IMPACT OF PSYCHOLOGICAL STRESS ON OBSTETRIC AND NEONATAL OUTCOMES AMONG WOMEN WITH PRETERM PREMATURE RUPTURE OF THE FETAL MEMBRANES

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Preterm premature rupture of the fetal membranes (PPROM) is one of the most significant causes of preterm birth. PPROM is complicated by infection of the placenta and membranes (chorioamnionitis) in about half the cases, increasing the likelihood of adverse infant outcomes. Because stress has been associated with both preterm birth and altered immune function, we hypothesized that stress would increase the risk of chorioamnionitis among women with PPROM who were not actively infected upon presentation. Stress was measured by both physiological [salivary cortisol and serum Corticotropin Releasing Hormone (CRH) levels] and psychological (stress questionnaires: the Psychiatric Epidemiology Research Questionnaire, the Daily Hassles Scale, the 14-item Perceived Stress Scale, and the Interpersonal Support Evaluation List) means. Logistic regression was used to determine the association between stress and the development of chorioamnionitis and between stress and a composite adverse neonatal outcome (death or abnormal cranial ultrasound finding). Linear regression was used to assess the association between stress and latency (time from rupture of membranes until delivery). A one SD increase in mean salivary cortisol concentration was associated with a 3-fold increased odds of developing chorioamnionitis (OR 3.17, 95% CI 0.88-11.46), and an 8.6 fold increased odds of adverse neonatal outcomes (OR 8.62, 95% CI 0.99-75.03). A 1 SD increase in CRH was

associated with a 33% increased odds of chorioamnionitis (OR 1.33, 95% CI 0.50-3.53) and a 3.5 fold increased odds of adverse neonatal outcomes (OR 3.45, 95% CI 0.55-21.56). Stress batteries were not associated with the development of either chorioamnionitis or adverse neonatal outcomes. There was little relationship between either physiologic or psychological measures of stress and latency. Physiologic measures of stress in women with PPROM may be associated with an increased risk of adverse obstetric and neonatal outcomes, specifically the development of chorioamnionitis and the composite newborn outcome of death or abnormal cranial ultrasound findings. Since prematurity is the most important causes of death and disability among children in developed countries, our finding is of significant public health importance. If a role for stress in the etiology of PPROM-associated chorioamnionitis is confirmed, then stress-reduction techniques may reduce adverse neonatal outcomes.

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INTRODUCTION

Preterm birth accounts for ~12% of all births in the United States today and is the most common cause of death and disability in developed countries (Hamilton, 2006). Of all causes of preterm birth, chorioamnionitis (infection and inflammation of the fetal membranes and placenta) has been associated most frequently with severe adverse neurodevelopmental outcomes, in particular cerebral palsy (O'Shea 1998, Yoon 1997). The pathophysiologic link between chorioamnionitis and cerebral palsy remains uncertain, but it is thought that circulating cytokines produced during the inflammatory response directly injure the developing fetal central nervous system.

In normal labor at term gestation, the amnion and chorion rupture sometime after the onset of labor. Preterm premature rupture of the fetal membranes (PPROM) is a condition in which the protective fetal membranes loose integrity and rupture before the onset of labor and at a gestational age of less than 34 weeks. PPROM, which accounts for 30% of preterm births (Kaltreider 1980), increases the likelihood of chorioamnionitis. Some women with PPROM are actively infected at the time of presentation, some develop chorioamnionitis in the subsequent days-to-weeks, and others never become infected. It is not clear why some women with PPROM develop chorioamnionitis whereas others do not.

Since psychological stress has been associated with immune impairment (Miller 2002; Yang 2000) and an increased risk of infection (Cohen, 1999), we hypothesized that psychological stress would increase the risk of chorioamnionitis among high risk women with

PPROM. In this pilot study, we prospectively evaluated the relationship between psychological stress and the development of chorioamnionitis in a group of women with PPROM who were not acutely infected at the time of enrollment. Stress was measured by both physiological [salivary cortisol and serum Corticotropin Releasing Hormone (CRH) levels] and psychological (stress questionnaires) means, and the correlation between the measures of stress was assessed. Specifically, we asked the following: 1) does stress increase the likelihood of chorioamnionitis among women with PPROM, 2) is stress associated with shorter latency periods (the time from rupture of membranes until delivery) among women with PPROM, and 3) does stress increase the risk of adverse neonatal outcomes among women with PPROM?

MATERIALS AND METHODS

This prospective cohort study was approved by the Institutional Review Board of the University of Pittsburgh School of Medicine. Written informed consent was obtained from all study participants

SUBJECTS

Women of any age or race admitted to Magee-Womens Hospital (Pittsburgh, PA) between July 1, 2006 and June 30, 2008 with the diagnosis of PPROM were eligible to participate if they were between 23 0/7 and 31 6/7 weeks gestation, carried a singleton or twin gestation, and did not deliver for at least 48 hours after hospital admission. We chose a 48-hour delay in order to limit the sample to women without active infection at the time of enrollment (since women with active infection at the time of presentation usually deliver promptly) and also allowed time for the acute psychological stress associated with the new diagnosis of PPROM to abate. Twins were allowed because the physiology of PPROM in twins at the young gestational age chosen is likely similar to that of singleton pregnancies, and less likely related to polyhydramnios. Exclusion criteria included overt signs of chorioamnionitis and complications of pregnancy which are

independently associated with a risk for adverse neonatal outcomes, including preeclampsia or pregnancy-induced hypertension, significant fetal growth restriction (estimated weight <3rd percentile for gestational age), and known congenital anomalies.

EXPERIMENTAL DESIGN

The experimental protocol is depicted in Figure 1, and each aspect described in detail below. All women admitted to the hospital with PPROM and sufficient amniotic fluid volume for sampling undergo diagnostic amniocentesis to test for chorioamnionitis. We did not approach women with positive amniotic fluid cultures for study participation.

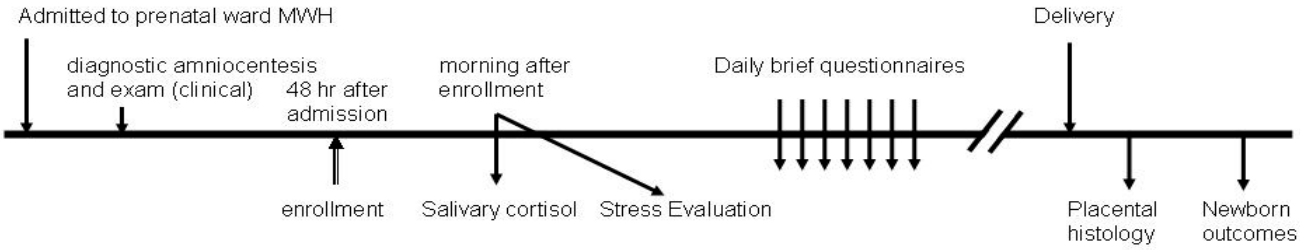

Figure 1. Study Design

STRESS EVALUATION

We assessed psychological stress by a variety of means, including both physiologic testing, [salivary cortisol secretion and serum concentrations of Corticotropin Releasing Hormone (CRH)] and self-report questionnaires.

Salivary Cortisol

Each subject collected her own saliva samples 5 times/day for 2 sequential days, beginning the morning of the day following enrollment. We designed sample collection, handling, and assay procedures to minimize the potential impact of extraneous factors on salivary cortisol assessments (Gibson 1999). Sample times on each day were: within one hour after waking (before breakfast and before the subject brushed her teeth), and 4, 9, 11, and 14 hours after waking. We gave subjects an alarm that reminded them when it was time to collect saliva, and instructed them to keep a log to verify collection times. If the sample time fell around a meal time, the subject was instructed to obtain the sample before eating or at least 1 hour after her meal. If she accidentally brushed their teeth or ate immediately prior to obtaining the sample, she was instructed to note this on her log. Smokers were instructed not to smoke during the 30 minutes prior to obtaining the sample.

Samples were collected using the Salivette device (Sarsedt, NC), which contains a small cotton wedge which the subject saturates with saliva by mouthing it for 2-3 minutes.

Saturated cotton swabs are sealed in air-tight storage container, and transported on ice to a -80^{o} freezer. Samples were shipped on dry-ice to the laboratory of Dr. Clemens Kirschbaum, who assayed for cortisol using a highly-sensitive luminescence immunoassay (IBL, Hamburg, Germany). Samples were exposed to a single freeze-thaw cycle before assaying. Cortisol results are reported as nmol/L.

Serum CRH Concentrations.

Serum CRH concentrations were drawn on two separate mornings 2-3 days apart, beginning the day after enrollment. Blood was collected into a chilled EDTA-treated tube which contained aprotonin (Sigma-Aldrich Corp., St. Louis, MO) at a concentration of 6 TIU aprotonin/ ml blood. Whole blood was centrifuged at 1,600xg for 15 minutes at 4°C. The plasma was removed and the serum stored at -80°C. Samples were shipped on dry ice to Phoenix Pharmaceuticals, Inc. (Burlingame, CA) for analysis. Samples did not undergo multiple freeze-thaw cycles. CRH results are reported as ug/dL.

Self-Report Stress Questionnaires

A research assistant administered the stress questionnaires to all study subjects. The specific batteries used included: a modified form of the Psychiatric Epidemiology Research Questionnaire (PERI, Dohrenwend, 1980); the Daily Hassles Scale (Lazarus 1989; DeLongis 1988); the 14-item Perceived Stress Scale (PSS, Cohen, 1983); the Interpersonal Support Evaluation List (ISEL, Cohen, 1985); and questions related to relationships, SES, demographics,

and health behaviors. Data for the PERI are presented as a) frequency of events occurring in the previous 6 months, b) subjective stress rating for the events that occurred at the time of the event, and c) current subjective stress rating for these same events. The Daily Hassles Scale is scored in areas of overall hassle, frequency of hassles and the intensity of the hassles. The items for the ISEL fall into 4 10-item subscales: the "appraisal" domain (the perceived availability of someone to talk to about one's problems): the "tangible" domain (the perceived availability of material supports); the "self-esteem" domain (the perceived positive feelings about one's self compared to others; and the "belonging" domain (the perceived availability of people to do things with). For the PERI, the Daily Hassles Scale, and the PSS, a higher score indicates higher levels of psychological stress. Since the ISEL evaluates perceived supports, a higher score on this scale indicates less psychological stress. Following the initial assessment, the research assistant returned on a daily basis until delivery to administer a brief questionnaire asking questions about the on-going stress of remaining in the hospital and support that the subject had received since hospitalization.

OUTCOMES

Maternal Outcomes

Chorioamnionitis

We determined the presence of chorioamnionitis using pathologic criteria because infection has been identified frequently by placental histology in women who deliver preterm without clinical signs of chorioamnionitis (Greig 1993, Hillier 1993, Hillier 1998). We used criteria adapted

from the recommendation of the Perinatal Section of the Society for Pediatric Pathology (Redline 2003) whereby chorioamnionitis was defined as the presence of neutrophils in the fibrous amnion and/or chorion, irrespective of severity grade. Placentas from all subjects were analyzed by a clinical perinatal pathologist who was unaware of the findings on psychophysiology testing.

Latency

We defined latency as the number of days from rupture of membranes until delivery (inclusive). Latency was evaluated as an outcome because subacute infection may present with the onset of labor and subsequent delivery.

Neonatal Outcomes

The primary neonatal outcome was a composite outcome of death or abnormal neonatal neurologic outcome. The latter was based on the results of the routine cranial ultrasound which at our institution is performed on all infants born at ≤32 weeks gestation and on infants born at 33-35 weeks gestation who have evidence of respiratory distress at birth. We graded cranial ultrasounds by the presence or absence of intraventricular hemorrhage [Papile (Papile 1978) grade], increased periventricular echogenicity, cystic periventricular leukomalacia, and significant ventriculomegaly.

CLINICAL DATA COLLECTION

A research nurse who was unaware of the placental histopathology results collected all maternal and newborn clinical information from the medical records. Data collected from the maternal chart included a) factors relating to possible of infection, including maternal temperature and heart rate, fetal heart rate, duration of preterm labor and rupture of fetal membranes, and the quality of amniotic fluid at delivery; b) underlying maternal conditions including hypertension and diabetes; c) vaginal bleeding, and d) drug and cigarette use during pregnancy; and e) medications administered before delivery including tocolytics, antibiotics, and betamethasone. Information extracted from the infant medical record included a) demographic and growth parameters; b) respiratory pathology - respiratory distress syndrome, pulmonary interstitial emphysema, pneumothoraces, and bronchopulmonary dysplasia; respiratory support (type and duration of ventilation, CPAP, oxygen and surfactant treatment); c) cardiovascular variables including vasopressor support, treatment for patent ductus arteriosus; d) parameters relating to infection, including antibiotic use and blood culture results; e) reports of the cranial ultrasound; and f) other morbidities.

BIOSTATISTICAL DESIGN AND DATA ANALYSIS

Logistic regression models were used to assess the association of stress with developing chorioamnionitis for the following measures of stress: mean salivary cortisol concentration (mean of all 10 samples), mean serum CRH concentration (average of 2 samples), and the score for each questionnaire scale or subscale. Logistic regression models were also used to assess the

association of stress with the composite neonatal outcome death or adverse neurologic outcome using the same set of stress measures. For these analyses only, when the woman was carrying twins, only data for the first born twin was considered in the analysis. Linear regression models were used to assess the association of the measures of stress with latency. Confounding variables in all models included gestational age at presentation and twin gestation. Race was considered as a possible confounding variable, but not included as the majority of women (21/25) were Caucasian. Instead, models were run with and without the inclusion of the Non-Caucasian women. Correlation between the physiological and psychological measures of stress was evaluated using Spearman's Correlation.

All data analysis was conducted using STATA v.10 (StataCorp, College Station, TX). Results of the logistic regression analyses are presented as the increase in the odds ratios for a 1 SD change in the stress measure. Results of the linear regression models are presented as the absolute change in latency for a 1 SD change in the stress measure.

RESULTS

Twenty-eight women were enrolled in the study. Of these, one delivered within 24 hours of enrollment and before she could complete the protocol, one completed initial portions of the protocol and then asked to withdraw from participation, and one could not complete the stress questionnaires due to a significant language barrier. Clinical characteristics of the remaining 25 women who make up the study cohort and their infants are shown in Table 1. No women admitted to illicit drug use. Two women smoked, and 1 woman was taking antidepressant medication. Eight of 22 women tested were GBS positive (Table 1); 1 of 19 tested was positive for *Chlamydia trachomatis*; none of 21 tested were positive for *Neisseria gonorrhoeae*. One woman was diagnosed with bacterial vaginosis; however, only three women had specific tests for bacterial vaginosis. All women were treated with "latency antibiotics" during their hospitalization. Fifteen women (60%) developed chorioamnionitis, as determined by placental pathology. While only one infant had a positive blood culture (for *Escherichia coli*), 16 infants were treated with antibiotics for at least one week for presumed sepsis.

The average mean cortisol value (±SD) was 4.36±3.36 nmol/L. The mean CRH concentration was 14.37±13.04 ug/dL. The mean scores for the different stress batteries are given in Table 2. For the PERI, the mean(±SD) frequency of life events was 13.8 ±7.6 (range of possible values 0-115). The mean subjective stress score rating for events at the time they occurred was 2.31±0.76 (on a scale of 1-4), while the mean current subjective stress rating for

events that occurred was 1.78±0.71 (possible range: 1-4). For the Daily Hassles Scale the overall score was 41.3±30.3 (possible range: 0-189), the frequency score was 23.4±12.3 (possible range 0-63), and the intensity score was 1.6±0.5 (possible range:0-3). The mean score on the 14-item PSS was 27.8±10.2 (possible range of scores 0-56). For the ISEL, scores in the 4 domains ranged from 22.2±5.7 for the "self-esteem domain" to 24.9±6.8 for the "tangible" domain (range for each domain is 0-30, with a score of 30 indicating the highest level of supports).

Associations of stress measures with chorioamnionitis, adverse neonatal outcomes, and latency are shown in Table 2. A one SD increase in mean salivary cortisol concentration was associated with a 3-fold increased odds of developing chorioamnionitis (OR 3.17, 95% CI 0.88-11.46), and an 8.6 fold increased odds of neonatal death or abnormal cranial ultrasound finding (OR 8.62, 95% CI 0.99-75.03). A 1 SD increase in CRH was associated with a 33% increased odds of chorioamnionitis (OR 1.33, 95% CI 0.50-3.53) and a 3.5 fold increased odds of neonatal death or abnormal cranial ultrasound finding (OR 3.45, 95% CI 0.55-21.56). Stress batteries were not associated with the development of either chorioamnionitis or adverse neonatal outcomes. There was little relationship between either physiologic or psychological measures of stress and latency. Results were similar when the logistic and linear regression models were run with the entire cohort and with only the Caucasian women. The one exception was the analysis of the intensity of Daily Hassles where odds ratio for the Caucasian-only analysis was twice the size of that for the group as a whole.

The relationship between stress measures is shown in Table 3. Salivary cortisol levels correlated poorly with stress batteries except for the Daily Hassles Scale. There was an inverse relationship between salivary cortisol levels and both the overall Daily Hassles score and the intensity score on the Daily Hassles Scale. Serum CRH concentrations correlated poorly with all

stress batteries. There was moderately good correlation between most stress batteries tested with positive correlations between scores on the PERI, the Daily Hassles Scale, and the PSS; and negative correlations between the ISEL and all other scales.

Table 1. Clinical characteristics of the study population (n=25).

Mother's Age [years; median (range)]	27 (16-39)
Gestational age at enrollment [weeks; median (range)]	28.3 (24.4-31.4)
Race (Caucasian)	21 (84)
Singleton Fetus	19 (76)
Gravida [median (range)]	2 (1-7)
Para [median (range)]	1 (0-3)
Group B Strep (+)	8 (32)
Amniocentesis (+)	0 (0)
Betamethasone doses [median (range)]	2 (2-4)
Latency [days; median (range)])	15 (6-113)
Chorioamnionitis	15 (60)
Gestational age at delivery [weeks; median (range)]	30.7 (25.1-34.1)
Birth weight [gm; median (range)]	1533 (564-2390)
Gender (male)	17 (68)
Respiratory distress syndrome	16 (64)
Positive blood culture at birth	1 (4)
Treatment with antibiotics for presumed sepsis	16 (64)
Intraventricular hemorrhage	
None or Grade I	19 (79)
Grade II	1 (4)
Grades III-IV	0 (0)
Not tested*	4 (17)
Periventricular Leukomalacia*	1 (4)
Ventriculomegaly*	0 (0)
Infant Demise	5 (20)

Results: n (%) unless otherwise noted.

* Two babies were not tested because they died before the cranial ultrasound could be completed. Two babies were not tested because they were born at 34 weeks gestational age, did not meet nursery protocol guidelines, and had no clinical indications for cranial ultrasound examination.

Table 2. Association of stress measures with chorioamnionitis, composite adverse neonatal outcome and latency.

		Chorioamnionitis		Composite Adverse Neonatal Outcome		Latency	
Measure	Mean Score (±SD)	Odds Ratio*	p-value	Odds Ratio*	p-value	Regression Coefficient*	p-value
Salivary Cortisol (nmol/L)	4.358±3.355	3.170	0.078	8.618	0.051	0.228	0.537
CRH (ug/dL)	14.366±13.039	1.33	0.569	3.447	0.186	0.174	0.622
PERI†							
a	13.80±7.62	0.737	0.494	0.399	0.277	0.369	0.304
b	2.31±0.76	0.715	0.501	0.810	0.721	0.402	0.226
c	1.78±0.71	1.014	0.970	0.713	0.619	0.260	0.448
Hassels‡							
overall	41.28±30.29	0.743	0.556	0.081	0.313	0.450	0.242
frequency	23.40±12.31	0.876	0.815	0.061	0.182	0.460	0.280
intensity	1.60±0.50	0.719	0.467	0.897	0.266	0.286	0.416
PSS§	27.80±10.17	0.850	0.773	0.389	0.312	0.141	0.740
ISEL‖							
appraisal	24.76±6.81	1.300	0.540	1.794	0.425	-0.260	0.451
tangible	24.88±6.81	0.863	0.773	0.756	0.637	-0.302	0.414
self esteem	22.16±5.72	0.695	0.451	0.770	0.669	-0.229	0.512
belonging	23.32±7.08	0.810	0.685	1.027	0.966	-0.061	0.987

Models are for subjects of all races, and contain confounding variables (twin and gestational age at enrollment).
* Odds ratios and regression coefficients given for one SD change in the predictor.
† Psychiatric Epidemiology Research Questionnaire (PERI, Dohrenwend, 1980), a=frequency of events, b=subjective stress the rating for events that occurred at the time of the event, c=current subjective stress rating.
‡ Daily Hassles Scale (Lazarus 1989; DeLongis 1988). § 14-item Perceived Stress Scale (Cohen, 1983).
|| Interpersonal Support Evaluation List (ISEL, Cohen, 1985), by domain.

Table 3. Spearman correlation coefficients (rho) for the relationship between different measures of stress.

	cortisol	CRH	PERIa*	PERIb	PERIc	Hassles† (overall)	Hassles (freq)	Hassles (intensity)	PSS_14‡	ISEL§ (appr)	ISEL (tang)	ISEL (self)	ISEL (belong)
cortisol	1.0000												
CRH	0.1292	1.0000											
PERIa	-0.0826	-0.1526	1.0000										
PERIb	0.1140	0.2035	**0.5007**	1.0000									
PERIc	-0.0433	0.0644	**0.5217**	**0.7507**	1.0000								
Hassels(o_all)	*-0.3465*	-0.1941	**0.5516**	0.1945	**0.4861**	1.0000							
Hassles(freq)	-0.2497	-0.2049	**0.4482**	0.1240	**0.4534**	**0.9491**	1.0000						
Hassles(inten)	*-0.4136*	-0.1007	**0.5464**	0.1173	0.3430	**0.8295**	**0.6604**	1.0000					
PSS_14	-0.2128	-0.1940	**0.6457**	0.3393	**0.5820**	**0.8147**	**0.7839**	**0.6296**	1.0000				
ISEL (apprs)	-0.0312	0.0937	**-0.5244**	*-0.3667*	**-0.4815**	**-0.6887**	**-0.6510**	**-0.5366**	**-0.7132**	1.0000			
ISEL(tang)	-0.0886	0.0426	**-0.4063**	**-0.4674**	**-0.7367**	**-0.6835**	**-0.6999**	**-0.5071**	**-0.6936**	**0.6871**	1.0000		
ISEL(self)	0.0321	-0.0096	-0.2399	-0.3130	**-0.5227**	-0.4915	**-0.5153**	-0.3319	**-0.7041**	**0.6653**	**0.5924**	1.0000	
ISEL(belong)	0.1771	0.2886	*-0.3603*	-0.2597	**-0.4654**	**-0.6757**	**-0.6898**	**-0.4693**	**-0.7602**	**0.8094**	**0.6623**	**0.8178**	1.0000

Numbers in bold have an associated p-value <0.05. Values in italic have p value <0.10.

* Psychiatric Epidemiology Research Questionaire (PERI, Dohrenwend, 1980), a=frequency of events, b=subjective stress rating for the events that occurred at the time of the event, c=current subjective stress rating

† Daily Hassles Scale (Lazarus 1989; DeLongis 1988)

‡ 14-item Perceived Stress Scale (Cohen, 1983),

§ Interpersonal Support Evaluation List (ISEL, Cohen, 1985), by domain

DISCUSSION

This is the first study to evaluate the association between stress during pregnancy and the risk of chorioamnionitis. Among our cohort of high-risk pregnant women with PPROM, we found that that an increase in salivary cortisol levels was associated with an increased odds of developing chorioamnionitis and the composite adverse neonatal outcome of death or abnormal cranial ultrasound findings. As opposed to this physiological measure, we did not find a relationship between psychological stress as measured by the 4 stress batteries tested (the Psychiatric Epidemiology Research Questionnaire, the Daily Hassles Scale, the 14-item Perceived Stress Scale, and the Interpersonal Support Evaluation List) and the development of chorioamnionitis, adverse neonatal outcomes or shortened latency.

Psychological stress has been associated with preterm birth among women with previously uncomplicated pregnancies. In the "Preterm Prediction Study" which enrolled 2593 women at or before 24 weeks gestation, the odds of birth before 35 weeks gestation was 1.16 ($p=0.003$) for each increase on the stress scale, after controlling for psychosocial and behavioral risk factors (Copper, 1996). Others have shown that the risk of preterm birth is highly associated with low socioeconomic status (Parker, 1994), a surrogate for increased stress. However, even within a low SES population, Lobel *et. al*. demonstrated that stress as measured by an abbreviated version of the Perceived Stress Scale is associated with preterm birth, whereas other traits such as anxiety and life events were not (Lobel 1992).

The mechanisms by which stress triggers preterm birth remains theoretical. Neuroendocrine and/or immune-mediated mechanisms may be involved. Corticotropin releasing hormone regulates the physiologic response to stress in the non-pregnant state. CRH is expressed in the placenta and may have a role in the initiation of labor (Ruiz 2002). In fact, CRH has been noted to be at extremely high levels in women who deliver preterm, and the elevations may be seen weeks before the onset of the preterm labor (Wadhwa 2001a). In contrast Ruiz *et. al*. did not find a difference between CRH levels in women whose pregnancies were complicated by genitourinary infections, and those whose pregnancies were not complicated by infection (Ruiz 2002). They hypothesized that the infectious process may be more related to early preterm births (such as in our study population), whereas CRH may be related to later preterm birth. Indeed, while chorioamnionitis may be responsible for the majority of very early preterm births, it is rarely seen in later preterm births (Wadhwa 2001b).

Our study differs from these previous studies in both the subject population and the outcome measures. Rather than survey a large low-risk population most of whom are not expected to deliver preterm, we studied a smaller, high risk population all of whom delivered at or before 34 weeks gestation. Thus, evaluating the effects of stress on the timing of delivery was not our focus. Instead, we chose our main outcome to be the development of chorioamnionitis. The relationship between stress and chorioamnionitis addresses specifically whether the risk of intrauterine infection is increased by stress.

Sixty percent of our cohort developed chorioamnionitis. This rate is consistent with rates in the literature for women with PPROM who did not have chorioamnionitis upon presentation and who were managed expectantly (Romero 1991). We found that development of chorioamnionitis was associated with increased salivary cortisol levels, and less-so with

increased CRH levels. Salivary cortisol concentrations may be the best marker for acute stress in this high-risk population. Although cortisol secretion is regulated by CRH, it may be that hypothalamic CRH responses are small relative to placental CRH production at this gestational age, so that small changes in CRH are masked by the larger placental load of CRH. Since all our subjects delivered preterm, it is also possible that the majority already had elevated CRH concentrations, making it difficult to distinguish well those with further elevations.

In an attempt to get at the different aspects of psycholological stress, we used 4 different stress batteries. Consistent with previous reports on the risk of preterm delivery (Parker 1994, Copper 1996), we did not find a relationship between life events, self-esteem and other measures of support with the development of chorioamnionitis. However, neither did we find a relationship between perceived stress and chorioamnionitis. The difference between our finding and the association between stress and preterm birth described in the epidemiologic literature is likely related to our choice of outcome. Since all our women delivered preterm, we could not evaluate the relationship between perceived stress and preterm birth in our cohort. Previous studies reporting an association between stress and preterm birth do not differentiate the specific etiology of the preterm birth (Lobel 1992, Copper 1996, Parker, 1994). Based on the lack of association between perceived stress and development of chorioamnionitis, we can hypothesize that the relationship between stress and preterm birth in the larger studies was not the result of an increased risk of infection-related preterm birth. Reanalysis of the epidemiologic data with the specific etiology of preterm birth may help elucidate the specific mechanism of stress-associated preterm birth.

Among our high-risk population of infants born to mothers with PPROM, we found that the risk of adverse neonatal outcomes increased as salivary cortisol levels in the mother

increased. Again, psychological measures of stress as assessed by the PERI, the Daily Hassles Scale, the PSS and the ISEL bore little relationship to this outcome. It has been reported that neonatal brain damage is no higher among infants with PPROM than among infants with spontaneous preterm delivery in the absence of PPROM (Park 2006). In the study by Park *et. al.* (Park 2006), the risk of cranial ultrasound abnormalities did not increase as latency increased, while the risk of chorioamnionitis did increase as latency increased. We did not have enough infants in our cohort to assess independently the effect of stress on cranial ultrasound abnormalities. However, the composite outcome of death or abnormal cranial ultrasound finding was seen only in infants who developed chorioamnionitis (Chi-square p=0.051, data not shown) and bore no relationship to latency.

Figure 2 depicts our proposed mechanism to explain the relationships that we found, in the context of what has been previously shown regarding the hypothalamic-pituitary-adrenal axis and preterm birth. In this model, PPROM stimulates the production of CRH from the placenta, irrespective o the degree of psychological stress. Since placental CRH production has a positive feedback loop, elevations in CRH in women with PPROM are primarily of from the placental source. Psychological stress acts on the hypothalamus to stimulate CRH production, but at smaller quantities than that produced by the placenta, and also with a self-limiting negative feedback loop. Psychological stress may have some effects on immune unction, but it is not clear that these effects predispose to the bacterial infection characteristic of chorioamnionitis. Thus, in women with PPROM CRH levels are elevated, irrespective of the degree of psychological stress. CRH then stimulates cortisol production, and women with higher levels of cortisol will manifest the stress-associated alterations in immune function that predispose to chorioamnionitis and adverse neonatal outcomes.

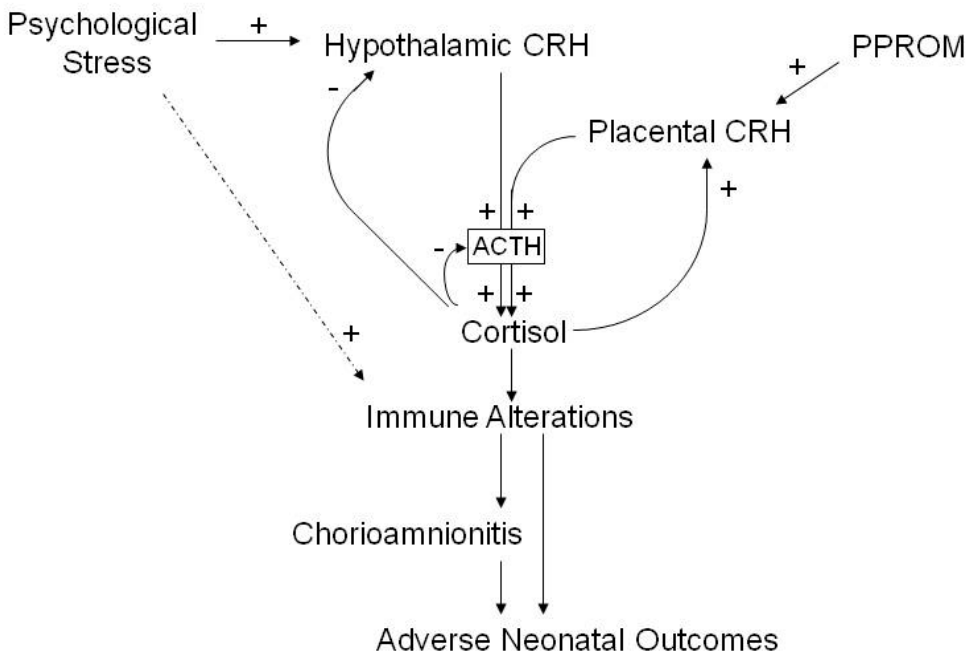

Figure 2. Proposed mechanism for PPROM-associated chorioamnionitis and adverse neonatal outcomes

The strengths of our study are 1) the completeness of the stress evaluation, including both physiological and psychological aspects of stress; 2) the recruitment of all subjects from a single institution, with fairly homogeneous management strategies; and 3) the restriction of the subject population to one cause of preterm delivery. The major weakness in this pilot study is the small sample size. Although we found strong associations between salivary cortisol and both the development of chorioamnionitis and adverse neonatal outcomes, the associations did not meet strict criteria of statistical significance using a p-value <0.05. Statistical significance may have been reached with a larger sample size. A larger sample size would also have enabled us to stratify by race, as stress levels and the physiologic response to stress may differ between

Caucasian and African American women. Next, since we did not dictate which vaginal cultures were performed on study subjects, we were unable to assess the interactions between stress measures and coincident bacterial vaginosis, *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. Future studies using larger sample sizes should incorporate evaluation for these organisms which may be pathogenic in the etiology of preterm birth. A final limitation is that we were unable to obtain a "first-awakening" cortisol value. With this in-patient population, most subjects were awakened early by nursing for routine hospital care, following which some women went back to sleep whereas others stayed awake. Since a consistent time to cortisol collection could not be assured, we elected not to collect awakening samples. Instead, the first cortisol value was collected within one hour of awakening, and the average of all 10 values from the two-day collection period was used for analysis. It remains possible that an even stronger association between cortisol values and chorioamnionitis would have been seen had we been able to reliably assess the cortisol awakening response (Clow 2004).

In summary, we found that among a cohort of high-risk women with PPROM who were not actively infected at the time of presentation, higher salivary cortisol concentrations were associated with the development of chorioamnionitis and with the composite outcome of neonatal death and/or abnormal cranial ultrasound findings. There was no relationship between psychological measures of stress and these adverse outcomes in this population. Further study into mechanisms by which stress may be associated with adverse maternal and infant outcomes should be explored in women with PPROM. For the larger group of women with preterm delivery, studies of psychological stress should differentiate among possible etiologies of preterm birth, because the mechanisms may differ for women with and without PPROM. Since preterm birth accounts for a large proportion of infant death and childhood disability the public

health significance of this research cannot be overstated. Programs or treatments which reduce stress among high-risk women may reduce the incidence of preterm birth and improve outcomes among those infants born prematurely.

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