

(n=5) (gWJ-MSC) pregnancies. To investigate the effect of gWJ-MSC on barrier integrity of human umbilical vein endothelial cell monolayer (HUVEC) leakage of albumin-FITC was measured at 0, 4 and 24 h time intervals after co-culture with nWJ-MSC or gWJ-MSCs. Sixty thousand HUVECs from a normal donor were seeded on 1% gelatin-coated 6.5mm transwell inserts (0.4µm pore). Upon confluence (24h), 5000 WJ-MSC (n or g) were placed on top of HUVEC. FITC-Albumin (1mg/ml) was added to the apical chamber and transfer to the lower compartment was assessed. The percentage of protein leakage was compared with controls (HUVEC alone). One-way ANOVA was used to test statistical differences; 6 replicates were performed.

Results: The tracer leakage values were undetectable at 0 h. No statistical difference was found at 4h. After 24h, tracer leakage was significantly different (HUVEC alone 20.1%±2.5, nWJ-MSC+HUVEC 19.5%±3.6 and gWJ-MSC+HUVEC 24.4%±5.8; ***P<0.001) between the groups. Tracer leakage increased when HUVEC was co-cultured with g-WJ-MSC.

Conclusion: These changes in vascular permeability suggest an impaired ability of WJ-MSC, even from well-controlled GDM, to maintain/increase endothelial integrity after transmigration.

P1.34.

EFFECT OF MATERNAL DIETS ENRICHED IN SUNFLOWER OR CHIA OIL ON THE GLYCOGENIC AREA AND THE UTERINE GLANDS SECRETORY GRANULES OF THE DECIDUA FROM DIABETIC RATS DURING EARLY POSTIMPLANTATION

Sabrina Lorena Roberti, Cintia Romina Gatti, Romina Higa, Alicia Jawerbaum. *Laboratory of Reproduction and Metabolism-CEFYO-UBA-CONICET, Buenos Aires, Argentina*

Objectives: Embryo development defects induced by maternal diabetes may start at early pregnancy when histotrophic nutrition occurs through the decidua, involving structures like the uterine glands and the glycogenic area. Prolactin is a marker of decidual function and the antioxidant enzyme manganese superoxide dismutase (MnSOD) regulates the decidual prooxidant state. Diets enriched in polyunsaturated fatty acids (PUFAs) have previously found to regulate MnSOD gene expression in different systems. Aim: To evaluate the effect of diets enriched in sunflower and chia oil (enriched in n-6 and n-3 PUFAs respectively) administered during early postimplantation on PAS staining of decidual glycogenic area and uterine glands as well as decidual prolactin and MnSOD expression in diabetic rats at day 9 of pregnancy.

Methods: Pregestational diabetes was induced in Wistar rats by streptozotocin administration (50 mg/kg). On days 7,8 and 9 of pregnancy diabetic rats received a standard diet or diets enriched in 6% of sunflower or chia oil. On day 9 of pregnancy PAS staining was performed to evaluate decidual glycogenic area and uterine glands and decidual prolactin and MnSOD mRNA levels were measured by RT-qPCR.

Results: We found a reduction in PAS staining of the glycogenic area and the uterine glands (p<0.05; 64%) in diabetic rats compared to controls, an alteration prevented by the maternal diets enriched in PUFAs. The prolactin (0.69%, p<0.05) and MnSOD (0.53%, p<0.05) mRNA levels were decreased in the decidua of diabetic rats compared to controls. Maternal diets prevented the MnSOD reduction but not prolactin reduction.

Conclusion: Maternal diabetes leads to alterations in decidual prolactin expression and decidual structures relevant for decidual histotrophic function, being the lasts prevented by diets enriched in PUFAs. As the decrease in MnSOD gene expression was also prevented by these diets, a link between metabolic and antioxidant pathways in the decidua is suggested and requires further research.

P1.35.

DCN PRODUCTION BY THE HUMAN DECIDUA: ROLE OF IL-1BETA

Chidambra Halari, Stephen Renaud, Peeyush Lala. *Western University, London, Canada*

Objectives: Our lab discovered that decorin (DCN), a proteoglycan made by decidual cells controls placental invasion and helps decidual cell

maturation, whereas decidual DCN over-production is associated with preeclampsia. Furthermore, DCN production increases during decidualization of human endometrial stromal cells (HESC) in vitro and in the first trimester decidua in vivo. What controls DCN production by the decidua is unknown. IL-1β, a pro-inflammatory cytokine essential for pregnancy success is reported to be upregulated in PE. By noting the effects of multiple cytokines we found that IL-1β is the key stimulus for DCN production by the HESC. Objective of our study was to define molecular pathway(s) of IL-1β induced DCN production by the HESC.

Methods: To test whether blocking IL-1Receptor (R) in HESC followed by decidualizing stimuli blocks/hinders IL-1β-induced DCN production, we measured DCN production (mRNA by qRT-PCR; secreted protein by ELISA) in the presence of a specific IL-1R antagonist. To clarify the role of IL-1β as inducer of DCN, we used inhibitors of 3 well-known IL-1β pathways. HESCs pre-treated with these inhibitors were cultured with fresh media containing IL-1β. DCN mRNA and secreted protein were measured. To measure activation of NF-kB by IL-1β we (a) measured phosphorylation of p65 at 15 min-6 hr. (b) conducted dual immuno-staining for p65 (to see nuclear translocation) in DAPI stained cells after IL-1β exposure for 15min, 30min and 24hrs and (c) luciferase reporter assay

Results: (a) Blocking IL-1R, reduced DCN production; (b) IL-1β-induced DCN production was reduced after exposure to NF-kB inhibitor alone (c) Western blots revealed p65 phosphorylation after exposure to IL-1β; (d) Dual immuno-staining for p65 in DAPI-stained cells revealed rapid nuclear translocation of p65. (e) NF-kB activation by IL-1β measured with NF-kB luciferase reporter assay showed an increased (2.5 fold) luminescence at 2.5hrs.

Conclusion: IL-1β induces DCN production by HESC using NF-kB pathway.

P1.36.

PLACENTAL DAGLβ REGULATES 2-ARACHIDONOYLGLYCEROL (2-AG) LEVELS AND IS INVOLVED IN PREGNANCY INDUCED INFLAMMATION

Natascha Berger¹, Thomas Bärnthaler², Jürgen Gindlhuber³, Nermeen Girgis⁴, Birgit Hirschmugl¹, Tom van der Wel⁵, Robert Zimmermann⁴, Ruth Birner-Gruenberger³, Mario van der Stelt⁵, Christian Wadsack¹. ¹Department of Obstetrics and Gynecology, Medical University of Graz, Graz, Austria; ²Otto Loewi Research Center, Division of Pharmacology, University of Graz, Graz, Austria; ³Gottfried Schatz Research Center, Division of Molecular Biology and Biochemistry, Medical University of Graz, Graz, Austria; ⁴Institute of Molecular Biosciences, University of Graz, Graz, Austria; ⁵Department of Molecular Physiology, Leiden Institute of Chemistry, Leiden University, Leiden, Netherlands

Objectives: Diacylglycerollipase alpha/beta (DAGLα/β) are two sequence-related serine hydrolases, biosynthesizing 2-arachidonoylglycerol (2-AG). 2-AG, one of the main endocannabinoids, and its esterified fatty acid arachidonic acid serve as substrates for pro-inflammatory cellular pathways. Chronic inflammation is one hallmark of placentas affected by preeclampsia (PE) which is often linked to an altered lipid metabolism. In this study, we aim to identify the specific function and regulation of DAGLα/β in healthy (CTRL) and PE placentas.

Methods: Lipid and fatty acid profiles of placental tissues and perfusates of tissue perfusions were measured by LC-MS. DAGLα/β transcripts were detected in situ and localized to placental cell types by immunohistochemical staining in CTRL and PE placentas. RNA and protein expression was examined by RT-qPCR and immunoblot analysis. DAGLβ activity was determined by activity-based protein profiling (ABPP) using a specific DAGL inhibitor.

Results: We identified DAGLβ as the predominant isoform (p<0.001) in placental tissue and detected DAGLα and β to varying degrees in different cell types of CTRL and PE placentas. In PE the number of DAGLβ transcripts and protein expression were significantly elevated (p=0.009, p=0.018, respectively). ABPP enabled us to detect DAGLβ activity which was significantly increased in PE tissues (p=0.003). The lipidomic profiling of CTRL and PE placentas revealed an altered lipid distribution in PE showing elevated levels of triacylglycerols containing polyunsaturated fatty acids. Ex vivo perfusion of placentas underlined the physiological relevance of

DAGL β activity by decreased 2-AG tissue levels upon pharmacological inhibition of the enzyme ($p=0.036$).

Conclusion: Our results suggest that placental DAGL β controls 2-AG levels, which in turn may impact the regulation of endocannabinoid signaling. Chronic inflammation during pregnancy as observed in PE, increases placental DAGL β expression and activity. We further provide evidence of dyslipidemia in PE placentas, thus counteracting inflammatory processes in the tissue.

P1.37.

BENEFICIAL IN VITRO EFFECTS OF THE PASSION FRUIT SEED EXTRACT AGAINST PLACENTAL ZIKA VIRUS INFECTION

Eloiza Tanabe¹, Jaqueline Santos¹, Keyla Pires¹, Monique Nova¹, Karen Borbely^{1,2}, Jadriane Xavier³, Flávia Santos³, Felipe Silva³, Júnia Barbosa³, Iara Valentim⁴, Josué Santos³, Marília Goulart³, Alexandre Borbely¹. ¹Cell Biology Laboratory, Institute of Health and Biological Sciences, Federal University of Alagoas, Maceió, Brazil; ²Faculty of Nutrition, Federal University of Alagoas, Maceió, Brazil; ³Institute of Chemistry and Biotechnology, Federal University of Alagoas, Maceió, Brazil; ⁴Federal Institute of Education, Science and Technology of Alagoas, Maceió, Brazil

Objectives: Brazil is the major producer of yellow passion fruit in the world. Its pulp is used by the food industry, and the oil from its seeds for cosmetic and medicinal purposes, consumed by native-Americans to improve health and prevent infections. We aimed to investigate the effects of passion fruit seed extract (PFSE) on term chorionic villi explants and HTR-8/SVneo cells infected by two strains of Zika virus (ZIKV), MR766 and PE243.

Methods: The PFSE antioxidant capacity was measured by DPPH, HOCl, lipid peroxidation, and mitoSOX staining. Cytotoxicity was measured by MTT. The infectivity and supernatant viral load were evaluated by immunofluorescence, flow cytometry, and RT-qPCR. Proliferation by Ki67 staining. Cytokines and angiogenic factors production were measured by flow cytometry. Cell death was evaluated by annexin V/propidium iodide and autophagy by LC3B staining. The PFSE showed low IC₅₀ values for DPPH and HOCl and excellent protection against lipid peroxidation. PFSE was well tolerated by cells and placental explants.

Results: The treatment with PFSE reduced supernatant viral load from HTR-8SV/neo infected by ZIKV ($p<0.01$ both strains), and for explants infected by PE243 ($p<0.01$). Flow cytometry showed a reduction of ZIKV+ cells ($p<0.05$), confirmed by immunofluorescence. Both treatments unchanged cell proliferation and cell cycle. In HTR-8/SVneo cells and placental explants, no differences were observed in IFN-I, II, and III production. The PFSE treatment increased FGF-basic ($p<0.05$) and ablated TNF- α production ($p<0.05$). The generation of superoxide radical anion and autophagy were inhibited by PFSE treatment as seen in the microscope, and cell death was greatly inhibited ($p<0.01$).

Conclusion: Thus, PFSE has a promising potential for protecting the placenta from ZIKV infection, and the development of biotechnological/medical products from industrial leftovers provides extra value to products and strong market value. Further tests will also be developed with promising compounds found in PFSE.

P1.38.

IN VITRO EVALUATION OF UVAOL TREATMENT IN TERM CHORIONIC VILLI EXPLANTS INCUBATED WITH GROUP B STREPTOCOCCUS

Ashelley Sousa¹, Ingredy Rodrigues¹, Larissa Almeida¹, Monique Nova¹, Maria Santos¹, Keyla Pires¹, Guillaume Sébire², Alexandre Borbely¹, Karen Borbely³. ¹Cell Biology Laboratory, Institute of Health and Biological Sciences, Federal University of Alagoas, Maceio, Brazil; ²Department of Pediatrics, McGill University, Montreal, Canada; ³Post-graduation in Nutrition, Federal University of Alagoas, Maceio, Brazil

Objectives: Group B Streptococcus (GBS) is the most common pathogen to colonize pregnant women and it can also cause chorioamnionitis, an infectious and inflammatory condition that affects the fetal membranes

during pregnancy and can lead to prematurity and neonatal sepsis. The maternal diet is a source of potentially beneficial molecules, such as uvaol, a triterpene found in olive oil, described to have cytoprotective, anti-oxidative and anti-inflammatory properties. As such, we aimed to evaluate uvaol treatment effects on term chorionic villi explants viability, and cytokines and angiogenic molecules after GBS incubation.

Methods: Five term chorionic villi explants from healthy women were cultured for 24 h, and the explants were treated with uvaol at 1, 10, 50 and 100 μ M for 1 h, further incubated with inactivated GBS at 10⁶, 10⁷ and 10⁸ CFU for further 24 h. Supernatants were collected to analyze cytokines and angiogenic factors by flow cytometry. The viability assay was performed with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) staining and measured with a spectrophotometer.

Results: GBS at concentrations of 10⁷ and 10⁸ CFU reduced the viability of placental explants ($p<0.05$), whereas 50 μ M uvaol prevented this viability reduction ($p<0.05$). From all analyzed molecules, GBS increased CXCL-8 ($p<0.001$), IFN- γ ($p<0.01$), IL-1 β ($p<0.05$), IL-2 ($p<0.001$), IL-5 ($p<0.05$) and IL-6 ($p<0.01$). Uvaol treatment was able to prevent CXCL-8 ($p<0.01$), IFN- γ ($p<0.05$), IL-1 β ($p<0.05$) and IL-6 ($p<0.01$) GBS-dependent increase. Additionally, uvaol alone increased IL-13 ($p<0.001$) and VEGF ($p<0.05$).

Conclusion: Our results show that uvaol provided placental protection from GBS deleterious effects, and could be an important natural product to be used through pregnancy in order to prevent non-desired pregnancy outcomes.

P1.39.

SCHINUS TEREBINTHIFOLIUS RADDI EXTRACTS PROTECT THE PLACENTA AGAINST ZIKA VIRUS INFECTION

Rayane M. Botelho¹, Jaqueline C. Santos¹, Keyla S.N. Pires¹, Monique A.V. Nova¹, Camilla M. Gonçalves¹, Karen S.C. Borbely¹, Mônica B.S. Oliveira², Iara B. Valentim³, Tauane S. Rocha², Marília O.F. Goulart², Alexandre U. Borbely¹. ¹Cell Biology Laboratory, Institute of Health and Biological Sciences, Federal University of Alagoas, Maceió, Brazil; ²Institute of Chemistry and Biotechnology, Federal University of Alagoas, Maceió, Brazil; ³Federal Institute of Education, Science and Technology of Alagoas, Maceió, Brazil

Objectives: Schinus terebinthifolius Raddi is a medicinal plant native from South America. This species already has demonstrated important biological activities. Recently reported, the ethanolic extracts from their peel (STPE) and from the whole fruits (STWFE) were able to reduce Zika virus (ZIKV) cytopathic effects on HTR-8/SVneo cells, reduced supernatant viral load, and reduced the amount of infected cells. As such, we aimed to investigate if these effects would be seen in term chorionic villi explants and if the extracts would interfere in cell proliferation, cytokines and angiogenic factor production.

Methods: The chemical composition of the extracts was evaluated by HPLC-UV/vis. The infectivity and supernatant viral load were evaluated by immunofluorescence, flow cytometry and RT-qPCR. Proliferation was visualized by Ki67 staining. Cytokines and angiogenic factors production was measured by flow cytometry. The chorionic villi explants were infected only by PE243 strain, whereas HTR-8/SVneo cells by both strains. The treatments with STPE or STWFE reduced supernatant viral load (both $p<0.05$) from the explants and cells.

Results: Both treatments unchanged cell proliferation and cell cycle. In HTR-8/SVneo cells, no differences were observed in IFN-I, II and III production with or without ZIKV strains and both treatments. The ZIKV PE243 infection of placental explants induced massive production of IL-6 ($p<0.05$) and CXCL-8 ($p<0.01$), increased production of Angiotensin-2 ($p<0.05$) and decreased production of FGF-basic ($p<0.05$). Both treatments prevented Angiotensin-2 increase ($p<0.05$), and utterly decreased TNF- α production ($p<0.05$), whereas only the STPE prevented CXCL-8 increase ($p<0.05$).

Conclusion: Both extracts could reduce ZIKV infection and modulate placental response, which seems to protect placentas from potential harms provoked by ZIKV infection. The development of biotechnological/medical products from industrial leftovers provide extra value to products from family farming, and strong market value. Further tests will be developed