Maternal Prenatal Depression in Pregnancies with Female and Male Fetuses and Developmental Associations With C-reactive Protein and Cortisol

Supplemental Information

Subject Assessment

Cortisol and Cortisone Hair Assay

Table S1. Differences between pregnancies with female or male fetuses

Statistical models:

Subject Assessment

Psychiatric diagnoses were made using the Structured Clinical Interview for DSM-IV Axis I Disorders with DSM-5 criteria. Together with the CESD, self-ratings on Adverse Childhood Experiences (ACE), State-Trait Anxiety Inventory-State Version (STAI-S), and the Perceived Stress Scale (PSS) were acquired in structured interviews by trained staff. Women who reported severe depression symptoms, including suicidal ideation, were referred to their clinicians for further assessment and treatment, including antidepressants. Translations were provided for consent and assessments for participants whose primary language was Spanish. Assessment of maternal infection and substance use in structured interviews were previously described (1-2). Mothers reported symptoms of infection, use of medications, prenatal vitamins, over-the-counter supplements and drugs, alcohol, cannabis, and nicotine at 16 weeks gestation and at 6-week successive intervals through 6 weeks postpartum. Maternal report of infection was positively correlated with clinical assessments in the prenatal clinic medical record, ρ = 0.96, P < .001 (1). No mother who denied marijuana use tested positive in urine toxicology (2). Labor, delivery, and neonatal parameters were taken from the medical record. At birth, 90% of women began breast feeding, but at the newborn P50 recording only 2% were still breast feeding.

Cortisol and Cortisone Hair Assay

The assay was conducted at iC42 Clinical Research and Development (University of Colorado, Aurora, CO) using a high-performance liquid chromatography- tandem mass spectrometry (LC-MS/MS) assay that was validated for this purpose.

Hair samples were washed, dried, pulverized, weighed and extracted by addition of 1.8 mL LC-MS-grade methanol and 200 µL of an internal standard solution in methanol containing 1 ng/mL of the deuterated internal standards (cortisol-d4) under light shaking at 37 °C for 18 hours. The extract was then dried in a Speedvac concentrator (Savant, Thermo Fisher, Waltham, MA) at 45°C for 2 hours. Residues were reconstituted in 400 µL 80% methanol/ 20% water (v/v), transferred into HPLC vials with 500-µL glass inserts and placed into the autosampler (Agilent 1100 HPLC system, Agilent Technologies, Santa Clara, CA) of the LC-MS/MS system. Calibrators

for cortisone and cortisol ranged from 0.0125 – 50 ng/mL were prepared in 80% methanol/ 20% water (v/v). For online extraction, one hundred microliter (100 µL) of the sample extracts were injected and loaded onto an Agilent Zorbax XDB-C18, 50·2.1mm (5µm). The mobile phase was 95% LC-MS grade water with 2 mM ammonium acetate (pH 4.5) and 5% of 2 mM ammonium acetate in LC-MS grade methanol at a flow rate of 1500 µL/min for 1 min. For sample analyses, analytes and their internal standards were then back-flushed onto a Shimadzu Shim-pack XR-ODS, 75·3.0mm (2.2µm particle size, Shimadzu, Kyoto, Japan) analytical column connected via a 6-port switching valve (Rheodyne, Cotati, CA). A gradient was run from 60% to 85% methanol within 5 min, to 95% methanol within an additional 4 min and was held at 95% for 6 min. The columns were then re-equilibrated to starting conditions. The total run time was 16.5 min. Both extraction and analytical columns were kept at 40^0C throughout the analysis. The detector was an AB Sciex API5000 triple stage quadrupole mass spectrometer (Sciex, Concord, ON, Canada). The LC-MS interface was an Atmospheric Pressure Ionization Source run in the positive ionization mode (+APCI) at a nebulizer current of 10 µA. The following ion transitions were monitored:

Compound	Q1 (m/z=)	Q3 (m/z=)	Declustering potential (DP)	Collision Energy (CE)
Cortisone [M+H]$^+$	361.1	91.0	136	69
Cortisol [M+H]$^+$	363.3	121.0	141	15
Cortisol-d4 [M+H]$^+$	367.3	121.0	141	15

The analytes were quantified based on the analyte/internal standard ratios. A deuterated cortisone sample was not available and therefore the cortisol standard was used. Calibration curves were fit using a 1/x weighting and a quadratic fit. The LC-MS/MS system was controlled and data was recorded and integrated using Analyst software (version 1.6.2, Sciex, Concord, ON, Canada). During the study sample runs, quality control samples met acceptance criteria for all analytes (imprecision < 15%, accuracy within 85-115% of the nominal concentrations).

Table S1. Differences between pregnancies with female or male fetuses.[1]

Mean (SD) or N (%)	Female N = 81	Male N = 81	*P*
Maternal age yrs	29.8 (5.7)	27.6 (6.3)	0.9
Minority status N	17 (23%)	16 (21%)	0.7
Adverse Childhood Experience rating	2.64 (2.43)	2.65 (2.45)	0.9
History of major depression N	10 (13%)	14 (18%)	0.5
Antidepressant use in pregnancy N	8 (12%)	12 (15%)	0.5
Gestational age at birth days	271.4 (18.6)	273.8 (15.7)	0.4
Birth weight g	3049.4 (607.7)	3266.9 (585.0)	0.02
Birth length cm	48.4 (5.3)	49.7 (4.2)	0.09
Birth head circumference cm	34.1 (2.7)	35.0 (2.7)	0.045
CESD depression rating16 wk	13.6 (8.6)	14.5 (10.3)	0.5
Maternal second trimester hair cortisol pg/mg	7.90 (3.27)	7.54 (2.31)	0.8
Maternal second trimester hair cortisone pg/mg	12.0 (1.8)	13.9 (2.1)	0.3
Neonatal hair cortisol birth pg/mg	128.2 (2.82)	139.5 (2.25)	0.6
Neonatal hair cortisone birth pg/mg	62.4 (2.8)	90.9 (1.8)	0.062
Maternal plasma CRP 16 wk gestation mg/L	9.68 (8.00)	7.75 (7.01)	0.12

[1]Values sorted by fetal sex and maternal CESD Depression rating are shown in the main text Table 1.

Table S2. Association of Newborn P50$_{S2}$µV with maternal gestational depression, stress, and anxiety

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Model	24.477[a]	13	1.883	7.239	.000
Intercept	.855	1	.855	3.286	.073
Child sex	.035	1	.035	.136	.713
Newborn P50$_{S1}$µV	18.463	1	18.463	70.986	<.0001
Depression CESD 16 weeks gestation	1.726	1	1.726	6.636	.011
Anxiety STAI 16 weeks	.069	1	.069	.264	.608
Stress PSS 16 weeks	.027	1	.027	.104	.747
CESD 22 weeks	.086	1	.086	.331	.566
CESD 28 weeks	.011	1	.011	.042	.838
CESD 34 weeks	.004	1	.004	.015	.903
CESD 40 weeks	.038	1	.038	.147	.702
CESD 46 weeks (postpartum 6 weeks)	.007	1	.007	.028	.866
Gestational age at P50 recording	.509	1	.509	1.957	.165
Maternal age	.181	1	.181	.694	.407
Gestational age at birth	.530	1	.530	2.036	.156
Error	28.871	111	.260		

a. R Squared = .441

Table S3. Association of Newborn $P50_{S2}\mu V$ with maternal gestational depression and other parameters

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Model	29.300[a]	14	2.093	10.244	.000
Intercept	.008	1	.008	.040	.843
Depression CESD 16 weeks gestation	1.816	1	1.816	8.887	.004
Infection	.009	1	.009	.042	.837
Tobacco use	.527	1	.527	2.578	.111
Alcohol use	.236	1	.236	1.154	.285
Marijuana use	.122	1	.122	.595	.442
Biological Father present	.253	1	.253	1.239	.268
Native American	.548	1	.548	2.681	.105
African American	.434	1	.434	2.126	.148
Obesity (pre-pregnancy BMI$\geq$30)	.567	1	.567	2.775	.099
Adverse Childhood Experiences (ACE)	.064	1	.064	.313	.577
Newborn $P50_{S1}\mu V$	17.358	1	17.358	84.961	.000
Gestational age at P50 recording	.479	1	.479	2.346	.129
Maternal age	.077	1	.077	.379	.539
Gestational age at birth	.011	1	.011	.055	.815
Error	20.431	100	.204		

Table S4. Association of Newborn P50$_{S2}$µV with maternal gestational depression and fetal sex

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Model	28.293[a]	7	4.042	18.159	.000
Intercept	.196	1	.196	.879	.350
Fetal sex * Depression CESD 16 weeks gestation	.187	1	.187	.841	.361
Fetal sex	.018	1	.018	.079	.779
Depression CESD 16 weeks gestation	2.864	1	2.864	12.866	<.001
Gestational age P50 recording	.240	1	.240	1.080	.300
Maternal age	.000	1	.000	.002	.969
Gestational age at birth	.088	1	.088	.397	.529
Newborn P50$_{S1}$µV	21.975	1	21.975	98.727	<.001
Error	34.278	154	.223		

a. R Squared = .451

Table S5. Association of Maternal C-Reactive Protein (CRP) 16 weeks gestation with maternal gestational depression, fetal sex, and other parameters

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Model	1103.949[a]	7	157.707	2.996	.006
Intercept	71.623	1	71.623	1.361	.245
Fetal sex * Depression CESD 16 weeks gestation	244.411	1	244.411	4.644	.033
Fetal sex * Infection	45.105	1	45.105	.857	.356
Fetal sex	311.724	1	311.724	5.923	.016
Depression CESD 16 weeks gestation	65.558	1	65.558	1.246	.266
Infection	326.770	1	326.770	6.209	.014
Obesity (pre-pregnancy BMI$\geq$30)	17.330	1	17.330	.329	.567
Maternal age	36.903	1	36.903	.701	.404
Error	7473.631	142	52.631		

a. R Squared = .129

Table S6. Association of Newborn P50$_{S2}$µV with maternal 16 week gestation CRP and fetal sex

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Model	23.293[a]	7	3.328	14.574	.000
Intercept	.081	1	.081	.353	.553
Child sex * Maternal CRP 16 weeks gestation	1.934	1	1.934	8.469	.004
Maternal CRP 16 weeks gestation	.005	1	.005	.020	.888
Child sex	.462	1	.462	2.025	.157
Gestational age P50 recording	.026	1	.026	.115	.734
Newborn P50$_{S1}$µV	21.467	1	21.467	94.024	.000
Maternal age	.012	1	.012	.053	.818
Gestational age at birth	.134	1	.134	.585	.445
Error	32.420	142	.228		
a. R Squared = .415					

Table S7. Association of Maternal 2nd trimester cortisol (log$_{10}$) with maternal gestational depression, fetal sex, and other parameters

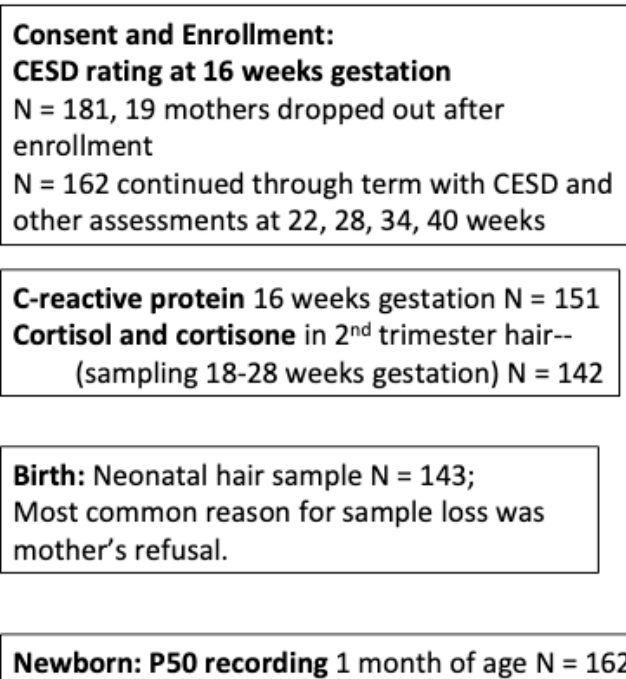| Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Model	1.926[a]	7	.275	1.372	.222
Intercept	1.957	1	1.957	9.763	.002
Fetal sex * Depression CESD 16 weeks gestation	1.100	1	1.100	5.490	.021
Fetal sex * Infection	.092	1	.092	.457	.500
Fetal sex	.556	1	.556	2.771	.098
Depression CESD 16 weeks gestation	.818	1	.818	4.078	.045
Infection	.018	1	.018	.089	.765
Obesity (pre-pregnancy BMI$\geq$30)	.033	1	.033	.167	.683
Maternal age	.029	1	.029	.144	.705
Error	26.260	131	.200		
a. R Squared = .068					

Table S8. Association of Newborn P50$_{S2}$μV with maternal 2nd trimester cortisol (log$_{10}$), child sex, and neonatal cortisone

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Model	8.654[a]	8	1.082	7.253	.000
Intercept	.001	1	.001	.008	.927
Child sex * Maternal 2nd trimester cortisol (log$_{10}$) * Neonatal cortisone	1.001	2	.500	3.355	.042
Child sex	.300	1	.300	2.010	.162
Maternal 2nd trimester cortisol (log$_{10}$)	.039	1	.039	.261	.611
Neonatal Cortisone	.880	1	.880	5.900	.018
Newborn P50$_{S1}$μV	5.185	1	5.185	34.761	.000
Gestational age P50 recording	1.630E-7	1	1.630E-7	.000	.999
Gestational age at birth	.002	1	.002	.016	.901
Maternal age at birth	.070	1	.070	.462	.499
Error	8.581	57	.151		

a. R Squared = .500

Table S9. Summary of findings of this study and other studies.

Finding	Novel finding of this study	Previously reported in this cohort	Replication of finding from another cohort	Previously reported by others
Maternal depression at 16 weeks associated with increased newborn P50$_{S2}$µV both sexes	Yes	No	No	No
Maternal depression associated with increased CRP in males	Yes	No	No	Partial: references 4-6, sex effect not investigated)
Maternal CRP associated with increased newborn P50$_{S2}$µV in males but not in females	No	Yes (reference 3)	No	No
Maternal depression associated with increased cortisol in females but not males	Yes	No	No	Partial: references 6-7 depression not investigated
Maternal cortisol associated with increased newborn P50$_{S2}$µV in females but not in males	Yes	No	No	No
Maternal cortisone increased relative to cortisol in pregnancies with males > females	Yes	No	No	No
Neonatal hair cortisone increased relative to cortisol in males > females	Yes	No	No	No
Antidepressants have no effect on newborn P50$_{S2}$µV	No	No	Yes (reference 8)	No

Figure S1. Diagram of subject flow through the study.

Consent and Enrollment:
CESD rating at 16 weeks gestation
N = 181, 19 mothers dropped out after enrollment
N = 162 continued through term with CESD and other assessments at 22, 28, 34, 40 weeks

C-reactive protein 16 weeks gestation N = 151
Cortisol and cortisone in 2nd trimester hair--
     (sampling 18-28 weeks gestation) N = 142

Birth: Neonatal hair sample N = 143;
Most common reason for sample loss was mother's refusal.

Newborn: P50 recording 1 month of age N = 162

Supplemental References

1. Freedman R, Hunter SK, Law AJ, Wagner BD, D'Alessandro A, Christians U *et al*. (2019): Higher gestational choline levels in maternal infection are protective for infant brain development. *J Pediatr* 208:198-206.e2.

2. Hoffman MC, Hunter SK, D'Alessandro A, Noonan K, Wyrwa A, Freedman R (2019): Interaction of maternal choline levels and prenatal Marijuana's effects on the offspring. *Psychol Med* doi:10.1017/S003329171900179X [Epub ahead of print]

3. Hunter SK, Hoffman MC, D'Alessandro A, Noonan K, Wyrwa A, Freedman R, *et al.* (2019): Male fetus susceptibility to maternal inflammation: C-reactive protein and brain development. *Psychol Med* doi.org/10.1017/S0033291719003313 [Epub ahead of print].

4. Cassidy-Bushrow AE, Peters RM, Johnson DA, Templin TN (2012): Association of depressive symptoms with inflammatory biomarkers among pregnant African-American women. *J Reprod Immunol* 94:202-209.

5. Azar R, Mercer D (2013): Mild depressive symptoms are associated with elevated C-reactive protein and proinflammatory cytokine levels during early to midgestation: a prospective pilot study. *J Womens Health* 22:385-389.

6. Bleker LS, Roseboom TJ, Vrijkotte TG, Reynolds RM, de Rooij SR (2017): Determinants of cortisol during pregnancy - The ABCD cohort. *Psychoneuroendocrinol* 83:172-181.

7. Giesbrecht GF, Campbell T, Letourneau N (2015): APrON Study Team. Sexually dimorphic adaptations in basal maternal stress physiology during pregnancy and implications for fetal development. *Psychoneuroendocrinol* 56:168-178.

8. Hunter SK, Mendoza JH, D'Anna K, Zerbe GO, McCarthy L, Hoffman C, *et al.* (2012): Antidepressants may mitigate the effects of prenatal maternal anxiety on infant auditory sensory gating. *Am J Psychiatry* 169: 616-624.