15 Assessing THC passage and the impact in human perfused placenta cotyledons

Emily Sheikh1, Aditya R. Kumar2, Katherine M. Leonard3, Joshua Monson4, Sarah McCormick1, Jason Pates5, Peter Napolitano6, Jashvant D. Unadkat6, Nicholas Ieronimakis7
1Madigan Army Medical Center, Lacey, WA, 2University of Washington, Steilacoom, WA, 3Womack Army Medical Center, Southern Pines, NC, 4Madigan Army Medical Center, Gig Harbor, WA, 5Madigan Army Medical Center, Steilacoom, WA, 6University of Washington, Seattle, WA, 7Madigan Army Medical Center, Tacoma, WA

OBJECTIVE: Maternal cannabis use in pregnancy continues to increase with legalization. Frequent cannabis use in pregnancy is associated with fetal growth restriction and low birth weight. The mechanism by which maternal cannabis use influences fetal growth remains unclear but is linked to the cannabinoid, Tetrahydrocannabinol (THC). Studies in animal models suggest exogenous THC crosses the placenta and observational human studies support these findings. The objectives of this study are to understand the mechanisms of THC transfer and its influence on fetal arterial vascular tone in human cotyledons.

STUDY DESIGN: A human placental dual-cotyledon, dual-perfusion model was utilized (Figure 1). For each experiment two cotyledons were isolated and compared from n=5 unlabored, uncomplicated cesarean placentas from pregnancies with no known tobacco, alcohol or drug use. To simulate maternal exposure experimental cotyledons were infused with 5μM THC into the intervillous space for two hours, control cotyledons received the vehicle. THC concentrations were analyzed in maternal fetal venous effluents. Fetal arterial pressures were monitored to assess viability and function. To confirm viability, lactate was also measured in fetal venous effluent at the end of each experiment. Values reflect mean and SEM.

RESULTS: From 5μM infused THC, the concentration in the fetal effluents was 0.1μM (+/- 0.04) (Figure 2A). In contrast, intervillous biopsy concentrations were 111μM (+/- 42). There were no significant differences in placenta fetal arterial pressures with THC exposure (Figure 2B). Lactate levels were not different between THC and vehicle infused cotyledons; THC 0.56 mmol/L (+/-0.023), vehicle 0.64 mmol/L (+/-0.015).

CONCLUSION: Approximately 2% of the infused THC concentration crosses the human placenta. A substantial proportion is retained in the tissue though this necessitates further examination. THC at concentrations infused did not alter vascular tone or viability within the timeframe of our experiments. Future directions include characterizing the mechanism of THC retention and transport through the placenta.

16 Comprehensive quantification of human milk oligosaccharides (HMOs) & sparse-but-true microbes in 2nd-trimester amniotic fluid (AF)

Kjersti M. Aagaard1, Maxim D. Seferovic2, Michael D. Jochum Jr.3, Anita Vinjamuri4, Sohini Banerjee3, Melissa Suter5, Alex Vidaeff5, Carilto Lebrilla3
1Texas Children’s and Baylor College of Medicine, Houston, TX, 2Baylor College of Medicine and Texas Children’s Hospital, Department of Obstetrics & Gynecology, Division of Maternal-Fetal Medicine, Houston, TX, 3Baylor College of Medicine, Houston, TX, 4Department of Chemistry, University of California Davis, Davis, CA

OBJECTIVE: There has been recent controversy as to whether mammalian fetuses normally develop in sterile intrauterine environments, or rather amidst sparse & low-abundance/low-diversity beneficial microbial communities. Despite supportive evidence from cultivation, metagenomics, molecular tagging & functional immune experiments, this controversy has persisted because of (1) presumptive contaminants, and (2) a lack of identifiable intrauterine substrates which could support beneficial microbial growth. Milk oligosaccharides are evolutionarily preserved complex glycans which...
are indigestible by mammals which produce them, but provide a growth advantage to beneficial microbes & promote a healthy infant gut. Based on initial detection in an unbiased AF metabolomics screen, we hypothesized that HMOs may be ubiquitous & serve as intrauterine beneficial microbial substrates. Our aims were to use unequivocal methodology to detect & correlate HMOs and metagenomes from 2nd trimester AF.

**STUDY DESIGN:** n=610 AF samples from 731 consenting subjects with ongoing and uncomplicated 2nd trimester gestations met QC for whole genome shotgun (WGS) metagenomics (microbiome) and HPLC-Chip/TOF-MS (HMOs). Robust and validated sequencing and computational approaches quantified and resolved high resolution microbial metagenomes and individual glycan structures, matched by accurate mass & retention time.

**RESULTS:** All known 34 HMOs and multiple non-pathogenic microbial metagenomes were detected in AF, with a strong correlation between HMO abundance and prokaryotic gene count (Fig.1). Metagenomes were distinct from contaminant controls, and microbial genomes from beneficial strains known to populate infant gut could be reconstructed (Fig.2)

**CONCLUSION:** We have demonstrated a strong correlation between HMOs and microbial metagenomes in 2nd trimester AF samples from a cohort of gravidae, of whom >85% go onto have largely uncomplicated term deliveries. We speculate that HMOs may serve as conserved bacterial substrates for sparse beneficial microbial communities in the intrauterine and postnatal environment of lactating mammals.