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J Pediatr. Author manuscript; available in PMC 2020 May 01.

Published in final edited form as:

J Pediatr. 2019 May ; 208: 198–206.e2. doi:10.1016/j.jpeds.2018.12.010.

Higher gestational choline levels protect fetal brain development in maternal infection

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Abstract

Objective—To assess whether choline decreases effects of maternal infections on fetal brain circuit development and on expression of infant behavior at 3 months of age.

Study Design—A case-control study was conducted in a public hospital obstetrics and midwifery service, with prenatal assessments of maternal infection, C-Reactive Protein (CRP), and choline levels and postnatal assessments of cerebral neuronal inhibition in 162 newborns. At 3 months, 136 parents completed reports of their child's behavior.

Results—Maternal infection at 16 weeks gestation, experienced by 41% of mothers, raised mean maternal CRP (d' = 0.47, $P = 0.002$) and decreased the development of cerebral inhibition of auditory response at 1 month of age (d' = 0.39, $P < 0.001$). Decreased newborn cerebral inhibition manifest as decreased behavioral self-regulation at 3 months. Higher choline levels in mothers with infections were associated with better newborn inhibition of auditory cerebral response, mitigating the infection effect ($\beta = -0.34$ [95% CI, −5.35 to −0.14], $P = 0.002$). At 3 months of

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A correction was made to this article on July 1, 2019. This version is the correction version.

In the article, "Higher Gestational Choline Levels in Maternal Infection Are Protective for Infant Brain Developlment," by Freedman et al, J Pediatr 2019;208:198–206e2, the authors incorrectly described the Infant Behavioral Questionnaire Revised-Short Form as completed at 1 year of age. The IBQ-R was completed at 52 weeks gestational age, generally 3 months post birth.

Data will be shared upon request.

Conflict of interest: The funders had no role in (1) study design; (2) the collection, analysis, and interpretation of data; (3) the writing of the report; and (4) the decision to submit the paper for publication. The authors have no conflicts of interest with commercial or other interests.

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age, children of mothers with infection and higher gestational choline levels had improved development of self-regulation, approaching the level of children of mothers without infection (β = 0.29 [95% CI, 0.05 to 0.54], $P = 0.03$).

Conclusion—Higher maternal choline levels, now recommended by the American Medical Association, decrease adverse effects of common maternal infections during gestation on children's early development of behavioral problems. These behavioral problems often lead to referrals to pediatricians and are associated with later serious mental illness.

Keywords

pregnancy exposure delayed effects; fetal development; choline; receptors nicotinic; sensory gating; child behavior

Maternal respiratory and genito-urinary infections generally do not infect the fetus. Nonetheless these seemingly benign infections significantly increase the risk that the child will develop mental illnesses including schizophrenia, autism, and ADHD.[1–7] Early second trimester is a particularly vulnerable time, when cerebro-cortical laminae form. [1,8] The mother's immune response activates macrophages that damage the placental chorionic villi and compromise fetal support.[9–11] Maternal C-Reactive Protein (CRP) levels are related to offsprings' subsequent autism or schizophrenia.[12–14] Puberty unmasks latent effects of such prenatal insults.[15] Although risk to the offspring from infection is less than risk from having a mentally ill parent, infection is more common and adds significantly to the familial genetic risk.[1,16]

In animal models of maternal immune activation, supplementing maternal dietary choline reduced interleukin-6 (IL-6) in the fetal brain and decreased offspring anxiety behaviors.[10] Choline's roles in fetal development include membrane synthesis, one carbon metabolism and DNA methylation, and, at higher concentrations, activation of α7-nicotinic cholinergic receptors,[17–18] which promote maturation of excitatory and inhibitory neuro-circuits.[19–21] Maturation of these neuro-circuits is not complete in schizophrenia.[22–23] Elimination of α7-nicotinic receptors by *CHRNA7* null mutation increases effects of immune activation and blocks effects of maternal choline supplementation on fetal brain development.[10,19]

Maternal phosphatidylcholine or choline supplementation and diets higher in choline improve childhood cognition and behavior.[24–30] However, no study has examined the relationship of maternal choline levels to the effects of infection in human pregnancy. We planned to observe their interaction on newborn cerebral auditory-evoked response inhibition, a biomarker of the prenatal development of inhibitory neuro-circuits that showed negative effects of familial risk and positive effects of choline in previous studies.[27,31]

Methods

Mothers and infants

Pregnant women were identified from admissions to Denver Health Medical Center, a public hospital prenatal clinic, before the 16th week of gestation, timed from the last menstrual period and verified by ultrasound (Figure 1). Exclusions were fetal anomaly, severe

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intrauterine growth restriction, and corticosteroid use. Women with asthma or allergies were otherwise included; no women had autoimmune disorders. After informed consent approved by the Colorado Multi-Institutional Review Board, diagnoses were made using the Structured Clinical Interview for DSM-IV Axis I Disorders, converted to DSM-5 criteria. Self-ratings on Center for Epidemiological Studies of Depression-R (CESD-R), State-Trait Anxiety Inventory-State Version (STAI-S), and the Perceived Stress Scale (PSS) were performed whenever maternal infection status was assessed, and acetaminophen, antibiotic, antidepressant and other psychotropics, and nicotine, alcohol, marijuana, and other substance use were recorded. Maternal status during pregnancy, including BMI, blood pressure, and pre-eclampsia were recorded.

Assessment of maternal infection

The medical record for all prenatal care was reviewed. A mother's report of infection was considered significant if it was entered as a problem in the medical record. Treatment was provided for all reported genito-urinary infections. Most respiratory infections were viral and were therefore not treated. In addition, mothers had an in person review of systems for symptoms of infection at 16, 22, 28, and 34 weeks by research personnel. The correlation between symptoms rated by the mother as moderate to severe in the interview and problems in the medical record is $r_s = 0.96$, $P < 0.001$.

Maternal choline levels

Maternal serum choline and its metabolite betaine at 16 weeks gestation were assayed by the Colorado Translational Research Center Metabolomics Core Laboratory, University of Colorado Denver. Serum was quickly separated by refrigerated centrifugation and stored at −80°C. Samples were extracted in methanol, acetonitrile, and water (5:3:2) and agitated for 30 minutes at 4°C. After centrifugation at 10,000g the supernatant was collected and stored at −80°C until analysis with an Ultra performance liquid chromatography-tandem mass spectrometer. Metabolites were assigned using Maven Metabolomic Analysis and Visualization Engine (Princeton, NJ).

Maternal C-Reactive Protein

Serum CRP at 16 weeks gestation was assayed by the Beckman-Coulter high sensitivity assay at the Colorado Translational Research Center, Colorado Children's Hospital.

Physiological recording of newborn cerebral inhibition

Newborns were studied at 1 month (44 weeks) after birth adjusted for gestational age.[32] Vertex electroencephalogram, electro-oculogram, submental electromyogram, and respiration were continuously recorded while infants napped. Recording of the cerebral auditory evoked potential P50 occurred in the second active sleep episode, the precursor of REM sleep, identified by low voltage desynchronized vertex activity with the absence of K-complexes, change in respirations, and large eye movements with submental atonia.[33] The second active sleep episode was reached 45 minutes after sleep onset. In adults, P50 inhibition in REM and waking are equivalent.[34]

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The P50 sensory gating paradigm assesses inhibition. The initial stimulus activates a $P50_{S1}$ response, which also activates collateral inhibitory interneurons. Strength of the inhibition is tested by the decrease in $P50_{S2}$ after a second stimulus.[35] For comparisons between individuals, control for variance in $P50_{S1}$ is desirable, which can reflect differences in excitability, but also technical factors like electrode impedance. Therefore, P50 inhibition is often assessed as amplitude ratios $P50_{S2}/P50_{S1}$ or $(P50_{S1}\text{-}P50_{S2})/P50_{S1}$.[35] However, the skew inherent in ratios limits their power for correlation with risk factors and outcomes. $P50_{S2}$ amplitude, covaried for $P50_{S1}$, which is normally distributed, has been previously proposed and was used here.[36] Lower $P50_{S2}$ amplitudes indicate increased inhibition. The assumption is that $P50_{S1}$ variance is small, compared to $P50_{S2}$ variance. In 151 newborns, effect sizes for $P50_{S1}$ differences between newborns whose mothers had no known risk versus women with depression or schizophrenia ranged from 0–0.16. Effect sizes for decrease in $P50_{S2}$ amplitude were 0.21–0.50.[31] The effect of maternal schizotypy on newborn P50 inhibition has been replicated by another group, who also found increased $P50_{S2}$ amplitudes.[37] Table 2 reports $P50_{S1}$, $P50_{S2}$, and $(P50_{S1}\text{-}P50_{S2})/P50_{S1}$. Technical aspects and reliability of recordings have been published.[32,38–39]

Behavioral assessment of the child

Parents completed the Infant Behavior Questionnaire-Revised Short Form (IBQ-R) when the infant was 52 weeks post gestation, generally 3 months of age.[40] Parents who were primarily Spanish-speaking completed the Questionnaire in Spanish. The 91-item IBQ-R Short Form has 3 standard indices developed by the scale originators using factor analysis to summarize its 14 components: Surgency (approach, vocal reactivity, pleasure in high stimulus intensity play like rough-housing, smiling/laughter, soothability, activity level, sensory sensitivity), Negativity (sadness, distress to limitation, fear, falling distress), and Regulation (pleasure in low stimulus intensity play like toys, cuddliness/affiliation, duration of orienting, smiling/laughter, soothability). Values in a reference sample of 12 month olds are: Surgency 5.08 (SD 0.78), Negativity 3.46 (SD 0.91), Regulation 5.47 (SD 0.63).[40]

Statistical analyses

Choline's effect size on P50 inhibition in a previous study was d' = 0.7.[27] We expected 20% of the women would have optimal choline levels (> 7 μM) and 33% attrition. [25,27] Therefore, we planned a sample of 200 women to have power $1\text{-}\beta = 0.95$, $\alpha = 0.05$, 1-tail.

Differences between mothers with and without infection were compared by Fisher's exact test or t-test. The Generalized Linear Model with a linear link analyzed effects of maternal infection and choline level on $P50_{S2}$, the primary physiological outcome. Multivariate General Linear Models were used for analysis of effects of maternal infection on IBQ-R indices. Infection was a categorical fixed effect, choline level was a random continuous variable, and infant sex and maternal age were covariates. Obesity (BMI > 30) and depression (CESD-R ≥ 16) were covariates because they have been associated with inflammation. The effect on CRP from infection is d' = 0.47, $P = 0.004$; from obesity, d' = 0.06 and from depression, d ' = 0.19, both not significant. Maternal age also reflected years of education (r = 0.32, $P < 0.001$, and Duncan Socioeconomic Index (r = 0.24, $P = 0.002$). Maternal smoking was a covariate for P50 analyses.[36] Other differences between mothers

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with and without infection were analyzed as possible covariates (Table 1). None significantly affected the interaction between infection and choline levels. Analyses were performed using the Statistical Package for the Social Sciences version 24 (IBM, Amonk, NY). Significance levels are two-tailed and Bonferroni-corrected for multivariate analyses, except for exploratory analysis of 14 individual IBQ-R components.

Results

The study enrolled 201 women before 16 weeks gestation. Of 162 who came with their infants for the 1 month post-gestation visit, 66 (41%) had reported an infection by 16 weeks gestation. Mothers who reported infection at 16 weeks gestation were younger and had less education and lower status occupations than those who did not report infection (Table 1). Eleven women had vaginal infections, 5 had urinary tract infections, 37 had viral respiratory infections, 11 had pharyngitis, 3 had influenza, and 4 had gastroenteritis. Five women had two infections. Type or number of infections had no effect on infant outcome; nor did infections later in gestation. Infection was accompanied by increases in maternal depression and anxiety symptoms and increased levels of CRP (d' = 0.47, $P < 0.004$; Table 2). Choline levels were not affected by maternal infection, depression, age, or socio-economic status.[41] Mothers lost to participation during their 18 months of study had no differences in infection rate or other variables (Figure 1). The principal reason for attrition was moving from Denver.

Effects of maternal infection and choline on newborn cerebral inhibition

Maternal infection at 16 weeks gestation was associated with significantly decreased inhibition of the cerebral auditory-evoked potential P50 in the 1-month-old newborn. $P50_{S2}$ increased by 27% in newborns of infected mothers, compared to newborns of uninfected mothers, indicative of less inhibition (d' = 0.39, $P<0.001$; Table 2). Effects of choline and infection on cerebral inhibition had a significant interaction (Wald $\chi^2 = 9.10$, df 1, $P = 0.003$, Table 3; online). Maternal choline levels at 16 weeks gestation were associated with significantly lower $P50_{S2}$ amplitudes in infants of mothers with infection ($\beta = -0.34$ [95% CI, −5.35 to −0.14], $P = 0.002$); there was no association in infants whose mothers were not infected (Figure 2). There were no effects of infection or choline on $P50_{S1}$ amplitude.

Effects of maternal infection and choline on infant behavior at 3 months of age

Infection decreased Regulation rating by 28%: −1.44 (SE 0.45), $P = 0.003$, d' = 0.28 (Table 2). There were no significant effects on Surgency or Negativity. Maternal infection and choline levels at 16 weeks had a significant interacting effect in a multivariate analysis of the 3 IBQ-R indices, specifically on Regulation ($F_{3,124} = 10.71$, $P = 0.003$, Table 4; online). The children of mothers with infections had increased Regulation associated with higher maternal choline levels; there was no such relationship for children whose mothers were not infected (infection $\beta = 0.29$ [95% CI, 0.05 to 0.54], $P = 0.03$; no infection $\beta = -0.14$ [95% CI, −0.31 to 0.04]). The IBQ-R component that showed most significant effects of choline in children of mothers with infection was in the Regulation index: pleasure in low stimulus intensity play ($\beta = 3.07$ [95% CI, 0.05 to 0.56], $P = 0.02$). IBQ-R Regulation in the children of mothers with infections was significantly associated with $P50_{S2}$ at 1 month of age ($\beta = -0.37$ [95% CI, −.014 to −0.86], $P = 0.04$).

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Maternal CRP levels at 16 weeks gestation decreased the child's IBQ-R Regulation index (β = −0.64 [95% CI, −1.25 to −0.034], $P = 0.04$). Maternal choline and CRP levels effects on Regulation had a significant interaction (Wald $\chi^2 = 4.79$, df 1, $P = 0.03$). The adverse effect of higher CRP levels was negated in women with choline levels > 7μM (β= 0.28 [95% CI, −0.01 to 0.59], $P = 0.06$).

IBQ-R Regulation does not have a minimum threshold considered abnormal, but children below the 5th percentile on early behavior and temperament ratings are often referred for clinical intervention. Five children of 53 mothers with infection (9.4%) had Regulation levels lower than the 95th percentile of the reference sample,[40] compared to 1 of 83 children of mothers without infection (1.2%, $P_{FET} = 0.03$). Four of these infected mothers with infection who had children with poor Regulation also had choline levels < 7μM.

Discussion

Higher maternal choline levels were associated with increased development of cerebral inhibition and newborns and behavioral regulation in 3-month-old infants, especially in mothers who experienced common infections early in pregnancy. The timing at 16 weeks gestation is consonant with the finding that choline levels are lowest in second trimester and with the epidemiological evidence that identifies 16 weeks gestation as a vulnerable period.[1,41] We did not find that the mother's infection, socioeconomic, or mental status influenced choline levels.[42]

Finding of 41% of mothers infected is consistent with 37% infection found in the second trimester for 4967 control mothers in the National Birth Defects Prevention Study.[43] The high frequency and unpredictability of many infections, notably respiratory infection, puts every pregnancy at potential risk of acquiring this complication. Lower socioeconomic status of women with infection has also been found in general hospital samples and attributed to stress and overcrowding, but the mechanism remains unclear.[44] The age disparity in pregnancy among women of different socioeconomic status observed in this study is nationwide, according to a *New York Times*-commissioned study with the National Center for Health Statistics.[45]

The mechanisms of choline's effects include direct activation of α7-nicotinic cholinergic receptors responsible for maturation of inhibitory and excitatory neurotransmission, as suggested by both animal models and *CHRNA7* pharmacogenomic effects in studies of phosphatidylcholine supplements.[10,19–21,27–28] Newborn P50 auditory evoked potential inhibition is a putative biomarker of this effect, because of its genetic relationship to *CHRNA7*.[46] Both P50 inhibition and *CHRNA7* are involved in the pathology of major mental illness. In schizophrenia, decreased P50 inhibition is associated with poor attention and executive function in schizophrenia,[47] and *CHRNA7* copy number variations and polymorphisms are associated with schizophrenia, autism, and ADHD.[48–50] In newborns, lower P50 inhibition predicts childhood behavior problems in attention and social withdrawal associated with ADHD and other mental illnesses.[51] Lower infant P50 inhibition is also associated with family history of psychotic disorder,.[31,37] In this study, decreased development of P50 inhibition presaged poorer self-regulation at 3 months of age.

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The P50 response is present in newborns at nearly adult amplitudes after 30 weeks gestation.[52–53] Inhibition of the $P50_{S2}$ is closely related to the development of theta activity, the hallmark of infant active sleep.[32] The 1 month of recording in the present study, mean gestational age 44.0 (SD 1.4) weeks. was chosen because infant active sleep patterns stabilize at this age (Harper 1981).[54] Inhibition of newborn $P50_{S2}$ has excellent test-retest reliability over 1.5 weeks (r_{icc} = 0.71, P<0.01) and is also closely correlated with recordings during REM sleep when the child is 4 years of age (r_{icc} = 0.42, P = 0.06).[38–39] In the present study, a second recording was performed at 3 month of age. $P50_{S2}$ amplitude at 1 month weeks predicted $P50_{S2}$ amplitude at 3 months, with covariance for $P50_{S1}$ at both ages (β = 2.43, 95%CI 0.74–4.25, P = 0.005).

α7-nicotinic cholinergic receptors are expressed in fetal cerebrum in large numbers early in gestation, but they do not receive acetylcholine synapses until just before birth.[55–57] In the absence of acetylcholine synapses, choline is a likely initial agonist.[18] Levels in the amniotic fluid are just sufficient to activate α7-nicotinic receptors.[58] The 50% effective concentration *in vitro* is 120μM.[59] However, many women are deficient in choline during pregnancy, in part because of the fetus's need for large amounts of choline for the synthesis of cell membranes.[17,60–61] In one study 56% of pregnant women and 54% in the present sample had plasma levels below 7μM at 16 weeks gestation, a level associated with liver damage from choline deprivation.[25,62]

Maternal plasma choline levels only indirectly reflect concentration at fetal α7-nicotinic receptors. Transport of choline is controlled by the placental choline transporter CLT1, which produces amniotic fluid levels approximately twice maternal plasma levels.[58,63] Uptake is proportional to plasma concentration, which suggests that higher peak levels may be important determinants of amniotic fluid levels.[64] Maternal levels obtained in non-fasting conditions, as in the present study, may be elevated after meals.[65–66] No women had choline levels outside the 2 SD of the mean, which might have indicated significant genetic effects.[67] Dietary history was not collected because of the low relationship of self-reported intake to maternal choline levels, r = 0.2.[25,68]

Higher levels of choline did not decrease CRP levels, which indicates that choline did not diminish maternal inflammation directly, although α7-receptors are involved in vagal regulation of immune response.[69] The significant interaction of inflammation and choline could have occurred in the fetal cerebrum or on the effect of the inflammatory response on the placenta, where α7-receptors are also expressed.[70]

In a human observational study, the effects of choline cannot be rigorously isolated from the multiple environmental and genetic influences that converge in fetal development. Vitamin D levels and folic acid levels were not obtained.[71] All women were in prenatal care where these supplements were strongly advised. In another study, choline and methionine levels were positively correlated, but folate and Vitamin B12 levels were not. Only choline levels affected infant outcome.[25] Obesity, acetaminophen, antidepressants, and marijuana use were common and had effects on the development of P50 inhibition and childhood behavior, but these effects were independent of the interaction between infection and choline levels.[72–75]

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Lower IBQ-R Regulation is associated with decreased reading readiness at age 4 years and decreased conscientiousness, organization, and increased distractibility at age 9 years.[76–77] As the child develops, Regulation moderates the child's Surgency and Negativity to meet cultural expectations.[78] Continuity between abnormalities appearing in the first year of life and the emergence of mental disorders such as schizophrenia in adulthood is also well established.[79–83] Childhood behavior does not fully predict later mental illness in any individual, but neither do interventions later in life restore function in individuals with early deficits from fetal brain development. Higher maternal choline positively affects child behavior for as long as 7 years, providing a potentially helpful continuity.[24]

Positive effects of higher choline levels in this study raise the issue of whether supplementation of choline in pregnancy is desirable. There have been 4 small randomized, placebo-controlled trials of choline or phosphatidylcholine supplementation.[26–30] Phosphatidylcholine is more resistant to bacterial degradation than choline.[65] The two forms are intraconvertable, and both have been used in the trials without significant adverse effects. We conducted a trial beginning at 17 weeks gestation of phosphatidylcholine 7300 mg (equivalent to 900 mg choline) versus placebo in which maternal infection was assessed.[27] The recommended dietary intake of 550 mg choline plus supplementation equivalent to 900 mg is less than half the 3500 mg maximum choline advised for pregnant women over 18 years of age (3000mg < 18 years of age).[84] The children were evaluated at 40 months of age using the Child Behavior Checklist.[28] Nine of 49 mothers had experienced infections during pregnancy; the lower prevalence of infection reflects FDA mandates that excluded some higher risk mothers. Phosphatidylcholine decreased the mean number of problems in attention and aggression in children of mothers who had infection (Table 5; online). Based on these trials, which also showed positive effects on behavior and cognition in children of mothers without specific risk factors, the AMA has recommended that mothers receive "evidence-based amounts of choline in all prenatal vitamins."[85] Prenatal vitamins currently contain as little as 10 mg, and therefore additional supplementation using phosphatidylcholine or choline might be required.

Acknowledgement:

This study was conceived and initiated by the late Randal G. Ross.

Supported by the National Institutes of Health NICHD K12HD001271–11 to MCH and NCATS UL1 TR001082 to all investigators and by the Institute for Children's Mental Disorders and the Anschutz Foundation to RF.

Abbreviations:

P50	Positive cerebral evoked potential, nominally 50 msec after auditory stimuli
S1,S2	Auditory Stimulus1 followed at 500 msec by Stimulus2
IBQ-R	Infant Behavior Questionnaire-Revised
CRP	C-Reactive Protein
IL-6	Interleukin-6

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CHRNA7	Gene for the α7-nicotinic cholinergic receptor peptide
CLT1	Choline transporter 1
DSM	American Psychiatric Association Diagnostic and Statistical Manual
REM	Rapid eye movement sleep
ADHD	Attention Deficit Disorder
CESD-R	Center for Epidemiological Studies Depression Scale Revised
STAI-S	State-Trait Anxiety Index-State
PSS	Perceived Stress Scale
FET	Fisher's Exact Test

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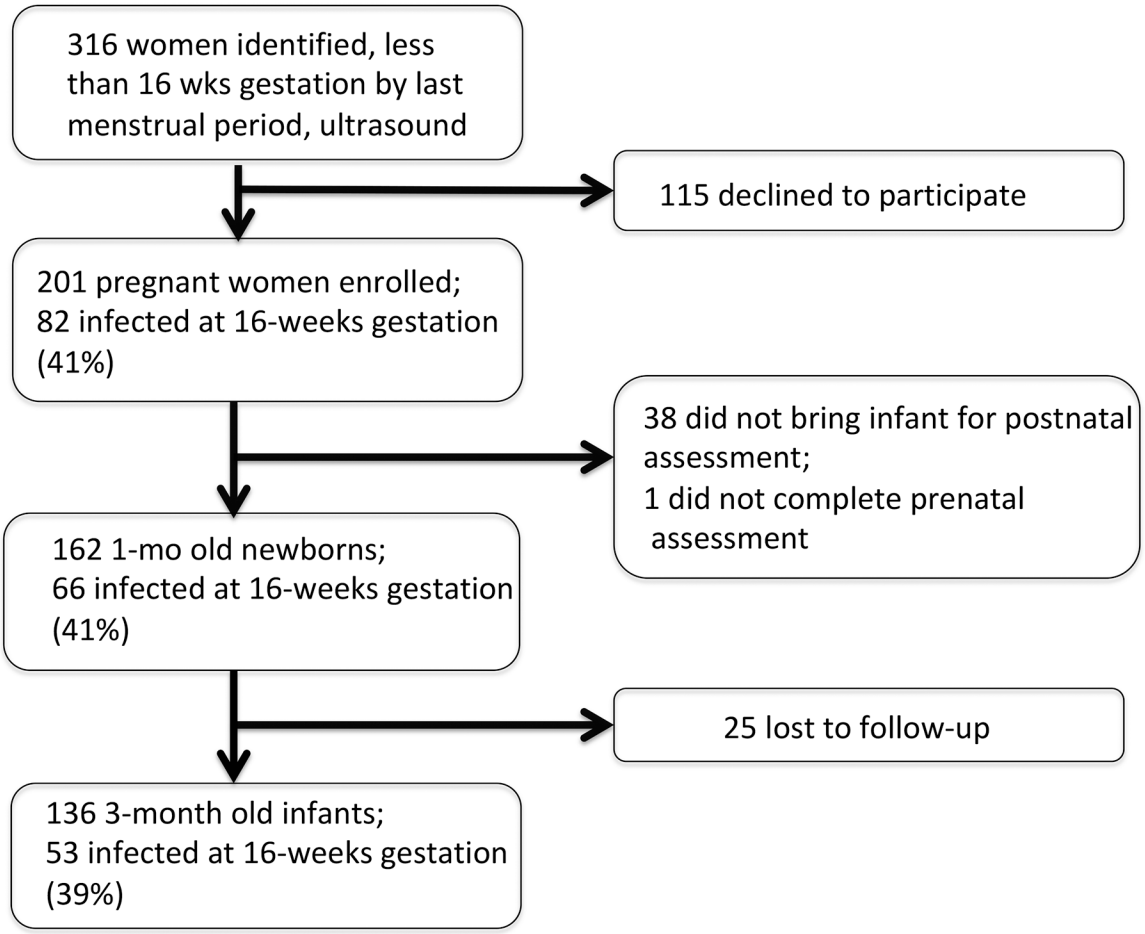

Figure 1.
Subject participation from 16 weeks gestation to 3 months post partum

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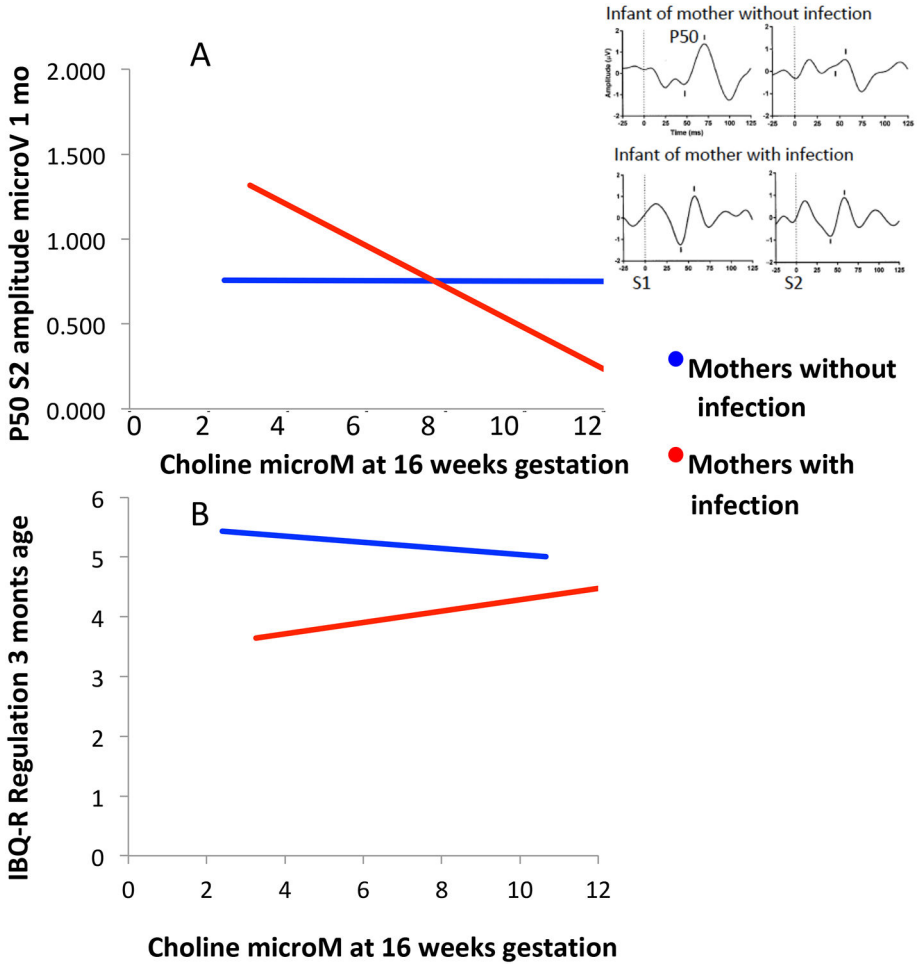

Figure 2.

Maternal infection and choline levels and development of newborn physiological inhibition and early childhood self-regulation. A. Maternal choline levels significantly improved cerebral inhibition, indicated by lower $P50_{S2}$ amplitude in newborns of mothers who reported infection. There was no significant effect in infants whose mothers were not infected.

B. Maternal choline levels significantly improved development of Regulation, measured on the Infant Behavior Questionnaire-Revised (IBQ-R) at 3 months of age in children of mothers who reported infection; effects for children of women with no infection were not significant.

Inset: P50 averaged evoked responses to paired auditory stimuli S1 and S2, delivered 500 ms apart. P50 amplitude is measured from the positive peak voltage to the preceding negative trough. For the infant of the uninfected mother, maternal choline level was 12.9 μM; $P50_{S2}$ is over 90% inhibited. For the infant of the infected mother, maternal choline level was 5.4 μM; there was no $P50_{S2}$ inhibition. Horizontal scale msec, vertical μV.

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Table 1.

Demographic, pregnancy, labor, and delivery differences between mother and newborn pairs by infection status at 16 weeks gestation

Mothers 16 wk gestation	**Un-infected N = 96**	**Infected N = 66**	**Significance**
Caucasian	80 (83%)	51 (77%)	0.4
African-American	6 (6%)	4 (6%)	0.9
Native American	4 (4%)	6 (9%)	0.3
Biracial	6 (6%)	5 (8%)	0.8
Hispanic	47 (49%)	31 (47%)	0.9
Married	49 (51%)	30 (45%)	0.5
Maternal age	30.6 (SD 6.0)	28.6 (SD 5.8)	0.03
Education years	14.0 (SD 3.2)	13.0 (SD 2.9)	0.03
Duncan Socio-Economic Index	49.3 (SD 21.5)	41.7 (SD 18.2)	0.02
Pre-pregnancy BMI	26.6 (5.9)	28.2 (7.6)	0.15
Obesity BMI ≥ 30	16 (24%)	31 (32%)	0.3
Bipolar disorder (DSM-5 296.5)	1 (1.0%)	6 (9.1%)[5]	0.02
Schizophrenia (295.9, 295.7)	2 (2.0%)	0	0.5
Major depressive disorder (296.21, 296.31)	10 (10.4%)	14 (18.2%)	0.07
Panic disorder, generalized anxiety disorder (300.01, 300,02)	4 (4.2%)	3 (4.5%)	0.9
Antidepressant	9 (9%)	13 (20%)	0.07
Acetaminophen	77 (80%)	56 (85%)	0.5
Cigarette smoking	7 (7.3%)	4 (6.1%)	0.9
Cannabis use	9 (9.4%)	16 (24.2%)	0.01
Alcohol use (>1 drink/wk)	0	3 (4.5%)	0.07
Labor and Delivery			
Diabetes	2 (2%)	7 (10%)	0.03
Hypertension	6 (6%)	4 (6%)	0.9
Preeclampsia	9 (9%)	5 (8%)	0.8
Proteinuria	7 (7%)	0	0.04
Edema	12 (12%)	14 (22%)	0.2
Chorioamniotis	8 (8%)	3 (5%)	0.5
Premature<37 weeks	5 (5%)	2 (3%)	0.7
Cesarean delivery	23 (24%)	19 (29%)	0.6
APGAR 5min	8.73 (1.17)	8.85 (0.41)	0.4
Newborn			
Sex male	46 (48%)	36 (54%)	0.4
Birth weight g	3116 (SD 663)	3229 (SD 514)	0.2
Birth weight %ile	60.9 (SD 24.6)	58.5 (SD 24.4)	0.5
Head circumference %ile	65.9 (SD 25.4)	70.1 (SD 21.9)	0.3
Birth length %ile	67.3 (SD 23.6)	68.7 (SD 22.9)	0.7

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Mothers 16 wk gestation	**Un-infected N = 96**	**Infected N = 66**	**Significance**
Gestational age birth	272 (SD 20)	274 (SD 13)	0.5
Large >90%ile for gestational age	11 (11%)	9 (13%)	0.8
Small <10%ile for gestational age	6 (6%)	1 (2%)	0.2
NICU admission>1 day	4 (4%)	7 (10%)	0.12
Formula fed only	12 (12%)	3 (5%)	0.10

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Table 2.

Maternal mental symptoms, choline levels, and inflammatory status at 16 weeks gestation

Maternal Symptoms	**No infection N = 96**	**Infection N = 66**	**Significance**
Center for Epidemiological Studies of Depression Scale-Revised	12.0 (SD 8.3)	17.0 (SD 10.2)	0.001
State-Trait Anxiety Inventory-State Version	33.7 (SD 9.5)	38.7 (SD 11.9)	0.004
Perceived Stress Scale	22.8 (SD 7.2)	24.8 (SD 9.0)	0.10
Maternal choline and metabolite	N = 96	N = 66	
Choline 16 weeks	6.50 (1.89)	6.20 (1.75)	0.3
Betaine 16 weeks	11.88 (SD .53)	10.99 (SD 3.62)	0.12
Maternal Cytokines	N = 90	N = 61	
C-reactive protein (CRP) mg/L	7.30 (SD 6.27)	10.90 (SD 8.77)	0.004
Newborn electrophysiology (1 month)	N = 96	N = 66	
P50 S1 amplitude (μV)	1.73 (SE 0.09)	1.67 (SE 0.11)	0.6
P50 S2 amplitude (μV)	0.75 (SE 0.05)	0.94 (SE 0.06)	< 0.001
P50 inhibition (S1–S2)/S1	0.56 (SE 0.03)	0.45 (SE 0.04)	0.003
Childhood IBQ-R rated behavior (3 months)	N = 84	N = 52	
Regulation	5.23 (SE 0.07)	3.79 (SE 0.09)	0.006[1]
Surgency	4.14 (SE 0.12)	3.25 (SE 0.16)	0.2
Negativity	3.05 (SE 0.10)	4.14 (SE 0.13)	0.5

[1]Bonferroni correction for 3 IBQ-R indices

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Table 3.

Effects of maternal infection and choline level at 16 weeks gestation on newborn P50 inhibition, measured as $P50_{S2}$ amplitude at 1 month post gestation

Source	Wald Chi-Square	Sig.
(Intercept)	5.685	.017
Maternal infection 16 wks	13.045	<.001
Child sex	.357	.500
Maternal age	.008	.930
Maternal smoking	.061	.805
Maternal obesity	2.044	.153
Maternal depression	.107	.744
$P50_{S1}$ amplitude	111.760	<.001
Choline 16wks	6.789	.009
Infection*choline	9.100	.003
Effect of infection 16 weeks gestation on $P50_{S2}$ amplitude	Marginal Mean μV	95% CI
No infection	.75 (SE .05)	.66 – .84
Infection	.94 (SE .06)	.83 – 1.05

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Table 4.

Effects of maternal infection and choline level at 16 weeks gestation on child's IBQ-R indices at 3 months of age

Source	Wilk's λ	IBQ-R Indices	F (df 1,124)	Sig.
Child sex	λ = .997	SURGENCY	.067	.797
	$F_{df3,124}$= .139	NEGATIVITY	.114	.736
	P= .937	REGULATION	.350	.555
Maternal age	λ = .975	SURGENCY	1.560	.214
	$F_{df3,124}$= 1.079	NEGATIVITY	.279	.598
	P= .361	REGULATION	.246	.621
Maternal obesity	λ = .939	SURGENCY	1.422	.235
	$F_{df3,124}$= 2.694	NEGATIVITY	7.620	.007[1]
	P= .049	REGULATION	.151	.699
Maternal depression	λ = .991	SURGENCY	.221	.639
	$F_{df3,124}$= .778	NEGATIVITY	.952	.331
	P= .937	REGULATION	.082	.776
Maternal infection 16 wks	λ = .924	SURGENCY	1.411	.237
	$F_{df3,124}$= 3.422	NEGATIVITY	.551	.459
	P= .019	REGULATION	10.184	.002[2]
Maternal choline 16 wks	λ = .997	SURGENCY	.059	.809
	$F_{df3,124}$= .116	NEGATIVITY	.003	.959
	P= .940	REGULATION	.103	.749
Infection* choline	λ = .922	SURGENCY	2.629	.107
	$F_{df3,124}$= 3.515	NEGATIVITY	.255	.614
	P= .017	REGULATION	10.709	.001[3]
Effect of maternal infection 16 wks, compared to uninfected: Difference (SE)		SURGENCY	−.887 (SE .747)	.237
		NEGATIVITY	.456 (SE .626)	.359
		REGULATION	−1.442 (SE .452)	.002[2]

Bonferroni correction

[1] *P* = .021;

[2] *P* = .006;

[3] *P* = .003

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Table 5.

Effects of maternal infection and phosphatidylcholine supplementation on Child Behavior Checklist Attention and Aggression Problems at 40 months of age

	No maternal infection		Maternal infection	
Infection* Phosphatidylcholine	**Placebo N = 22**	**Phosphatidylcholine N = 18**	**Placebo N=4**	**Phosphatidylcholine N=5**
Attention $F_{1,45} = 7.79$ P = 0.008	2.41 (SD 1.33)	2.06 (SD 1.73)	4.75 (SD 3.10)	0.83 (SD 1.33)[1]
Aggression $F_{1,45} = 14.07$, P < 0.001	5.91 (SD 4.39)	6.78 (SD 1.41)	19.5 (SD 7.33)	6.00 (SD 5.24)[1]

[1]Tukey's HSD P < 0.01 for comparison with placebo in mothers with infection