255 THE DEGREE OF EXPRESSION OF SERINE PROTEASE HTRA1 AND ITS AFFECTS ON TROPHOBLAST CELL INVASION IN NORMAL AND ABNORMAL PLACENTATION DENNIS MCWEENEY[1], FUNMINIYI AJAYI[2], NICHOLAS KONGOASA[3], THOMAS GAFFEY[4], VIJI SHRIDHAR[3], BRIAN BROST[1], JEREMY CHIEN[3], [1]Mayo Clinic College of Medicine, Maternal Fetal Medicine Division, Rochester, Minnesota, [2]The Ohio State University, Maternal Fetal Medicine Division, Columbus, Ohio, [3]Mayo Foundation for Medical Education and Research, Rochester, Minnesota, [4]Mayo Clinic College of Medicine, Anatomic Pathology, Rochester, Minnesota

OBJECTIVE: Previous work by Ajayi et al demonstrated that serine protease HtrA1 (high temperature requirement factor A1) is overexpressed in trophoblastic tissue in severe preeclamptic women and limits the degree of migration and invasion of trophoblast cells. We hypothesize that aberrant expression of HtrA1 may play a role in pregnancies complicated by placenta accreta, increta or percreta.

STUDY DESIGN: All women who underwent cesarean/postpartum hysterectomy at Mayo Clinic were identified over a 15 year period. Tissue blocks from the placental-uterine interface were immunohistochemical stained with affinity-purified anti-HtrA1 antibody as previously described. The level of HtrA1 expression was quantitatively scored via light microscopy by a placental pathologist blinded to the clinical history and assigned a ranking score of 0 (no staining) to 3 (marked staining). A similar preparation was performed by Ajayi et al analyzing specimens of women with severe preeclampsia for comparision. Fischer's Exact test was performed with statistical significance defined as $p<0.05$.

RESULTS: Fourteen patients underwent hysterectomy for abnormal placentation (accreta-6, increta-4, percreta-4) while twenty patients underwent hysterectomy for other conditions. When HtrA1 expression in accreta, increta or percreta group was compared to those of the preeclampsia group, the difference was statistically significant for villous ($p=0.0258$) and extravillous ($p=0.0026$) trophoblastic samples. However, when comparing samples from the normal placentation group to the accreta, increta or percreta or preeclampsia group, no difference was found.

CONCLUSION: Our results suggest that underexpression of HtrA1 in villous and extravillous trophoblasts may be responsible for the excessive depth of invasion in pregnancies complicated by placenta accreta, increta, or percreta. In fact, one can view these findings as part of the continuum or spectrum of disease severity with HtrA1 overepression resulting in shallow trophoblast invasion in preeclampsia and underexpression in cases of abnormally adherent invasion.

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256 IDO AND SFLT-1: A POTENTIAL MOLECULAR LINK BETWEEN THE IMMUNE SYSTEM AND PREECLAMPSIA. MARK SANTILLAN[1], DONNA SANTILLAN[1], CURT SIGMUND[1], STEPHEN HUNTER[1], [1]University of Iowa, Iowa City, Iowa

OBJECTIVE: Many studies strongly suggest that an immune response to pregnancy triggers the cascade of physiologic effects resulting in preeclampsia (PreE). One candidate immunogenic protein in this theory is indoleamine 2,3-dioxygenase (IDO) which is essential to the catabolism of tryptophan, an amino acid important in T cell function. IDO deficiency in placentas from preeclamptics is linked to increased placental rejection and dysfunction. IDO deficiency may also contribute to the vascular oxidative stress characteristic of PreE as IDO utilizes superoxide anion to catabolize tryptophan. The objective of this study is to explore a potential molecular link between the immune system and the vasculature, by investigating IDO and sFLT-1, an important soluble anti-angiogenic PreE effector protein, using a human cell culture model.

STUDY DESIGN: HeLa cells were plated and stimulated with Interferon-γ (IFN-γ) for 24 hours. From these cells, IDO and sFLT-1 expression were examined by reverse transcriptase PCR and/or immunoblotting. Under the same stimulation conditions, HeLa cells were also exposed to 1-methyl tryptophan (1-MT), an IDO inhibitor, or L-NAME, a nitric oxide synthetase (NOS) inhibitor, for 24 hours. IDO and sFLT-1 expression were also determined.

RESULTS: Interestingly, IFN-γ induced ***both*** IDO and sFLT-1 mRNA expression. The addition of 1-MT to the IFN-γ stimulated cultures inhibits IDO protein expression. The addition of L-NAME to IFN-γ stimulated cultures does not inhibit the induction of IDO mRNA and protein expression in HeLa cells.

CONCLUSION: Both IDO and sFLT-1 have been implicated in the etiology of preeclampsia. INF-γ induction of sFLT-1 has not been previously described. Because INF-γ induced expression of ***both*** IDO and sFLT-1 and L-NAME did not inhibit IDO expression, we postulate that there is a mutual mechanism regulating IDO and sFLT-1 expression. In future studies, we will test our hypothesis that NOS and INF-γ are critical molecular links between IDO and sFLT-1.

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257 CHORION FETAL CELL LAYER IS PREMATURELY DESTROYED IN PPROM COMPARED TO PTL AND TERM PATIENTS BERNARD CANZONERI[1], LIPING FENG[1], REX BENTLEY[1], PHILLIP HEINE[1], AMY MURTHA[1], [1]Duke University, Durham, North Carolina

OBJECTIVE: The etiology of preterm premature rupture of membranes (PPROM) remains unclear. We hypothesize that tissue remodeling in fetal membranes, specifically the chorion, results in weakening and rupture. We sought to quantify the chorion cell layer in fetal membranes from PPROM, preterm labor (PTL), and term subjects.

STUDY DESIGN: A retrospective investigation was performed on clinically collected fetal membrane samples from 75 subjects delivered at Duke Medical Center (25 in each group: PPROM, PTL, and term labor). Paraffin embedded fetal membrane histologic sections were obtained. Immunohistochemistry was performed using a primary antibody against cytokeratin. A Zeiss Axio Imager fluorescence microscope was used to take 2 digital images of each quadrant of fetal membranes on each slide (8 images per slide). ImageJ software was used to measure the chorion and decidua in 4 distinct regions of the image (32 measurements/subject). The average chorion thickness and the % chorion of choriodecidua thickness were calculated and compared among the 3 groups. Statistical analysis was performed by Kruskal Wallis and chi square where appropriate.

RESULTS: There was no difference in maternal age or race between groups. Chorion cell layer thickness in PPROM subjects was significantly smaller when compared to PTL and term subjects (70.8 vs. 92.0 vs. 160.4.4 μm, $p<.0001$). Also, the % chorion of choriodecidua was significantly less in PPROM compared to PTL and term subjects (15.7 vs. 25.1 vs. 35.7%, $p<.0001$). For all subgroups analyzed, there appeared to be a dose response effect which was demonstrated least in term subjects and greatest in PPROM subjects. Further, when severe histologic chorioamnionitis was excluded from analysis, the results were similar (76.6 vs. 81.8 vs. 160.4 μm, $p=.0006$)

CONCLUSION: Premature destruction of the chorion cell layer of fetal membranes appears to be more common in PPROM compared to PTL and term subjects. Furthermore, when severe chorioamnionitis is excluded, the findings were unchanged, suggesting that this finding may be independent of infection.

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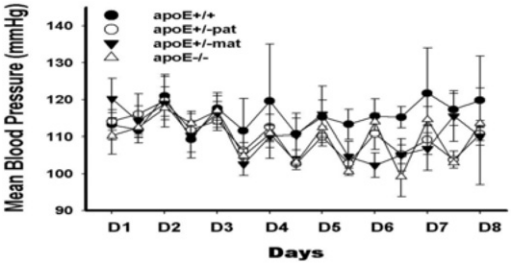
258 FETAL PROGRAMMING OF ATHEROSCLEROSIS: EFFECT ON ACTIVITY AND BLOOD PRESSURE IN YOUNG ADULT MICE. NIMA GOHARKHAY[1], FANGXIAN LU[1], EGLE BYTAUTIENE[1], ESTHER TAMAYO[1], ANCIZAR BETANCOURT[1], PHYLLIS GAMBLE[1] GARY HANKINS[1], MONICA LONGO[1], GEORGE SAADE[1], [1]University of Texas Medical Branch, Department of Obstetrics and Gynecology, Galveston, Texas

OBJECTIVE: We have previously described a model of developmental programming of atherosclerosis in heterozygous mice offspring born to apoE-knockout mothers. These animals displayed hypercholesterolemia, atherosclerotic plaques, and renal and hepatic lesions. Our objective in this study was to determine if the programmed atherogenic state is also associated with altered blood pressure and activity indices in the young adult offspring.

STUDY DESIGN: We crossbred apoE knockout (apoE−/−) and wild-type C57BL/6J (apoE+/+) mice to obtain heterozygous offspring born to hypercholesterolemic apoE−/− mothers (apoE+/−mat), heterozygous offspring born to wild-type mothers (apoE+/−pat), homozygous knockout offspring (apoE−/−), and homozygous wild-type offspring (apoE+/+). The resulting pups were followed until 4 months of age, at which time blood pressure (BP) catheters were inserted through the left carotid artery into the aortic arch and connected to a telemetric transmitter. BP parameters and activity levels were recorded continuously for up to 7 days in conscious unrestrained offspring. All parameters were averaged over 6-hour intervals and compared among the study groups.

RESULTS: At 4 months of age, no significant difference was found in mean, systolic or diastolic BP or in pulse pressure among the 4 study groups. The activity level in all mice followed a diurnal pattern as expected but was not different among the various groups.



CONCLUSION: Fetal programming of atherosclerosis does not lead to altered physical activity and blood pressure in young adult offspring. It is still possible that these parameters are not altered until later in life.

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