Treatment with intravenous immunoglobulin increases the level of small EVs in plasma of pregnant women with recurrent spontaneous abortions

Bæk, Rikke ; Jørgensen, Malene Møller; Varming, Kim; Christiansen, Ole Bjarne

Published in:
Journal of Extracellular Vesicles

DOI (link to publication from Publisher):
10.1080/20013078.2018.1461450

Publication date:
2018

Document Version
Publisher's PDF, also known as Version of record

PT02.13

Protein profiling of extracellular vesicles from the oviductal fluid of sows before and after ovulation

Inga Weiss; Sergio E. Palma-Vera; Andreas Vernunft; Jennifer Schoen; Shuai Chen

Institute of Reproductive Biology, Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany, Dummerstorf, Germany

Background: Extracellular vesicles (EV) present in the oviduct fluid (OF) have been suggested to deliver oviductal signals to gametes/embryo, thereby mediating the gamete/embryo–maternal crosstalk. Aim of this pilot study was to characterize the protein profile of oviductal EVs collected from sows before and after ovulation.
Methods: We isolated EVs from OF collected from sows in mid and late estrus (n = 2/group) and in metestrus (d2 post ovulation, n = 4), using a two-step polyethylene glycol precipitation followed by ultracentrifugation. EVs were visualized by transmission electron microscopy (TEM). BCA assay and mass spectrometry were performed to analyse their protein content. Differential protein expression analysis was performed using the R/Bioconductor package "Differential Enrichment analysis of Proteomics data" (DEP).
Results: TEM proved the presence of EVs (cup-shaped structure and size smaller than 150 nm) after isolation. Mass spectrometry analysis identified 1002 proteins which were expressed in all samples. The top 500 variable proteins produced cycle stage-dependent clusters after principal component analysis, which was corroborated by hierarchical clustering analysis of the differentially expressed proteins (5% FDR). Due to the low number of biological replicates, we observed a small number of differentially expressed proteins. The comparison between late-estrus (around LH peak) and metestrus showed eight up-regulated and three downregulated proteins, while the comparison between mid-estrus (before LH peak) and metestrus indicated six upregulated and six downregulated proteins. Seven significantly up-regulated proteins were detected when comparing the two estrus stages. Interestingly, upregulated proteins of the contrasts late-estrus versus mid-estrus and late-estrus versus metestrus intersected in six common proteins CRYM, RHOA, SP17, RS20, AKAP9 and ELMO3. In comparison to both other stages, HEM2 was upregulated in metestrus.
Summary/conclusion: These first results indicate that the protein content of oviductal EVs is dynamically adapted during the periovulatory period. These regulated EV proteins are potentially involved in gamete/embryo–maternal interactions.
Funding: Inga Weiss is supported by the H. Wilhelm Schaumann Foundation, Hamburg, Germany.

PT02.14

Treatment with intravenous immunoglobulin increases the level of small EVs in plasma of pregnant women with recurrent spontaneous abortions

Rikke Baek[1]; Malene M. Jørgensen[1]; Kim Varming[1]; Ole Bjarne Christiansen[2]

[1]Department of Clinical Immunology, Aalborg University Hospital, Aalborg, Denmark; [3]Department of Obstetrics and Gynecology, Aalborg University Hospital, Aalborg, Denmark

Background: Recurrent spontaneous abortion (RSA) is the cause of childlessness in 2–5% of reproducing couples. Immunological mechanisms have been proposed as an aetiology in some cases of RSA. Various forms of immunotherapy have been attempted in individuals thought to have an immunologic mechanism associated with RSA. Intravenous immunoglobulin has been tested in a placebo-controlled trial of women with RSA, and the effect of plasma small EV (sEV) phenotypes and levels were investigated during the pregnancy.
Methods: Twelve pregnant women with RSA who participated in the aforementioned trial were included in this study. In a blinded set-up, five of the women were given treatment with intravenous immunoglobulin and the rest were given placebo (human albumin). Venous peripheral blood (EDTA) was obtained from the women at several time points during their pregnancy.
Small EV concentration and composition were analysed by the EV Array (Jørgensen et al., 2013, JEV) using 29 selected surface markers. The antibodies used to capture the EVs included antibodies against EVs in general (CD9, CD63, CD81, Alix, Flotilin-1 etc.) and placental and immunological markers (PLAP, HLA ABC, HLA DR/DP/DQ, HLA G, FSHR, LHR, TSHR etc.).
Results: The first of the sequential samples (obtained before the first infusion in pregnancy week 5) from each woman were used as reference point to which the rest of the samples were normalized in order to detect the change over time. Already at the second sampling point (after 11–21 days), the level of sEVs carrying CD9 and CD81 increased massively (2–4 fold). After 30–40 days, this increase stops and remains stable during the rest of the pregnancy.
Summary/conclusion: A larger cohort/study is needed for increasing the statistical power. However, the tendencies are notably that the treatment with intravenous immunoglobulin has an effect on the level of sEVs in plasma.

PT02.15

The role of extracellular vesicles in mediating placental responses to maternal cellular stress

Catherine Evans; Thomas Rice; Beate Kampmann; Beth Holder

IMPERIAL COLLEGE LONDON, London, United Kingdom

Background: During pregnancy, the placenta acts as the interface between the maternal and foetal circulations. The placenta sheds extracellular vesicles (EVs), including exosomes, into the maternal circulation, which interact with maternal immune cells. We have recently demonstrated that this trafficking of EVs is bidirectional, with trafficking of EVs from immune cells to the placenta. EVs shed by stressed cells can elicit a "bystander effect" in recipient cells. We therefore investigated the functional impact of EVs released by stressed monocytes on placental trophoblast cells.
Methods: THP-1 cells were exposed to oxidative stress by hydrogen peroxide treatment. EVs were isolated by differential centrifugation and characterized by nanosight tracking analysis. EVs were added to BeWo trophoblast cells, which were then either left unstressed, or were subjected to oxidative stress.
Results: Oxidative stress induced by hydrogen peroxide had no effect on EV size nor concentration. Pretreatment with EVs from stressed or unstressed cells caused a small reverse in reduction of trophoblast viability in response to oxidative stress.
Summary/conclusion: EVs from maternal immune cells may help increase placental resistance to oxidative stress.
Funding: NIHR Imperial Biomedical Research Centre
MRC The Gambia