STRESS AND IMMUNE RESPONSES DURING PREGNANCY

I. OBJECTIVES

This study will examine effects of everyday life stress and obesity on immune responses to influenza virus vaccine (the flu shot) during pregnancy. Antibody levels increase after vaccination. Higher antibody levels indicate better immune protection from influenza (the flu). In addition to providing protection from the flu for the mother, being vaccinated during pregnancy may protect infants from the flu during the first six months of life during which time infants cannot be vaccinated. Our goal is to determine whether greater life stress and obesity reduce 1) antibody responses to the flu shot in women and 2) antibody levels in the newborn at the time of delivery.

II. BACKGROUND AND SIGNIFICANCE

Stress and Antibody Response to Influenza Vaccination

Evidence supports an association between stress and poorer antibody response to influenza vaccination. In a sample of older adults, individuals who were caregiving for a spouse with dementia (a chronically stressed group) showed poorer response to influenza vaccine than control subjects matched for age, sex, and socioeconomic status [1]. Subsequent replication of these findings showed that both former caregivers (who had lost their spouses within the past few years) and current caregivers showed less antibody in response to vaccination than did control subjects [2]. Similarly, Vedhara et al. [3] found that caregiver stress was associated with poorer response to influenza vaccine; caregivers were less likely to show a four-fold increase in antibody production than were non-caregivers. Furthermore, Vedhara et al. (1999) found that among individuals showing adequate response, non-caregivers showed a more robust response. Many studies of the effects of stress on influenza vaccine to date have focused on older populations. However, stress has also affects immune response to influenza vaccination in a younger groups[4] [5]. Similarly, academic stress has been shown to affect antibody response to Hepatitis B vaccination in medical students, also a younger population [6, 7]. To our knowledge, no study has explored the effects of stress on response to any vaccine during pregnancy.

Although stress has been studied in association with response to other vaccines including, but not limited to, hepatitis B [8] and pneumococcal pneumonia [9] assessment of antibody response to influenza vaccination is an ideal model for exploring stress and immune function during pregnancy. As noted, this vaccination that is recommended to pregnant women [10]. Furthermore, the effectiveness of influenza vaccination in pregnant women has important health implications; influenza is more likely to result in serious medical complications among pregnant women than the general population. Relatedly, pregnant women are more likely to undergo hospitalization as a result of influenza than non-pregnant individuals of similar age. Therefore, understanding the effects of stress on response to this particular vaccine has important implications for potentially severe health outcomes. **The primary goal of this investigation is to examine whether pregnant women who report greater life stress show poorer antibody responses to influenza vaccination measured at one month post-vaccination and poorer antibody transfer to the neonate as measured in cord blood at the time of delivery. This study will also examine the role of obesity in these relationships. It is predicted that obesity will exacerbate effects of stress, resulting in the poorest outcomes in women with high stress and obesity.**

III. PROCEDURES

A. Research Design

The current study is designed to evaluate the relationship between psychosocial factors on one hand and antibody responses to influenza vaccination on the other. Participants will complete two study visits: pre-

vaccination and one month post-vaccination. Maternal a maternal serum sample will be collected by hospital staff during the hospital stay at the time of delivery and we will obtain a sample of cord blood. Cord blood is standardly collected and stored following every delivery at OSUMC. If the sample is not needed for medical reasons, it is discarded after a few days. We will obtain samples that would otherwise be discarded. If maternal serum is not collected at the time of hospital stay due to error in coordinating with hospital staff, we will arrange for a serum sample to be collected at the participant's home, a visit to our laboratory or CRC, or we will coordinate with the woman to collect this sample at the time she nexts visits her physician for a post-partum visit. If cord blood is not available from the hospital due to not being collected at delivery or used for testing purposes for infant care, we will arrange for infant blood to be collected by heel stick at the participant's home, a visit to our laboratory or CRC.

B. Sample

Pregnant women ages 18 and older will be recruited. As a part of routine care, pregnant women are encouraged to receive influenza vaccination. Women will primarily be recruited at their regular prenatal or gynecology visits at McCampbell Clinic. Those who are interested will be asked questions related to inclusion/exclusion criteria (i.e. age, health conditions, medications, and fetal health) to determine if they are eligible. Participant medications and health status (e.g., presence of chronic health conditions) will also be determined via chart review to confirm eligibility. Eligible individuals will be consented and the study will be described verbally to them.

Inclusion criteria for participants are adult (18 years and older) pregnant women who choose to have the influenza vaccination and who give informed consent. Exclusion criteria are fetal anomaly, health conditions or medications with a major immune component (e.g., arthritis, cancer, lupus) and inability to give informed consent.

Participants will be recruited from the prenatal clinic and general gynecology clinic at McCampbell Hall, OSU. Participants will also be recruited through flyers at other Ob/Gyn clinics and advertisements in local newspapers and campus newsletters (e.g., OSU Today) and ads posted online (e.g., on Campus newsletters; Stressandpregnancy.osumc.edu and other relevant websites for advertising/recruiting including ResearchMatch.org). Study visits will be completed at McCampbell Clinic, the OSU Clinical Research Center at Dodd Hall, or at Dr. Christian's clinical laboratory at the Institute for Behavioral Medicine Research. If women are unable to travel to OSU for study visits (e.g., due to child care obligations), they may be given the option, funding permitting, of having a nurse or phlebotomist visit their home to complete follow up visits. (All Visit 1s will be conducted at either McCampbell Clinic or the CRC as this study visit requires the administration of the influenza virus vaccine).

C. Vaccination/ Measurement / Instrumentation

Influenza Vaccination. Each woman will be administered a standard influenza vaccine produced by GlaskoSmithKline or SanofiPasteur as available from the OSU Pharmacy which supplies McCampbell Clinic. The vaccine will be administered by a clinic nurse or a Clinical Research Center nurse. Each .05mL dose contains 45 µg of hemaglutinin of each of virus strain. All women vaccinated during the same influenza season will receive the vaccine from the same manufacturer and same lot whenever possible.

Serum samples. At each visit, whole blood will be collected into vacutainer tubes while the subject is in a seated position. Samples will be immediately centrifuged and placed in freezer storage until analysis. These samples will be stored at the laboratory of Dr. Lisa Christian.

Self-report Measures. Questionnaire measures will be used assess how psychosocial factors (including stress, mood, and social support) relate to immune parameters of interest. Each of the following will be completed at one or more study visit.

The **Center for Epidemiological Studies Depression Scale (CES-D)** has been used extensively as a brief measure of depressive symptomatology [11,12]. Studies have shown acceptable test-retest reliability and excellent construct validity [12]. The 10-item version of the **Perceived Stress Scale (PSS)** will be used to measure the subjective experiences of stress and coping with stress using the past month as timeframe [13]. **Parental Attitudes** regarding happiness about pregnancy have been associated with birth weight [14]. Two questions adapted from the National Survey for Family Growth will be used in the current study [15]. Each participant will be asked to describe how she felt about her pregnancy at the moment of discovery from 1 to 10, with 1 signifying unhappiness and 10 signifying happiness. Each participant will also rate her perception of her partner's happiness. This measure will be given to pregnant women only. **The Perceived Social Support Scale (PSSS)** will be used to assess support from family, friends, and a significant other. The **Prenatal Life Events Scale (PLES)** will be used to assess major life events occurring in the past year. This measure is appropriate for pregnant and nonpregnant women. The **Prenatal Health Behavior Scale** will be used to assess health behaviors including health eating, unhealthy eating, vitamin use, smoking, and exercise. This scale is appropriate for pregnant as well as nonpregnant women. The **Pregnancy Distress Questionnaire (NUPDQ)** will be used to assess anxiety about pregnancy, childbirth, and the strains of an additional child. This questionnaire will be completed by pregnant women only. The **Life Difficulties** questionnaire will be used to measure economic strain and living difficulties among both pregnant and nonpregnant women. The **Pittsburgh Sleep Quality Index (PSQI)** will be used to measure subjective sleep quality. We will assess **Fatty Acid** consumption via questionnaire assessing fish intake and fish oil supplement usage.

D. Detailed Study Procedures.

The following is the description of study procedures as provided to participants in the informed consent:

Visit 1 will take 60-90 minutes. You will answer questionnaires about your health, health behaviors, stressful life events, mood (e.g., anxiety and depressive symptoms), and social support. These include questions about positive and negative interactions with your friends/family as well as questions regarding stressful or traumatic events you may have experienced growing up. You will have 35 mL of blood drawn. Finally, you will receive an influenza virus vaccination (flu shot).

Visits 2 will take about 30 minutes and will occur one month after Visit 1. You will answer questions about your recent health behaviors, life stress, and mood and have 30 mL of blood drawn.

During your hospital stay at the time of the delivery of your baby, hospital staff will obtain a 30mL blood sample for our study. If this sample is not collected during your hospital stay, or if you do not deliver in a hospital, we will schedule a visit to your home or at our office soon after your delivery at a time that is convenient for you in order to collect this blood sample.

Finally, cord blood is standardly collected at every delivery at OSUMC. If it is not needed for medical reasons, it is discarded after a few days. If you deliver at OSUMC and your baby's cord blood is not needed for medical reasons, it will be given to our study personnel rather than being thrown away. If cord blood is not available for your baby, we will schedule a visit to our office soon after your delivery at a time that is convenient for you, in order to collect this blood sample. If you cannot come to our office, we will work with you to schedule a visit to your home. This blood sample will be collected from your baby by heel stick which is similar to a finger stick in adults.

Also, after your delivery, researchers will access your medical records to obtain the following information related to your pregnancy: your date of delivery, the birth weight and sex of your baby, how much weight you gained during your pregnancy, your blood pressure at clinic visits, the results of your clinical screenings for sexually transmitted diseases, and whether you had any complications during pregnancy or delivery.

Use of REDCap

We will use REDCap Survey for the purposes of online screening of participants. In the future, we may also use REDCap Database for collection and maintenance of study data. Data collected from enrolled participants is currently collected via teleform. The OSU CCTS Research Informatics Services Core will be used as a central location for data processing and management of data obtained via REDCap Survey and, if utilized in the future, REDCap Databas. Vanderbilt University, with collaboration from a consortium of institutional partners (including OSU) and the NIH National Center for Research Resources, has developed a software toolset and workflow methodology for electronic collection and management of research and clinical trial data. REDCap (Research Electronic Data Capture) data collection projects rely on a thorough study-specific data dictionary defined in an iterative self-documenting process by all members of the research team with planning assistance from the CCTS Research Informatics Services Core. As part of the data dictionary development process, individual fields can be denoted as "identifiers". When exporting a de-identified dataset, these variables are omitted. Additionally, the data export tool also allows for the shifted of dates for a limited data set export. REDCap provides a secure, web-based application that is flexible enough to be used for a variety of types of research, provides an intuitive interface for users to enter data and has real time validation rules (with automated data type and range checks) at the time of entry. It offers easy data manipulation with audit trails and ad hoc reporting functionality for reporting, monitoring and querying patient records, and an automated export mechanism to common statistical packages (SPSS, SAS, Stata, R/S-Plus). REDCap is 21 CRF Part 11 capable. Currently, REDCap installations support electronic signatures by positively identifying the user through a unique username and password combination. The provisioning of accounts and user access to specific database(s) is integrated with the OSU Medical Center LDAP authentication service, and the provisioning of access and specific user rights are managed by CCTS staff.

E. Data Analysis

Analysis of influenza antibody titers. Antibody titers will be determined using an ELISA for the complete vaccine and each of the HA proteins included in the vaccine in the given year. Briefly, 96-well plates will be coated overnight at 4°C with vaccine (0.9 mgyml) or purified HA protein (0.3 mgyml), washed, and incubated 2 hr at 37°C; serum dilutions prepared in phosphate-buffered saline supplemented with 10% fetal bovine serum will then be added. Plates will be rewashed and goat anti-human IgG antibody and then alkaline phosphatase conjugate will be added and plates will be incubated 2 hr at 37°C. The secondary antibody will be detected using Sigma 10 phosphatase substrate tablets (Sigma) in diethanolamine buffer and absorbance was read at 405 nm. Titers were determined by identifying the serum dilution at which the titration curve became asymptotic.

Statistical Considerations.

Sample size calculation.

Sample size was based on ≥80% power to examine each hypothesis. Although Holm's stepdown procedure will be used in analyses, the more conservative Bonferroni adjustment was used for calculating sample size. For testing associations of composite stress and obesity with maternal antibody responses (Aim 1), our analyses will focus primarily on A/H1N1 and secondarily on influenza B; a 2009 meta-analyses concluded that responses to A/H3N2 strains are generally not susceptible to stress.[10] A

sample size of 60 in a given year will provide ≥90% power to detect an odds ratio of 0.4 for 4-fold antibody increase for one SD increase in composite stress, at the Bonferroni-adjusted significance level α=.025. A sample size of 180 across 3 years will provide ≥82% power to detect an odds ratio of 0.38 for obese vs. non-obese, also at α=0.025. These effect sizes are shown in our preliminary data. We will have ≥80% power to detect these effects for influenza B, assuming similar effect sizes. For testing associations of composite stress and obesity with cord blood antibody levels (Aim 2), a sample size of 60 in a given year will provide ≥80% power to detect an odds ratio of <0.45 for adequate cord blood antibody level for one SD increase in composite stress, at α=0.025. A sample of 180 across 3 years will provide ≥80% power to detect an odds ratio of 0.4 or lower for adequate cord blood antibody level in newborns from obese versus non-obese mothers, at α=0.025.

Statistical Analyses.

Specific Aim 1. Effects of stress on maternal antibody responses will be tested independently for each vaccination year. Thus, our data will provide valuable data regarding the extent to which effects of stress are consistent and reproducible across flu seasons. We will test the association between obesity and maternal antibody responses using data across all three vaccination years. The primary and supporting endpoints will be the same as above. The independent variable will be obesity status. A secondary analysis will explore the interaction between obesity and stress; these analyses will focus on depressive symptoms and the composite stress measure (CES-D, PSS, and STAI). We expect that stress and obesity will interact, with more pronounced effects of obesity among the highly stressed. Although the study is not specifically powered for the detection of interaction effects, based on our preliminary findings we expect to obtain useful estimates.

Specfic Aim 2. The primary analysis will be logistic regression testing whether stress and obesity are associated with attenuated cord blood antibody levels. The primary endpoint will be adequacy of cord blood antibody level (yes/no), with an adequate antibody level defined as a serum HAI antibody titer > 40. The same models will be used as described in Specific Aim 1, except for the change in dependent variable. A supporting analysis will test cord blood antibody levels as a continuous measure. Linear regression models will be used to test this supporting outcome, using the same independent variables as the logistic regression models.

Secondary meditational analyses will examine four potential pathways linking stress and obesity with low cord blood antibody titers: 1) the magnitude of maternal response at one month post-vaccination, 2) the maintenance of maternal antibody levels over time (maternal antibody at one month post-vaccination / maternal antibody at delivery) controlling for weeks from vaccination to delivery, 3) efficiency of antibody transfer (ratio of maternal antibody at time of delivery / newborn antibody level), and 4) the frequency of preterm birth (expected to be 15% overall). We expect factors 1 & 3 to exert the greatest effects. Our study is powered to detect overall effects of maternal stress and obesity on newborn antibody levels, but is not powered to detect the relative influence of each of these specific pathways. However, these analyses will provide a critical foundation for future studies in this line of investigation which will include this focus.

We do not expect racial differences in Aim 1. Due to higher rates of preterm birth in Blacks than Whites (18% vs 12%), this pathway is likely to contribute more strongly to the association between stress and lower cord blood titers for Blacks. We will use two approaches to examine effects of race: 1) inclusion of race as a predictive variable and 2) testing the secondary analysis model above separately for Blacks and Whites.

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