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Early signals of embryo presence during preimplantation period of pregnancy in mouse

DOCTORAL THESIS

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Summary

Pregnancy is one of the most fascinating biological phenomena. Its establishment and maintenance depends on embryo-maternal dialogue. Crucial phase of pregnancy is the pre-implantation period during which many developmental stages are achieved and the free-floating embryo is exposed to dynamic changes of the surrounding micro-environment. In humans, the incidence of early embryo death and pregnancy loss is estimated to be 30%. Disrupted embryo-maternal communication in the period directly preceding implantation may be one of the most important factors influencing reproductive efficiency. Moreover, high pre-implantation embryo death rate indicates the presence of an intensive selection process and suggests that failure in pregnancy establishment results from disturbances in the interaction between mother and embryo, recognized as a synchronic embryo development and the preparation of uterine tissue (mucosa) for its reception and implantation. Pre-implantation communication is achieved through secretory activity and direct interaction of reproductive tract (fallopian tubes, uterus) and the embryo, and can be defined as two types of signals - interacting locally (short signals) and peripherally (long signals). Short signals are represented by local change in transcriptional and/ or secretory activity of endometrial cells due to the presence of embryos in the reproductive system, activating multiple signaling pathways. There are also indications that the embryo-induced systemic immunoregulation (long signals) acts by the amplification of the signal through the uterus and fallopian tubes into the peripheral immune cells. However, during the pre-implantation period, these effects remain elusive. The main objective of the current research is to study the cellular and molecular processes involved in embryo implantation and trophoblast invasion to the endometrium. Such investigation will be helpful in understanding this complicated dialogue and its implications in the pathologies of pregnancy. Despite numerous studies on global gene profiling in the endometrium in response to the developing embryo, the hierarchy of those molecular interactions remains poorly understood.

The hypothesis of this study was that the embryonic signals measured in two compartments: in the uterus and peripheral immune cells, will be effective if the corresponding number of signaling pathways would be activated. The first goal was to answer the question which signaling pathways govern the pre-implantation period and do they differ in terms of reduced embryo development potential. The second objective of the study was to

compare T $CD4^+$ -lymphocytes' proteome during pre-implantation pregnancy, as a result of effective peripheral signaling, as well as to determine whether the biological quality of the embryo is recognized by peripheral immune cells. Therefore, two experiments were performed.

The first experiment was performed on two groups of mice: mice in pre-implantation period of pregnancy (3.5 dpc) and pseudopregnancy (3.5 dpc, control group) induced by natural mating. Material for further analysis was collected on 3.5 dpc: uteri for gene expression analysis and spleen for proteomic studies of splenic $TCD4^+$ lymphocytes.

The second experiment was performed using two groups of female mice after non-surgical embryo transplantation (obtained from female donors and cultured *in vitro*), used as a model for investigation signals from embryos of different developmental potential. Embryos which were transferred to uteri of recipient mice were: normal embryos (control) and embryos cultured in the presence of TNFα (embryos with reduced biological potential). Material for further analysis was collected on 3.5 dpc: uteri for gene expression analysis and spleen for proteomic studies of splenic $TCD4^+$ lymphocytes.

Based on the presented results, the following conclusions regarding early embryo signaling in the preimplantation period of pregnancy in mice can be derived. Presence of the embryo in the uterus during the pre-implantation period results in transcriptional silencing in the uterus: gene expression down-regulation in majority of investigated signaling pathways. Obtained results support the hypothesis that embryo triggers a local response in the uterus by negatively regulating the activity of endometrial signaling pathways. Signal- transduction pathways that are actively regulated by the presence of the embryo in pre-implantation pregnancy are NFκB, TGFβ and WNT. The NFκB pathway seems to be particularly important due to its altered regulation observed both during natural pregnancy and after transfer of embryos treated with TNFα. In addition, the results present for the first time that the expression of Ptgs2 gene and its protein product PTGS2 is induced shortly before implantation. This may be an indication that Ptgs2 expression is the earliest positive embryo signal of implantation preparation in mice.

Another result of this study was proteomic map of splenic $TCD4^+$ lymphocytes in mice. Additionaly, it has been shown that embryo signaling in the immunological peripheral compartment is recognized before implantation and is related to the expression of cytoskeletal and immunoregulatory proteins in $TCD4^+$ lymphocytes. Among the proteins with altered expression induced by the presence of TNFα-treated embryos were: proteins of cellular stress, proteins involved in the regulation of cytoskeleton and lymphocyte mobility, and proteins

with immunomodulatory function. The quality of embryo signals before implantation varies depending on the "quality" of the embryo, which is visible both in the local compartment (uterus) and at the periphery (peripheral T lymphocytes).