or PD1 could have contributed to miscarriage in mice previously giving birth properly. Research to date identifies only individual molecules that control the immune system on individual subpopulations of lymphocytes. At the

moment, the amount of therapy for women with recurrent miscarriages is slight. One of the treatments is to administer progesterone to patients, but the mechanism of its action on improving the condition of pregnancy in these women is still unknown, and confirmation of the effectiveness of the therapy is still under investigation. Sex hormones are known to influence both humoral and cellular responses in the immune system. Literature data indicate that the administration of sex hormones has a significant impact on the functioning of T lymphocytes or NK cells.

To better understand the role and function of immune-controlling molecules in recurrent miscarriages and normal pregnancy, this project assessed the expression of an extended set of immune checkpoints: PD1, TIM-3, LAG-3, VISTA and TIGIT. These studies were performed simultaneously on subpopulations of helper, cytotoxic and regulatory T lymphocytes, as well as NK and NKT cells. Additionally, the project investigated the soluble forms of immune checkpoints and their ligands. The obtained data may contribute to the determination of parameters that can be used in the diagnosis of habitual miscarriages. The project also investigated the effect of sex hormones such as: progesterone, 17β-estradiol and dihydrotestosterone on the expression of immune checkpoints on T lymphocytes as well as NK and NKT cells. The obtained data may allow to indicate the direction that will bring us closer to finding an effective therapy for women with recurrent miscarriages or explain the mechanism in which hormone therapy works in women with recurrent miscarriages.

Materials and methods: Three groups of women participated in the research: women with recurrent miscarriages, pregnant women and non-pregnant women, whose pregnancies previously developed properly. Flow cytometry was used to assess the presence of immune checkpoints on cells of the immune system. On the other hand, the concentrations of soluble forms of immune checkpoints and their ligands were determined using the Luminex platform. Sex hormone-stimulated cell cultures were established from mononuclear cells isolated from peripheral blood, collected from study participants. After 48 hours of stimulation at 37° C in an incubator, the presence of immune checkpoints was determined by flow cytometry.

Results: As a result of the conducted research, the expression of PD1, LAG-3 and VISTA was shown differently on T lymphocyte subpopulations, we additionally showed a difference in the expression of TIM-3 on NK cells and cytotoxic T lymphocytes between women with recurrent miscarriages and pregnant women. Also, the concentrations of soluble forms of immune checkpoints, such as sGal-9, sTIM-3, sLAG-3, sCD80, sCD86, sCD112, sCD155, sVISTA turned

out to be different between the studied groups. It has also been shown that progesterone at a concentration of 500 ng / ml has the greatest influence on the expression of immune checkpoints among the tested sex hormones.

Conclusions: The conducted research may contribute to the establishment of new parameters (biomarkers), the evaluation of which may allow for better diagnosis in women with recurrent miscarriages, thus increasing the chance for the proper development of pregnancy. The demonstrated changes after stimulation with sex hormones show a tendency to eliminate the detected disturbances in the expression of immune checkpoints on the cells of women with recurrent miscarriages in relation to women with properly developing pregnancy. The collected data on the influence of sex hormones on the cells of the immune system may allow the development of a therapy for women with recurrent miscarriage and explain how sex hormones influence the cells of the immune system during the development of pregnancy.