

should be screened for thyroid disorders when the male is not identified for being the cause of infertility. When clinical or subclinical thyroid hypo activity is detected (TSH >2.5 mIU/l), thyroxin should be administered. In the case of polycystic ovary syndrome patients, thyroxin should be administered even when TSH is over 2.0 mIU/l. Hyperthyroidism should be treated by antithyroidals or surgery. The presence of thyroid antibodies alone is not responsible for a lower pregnancy rate. When these women get pregnant, they should be further controlled and treated when necessary.

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Circulating cytokines influence the fetal growth in pregnant women with rheumatoid arthritis

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Introduction: High rheumatoid arthritis (RA) disease activity during pregnancy is associated with lower birth weight. Lower birth weight, even within the normal range, has been linked with cardiovascular diseases and metabolic syndromes later in life. Why high disease activity results in lower birth weight is not known. Outside pregnancy, active RA is associated with high levels of circulating cytokines. We therefore aim to assess the serum levels of IL-10, IL-6 and TNF α in pregnant women with RA and to evaluate correlations between these levels, disease activity, and birth weight.

Methods: Current study is embedded in the PARA-study, a prospective study on RA and pregnancy. 134 pregnant RA patients are enrolled in first and 168 in third trimester. 33 healthy pregnant women served as control. We analysed data using birth weight standard deviation scores (bwsds). Maternal disease activity is based on the disease activity score for 28 joints (DAS28) (range 0–10). IL-10, IL-6 and TNF α were determined on an Immulite 1000 system.

Results: In RA patients levels of IL-10, IL-6 and TNF α decrease during pregnancy, most likely as a results of declining disease activity. Strong correlations were found between these cytokines in first and third trimester and between DAS28 and IL-10, IL-6.

First trimester patients with detectable IL-10 ($n=12$) showed a higher disease activity than the IL-10 negative patients, a mean DAS28 4.4 (SD 1.2) and 3.6 (SD 1.1) respec-

tively. To diminish the influence of disease activity on birth weight we matched the IL-10 positive to an IL-10 negative group. Matching was on disease activity, parity and prednisone use. Mean bwsds was significantly higher ($p=0.02$) in the IL-10 positive group 0.92 (SD 0.7) than in the IL-10 negative match, 0.15 (SD 0.7). No such association was found in third trimester.

To determine the additional effect of IL-6 to disease activity on bwsds stratification was done. In first trimester we stratified IL-6 and DAS28 in a high and low group based on their median, resulting in 4 groups. In the two high DAS28 groups (defined median over 3.8), bwsds was significantly lower in high IL-6 than in low IL-6 ($p<0.05$). In the high and low IL-6 groups bwsds was -0.19 (SD 1.12) and 0.36 (SD 0.93), respectively. No such association was found in third trimester.

TNF α was stratified the same way. In third trimester, only in patients with low disease activity, bwsds was lower in the low TNF α group than in the high TNF α group, bwsds of 0.05 (SD 0.97) and 0.52 (SD 0.96) respectively ($p<0.05$). Same trend was seen in the healthy cohort group, although not significant. No such association was found in first trimester or in patients with high RA disease activity.

Conclusions: Circulating cytokines influence fetal growth in pregnant women with RA. In first trimester elevated IL-10 seems to protect against the negative influence of RA disease activity on birth weight, IL-6 seems to amplify this negative influence. Both cytokines create a bwsds deviation of more than 0.50 which is considered clinically relevant. In third trimester there is no influence suggesting an early critical window.

Finally, our data might indicate a physiological involvement of TNF α in fetal growth during third trimester in healthy controls and patients with low disease activity. This implicates that TNF-blockers, which are more and more prescribed during pregnancy to treat RA, should be used with caution.

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Allergen induced release of immune mediators in human placenta—A possible role in fetal programming of allergies?

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Introduction: Previous animal experiments indicated the transmissibility of general allergic diathesis from the mother to the fetus. All major cell types known to be involved in allergic processes, including dendritic cells, T cells, B cells and mast cells are present in the decidua. Thus, allergen induced mediator release may prime the fetus for easier sensitization in later life.

Material and methods: To investigate possible differences in cytokine and chemokine production from

placental tissue of atopic and healthy mothers after contact with a relevant allergen, we developed a one sided open placenta perfusion model with an internal standard to perfuse two separate cotyledons from the same placenta simultaneously with and without addition of relevant allergens. Hence, each control cotyledon provides the respective baseline values for quantitative analysis of allergen induced mediator release. Outflow samples were collected and analyzed for immune mediator release. Histamine concentration was determined spectrophotofluorometrically after extraction and derivatization with o-phthalaldehyde. The cytokines IL-2, IL-4, IL-6, IL-10, TNF α , IFN γ and IL-13, as well as the chemokines CXCL10, CXCL11, CCL17 and CCL22 were analyzed by using a cytometric multiplex bead array. By immuno-histochemical analysis IL-6 and TNF α were visualised in the perfused tissue sections.

Results: After application of relevant allergens in perfusion medium, a significant time-dependent release of TNF α and IL-6 was detectable in placenta of mothers suffering apple allergy compared to those from healthy mothers. The expression of the other cytokines and histamine was not remarkably altered. IL-13 was not detectable at all.

Conclusion: Allergens can induce allergy related effects, which may disturb the immunological balance at the fetomaternal interface and might prime the developing fetal immune system for facilitated later susceptibility for allergic sensitizations.

Keywords: Placenta; Perfusion; Cytokine

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Influence of galectin-1 on syncytium formation, proliferation and signal processes in trophoblast tumor cells BeWo

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Introduction: Galectin-1 (gal-1), a member of the mammalian β -galactoside-binding proteins, exerts biological effects by the recognition of glycan ligands, including those involved in cell adhesion metastasis, cell growth regulation and apoptosis. It has been demonstrated that gal-1 recognizes appropriate glycotopes on choriocarcinoma cells BeWo, which reveal two coexisting phenotypes, the cytotrophoblast-like and the syncytiotrophoblast-like phenotype. This information led to the question if gal-1, via its two carbohydrate binding sites, could influence the fusion formation in trophoblast cells. Although it has been demonstrated that gal-1 induces cell differentiation processes in trophoblast cells, no information on

its signal transduction processes is available so far. As phosphorylation is a major mechanism that controls multiple biological processes including cell differentiation, survival and proliferation, we examined which receptor tyrosine kinases (RTKs) and mitogen-activated protein kinases (MAPKs) are influenced by gal-1 in BeWo cells. Furthermore, we investigated the effect of gal-1 on syncytium formation in BeWo and human villous trophoblasts (HVT) cells.

Materials and methods The effect of gal-1 on syncytium formation was investigated with immunocytochemical and double immunofluorescence stainings, cell-labelling and real-time RT-PCR. BeWo choriocarcinoma and HVT cells were incubated *in vitro* for 24 and 48 h in the absence (controls) and presence of gal-1 and forskolin and stained with antibodies against Ki67, β -catenin, E-cadherin and syncytin. BeWo and HVT cells were incubated with 60 μ g/ml gal-1 for 48 h (BeWo) or 96 h (HVT) and cell fusion was detected by fluorescent cell-labelling solution. Finally, BeWo cells were incubated for 1 h or 48 h in the absence and presence of 60 μ g/ml gal-1 and real-time RT-PCR was performed.

BeWo cells were incubated for 24 h in the absence (controls) and presence of 60 μ g/ml gal-1. With the RayBio[®] Human RTK Phosphorylation Antibody Array 1, the relative levels of phosphorylation of different human RTKs could be detected simultaneously. The Human Phospho-MAPK Array Kit was used to determine the relative levels of phosphorylation of different human MAPKs and other serine/threonine kinases simultaneously. The signal intensities were compared and quantified with the Quantity One Version 4.5.2 program.

Gal-1-treated and non-treated cells were incubated with antibodies against REarranged during Transfection (RET) and phosphorylated RET^{Y905}. In addition, the TransAM[™] Elk1 Transcription Factor Assay Kit was used for quantification of relative Elk1 activation.

Results: We showed with immunocytochemical staining a downregulation of β -catenin expression in the 24 h BeWo cell culture and with double immunofluorescence staining an inhibition of the β -catenin and E-cadherin expression in the 48 h BeWo cell culture stimulated with gal-1 or forskolin. The inhibition of E-cadherin was demonstrated on mRNA level in the 1 h BeWo cell culture too. Increased cell fusion was also showed with DiO and Dil fluorescent cell-labelling solution in the 48 h BeWo cell culture. In addition, we demonstrated the downregulation of Ki67 protein expression in the 24 h BeWo cell culture and on mRNA level in the 1 h BeWo cell culture. We also showed the upregulation of syncytin protein and mRNA expression after incubation of the 48 h BeWo cell culture with gal-1 or forskolin.

Similar results were obtained with HVT cells: the amount of cell fusion was significantly increased in the gal-1 treated 48 h cell culture *in vitro* compared to untreated cells as demonstrated with β -catenin and E-cadherin double immunofluorescence staining. This increase was also shown by fluorescent cell-labelling with DiO and Dil in the 96 h cell culture compared to untreated cells.