38 Hyperoxygenation in pregnancy exerts a more profound effect on hemodynamics than in a non-pregnant state

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OBJECTIVE: The hemodynamic effects of oxygen therapy are under recognized and the impact of hyperoxygenation (HO) on maternal hemodynamics is currently unknown. Using transthoracic bioreactance (NICOM), we sought to examine the effect of brief hyperoxygenation on cardiac index (CI), systemic vascular resistance (SVR), blood pressure (BP), stroke volume (SV) and heart rate (HR) in pregnant mothers during the third trimester as compared to those observed in a non-pregnant population subjected to the same period of hyperoxygenation.

STUDY DESIGN: Hemodynamic monitoring was performed in a continuous manner over a 30-minute period using NICOM. HO (O2 100% v/v inhalational gas) was carried out at a rate of 12L/min via a partial non-rebreather mask for 10-minutes. CI, SVR, SV, HR, and BP were recorded prior to HO (Time 1), at the end of HO (Time 2), and 10 minutes following the cessation of HO (Time 3). Data were presented as means ±SD or medians [IQR]. Two-way ANOVA with repeated measures was used to assess the change in hemodynamic indices over time, and the differences between the two groups.

RESULTS: Forty six pregnant and twenty non-pregnant females were prospectively recruited with a median age of 33[26 − 38] and 32 [28-37] years respectively (p=0.82). The median gestation was 35 [33 − 37] weeks. Baseline hemodynamic measurements are seen in Table 1 (mean ±SD). In the pregnant group there was a fall in CI over the study period coupled with a rise in SVR with no recovery by time 3 (Figure 1). HR decreased in Time 2, returning to baseline levels by 10 minutes post-cessation of HO. There was a decrease in SV over the study period. There was no change in systolic or diastolic BP over the study period. In the non pregnant group there was no significant change in the CI, TPR, SV, HR, systolic or diastolic BP over the study period.

CONCLUSION: Hyperoxygenation during the third trimester is associated with a fall in CI, mediated by a decrease in HR, and a rise in SVR without recovery to baseline levels at 10 minutes following cessation of HO. Similar hemodynamic changes have been documented in other patient populations in response to hyperoxia and counteract any proposed increase in oxygen delivery, limiting the benefit of oxygen therapy in the absence of hypoxia. In light of these cardiovascular effects, the role of hyperoxygenation in obstetric patients, especially those with cardiovascular disease, requires further investigation.

Table 1:
Baseline Hemodynamic Measurements in pregnant vs non-pregnant

Baseline measurements (mean ±SD)	Pregnant	Non-Pregnant	p-value
Cardiac Index (L/min/m^2)	3.3 ± 0.5	2.8 ± 0.6	0.004
Systemic Vascular Resistance (dynes/sec/cm^{-5})	1236 ± 286	1509 ± 312	0.002
Stroke Volume (mL)	73 ± 13	68 ± 13	0.16
Heart Rate (beats per minute)	87 ± 10	72 ± 9	0.001
Systolic Blood Pressure (mmHg)	121 ± 17	114 ± 8	0.083

Figure 1:
Changes in hemodynamics over time in pregnant vs non-pregnant

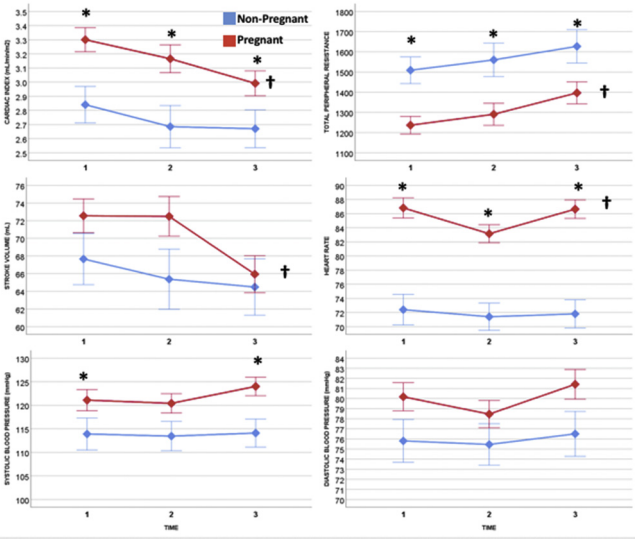

Figure1
* = p <0.05 between groups at time point
† = p <0.05 within group Time 3 vs. Time 1

39 Amniotic fluid contains detectable microbial DNA that significantly differs from appropriate contamination controls

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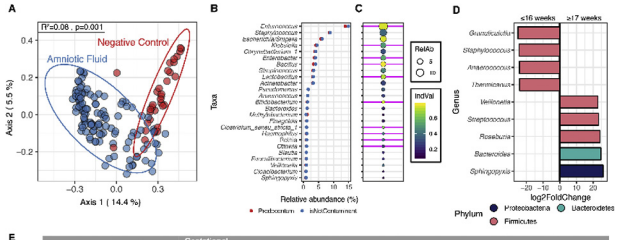
OBJECTIVE: A growing body of literature, including data from multiple labs, is emerging that suggests the intrauterine environment, including amniotic fluid, may contain unique microbial communities. However, despite use of appropriate controls, this work is criticized as being confounded by contaminants. Using sterile techniques and negative controls, we sought to assess whether a

microbial signature exists in a large prospective cohort of subjects amniotic fluid obtained during amniocentesis during early-to-mid second trimester.

STUDY DESIGN: As part of a feasibility study we performed targeted amplicon analysis of the V4 region of the 16S rRNA gene on amniotic fluid collected from gravidae at the time of genetic amniocentesis (gestational ages 16- 22wks; feasibility n=95, total cohort n=731). Microbial DNA was extracted from samples and negative controls (processed in parallel or archival, n=32), and submitted for Illumina MiSeq high-throughput sequencing. Sequences were processed with DADA2 to generate an amplicon sequence variant (ASV) table that contains highly resolved taxonomic classifications of microbes. Prevalence-based filtering of putative DNA contaminant sequences using decontam was performed.

RESULTS: We initially identified 439 genera present in amniotic fluid. Multidimensional scaling (Bray-Curtis) revealed amniotic fluid samples to discretely separate from negative controls (PERMANOVA, $p<0.001$)(**Fig 1A**). Using prevalence-based filtering we identified up to 44 genera that represent putative contaminants. Removal of contaminant ASVs resulted in minimal alterations in the abundance of the top twenty-five most abundant taxa or overall microbial community structure (**Fig 1B**). Post-filtering, when compared to DNA extraction kit negatives, ten of the twenty-five most abundant taxa were classified as indicator taxa ($p<0.05$) including *Staphylococcus*, *Streptococcus*, *Lactobacillus*, and *Bifidobacterium* spp. (**Fig 1C**). Differential abundance testing revealed 9 ASVs that were significantly enriched based on gestational timepoint at which the fetal urine begins contributing to the amniotic fluid (**Fig 1D**).

CONCLUSION: Amniotic fluid contains quantifiable levels of microbial DNA and clusters separately from negative controls. Furthermore, we identified highly prevalent ASVs belonging to biologically relevant taxa implicated as important in the postnatal gut health of the newborn, including *Lactobacillus* and *Bifidobacterium* spp.



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Reference	n	Gestational age at sampling (weeks GA)	Sampling method	Sequencing method	Controlled for contaminants	Predominant genera
Current Study	95	16-22	Genetic amniocentesis	V4 16S Illumina MiSeq	Yes - Extraction blanks	*Enterococcus, Klebsiella, Enterobacter, **Lactobacillus, Bifidobacterium***
Rehbinder et al., 2018, AJOG	24	37-40	Sterile elective/acute caesarean section	V3 16S Illumina MiSeq	Yes - Laboratory/ operating room extractions	*Sneathia, Caulobacteraceae (other), **Lactobacillus, Pseudomonas, Acinonacus, Bifidobacterium***
Collado et al., 2016, Sci Rep.	15	>37	Sterile elective caesarean section	V1V3 16S Illumina MiSeq	No	*Adlercreutzia, **Lactobacillus, Sphingomonas, Granulicatella, Bifidobacterium***
Rautava et al., 2012, Neonatology	14	>37	Sterile elective caesarean section	qPCR	No	***Bifidobacterium, Lactobacillus***

Figure 1. Amniotic fluid contains a distinct microbial community structure compared to DNA extraction kit negative controls. **A.** MDS plot of the Bray-Curtis distance for amniotic fluid (n=116) and DNA extraction kit negative controls (n=32). Ellipses represent 95% confidence intervals. R-squared and p-values were determined via PERMANOVA test. **B.** Relative abundance dot plots of the top 25 most abundant genera present in amniotic fluid samples (representing 69% of overall genera) before and after filtering putative contaminant ASVs present in DNA extraction kit negative controls using the decontam functions isContaminant and isNotContaminant. The isContaminant function resulted in a high degree of conservation of the relative abundance of the top 25 most abundant genera (69% of overall genera and removal of 12 genera) compared to the isNotContaminant function (31.8% of overall genera and removal of 44 genera). **C.** Bubble plot projecting the relative abundance (%, RelAb) of 25 taxa from panel B and their associated indicator values in amniotic fluid samples. Indicator values were calculated by grouping amniotic fluid and DNA extraction kit negative controls after putative contaminant ASVs determined via isNotContaminant were removed from the dataset. A larger indicator value indicates greater specificity within the group. Amniotic fluid associated taxa with significant indicator values ($p<0.05$) are denoted with magenta lines. **D.** DESeq2 was used to perform a variance stabilizing transformation on the ASVs and test for the significant differential enrichment of taxa. Nine ASVs were identified as differentially enriched (Benjamini-Hochberg FDR corrected p-value <0.01) based on the binned gestational age of samples (≤16 weeks or ≥17 weeks gestational age). **E.** Summary of current and recent studies on the amniotic microbiome. Genera that are consistantly identified across studies are bolded.

40 The importance of fetal expression of *Npas2* in regulating lifelong satiety

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OBJECTIVE: Despite being in an environment absent of light-dark signals, under maternal influence the fetus establishes and then maintains its circadian rhythms. Previously, we have demonstrated that this occurs largely in the fetal liver and not in the fetal brain. Moreover, it is under the influence of the maternal diet with a high fat maternal diet being disruptive. In order to understand the precise mechanisms with which this occurs, we generated two novel *Npas2* cKO mouse models of the peripheral circadian clock (liver) with loss of *Npas2* occurring at distinctive developmental time points (fetal and neonatal) and have shown that *Npas2* cKO mice gain significantly more weight (fetal and neonatal) and alter their glucose tolerance (fetal) when compared with controls (wildtype; WT). In this study we aimed to test the hypothesis that loss of hepatic *Npas2* and the developmental timing of the loss significantly alters the gene expression of the remaining core circadian clock genes.

STUDY DESIGN: Neonatal *Npas2* cKO (n=120) and control mice (WT, n=132) fed a control diet (CD), neonatal *Npas2* cKO (n=82) and control mice (WT, n=76) fed a high-fat diet (HFD), and fetal *Npas2* cKO (n=121) and control mice (WT, n=111) fed a high-fat diet (HFD) were generated for this study. At 16 weeks post-weaning mice were euthanized at 4-hour intervals over a 24-hour period (11AM, 3PM, 7PM, 11PM, 3AM, 7AM, 11AM). RNA was extracted from mouse livers and converted to cDNA for qPCR analysis. Taqman probes for circadian genes *Bmal1*, *Clock*, *Cry1*, *Cry2*, *Per1* and *Per2* were used to determine relative abundance of these genes.

RESULTS: qPCR analysis reveals that neonatal *Npas2* cKO mice fed a CD gene expression is unaltered for all circadian genes tested in the liver compared to WT (Figure 1).

CONCLUSION: We have deleted a circadian gene in the liver during fetal development or neonatal life which results in additional weight gain under metabolic stress (HFD), but does not generally disrupt the circadian machinery. Taken together, our findings collectively suggest that disruptions to *Npas2* hepatic expression during fetal development result in lifelong metabolic disease risk.

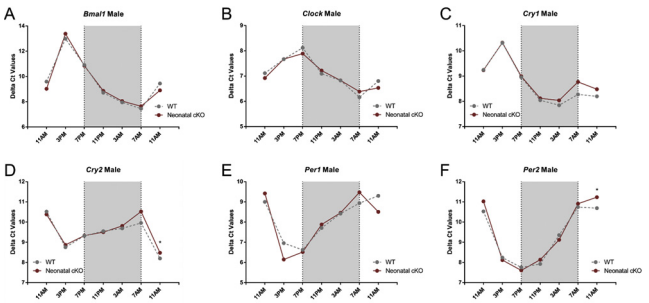

Figure 1. CD fed male neonatal *Npas2* cKO mice hepatic circadian clock gene expression is not significantly altered. qPCR analysis of circadian gene expression in CD fed male neonatal *Npas2* cKO (red, solid line) and WT (grey, broken line) mouse livers. **(A)** *Bmal1*. **(B)** *Clock*. **(C)** *Cry1*. **(D)** *Cry2*. **(E)** *Per1*. **(F)** *Per2*. * = $p < 0.05$ (student t-test).