

to test the hypothesis that placental DNA is able to stimulate a robust proinflammatory cytokine response after enzymatic cleavage of telomere regions.

**STUDY DESIGN:** Genomic DNA was obtained from maternal, fetal and placental tissues from pregnant CD-1 mice (DNA extraction kit from Roche). The DNA was then incubated with the exonuclease BAL-31 (which cleaves the telomere ends of the chromosomal DNA). The BAL-31 treated DNA was incubated with mouse peritoneal macrophages (RAW 264.7 cells from ATCC) to assess TLR9 stimulation. Positive control incubations were performed using ODN2395 (a TLR9 agonist). IL6 concentration in the incubation media was assayed by ELISA (BioLegend), normalized for cellular protein, and reported as picograms (pg) per milligram (mg) protein. Each experiment N = 4.

**RESULTS:** The optimal BAL-31 digestion time was 2 hours. The maximal stimulation occurred with 1 - 5 mcg/mL BAL-31 treated placental DNA (result = 11,431 - 15,140 pg IL6/mg protein); results significantly ( $p < 0.01$ ) higher than found with untreated DNA (= 5.7 pg/mg) or ODN2395 (= 944 pg/mg). Timed studies demonstrated increased IL6 at 6 hours which peaked at 24 hours after the DNA addition. Compared to placental DNA, BAL-31 treated fetal DNA produced a similar robust IL6 response (= 13,654 pg/mL), whereas BAL-31 treated DNA from maternal liver produced a significantly lower IL6 response (= 6,313 ng/mg,  $p < 0.01$ ).

**CONCLUSION:** During apoptosis, DNA undergoes degradation, including removal of telomere sequences. These studies have demonstrated that placental DNA depleted of telomeres stimulates robust IL6 production by macrophage cells. Inflammation appears to play a key role during the onset of spontaneous parturition; these studies have provided additional support for the hypothesis that cell-free fetal DNA, generated during placental apoptosis, is able to trigger these events. (Funded by VCRB at MGH)

**205 Maternal outcomes in pregnancies with pulmonary hypertension: A cohort of 89 cases**

Shayna N. Conner, Kathryn J. Lindley, Alison G. Cahill

<sup>1</sup>Washington University in St. Louis, St. Louis, MO

**OBJECTIVE:** Maternal pulmonary hypertension (pHTN) has historically been associated with prohibitively high maternal morbidity and mortality. However, data is limited to case reports and case series of the most severe cases, restricting our ability to adequately counsel and care for these patients. We sought to examine maternal outcomes in a large cohort of women with pHTN of various etiologies and severity.

**STUDY DESIGN:** We performed a retrospective cohort study of all pregnancies in women with pHTN delivering at a single tertiary center over a 10 year period (2004-2014). pHTN was defined as pulmonary artery systolic pressure  $\geq 40$ mmHg prior to or during pregnancy estimated by Echocardiography or directly measured by right heart catheterization. Patients with pHTN due to congenital heart disease (CHD) were compared to those with pHTN from other causes. Additionally, patients were compared according to degree of pHTN (mild, moderate, or severe). Primary outcomes were intensive care admission (ICU), hospital readmission after delivery, heart failure, respiratory failure, pre-eclampsia, arrhythmia during pregnancy, death, and a composite of severe morbidity/mortality. Overall results were analyzed in three ways: 1) as a whole, 2) including only births after 20 weeks, 3) including only first pregnancy since diagnosis of pHTN.

**RESULTS:** Our cohort included 89 pregnancies in 68 women. 16 cases were associated with CHD, while 73 were due to other causes. The

cohort included 58 mild, 19 moderate, and 12 severe cases of pHTN. There were only three cases of maternal death, all during the first pregnancy since diagnosis. In continuing pregnancies, rates of heart failure, respiratory failure, and ICU admission were 21.1%, 23.7%, and 29% respectively. Composite severe morbidity and mortality was 42.1% overall in continuing pregnancies and increased to 50% in patients with severe pHTN.

**CONCLUSION:** In the largest cohort of pHTN cases to date, we identified that the risk for major maternal morbidity is high, 40.7% among those with mild disease up to 50% for those with severe disease. Two of the three deaths occurred in women with mild disease.

	Overall*	Overall†	Overall‡	pHTN-CHD associated	pHTN-Other etiology	Mild (PASP 40-54)	Moderate (PASP 55-69)	Severe (PASP $\geq 70$ )
	N=89	N=76	N=68	N=16*	N=73*	N=54†	N=14†	N=8†
ICU	25.8% (23)	29.0% (22)	27.9% (19)	18.8% (3)	27.4% (20)	27.8% (15)	35.7% (5)	25% (2)
Readmission	21.4% (19)	23.7% (18)	22.1% (15)	12.5% (2)	23.3% (17)	24.1% (13)	28.6% (4)	12.5% (1)
Heart failure	19.1% (17)	21.1% (16)	25% (17)	12.5% (2)	20.1% (15)	25.9% (14)	7.1% (1)	12.5% (1)
Respiratory failure	20.2% (18)	23.7% (18)	23.5% (16)	18.8% (3)	20.1% (15)	24.1% (13)	28.6% (4)	12.5% (1)
Pre-Eclampsia	20.2% (18)	23.7% (18)	19.1% (13)	25% (4)	19.2% (14)	18.5% (10)	42.9% (6)	25% (2)
Arrhythmia	6.7% (6)	7.9% (6)	5.9% (4)	12.5% (2)	5.5% (4)	7.4% (4)	7.1% (1)	12.5% (1)
Maternal Death	3.4% (3)	2.6% (2)	4.4% (3)	6.3% (1)	2.7% (2)	3.5% (2/58)*	0% (0/19)*	8.3% (1/12)*
Composite morbidity/mortality‡	38.2% (34)	42.1% (32)	42.7% (29)	31.3% (5)	39.7% (29)	41.4% (24/58)*	31.6% (6/19)*	33.3% (4/12)*
						40.7% (22/54) †	42.9% (6/14) †	50% (4/8) †

Data is presented as % (n)  
 \* Including all cases  
 † Including all births after 20 weeks  
 ‡ Including only first pregnancy since diagnosis of pHTN  
 § Composite of maternal death, heart failure, respiratory failure, intensive care unit admission, and invasive cardiac procedure required during pregnancy or 6 weeks post-partum.

**206 Sex-specific effects of maternal obesity on embryo size and fetal brain oxidative stress**

Andrea G. Edlow, Faycal Guedj, Deanna Y. Sverdlov, Caterina Neri, Sanaya T. Daruvala, Diana W. Bianchi

<sup>1</sup>Tufts Medical Center, Mother Infant Research Institute, Boston, MA, <sup>2</sup>Mother Infant Research Institute, Boston, MA, <sup>3</sup>Tufts University School of Medicine, Boston, MA

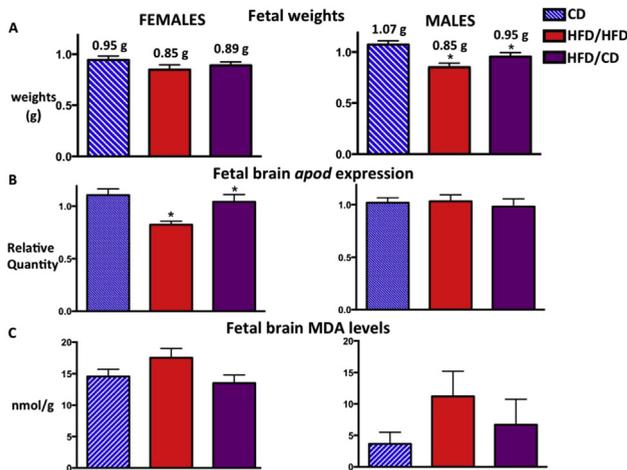
**OBJECTIVE:** Maternal obesity (MATOB) is associated with adverse neurodevelopmental outcomes in children, including autism spectrum disorder, developmental delay, and ADHD. Underlying mechanisms remain unclear. In amniotic fluid of obese women, we previously identified gene expression patterns consistent with dysregulated fetal brain development and significant upregulation of *APOD*, a CNS-specific gene implicated in response to oxidative stress. Here, we directly examined fetal brains in a mouse model of maternal diet-induced obesity.

**STUDY DESIGN:** Female (F) C57BL/6J mice were fed a 60% fat high-fat diet (HFD) or a 10% fat control diet (CD) for 10-12 weeks prior to mating. In pregnancy, obese dams continued on the HFD (HFD/HFD), or transitioned to a CD (HFD/CD). Lean dams stayed on the CD (CD/CD). On embryonic day 17.5, fetal brains were snap frozen. RNA and protein were extracted from 25-35 embryonic brains/diet group. Quantitative RT-PCR was performed for *apod*. Fetal brain malondialdehyde (MDA), a marker of oxidative stress, was quantified using HPLC. Kruskal-Wallis and Mann-Whitney testing determined significant differences between groups ( $p < 0.05$ ).

**RESULTS:** Embryos of HFD/HFD dams were significantly smaller than controls, with males (M) more severely affected than Fs ( $p < 0.001$ , Fig 1A). Brain *apod* expression was significantly decreased in F embryos of HFD/HFD dams compared to controls ( $p = 0.003$ , Fig 1B). When obese dams were switched to a CD in pregnancy, brain *apod* expression in Fs increased to levels similar to controls ( $p = 0.04$ ). These changes were not observed in M brains. F brains

exposed to MATOB (HFD/HFD and HFD/CD) had significantly higher MDA levels than Ms ( $p=0.02$ , Fig 1C).

**CONCLUSION:** MATOB had a more significant effect on M embryo size. However, MATOB had more significant effects on F brain *apod* expression and MDA levels, two parameters related to brain oxidative stress. Maternal diet in pregnancy also impacted fetal brain gene expression of *apod* in Fs only. MATOB and diet in pregnancy appear to have sex-specific influences on embryo size and on the fetal brain. Future evaluation of the effects of MATOB on fetal neurodevelopment should consider fetal sex and maternal diet.



## 207 The intestinal microbiome is regulated by diet in a novel primate model of polycystic ovarian syndrome (PCOS)

Amanda Prince, Jun Ma, Christina Megli, Diana Takahashi, Kevin Grove, Richard Stouffer, Kjersti Aagaard

<sup>1</sup>Baylor College of Medicine, Houston, TX, <sup>2</sup>Oregon National Primate Research Center, Beaverton, OR

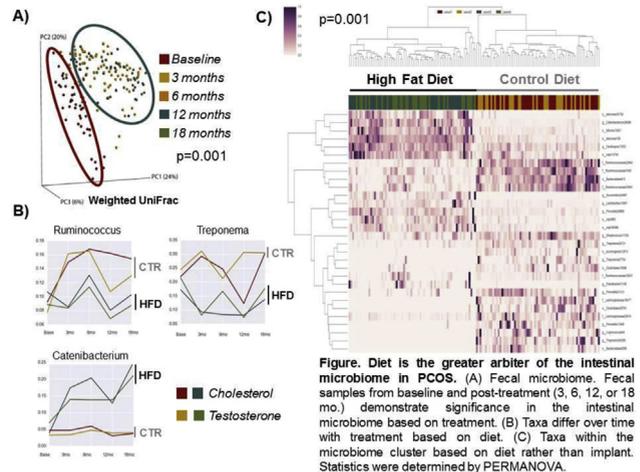
**OBJECTIVE:** Polycystic ovarian syndrome (PCOS) is a disorder characterized by oligo-ovulation and hyperandrogenism. PCOS confers risk of infertility, obesity, insulin resistance and diabetes. Afflicted gravidae are at risk of perinatal complications (i.e. gestational diabetes, preeclampsia, hypertension, cesarean delivery and preterm birth). Further, hyperandrogenism confers insulin resistance upon consumption of a high fat diet. The microbiome is altered in obesity and diabetes and is implicated in childhood obesity via cesarean delivery. However, it is unclear if the microbiome is a cause or symptom of altered metabolism. Thus, in order to examine the role of the microbiome in insulin resistance and obesity in PCOS, we examined the influence of diet and androgens on the microbiome in a novel non-human primate model.

**STUDY DESIGN:** Young female Rhesus macaques received either a cholesterol (control, n=20) or testosterone (experimental, n=20) implant. Additionally, these groups were further distinguished by diet, which resulted in four distinct cohorts (n=10 per cohort, Table 1). Oral and anal swabs and stool were collected before (0) and at 3, 6, 12, and 18 months post-treatment. 16S sequencing was performed on isolated DNA.

**RESULTS:** Within the oral and intestinal microbiome, we found differences between baseline and all treatment time points ( $p=0.001$ , Fig A). For instance, Ruminococcus, Catenibacterium, and Treponema species in the intestinal microbiome are similar between cohorts at baseline, but these bacteria separate the cohorts based on diet as treatment progresses (Fig B). To further investigate

cohort separation based on diet, we generated a heatmap showing that multiple taxa are altered by diet ( $p=0.001$ ) rather than by testosterone treatment ( $p\leq 0.52$ ) within the intestinal microbiome (Fig C).

**CONCLUSION:** These results demonstrate a minimal role for testosterone in altering the intestinal microbiome, whereas a high fat diet significantly alters the microbiome of exposed female macaques. These data further demonstrate that diet influences the microbiome rather than the microbiome acting independently of diet.



	Implant	Diet
Cohort 1	Cholesterol	Control
Cohort 2	Testosterone	Control
Cohort 3	Cholesterol	High fat
Cohort 4	Testosterone	High fat

Table 1. Non-human Primate Model of PCOS.

## 208 Obesity delays the onset of lactation and alters the fatty acid composition of milk

Justin Bohrer

<sup>1</sup>University of Wisconsin, Madison, WI

**OBJECTIVE:** Obesity is a known risk factor for delayed onset of stage II lactogenesis in epidemiologic studies. The underlying mechanism of this association remains elusive. Serotonin is a regulator of mammary gland function and is elevated in obese individuals. We hypothesized that obesity promotes inflammation resulting in early mammary gland involution through a serotonin dependent mechanism.

**STUDY DESIGN:** Mice fed a high fat diet (HFD) starting at 5 weeks of age were compared to mice fed a control diet (LFD). Wild type (WT) mice were compared to those genetically deficient for TPH-1, the rate-limiting enzyme in peripheral serotonin synthesis. Serum serotonin concentrations were collected prior to diet initiation, and on d 1 and 10 of lactation. Milk yields were measured daily from d1-10 of lactation. Milk samples were collected daily and analyzed using gas chromatography to determine fatty acid profiles. Mice were euthanized on d 10 of lactation and mammary glands were harvested. The number of intact alveoli and mean alveolar diameter were measured from histologic sections. Cytokine expression profiles were determined using PCR arrays.

**RESULTS:** Twelve wild type (WT) mice (HFD=4, LFD=8) were compared with sixteen mice deficient for TPH-1 (HFD=6, LFD=10). Total pup mortality was higher in the HFD (86%) than the LFD (37.3%) dams ( $p<.001$ ). Milk yield was decreased on d 1 in