S13.
DESCRIPTIVE AND FUNCTIONAL ANALYSES RELATE ERBB2/ERBB3 HETERODIMERS TO EVT FUNCTION AND CORRELATE EGFR/ERBB4 WITH CELL CYCLE PROGRESSION IN HEALTHY AND COMPLETE HYDATIDIFORM MOLE PLACENTAE

Kerstin Plessl, Philipp Velicky, Martin Knöfler, Jürgen Pollheimer. *Department of Obstetrics and Fetal-Maternal Medicine, Reproductive Biology Unit, Medical University of Vienna, Austria*

Objective: Our aim was to characterize the expression and activation pattern of ERBB receptors in human first trimester trophoblast subtypes. Second, we studied ERBB-specific downstream signaling and function in EGFR+ as well as HLA-G+ trophoblasts.

Methods: ERBB receptor expression was analyzed in primary trophoblast cell isolates by means of microarray, CISH, FISH, qRT-PCR and Western blotting, as well as immunofluorescence stainings of placental and decidual tissue sections. In addition, isolated trophoblasts were stimulated with ERBB-specific ligands including EGF, HB-EGF and NRG1 to evaluate the phosphorylation status of ERBB receptors. Functional assays were performed to study ERBB-related function in trophoblasts including proliferation, invasion or apoptotic resistance. Finally, the average number of EGFR+ trophoblast layers was determined in complete hydatidiform mole placentae and compared with healthy age-matched controls.

Results: EGFR+ trophoblasts co-express ERBB4, but are devoid of ERBB2 and ERBB3. In contrast, HLA-G+ trophoblast subtypes exhibit an EGFR/ERBB4- and ERBB2/ERBB3+ phenotype. The ERBB1/4-specific ligands EGF and HB-EGF induce proliferation in EGFR+ positive trophoblast subtypes. Consistently, EGFR+ trophoblastic layers are significantly expanded in CHM placentae when compared with healthy controls. Stimulation with conditioned medium from primary decidual cells or rhu NRG1 induced the phosphorylation of ERBB2/ERBB3 and Akt, ERK, s6 kinase as well as mTOR. Further, NRG1-mediated activation of ERBB2/3 suppresses camptothecin-induced apoptosis in primary trophoblasts. Finally, we were able to detect amplification of the ERBB2 gene as well as numerical aberrations of chromosome 17 by FISH analyses in situ and in isolated EGFR/HLA-G-sorted trophoblasts.

Conclusions: Our data clearly indicate that EGFR and ERBB4 are specifically expressed by proliferative trophoblasts whereas ERBB2/3 are exclusively found in EVTs. Accordingly, EGF and HB-EGF induce cell cycle progression in primary trophoblasts and placental explants.On the other hand, decidual cell derived NRG1 induces apoptotic resistance in EVTs by activating ERBB2/3. Furthermore, we propose EGFR as marker for proliferative trophoblasts and as therapeutic target in CHM placentae.

S14.
EFFECTS OF SHIGA TOXIN TYPE 2 ON RATS IN THE EARLY AND LATE STAGES OF PREGNANCY

C. Ibarra [1], A.M. Franchi [2]. [1] *IFIBIO-CONICET, Universidad de Buenos Aires, Buenos Aires, Argentina*; [2] *CEFYBO-CONICET, Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina*

Shiga toxin type 2 (Stx2) is the leading cause of Hemolytic Uremic Syndrome (HUS), a systemic complication characterized by thrombocytopenia, microangiopathic hemolytic anemia and acute renal failure. In Argentina, HUS is endemic and affects around 400 new patients per year. Maternal bacterial infections during pregnancy are associated with an increased incidence of fetal death, congenital malformations, placental abruption, premature rupture of membranes and prematurity in humans and animals. We propose that Stx2 might be one of the causes of feto-maternal morbimortality not yet investigated. We used a model of HUS in rats by intraperitoneal injection of Stx2. In rats in the late stage of pregnancy, Stx2 produced maternal lethality in a dose-dependent manner, fetoplacental resorption, placental abruption, intrauterine hemorrhage and premature delivery of dead fetuses. The high expression of iNOS and TNF-⟨ are the main responsible for these effects since the combined action of aminoguanidine and etanercept prevented Stx2-induced preterm delivery by roughly 70%. In rats in the early stage of pregnancy, Stx2 induced vaginal bleeding, structural alterations in the utero-placental unit and spontaneous abortion. Stx2 was immunolocalized in the microvasculature and decidual cells of Stx2-treated rats where the globotriaosylceramide (Gb3) receptor of Stx2 has been detected. Alteration in the utero-placental vasculature, hypoxia in decidua cells and leukocyte infiltration with high expression of TNF-α at 2 h post Stx2 treatment were detected. These results indicate that spontaneous abortion occurs by a direct cytotoxic effect of Stx2 on the utero-placental unit, deficient utero-placental perfusion and abnormal maternal inflammation.

S15.
CONGENITAL TRANSMISSION OF PROTOZOA: PLACENTAL INFECTION BY *TRYPANOSOMA CRUZI*

C. Díaz-Luján [1,*], M.F. Triquell [1,*], E. Bolatti [1], M.J. Moreira Espinoza [1], D. Hardisson [3], C. Castillo [4], U. Kemmerling [4], R.E. Fretes [1,2]. [1] *Department of Cell Biology, Histology and Embryology, Medicine School, Universidad Nacional de Cordoba-INICSA (CONICET), Argentina*; [2] *Histology and Embryology-IICSHUM-Universidad Nacional de La Rioja, Argentina*; [3] *Hospital La Paz, Facultad de Medicina, Universidad Autónoma de Madrid, Spain*; [4] *Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile, Chile*

Chagas disease and its congenital transmission are caused by the protozoan *Trypanosoma cruzi*. The maternal-fetal transmission has spread the disease into non-endemic countries. Its incidence is low and the complete placental barrier limits the placental infection. Since discontinuities in the placental barrier have been described, the **Objectives** and **Methods** were to analyze the susceptibility of placental barrier cells to infection by T. cruzi and the role of nitric oxide by using monolayers of trophoblasts in vitro, placental explants in an *in vitro-ex vivo* experimental design and placentas from chagasic women.

Results: The syncytiotrophoblast (STB) was significantly less infected than the cytotrophoblast (CTB), indicating that the inner cells of chorionic villi are more susceptible to infection than the cells which are in contact with the maternal blood, explaining at least in part the resistance of transmission to the fetus. Nitric oxide (NO) levels increased when STB was present and correlated significantly with decreased viability of parasite cells in the culture media (r=0.93). Moreover, expression of endothelial Nitric Oxide Synthase (eNOS) by STB, one of the isoenzymes that produces NO, decreased significantly in placentas with maternal-fetal transmission of Chagas, which were also positive to *T. cruzi* DNA by PCR technique, indicating that this gas plays an important role in the clearance of parasites in the placental environment, in placental infection and/or congenital transmission.

Conclusion: These results support that STB plays a key role limiting the infection of chorionic villi by *T. cruzi*, via clearing parasites out of the placental environment and via NO producing enzymes. Thus, these results establish that there was a placental environment deleterious to *T. cruzi* and that discontinuities in the placental barrier and/or deficiencies in placental defenses, such as eNOS, facilitate the infection of placental tissue and maternal-fetal transmission of Chagas.

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S16.
CONGENITAL CHAGAS DISEASE: THE TROPHOBLAST TURNOVER AS A POSSIBLE LOCAL DEFENSE MECHANISM OF THE HUMAN PLACENTA

C. Castillo, A. Liempi, D. Droguett, I. Carillo, F. Gomez, M. Negrete, K. Barahona, A. Hernández, J.D. Maya, N. Galanti, U. Kemmerling. *Institute for Biomedical Sciences, Faculty of Medicine, University of Chile, Santiago, Chile*

[*] Equal contribution