Title: Effects of Pregnancy-Specific Anxiety on Placental Inflammatory and Oxidative Stress Response and Birth Outcomes (MOMS ™ Placenta)

Date: January 15, 2021

NCT#: Pending

EIRB Protocol Template (Version 1.10)

1.0 General Information

***Please enter the full title of your study:**

Effects of Pregnancy-Specific Anxiety on Placental Inflammatory and Oxidative Stress Response and Birth Outcomes

***Please enter the Protocol Number you would like to use to reference the protocol:**

FWH20190163H
* This field allows you to enter an abbreviated version of the Protocol Title to quickly identify this protocol.

Is this a multi-site study (i.e. Each site has their own Principal Investigator)?

No

Does this protocol involve the use of animals?

○ Yes ◉ No

2.0 Add Site(s)

2.1 List sites associated with this study:

Primary Dept?	Department Name
○	**USAF** - 59th Medical Wing (59 MDW)

3.0 Assign project personnel access to the project

3.1 *Please add a Principal Investigator for the study:

Brady, Robert O, MD Lt Col

Select if applicable

☐ Student ☐ Site Chair
☐ Resident ☐ Fellow

3.2 If applicable, please select the Research Staff personnel:

A) Additional Investigators

Yuan, Tony Tao
Associate Investigator

B) Research Support Staff

Armstrong-Spenrath, LA'QUITA L
Team Member
Farhat, Julie Kristine, MBA
Team Member
Walker, Katherine C, MSN RN
Research Coordinator

3.3 *Please add a Protocol Contact:

Brady, Robert O, MD Lt Col
Walker, Katherine C, MSN RN
Yuan, Tony Tao

The Protocol Contact(s) will receive all important system notifications along with the Principal Investigator. (i.e. The protocol contact(s) are typically either the Protocol Coordinator or the Principal Investigator themselves).

3.4 If applicable, please select the Designated Site Approval(s):

Add the name of the individual authorized to approve and sign off on this protocol from your Site (e.g. the Site Chair).

4.0 Project Information

4.1 * Has another IRB/HRPP reviewed this study or will another IRB/HRPP be reviewing this study? If Yes, answer the questions according to the IRB/HRPP Determination.

○ Yes ◉ No

IRB Name	Review Date	Determination
No records have been added		

4.2 * Is this a research study or a Compassionate Use/Emergency Use/HUD project?

◉ Yes ○ No

4.3 What type of research is this?

- ☑ Biomedical Research
- ☐ Clinical trial (FDA regulated)
- ☐ Behavioral Research
- ☐ Educational Research
- ☑ Psychosocial Research
- ☐ Oral History
- ☐ Other

4.4 Are you conducting this project in pursuit of a personal degree?

○ Yes ◉ No

4.6 * Is this human subjects research? (As defined by 32 CFR 219)

Human subject means a living individual about whom an investigator (whether professional or student) conducting research:
(i) Obtains information or biospecimens through intervention or interaction with the individual, and uses, studies, or analyzes the information or biospecimens; or
(ii) Obtains, uses, studies, analyzes or generates identifiable private information or identifiable biospecimens.

◉ Yes ○ No

4.7 * Do you believe this human subjects research is exempt from IRB review?

○ Yes ◉ No

5.0 Personnel Details

5.1 List any Research Team members without EIRB access that are not previously entered in the protocol:

Name: (Last, First, M.I.)	Phone Number:	Email Address:	Associated Institution:
Weis, Karen L. Role on Protocol: Principal Investigator	210-829-3987	weis@uiwtx.edu	University of the Incarnate Word
Name: (Last, First, M.I.) Yuan, Tony T. Role on Protocol: Associate Investigator	Phone Number: 214-437-0541	Email Address: tony.t.yuan.ctr@mail.mil	Associated Institution: USAF CAMD, Lackland AFB
Name: (Last, First, M.I.) Bustamante, Blandine Role on Protocol: Associate Investigator	Phone Number: 210-283-6374	Email Address: bhelfric@uiwtx.edu	Associated Institution: University of the Incarnate Word
Name: (Last, First, M.I.) Graham, Brittney S. Role on Protocol: Associate Investigator GME Pathology Resident	Phone Number: 210-916-6309	Email Address: brittney.s.graham.mil@mail.mil	Associated Institution: BAMC - Pathology Residency

Name: (Last, First, M.I.)	Phone Number:	Email Address:	Associated Institution:
Farhat, Julie K	210-841-7257	julie.k.farhart.ctr@mail.mil	University of the Incarnate Word
Role on Protocol:			
Research Assistant			

5.2 Will you have a Research Monitor for this study?

- ○ Yes
- ◉ No
- ○ N/A

6.0 Data/Specimens

6.1 Does the study involve the use of existing data or specimens only (no interaction with human subjects)?

○ Yes ◉ No

7.0 Funding and Disclosures

7.1 Source of Funding:

Funding Source	Funding Type	Amount
: Military Operational Medicine Research Program (MOMRP) (JPC-5) Using current MOMS funding FA8650-17-2-6817	: Operations and Maintenance (O&M) UIW personnel conducting recruitment & follow-up funded by current MOMS funding	1845943
: Other Intramural (GME) funding - Funding pending	: Other off-set costs associated with histologic specimens and the biomarker data.	0

Total amount of funding:

1845943

7.2 Do you or any other Investigator(s) have a disclosure of a personal interest or financial nature significant with sponsor(s), product(s), instrument(s) and/or company(ies) involved in this study?

○ Yes ◉ No

If Yes, complete and attach Conflict of Interest forms for all key personnel

8.0 Study Locations

8.1 Is this a collaborative or multi-site study? (e.g., are there any other institutions involved?)

(•) Yes ( ) No

8.2 Study Facilities and Locations:

Institution	Site Name	Site Role	FWA or DoD Assurance Number	Assurance Expiration Date	Is there an agreement?	IRB Reviewing for Site
Army	Brooke Army Medical Center	Performance site	FWA00004092	06/07/2022	: IAIR	: WHASC IRB Protocol Office
	University of the Incarnate Word	Coordinating center	FWA00009201	10/15/2023	: IAIR	: WHASC IRB Protocol Office

Other:

Other Institution Site	Site Role	FWA or DoD Assurance Number	FWA or DoD Expiration Date	Is there an agreement?	IRB Reviewing for Site
No records have been added					

8.3 Are there international sites?

Attach international approval documents, if applicable, when prompted. Note: Ensure local research context has been considered

( ) Yes (•) No

8.4 Is this an OCONUS (Outside Continental United States) study?

( ) Yes (•) No

Select the area of responsibility:

Have you obtained permission from that area of responsibility? (This is a requirement prior to study approval)

( ) Yes ( ) No

9.0 Study Details

9.1 Key Words:

Provide up to 5 key words that identify the broad topic(s) of your study

Placenta, Biomarkers, MOMS, Inflammatory Response, Maternal

9.2 Background and Significance:

Include a literature review that describes in detail the rationale for conducting the study. Include descriptions of any preliminary studies and findings that led to the development of the protocol. The background section should clearly support the choice of study variables and explain the basis for the research questions and/or study hypotheses. This section establishes the relevance of the study and explains the applicability of its findings

Significance:
Prenatal maternal anxiety and depression have been implicated as possible risk factors for preterm birth (PTB) and other poor birth outcomes (Ding et al., 2014; Doktorchik et al., 2018; Reck et al., 2013). Preterm deliveries are considered to be one of the key indicators for national health and is the leading cause of neonatal death as well as the second most frequent cause of death in children aged < 5 years worldwide (WHO, 2012). Every year, approximately 15 million babies are born prematurely worldwide, which equates to 1 out of every 10 babies (WHO, 2012). In the U.S., the estimated annual cost of PTB is approximately 26 billon dollars (IOM, 2007), which includes maternal delivery costs, medical care for the infant up to age 5 years, early intervention, and disability-specific lifetime medical costs. In addition, PTB outcomes are closely related to low birthweights (LBW), which accounted for 22 million newborns worldwide in 2015. Following this report, the World Health Organization established new goals for 2025 to decrease low birthweight (LBW) by 30% and improve maternal health (WHO, 2014). Overall, etiology underlying maternal mental health and its relationship to PTB and LBW is poorly understood. Literature has theorized that stress-associated chronic inflammation and oxidative stress leads to changes that disrupts immunological homestasis that then triggers preterm labor. Concurrently, stress-associated cellular damage can result in an acute inflammatory response and significant cytokine upregulation (Tsiartias, 2012). Alternatively, the disruption of normal placental development can lead to alterations in placental vasculogenesis and angiogenesis as well as changes in hormone and enzyme activity results in placental insufficiency leading to impaired fetal growth and reduced birth weight (Parra-Saavedra et al., 2013).

Background and Review of Literature:
Research efforts in the investigation of reproductive health, especially as it relates to mental health of active-duty servicewomen, female veterans, or wives of service members is limited (Ganzer, 2016). As operational conditions have intensified so has the stress and anxiety experienced by the active duty member and their spouses (Verdeli et al., 2011). As result of these stresses, many veterans are reported to have long-term mental health issues associated with their service. Mental health along with physical condition, socioeconomic status, and the availability of social support can siginficiantly impact pregnancies. As operational stress and anxiety rise for active duty women coupled with lack of necessary social support increases the likelihood of pregnancy complications and poor birth outcomes (Dunkel-Schetter, 2011). To better elucidate the roles of mental health and social support on birth outcomes, it is important to the examine how these psychological factors are manifested physiologically. Although literature has shown that the biopsychosocial mechanisms PTB are driven by an inflammatory/immune response resulting from the activation of the hypothalamic-pituitary-adrenal (HPA) axis, there are limited understanding the mechanisms that leads to LBW. However, it is hypothesized that elevated proinflammatory cytokines and C-reactive proteins (Coussons-Read et al., 2007) that is seen in PTB might also play role in LBW. Conceivably, inflammatory/oxidative stress responses leading to alteration in cellular functions and placental development affects fetal nutrient transport, which ultimately results in lower birth weights. Prospective studies are, in which pregnant women are followed aross pregnancy, with repeated measures of anxiety and symptoms of depression are needed to be compared to pathological placental changes and molecular biomarkers associated with poor birth outcomes.

9.3 Objectives/Specific Aims/Research Questions:

Describe the purpose and objective(s) of the study, specific aims, and/or research questions/hypotheses

Purpose of Study:
1). Explore the associations of prenatal maternal anxiety to placental histological findings
2). Explore the associations between prenatal maternal anxiety and the function of pro-inflammatory, anti-inflammatory, and immunoregulatory cells found in placental tissue.

3) Determine the effect of maternal anxiety on the association between placental molecular changes on pregnancy and birth and infant outcomes.

Hypotheses:
1a) Increased values for measures of prenatal maternal anxiety, stress, and depression will be associated with increased placental pathology.

2a) Increased values for measures of prenatal maternal anxiety, stress, and depression will be associated with increased Th1 response in respect to both Th2 and Th17 immunological response.

1b & 2b) Increased values for measures of prenatal maternal anxiety, stress, and depression will correspond with both increased oxidative stress and general stress hormones.

9.4 Study Design:

Describe study design in one to two sentences (e.g., prospective, use of existing records/data /specimens, observational, cross-sectional, interventional, randomized, placebo-controlled, cohort, etc.). Specify the phase – Phase I, II, III, or IV – for FDA-regulated investigational drug research

The study is a repeated measures, longitudinal design in which Active duty and DoD beneficiary pregnant women, 18 years of age and older, that are currently being recruited and consented for the Mentors Offering Maternal Support (M-O-M-S) research program at the Brooke Army Medical Center (BAMC). They will also be invited to participate in the placental study, as well as pregnant women entering prenatal care in the first trimester, who are not in the M-O-M-S program. Women choosing not to participate in the M-O-M-S study can elect to participate only in the placental study. Psychosocial measures of prenatal anxiety, depression and resilience will be collected at study entry and 12, 16, 24, and 32 weeks gestation. Placentas, of women consented to this study will be obtained following delivery.

No inducements, monetary or otherwise, will be offered to terminate a pregnancy [45 CFR 46.204 (h)]. Individuals engaged in the research will have no part in any decisions as to the timing, method, or procedures used to terminate a pregnancy [45 CFR 46.204(i)]. Individuals engaged in the research will have no part in determining the viability of a neonate [45 CFR 46.204(j)].

9.5 Target Population:

Describe the population to whom the study findings will be generalized

The target population is ALL pregnant women within the San Antonio Military Community, receiving their OB orientation and prenatal care at the Brooke Army Medical Center (BAMC). Military prenatal services are now consolidated and provided at BAMC. The San Antonio military population includes Air Force, Army, Navy, and Marine active-duty servicewomen, retired service women, and female military beneficiaries of active duty, and retirees. The intent is to generalize the findings to all military branches, recognizing that some of the branches may not have broad sampling within this location. On-going studies/data collection at 3 other military installations will provide baseline data for comparisons. The findings may be extrapolated to pregnancy in general.

All women attending OB orientation have had their pregnancies verified through the OB clinic. Therefore, additional screening is not required to confirm they are pregnant. The study inclusion criteria includes, being a pregnant woman, in the first trimester, Active Duty or a DoD beneficiary. The women may also be participants in the Mentors Offering Maternal Support study at BAMC.

9.6 Benefit to the DoD:

State how this study will impact or be of benefit to the Department of Defense

Military Relevance:
The vast majority of women (97.6%) serving in the active component of the military are women of childbearing potential (Stahlman, Witkop, Clark, & Taubman, 2017). At the end of 2016, there were 202,849 women serving in the active service components (Army, Navy, Air Force, or Marines), and of these, 197, 947 were considered women of child-bearing potential. Maternal conditions (including

pregnancy complications and delivery) accounted for 15.3% of all hospital bed days, and is the second most frequent diagnoses for hospitalization among active duty females behind mental disorders (MSMR, 2015). As the number of women serving in the military increases, it is particularly important to understand the relationship between mental health during pregnancy and its complications leading to poor birth outcomes.

10.0 Study Procedures, Data Management, and Privacy

10.1 Study Procedures:

Describe step-by-step how the study will be conducted from beginning to end

Subjects:
All Active Duty and DoD beneficiary gravid women, 18 years of age or older, receiving prenatal care at BAMC. They will be consented for the placental study and will sign a written Informed Consent Document and HIPAA Authorization Document for study participation.

Research Design and Step-by-Step Methods:
The target population is ALL pregnant women within the San Antonio Military Community, receiving their OB orientation and prenatal care at the Brooke Army Medical Center (BAMC) and who meet all study inclusion criteria, will be invited to participate in this placental study. Placentas, of women consented to this study will be obtained following delivery. The placental study will also include psychosocial measures of maternal prenatal anxiety and symptoms of depression, self-esteem, resilience and support obtained at various time points in the first, second and third trimester, as presented in Table 2.

Table 2.

Variable	Measurement	Collection Timepoints
Prenatal Maternal Stress and Anxiety – (Prenatal Adaptation)	Lederman Prenatal Self-Evaluation Questionnaire (PSEQ-sf), 53 items (7 scales). Higher scores on a scale indicate greater anxiety related to the formulation of the motherhood role. Biochemical markers of stress in labor, and labor and postpartum outcomes were used to provide convergent and divergent construct validity; Cronbach's alpha coefficients for the scales range from α = .75 to .92. Weis obtained similar coefficients in her military populations. The instrument has been factored with a military population and all 7 scales remained distinct. The scales measure the level of anxiety the woman is experiencing relative to the particular dimension.	Pregnancy: Initial, 12, 16, 24, & 32weeks
Self-Esteem	Rosenberg Self-Esteem Scale (RSE), 10-item scale measuring the degree to which one values oneself. The possible range of scores is 10 to 40, with higher scores indicating higher self-acceptance. The Cronbach's alpha reliabilities range from .84 to .90. The construct validity of the instrument was demonstrated by examining its conformity to theoretical predictions. The test-retest reliability was found to be r = 0.85. This instrument was used in the pilot M.O.M.S. project with good results.	Pregnancy: Initial, 12, 16, 24, & 32 weeks
Family Adaptability and Cohesion	Family Adaptability and Cohesion Evaluation Scales (FACES II) 30-item (2 scales) measuring the ability of the family (couple) to change its roles, rules, and power structure. High scores on both scales indicate extreme (dysfunctional) responses and low scores indicate flexibility, adaptability, or balance. Cronbach's alpha coefficients for FACES II couples version are 0.87 for cohesion and 0.78 for adaptability from a National Survey of 1000 families. Weis has used this instrument with good results.	Pregnancy: Initial, 12, 16, 24, & 32 weeks
Prenatal Depression	Edinburgh Postnatal Depression Scale (EPDS) is a 10-item self-report scale validated for use during pregnancy and the postpartum period. Scores range from 0-30; higher scores are associated with higher depression.	Pregnancy: Initial, 12, 16, 24, & 32 weeks

	Scores of 12 or greater will be associated with prenatal depression. The Cronbach's alpha has been established as 0.80.	
Perceived Support	Social Support Index (SSI) is a 17-item instrument designed to measure the degree to which families are integrated into the community, view the community as a source of support, and feel that the community can provide emotional, esteem, and network support. The instrument uses a 5-point Likert scale with higher scores indicating more perceived, anticipated social support. The SSI had a .40 validity coefficient with the criterion measure of family well-being. Construct validity was assessed in a study with over 1,000 families and the perceived support was positively correlated with a family' s sense of fit within the community (r = .40). The internal reliability of the SSI was reported as α = .82 and test-retest reliability as α = .83.	Pregnancy: Initial, 12, 16, 24, & 32 weeks
Military Family Commitment and Stress	Family Index Coherence (FIC) is a 17-item instrument developed to measure the degree to which families feel committed to the military mission and identify a subscale of family coping to manage life changes and stresses. The instrument uses a 4-point Likert scale ranging from "Strongly Agree" (3) to "Strongly Disagree" (0). The internal reliability of FIC has been established as α = .82 and test-retest reliability of α = . 86.	Pregnancy: Initial, 12, 16, 24, & 32 weeks
Resilience	Brief Resilience Scale (BRS) is a 6-item instrument developed to measure the concept of "bouncing back from stress." The instrument uses a 5-point Likert scale ranging from "Strongly Agree" (5) to "Strongly Disagree" (1) with a neutral measure (3). The internal reliability for the BRS has been established as .80-.91.[27] The test-retest for one month ranged from .63-.69 in two samples.	Pregnancy: Initial, 12, 16, 24, & 32 weeks
Pandemic Related Stress /Anxiety	The Pandemic Related Pregnancy Stress Scale (PREPS), 17 item instrument developed to measure the thoughts and concerns pregnant women might have due to the COVID-19 pandemic using 3 constituent factors: preparedness stress, perinatal infection stress, and positive appraisal. The response scale ranges from 1= Very Little to 5= Very Much. Stress scales are internally consistent with Cronbach's alpha coefficient is >0.80.	Pregnancy: Initial, 12, 16, 24, & 32 weeks

Placental tissue collected after birth will be used to obtain histologic, microscopic, and molecular data. Pregnancy and neonatal outcome data including gestational age at delivery, infant birthweight, mode of delivery, and pregnancy complications, including pre-eclampsia, gestational hypertension, gestational diabetes, and perinatal and neonatal infections will be collected from the maternal and infant medical records. If missing from the demographic data form, maternal parity, gestational age at first prenatal appointment, ethnicity, beneficiary status (active duty vs. dependent), smoking history, and other medical history will be obtained from the obstetrical record. Participants will sign a HIPAA form providing permission to access their obstetrical records (prenatal and inpatient) for the pregnancy complications and neonatal/birth outcomes data.

Source of Research Material	**Standard Care? (Y/N)**
Placental tissue	Y
Prenatal questionnaire booklet	N
Outpatient Record Data (prenatal EDC verification/pregnancy complications)	Y
Inpatient Record Data (birth outcomes)	Y

Setting:
All women attending OB orientation have had their pregnancies verified through the OB clinic. Therefore, additional screening is not required to confirm they are pregnant. The study inclusion criteria includes, being a pregnant woman, in the first trimester, Active Duty or a DoD beneficiary. The women may also be participants in the Mentors Offering Maternal Support study at BAMC.

Women choosing not to participate in the MOMS study can elect to participate only in the Placenta Study.

Date(s):
Upon final approval from the IRB and deferral from the BAMC HRPO, recruitment of participants is anticipated to begin in November 2019 and continue through March 2021. Birth and outcomes data will be retrieved from both the mother's and newborn infant's electronic medical record through December 2021, with the study ending February 28, 2022.

10.2 Data Collection:

Describe all the data variables, information to be collected, the source of the data, and how the data will be operationally measured.

Data Collection and Processing:
For women who have consented to participate in the study, following delivery, all placentas transported to Pathology are placed in a labeled tissue container and "Labor and Delivery Placenta Pathology Submission Form" is attached per normal standard procedures.

The obstetrical provider will place the order for histology, annotating on the order submission that the placenta is designated as a MOMS study placenta for pathologic evaluation. Once the placenta specimens for this study arrive in the pathology laboratory, the appropriate lab research personnel will be notified and the specimen will be given a unique code for study purposes and tissue specific collection will occur. The placenta will undergo the usual examination in accordance with local SOPs. Additionally, unlabeled and coded slides of each case will be processed concurrently and collected by BAMC research personnel. These coded slides will be sent to Dr. Bustamante at UIW for review.

During the gross examination selected portions of the placenta will be taken and flash frozen, in addition to the usual sections taken for histology processing. The placental specimens will be reviewed by Dr. Brady for diagnosis based on histology. The flash frozen tissue will then be prepared for shipment to 59 MDW Center for Advance Molecular Detection (CAMD), for the identification of immunological, inflammatory, and hormone biomarkers.

Dr. Brady, BAMC/PI will conduct placental gross and microscopic evaluations. Frozen tissue biopsies for CAMD will be used for characterization and identification of specific immunological, inflammatory, and hormone biomarkers changes, Tissue specimens will be homogenized and divided into two groups for analysis: (1) RT-PCR for RNA expression; (2) Multiplexed panel for protein expression. Response groups will be divided into: (1) Th1, (2) Th2, (3) Hormonal, and (4) Oxidative Stress, and some of their specific factors.

The coded, flash frozen placental tissue will be stored in a -80°C freezer until they are packed by the PI and/or pathology resident into storage boxes appropriate for transport to the Center for Advance Molecular Detection (CAMD). Dry ice will not be required for immediate transport between BAMC pathology lab and CAMD. Project coordinator and/or the research personnel will sign for the specimens and verify packaging. They will be responsible to ensure the specimens are taken directly to the CAMD lab, where they will be signed in accordance to CAMD SOP 4-4. Specimens will be stored at -80°C, until sufficient specimens are available for a trimester batch run. Dr. Yuan, the senior scientist at the 59 MDW S&T, and a co-investigator on the study will be responsible for all tissue specimens and the requisite data.

Patients' will be consented for the use of their placental tissue. Once the analysis has been run and the data is analyzed with the psychosocial and microscopic data, one of three things will occur according to the participant consent: 1) the tissue will be destroyed, 2) the tissue will be maintained as a coded specimen within a tissue repository at CAMD, and/or 3) coded slides will be maintained for medical student use at UIW School of Medicine. The tissue will be maintained until the completion of all data analyses as re-analysis might occur. In the process of data cleaning and analysis, questions may arise regarding the results requiring re-running of that "batch." At which time it is determined that there is no further need for the tissue, all coded specimens and data kept at 59MDW, BAMC, and UIW will be handled and disposed of in accordance with federal regulations and will be destroyed at the end of all data mergers, or no later than at the closure of the study, based on AFI 33-332, "The Air Force Privacy and Civil Liberties Program" and the National Institute Standards and Technology Special Publication (NIST SP 800-88) for the approved methods to destroy PII. Note, the only items maintained at UIW, are the coded histology slides (until completion of the study, at which time, the slides will no longer be coded but maintained within a slide "library" for student review purposes. There will be no patient information associated with the slides or tissue specimens. Results of the questionnaires (maintained in an SPSS de-identified dataset), and de-identified outcomes data IF the CRADA is obtained will be utilized by study PIs for completion of reports and publications. UIW will not be maintaining or storing any tissue samples or copies of patient medical records.

10.3 At any point in the study, will you request, use, or access health information in any form, including verbal, hard copy and electronic?

- (•) Yes
- ( ) No

10.4 Review the definitions below and respond to the following two questions. If you are not sure of the answers, email DHA.PrivacyBoard@mail.mil for assistance.

The *Military Health System (MHS)* is defined as all DoD health plans and DoD health care providers that are organized under the management authority of, or in the case of covered individual providers, assigned to or employed by, the Defense Health Agency (DHA), the Army, the Navy, or the Air Force

***MHS workforce members* are employees, volunteers, trainees, and other persons whose conduct, in the performance of work for the MHS, is under the direct control of the MHS, whether or not they are paid by the MHS.**

***MHS business associates* are persons or entities that provide a service to the MHS and require protected health information (PHI) to provide the service.**

Are you an MHS workforce member?

- (•) Yes, I am an MHS workforce member
- ( ) No, I am not an MHS workforce member

Are you an MHS business associate?

- ( ) Yes, I am an MHS business associate
- (•) No, I am not an MHS business associate

10.5 Have you consulted with an MHS data expert to determine the data elements required for your study?

Consulting with a data expert often saves time later in the compliance process because the data expert can advise on the data available in the numerous MHS information systems, the quality of that data and the methods for encrypting and collapsing data. To schedule a consult with an MHS data expert, send an email to: (**DHA.PrivacyBoard@mail.mil**)

- ( ) Yes, then complete the questions below according to the data consult
- (•) No, then complete the questions below according to the best of your knowledge

10.6 Indicate how you will request data from the MHS. Select all that apply.

- [ ] Talking with MHS health care providers or MHS health plans about specific research participants
- [ ] Obtaining MHS hard copy records specific to research participants
- [x] Obtaining data from an MHS information system(s)

10.7 If you are obtaining data from an MHS information system(s), indicate whether you plan to receive a data extract or whether you plan to access an MHS information system directly to create a data set.

A data extract is when the MHS or a contractor provides the data set directly to the researcher. When receiving a data set through data extract, the researcher may indicate whether the data elements should be provided as is, encrypted or collapsed. In contrast to a data extract, access to an information system means that the researcher may directly access an MHS information system and create a data set for the research study

- [ ] Data Extract
- [x] Access

10.8 Do you intend to request de-identified data from the MHS in your research study?

There are different two methods for de-identifying data pursuant to HIPAA:
1) Safe Harbor Method: Removing all of the identifiers listed in Table 1 below, provided that the researcher does not have actual knowledge that the remaining data can be used alone or in combination with other information to identify the individual who is the subject of the information
2) Statistical Method: An expert, with appropriate knowledge of and experience with generally accepted statistical and scientific principles and methods for rendering information not individually identifiable, determines that the data is not individually identifiable

○ Yes ◉ No

10.9 Indicate the MHS information system(s) from which you will seek to obtain data

If you do not know which system(s) contains the data elements you need, refer to the Guide for DoD Researchers on Using MHS Data or request guidance from an MHS data expert at: **DHA.PrivacyBoard@mail.mil**.

Below is a list of commonly used MHS systems. If the system from which you seek to obtain data is not listed below, list the name of the system in the "Other MHS Systems" category below
PHI Systems:

MHS Information System	Requesting Data
AHLTA	Yes
CHCS	Yes
ESSENTRIS	Yes

PII-Only Systems:

MHS Information System	Requesting Data
No records have been added	

De-Identified Data & Other Systems:

Information System	Requesting Data
No records have been added	

10.10 Do you intend to merge or otherwise associate the requested data with data from any sources outside of the MHS, including other DoD systems that are not part of the MHS?

○ Yes, will merge data
◉ No, will not merge data

10.11 Indicate the data elements about research participants or relatives, employers, or household members of the research participants that you will request from MHS hard copies or from MHS information systems.

If you will merge data, also indicate non-MHS data elements about research participants or relatives, employers, or household members of the research participants that you will have access to in any form or medium.

Data Element(s)	MHS	Non-MHS Systems	MHS Hard Copies
1. Names	☑	☐	☐

2. Postal address with only town, city, state and zip code	☑	☐	☐
3. Postal address with all geographic subdivisions smaller than a state, including street address, city, county, precinct, zip code and their equivalent geocodes, except for the initial three digits of a zip code if, according to the current publicly available data from the Bureau of Census: 1) the geographic unit formed by combining all zip codes with the same three initial digits contains more than 20,000 people; and 2) the initial three digits of a zip code for all such geographic units containing 20,000 or fewer people is changed to 000	☐	☐	☐
4. Dates including all elements (except year) directly related to an individual, including birth date, admission date, discharge date, and date of death	☑	☐	☐
5. Ages over 89 and all elements of dates (including year) indicative of such age, unless you will only request a single category of "age 90 or older"	☐	☐	☐
6. Telephone numbers	☑	☐	☐
7. Fax numbers	☐	☐	☐
8. Electronic mail addresses	☑	☐	☐
9. Social Security numbers (SSNs)	☑	☐	☐
10. Medical record numbers	☐	☐	☐

11. Health plan beneficiary numbers	☑	☐	☐
12. Account numbers	☐	☐	☐
13. Certificate/license numbers	☐	☐	☐
14. Vehicle identifiers and serial numbers, including license plate numbers	☐	☐	☐
15. Device identifiers and serial numbers	☐	☐	☐
16. Web Universal Resource Locators (URLs)	☐	☐	☐
17. Internet Protocol (IP) address numbers	☐	☐	☐
18. Biometric identifiers, including finger and voice prints	☐	☐	☐
19. Full-face photographic images and any comparable images	☐	☐	☐
20. Any other unique identifying number, characteristic, or code (Diagnosis, DEERS ID, EDI-PI, Rank)	☐	☐	☐

If you are obtaining SSNs, provide a justification as to why and explain why a substitute cannot be used

The last four of SSNs will only be on the participant log (key). The participant log will be password protected on each computer. The transfer of participant log information between sites will require a locked and encrypted file. The column with the last four of SSNs will be eliminated prior to any transfer or participant "key" information between sites.

10.12 Do you believe it is possible for the MHS data to become identifiable because of triangulation, a small cell size, or any unique data element(s)?

Triangulation means using different data elements that are not themselves identifiable but that when combined can be used to identify an individual. For example, triangulation would use rank and race together to determine the identity of an individual with a particular health condition.

Small cell size means that there is only a small number of eligible individuals that satisfy the category description. Guidance for acceptable cell size is available from the Centers for Medicare and Medicaid Services. For example, the rank category of four star generals with a particular diagnosis may be less than 30, so the rank category may need to be expanded to include lower ranks.

A unique data element includes any unique features that are not explicitly enumerated in the categories of data in rows 1 – 20 of the table above (in Section 10.10), but that could be used to identify an individual. Unique data elements include characteristics that are not themselves identifying, such as the rank of general or admiral, or a race or gender, but within the context of other information could be identifiable.

- ○ Yes, I believe there is a reasonable possibility the MHS data will become identifiable
- ◉ No, I believe there is no reasonable possibility the MHS data will become identifiable

10.13 Have you completed and uploaded an appropriate HIPAA document (i.e. HIPAA Authorization will be obtained or Waiver/alteration of HIPAA Authorization is being requested)?

- ◉ Yes
- ○ No
- ○ N/A

10.14 Managing Data (Data Management and/or Sharing Plan) and/or Human Biological Specimens for this Study:

Include in this section the plan for acquiring data (both electronic and hard copy), access during the study, data/specimen storage and length of time stored, shipment/transmission, and the plan for storage and final disposition at the conclusion of the study. Describe any data agreements in place for accessing data within and/or outside of your institution (e.g., Data Sharing Agreement, Data Use Agreement, Business Agreements, etc.)

A copy of the consent will be stored by the investigator in a locked file cabinet in a locked office, as part of the research record. All information about the participant, collected on this study, will be kept in an electronic database, which will be double password-protected, firewall-protected, and access-restricted to people involved in this study. Dr. Weis, the co-investigator will not have access to this dataset until which time the CRADA is signed and in place. As soon as possible, any link between identity and the research information will be destroyed which means research information about the participant will be permanently de-identified. Personal identifying information and identifiable bio-specimens will be destroyed no later than at the closure of the study. The research information and bio-specimens collected will not be used for any additional research activity beyond what has been described in the consent.

All bio-specimens kept at the *59 MDW, BAMC or University of the Incarnate Word* will be handled and disposed of in accordance with Federal regulations and local laboratory procedures. In accordance with the authorization within the patient consent, once the tissue has been analyzed and run, one of three things will occur, 1) tissue will be destroyed, 2) the tissue will be maintained as a coded specimen within a tissue repository at CAMD, and/or 3) coded slides will be maintained for medical student use at UIW, School of Medicine.

Histology slides for review by Dr. Blandine Bustamante will be packed by the PI and/or pathology resident on the study in accordance with bio-specimen requirements. The histology slide samples will be coded and transported from BAMC pathology to UIW School of Medicine at Brooks-City-Base, TX.

All coded tissue specimens stored as a part of the CAMD Data/Tissue Repository for the purposes of this study - (FWH20130092H) at the *Center for Advance Molecular Detection (CAMD) at Joint Ba se San Antonio Lackland* and will be handled and disposed of in accordance with Federal regulations upon termination of the repository. No individual, investigator, institution or agency will have access to this database without permission of the Pl or Repository Manager, *Dr. Tony Yuan,* and where applicable, the 59 MDW Institutional Review Board (IRB). The Repository Manager, Dr. Tony Yuan, is responsible for all PHI and PII data stored in the repository.

Only de-identified data (data that meets the HIPAA standards for removing direct identifiers or information) will be released to investigators with an IRB-approved research study, so tracing specific information back to the donor of the data should not be possible. Recipient investigators may only receive limited data sets under a Data Use Agreement (DUA) including the information necessary to conduct their IRB-approved research. All recipient investigators requesting data from the repository must have approval from the Repository Manager and must have a research study approved through a DoD Institutional Review Board (IRB) and/or the 59 MDW IRB.

Once the data is collected, an individual's data will not be released or shared with anyone outside the project. Pregnancy and birth outcomes data will be collected from the participant's prenatal record via the electronic medical record. All participants will complete a HIPAA form in addition to the consent form indicating their knowledge of the study personnel obtaining this outcome data from their medical

records. Research coordinators, PIs, AIs will complete CITI training and HIPAA training. The procedures outlined above will be covered during team orientation and included within the study binders. Participant questionnaire booklets will be shredded once all the data has been entered into the database twice, cleaned and verified for correctness. Participant log (key) will be needed until all final reports are completed and then shredded.

The study also involves the collection of psychosocial data. The collection of longitudinal psychosocial data requires the collection of some PHI. The information needed to sustain contact with the participants is maintained on a separate patient identification log and is secured within a double-password protected folder apart from any of the patient data and tissue samples, accessible only by the PI and the project coordinator.

10.15 Managing Data (Data Management and/or Sharing Plan) and/or Human Biological Specimens for Future Research:

If the study involves collecting, storing, or banking human specimens, data, or documents (either by the Investigator or through an established repository) for FUTURE research, address. How the specimens /data will be used, where and how data/specimens will be stored (including shipping procedures, storage plan, etc.), whether and how consent will be obtained, procedures that will fulfill subjects' request as stated in the consent, whether subjects may withdraw their data/specimens from storage, whether and how subjects may be recontacted for future research and given the option to decline, whether there will be genetic testing on the specimens, who will have access to the data/specimens, and the linkage, the length of time that data/specimens will be stored and conditions under which data/specimens will be destroyed.

See above section 10.14

11.0 Statistical/Data Analysis Plan

11.1 Statistical Considerations:

List the statistical methods to be used to address the primary and secondary objectives, specific aims, and/or research hypotheses. Explain how missing data and outliers will be handled in the analysis. The analysis plan should be consistent with the study objectives. Include any sub-group analyses (e.g., gender or age group). Specify statistical methods and variables for each analysis. Describe how confounding variables will be controlled in the data analysis

For characterization and identification of specific immunological, inflammatory, and hormone biomarkers changes, placental tissue biopsies taken from the same locations as the tissue for the paraffin blocks, will be flash-frozen in liquid nitrogen prior to being sent (by courier) to the 59th MDW Center of Advanced Molecular Detection (CAMD) at JBSA-Lackland. Once received, each biopsy will be homogenized and divided into two groups for analysis: (1) RT-PCR for RNA expression; (2) Multiplexed panel for protein expression. Response groups will be divided into: (1) Th1, (2) Th2, (3) Hormonal, and (4) Oxidative Stress, and some of their specific factors are summaried in **Table 1.**

Table 1: Select Immunological, Hormonal, and Oxidative Stress Factor for Analysis.

Th1	Th2	Th17 (Treg)	Oxidative Stress	Stress Hormone
IFN-y	IL-3	IL-17	Hydrogen Peroxide	Prostaglandin E2
TNF- β	IL-4	IL-23	Nitric Oxide Synthase Family	Cortisol
TNF-α	IL-5	TGF-β	S-glutathionylation	CRH (HPA Axis)
IL-12p70	IL-10		C-reactive Protein	ATCH
IL-18	IL-13			DHEA
IL-1β	LIF			
IL-6				
IL-2				
MIP-1α				

MIP-1β

Specifically, analysis will explore the physiological correlations between the biomarker groups as a response to psychosocial factors and in relation to histopathological analysis. The psychosocial measures obtained at various timepoints in first, second, and third trimester are presented in Table 2.

Table 2.

Variable	Measurement	Collection Timepoints
Prenatal Maternal Stress and Anxiety – (Prenatal Adaptation)	Lederman Prenatal Self-Evaluation Questionnaire (PSEQ-sf), 53 items (7 scales). Higher scores on a scale indicate greater anxiety related to the formulation of the motherhood role. Biochemical markers of stress in labor, and labor and postpartum outcomes were used to provide convergent and divergent construct validity; Cronbach's alpha coefficients for the scales range from α = .75 to .92. Weis obtained similar coefficients in her military populations. The instrument has been factored with a military population and all 7 scales remained distinct. The scales measure the level of anxiety the woman is experiencing relative to the particular dimension.	Pregnancy: Initial, 12, 16, 24, & 32weeks
Self-Esteem	Rosenberg Self-Esteem Scale (RSE), 10-item scale measuring the degree to which one values oneself. The possible range of scores is 10 to 40, with higher scores indicating higher self-acceptance. The Cronbach's alpha reliabilities range from .84 to .90. The construct validity of the instrument was demonstrated by examining its conformity to theoretical predictions. The test-retest reliability was found to be r = 0.85. This instrument was used in the pilot M.O.M.S. project with good results.	Pregnancy: Initial, 12, 16, 24, & 32 weeks
Family Adaptability and Cohesion	Family Adaptability and Cohesion Evaluation Scales (FACES II) 30-item (2 scales) measuring the ability of the family (couple) to change its roles, rules, and power structure. High scores on both scales indicate extreme (dysfunctional) responses and low scores indicate flexibility, adaptability, or balance. Cronbach's alpha coefficients for FACES II couples version are 0.87 for cohesion and 0.78 for adaptability from a National Survey of 1000 families. Weis has used this instrument with good results.	Pregnancy: Initial, 12, 16, 24, & 32 weeks
Prenatal Depression	Edinburgh Postnatal Depression Scale (EPDS) is a 10-item self-report scale validated for use during pregnancy and the postpartum period. Scores range from 0-30; higher scores are associated with higher depression. Scores of 12 or greater will be associated with prenatal depression. The Cronbach's alpha has been established as 0.80.	Pregnancy: Initial, 12, 16, 24, & 32 weeks
Perceived Support	Social Support Index (SSI) is a 17-item instrument designed to measure the degree to which families are integrated into the community, view the community as a source of support, and feel that the community can provide emotional, esteem, and network support. The instrument uses a 5-point Likert scale with higher scores indicating more perceived, anticipated social support. The SSI had a .40 validity coefficient with the criterion measure of family well-being. Construct validity was assessed in a study with over 1,000 families and the	Pregnancy: Initial, 12, 16, 24, & 32 weeks

	perceived support was positively correlated with a family's sense of fit within the community (r = .40). The internal reliability of the SSI was reported as α = .82 and test-retest reliability as α = .83.	
Military Family Commitment and Stress	Family Index Coherence (FIC) is a 17-item instrument developed to measure the degree to which families feel committed to the military mission and identify a subscale of family coping to manage life changes and stresses. The instrument uses a 4-point Likert scale ranging from "Strongly Agree" (3) to "Strongly Disagree" (0). The internal reliability of FIC has been established as α = .82 and test-retest reliability of α = .86.	Pregnancy: Initial, 12, 16, 24, & 32 weeks
Resilience	Brief Resilience Scale (BRS) is a 6-item instrument developed to measure the concept of "bouncing back from stress." The instrument uses a 5-point Likert scale ranging from "Strongly Agree" (5) to "Strongly Disagree" (1) with a neutral measure (3). The internal reliability for the BRS has been established as .80-.91.[27] The test-retest for one month ranged from .63-.69 in two samples.	Pregnancy: Initial, 12, 16, 24, & 32 weeks
Pandemic Related Stress /Anxiety	The Pandemic Related Pregnancy Stress Scale (PREPS), 17 item instrument developed to measure the thoughts and concerns pregnant women might have due to the COVID-19 pandemic using 3 constituent factors: preparedness stress, perinatal infection stress, and positive appraisal. The response scale ranges from 1= Very Little to 5= Very Much. Stress scales are internally consistent with Cronbach's alpha coefficient is >0.80.	Pregnancy: Initial, 12, 16, 24, & 32 weeks

Pregnancy and neonatal outcome data including gestational age at delivery, infant birthweight, mode of delivery, and pregnancy complications, including pre-eclampsia, gestational hypertension, gestational diabetes, and perinatal and neonatal infections, will be collected from the maternal and infant medical records. If missing from the demographic data form, maternal parity, gestational age at first prenatal appointment, ethnicity, beneficiary status (active duty vs. dependent), smoking history, and other medical history will be obtained from the obstetrical record. The participants have signed a HIPPA form as part of the MOMS study and all data mentioned is being collected as a part of this study.

11.2 Sample Size:

1200

11.3 Total number of subjects requested (including records and specimens):

1200

11.4 If you are recruiting by study arm, please identify the arms of the study and how many subjects will be enrolled in each arm

N/A

11.5 Please provide a justification for your sample size

There are approximately 1500 deliveries a year at the San Antonio Military Medical Center. Approximately 1100 (73%) of delivered placentas are sent for pathologic examination. Approximately 45% of the placentas are found to have some type of pathology. Given the percentage of placental pathology within this population, 1200 deliveries would be required to compare differences in placental tissue to psychosocial measures with a power of 80% and significance of .05.

11.6 Data Analysis Plan: Complete description: Background, Objectives, Design, Step by Step how the project is going to be done, Data analysis plan:

Data Analysis Plan:
Statistical Analysis System (SAS) version 9.4 will be used for all analyses. Descriptive statistics (mean, sd, median for each continuous variable and proportions for each discrete variable) will be used to describe the sample. Linear or generalized linear mixed models will be developed for all variables, prenatal anxiety, self-esteem, family adaptability/cohesion, prenatal depression and perceived support, as well as the protein and RNA values, in order to examine their association with placental metrics across pregnancy. The linear (mixed) model will be used for all normally distributed continuous data. In the case of skewed continuous outcome data, the generalized linear (mixed) model with gamma distribution and log-link will be chosen. For binary or dichotomized outcomes, a mixed model logistic regression will be chosen. Measurement times (dates of data collection by gestational age), will be included in the models as predictors when available with and without adjusting for the participant's age, employment status, prior deliveries, marital/partner status, education, deployment of partner during pregnancy, military branch of service of participant or partner, ethnicity, military rank of participant or partner and MOMS group. In the case of dual military couples, the rank and service of the active duty woman will be used. The final models will be determined with backward elimination procedure. Timing of data collection (weeks gestation), may also be chosen as a random effect within the model. Additionally, each prenatal variable (prenatal anxiety, self-esteem, depression, family adaptability/cohesion and resilience) will be longitudinally regressed on measurement time to obtain the slope through best linear unbiased predictor (BLUP). The resulted individual slopes will be used to determine any significant effects for prenatal psychosocial scores to pregnancy complications, birth and infant outcomes, and to the physiologic data (both placental scores and biomarker data).

To examine if anxiety mediates the relationship between biomarkers and birth outcomes, we will develop a Baron and Kenny (1986) mediation model assess the relationships as in Bauer and Preacher (2006). We will conduct three mixed regression models adjusted for all appropriate covariates:

1. Regress birth outcome on biomarker.
2. Regress birth outcome on biomarker and anxiety.
3. Regress anxiety on biomarker.

For the above regression models, (1) and (2) will use logistic regression and (3) will use linear or a generalized linear model.

12.0 Participant Information

12.1 Subject Population:

The target population is ALL pregnant women within the San Antonio Military Community, receiving their OB orientation and prenatal care at the Brooke Army Medical Center (BAMC). Military prenatal services are now consolidated and provided at BAMC. The San Antonio military population includes Air Force, Army, Navy, and Marine active-duty servicewomen, retired service women, and female military beneficiaries of active duty, and retirees. The intent is to generalize the findings to all military branches, recognizing that some of the branches may not have broad sampling within this location. On-going studies/data collection at 3 other military installations will provide baseline data for comparisons. The findings may be extrapolated to pregnancy in general.

All women attending OB orientation have had their pregnancies verified through the OB clinic. Therefore, additional screening is not required to confirm they are pregnant. The study inclusion criteria includes, being a pregnant woman, in the first trimester, Active Duty or a DoD beneficiary. The women may also be participants in the Mentors Offering Maternal Support study at BAMC.

12.2 Age Range:

Check all the boxes that apply. if the age range of potential subjects (specimens, records) does not match the range(s) selected, please specify in the text box.

☐ 0-17

- [x] 18-24
- [x] 25-34
- [x] 35-44
- [x] 45-54
- [ ] 55-64
- [ ] 65-74
- [ ] 75+

12.3 Gender:

- [ ] Male
- [x] Female
- [ ] Other

12.4 Special categories, check all that apply

- [ ] Minors /Children
- [ ] Students
- [ ] Employees - Civilian
- [ ] Employees - Contractor
- [ ] Resident/trainee
- [ ] Cadets /Midshipmen
- [ ] Active Duty Military Personnel
- [ ] Wounded Warriors
- [ ] Economically Disadvantaged Persons
- [ ] Educationally Disadvantaged Persons
- [ ] Physically Challenged (Physical challenges include visual and/or auditory impairment)
- [ ] Persons with Impaired Decisional Capacity
- [ ] Prisoners
- [x] Pregnant Women, Fetuses, and Neonates
- [ ] Non-English Speakers
- [ ] International Research involving Foreign Nationals - Headquarters Review is necessary

12.5 Inclusion Criteria:

Order Number	Criteria
1	**Inclusion:** Prima or multigravida; 1st trimester of pregnancy at consent; ≥ 18 years old; wife or DoD beneficiary (Active Duty or retiree) partner to a military service member or retiree or a DoD beneficiary member (Active Duty, retiree). If the pregnant woman is not a DoD or TRICARE beneficiary, than Secretarial Designee (SECDES) status approval must be obtained in order for her to be eligible to participate in the research study, based on DoDI 6025.23.

12.6 Exclusion Criteria:

Order Number	Criteria
1	**Exclusion:** Anticipated permanent change of station during study; dependent daughter of active duty or retired military member; > 12 weeks gestation at consent; < 18 years of age; inability to understand or speak English; non-sponsored DoD members (civilians).

13.0 Recruitment and Consent

13.1 Please describe the recruitment process, including how subjects will be identified and selected for the study.

The study inclusion criteria includes, being a pregnant woman, in the first trimester, Active Duty or a DoD beneficiary. The women may also be participants in the Mentors Offering Maternal Support study at BAMC. All women attending OB orientation have had their pregnancies verified through the OB clinic. Therefore, additional screening is not required.

Identification of participants will occur in the following ways:
1) Women entering OB care within the first trimester of pregnancy (≤ 12 weeks) will be introduced to the study by research team members in person or by phone. For those women indicating interest in participating they will be provided the study information, a review of the inclusion/exclusion criteria for participation and the informed consent documents.

2) Women who are not contacted by phone may be met in person during OB orientation to discuss the study or have the option of setting up another more convenient time to meet with the research team members.

3) Potential participants may also be accessed in the OB clinic after the OB orientation appointment if they express a desire to hear more about the study/meet with a team member. If the patient does not have time to stay after the OB orientation to meet with a study member they may elect to complete the Inclusion /Exclusion screening form with contact information and the research team members will follow-up with a phone call.

4) Potential participants will also be given the option to receive their ICD and HIPAA documents via email form which will be signed by wet signature or electronically signed and returned to a research team member who will review the ICD and the HIPPA forms.
a) Each electronic signature will have a date stamp incorporated with it at the time of signature. [NOTE: without a date stamp, the electronic signature is not valid]
b) The investigator will have some means to personally verify the subject's identification, e.g., compare with a State-issued or government-issued ID; a driver's license; use of personal security questions; etc. This must be done BEFORE the investigator sanctions the acceptance of an individual's electronic signature. [The investigator must state the means of verification in their research files for audit purposes.]
c) After the research team member administering the consent, signs and dates the ICD, a copy of the signed ICD will be given to the subject. The ICD and HIPPA form will be signed prior to any data collection.

Once consented, the participant will then be asked to complete the first study booklet. If the participant chooses to review/complete the study documents at home rather than at their appointment they can complete them, through the web-based, Qualtrics system, specifically designed for collecting anonymous research content.

Participation in the study is a voluntary activity. Every participant indicating a desire to participate in the study will be consented in person prior to completion of any study documents or interview. The time and place of consent will vary depending on the means of recruitment (see options above). If the participant is recruited in person, then the consent process can be conducted at the same time. However, if the participant has called indicating interest in the study or been recruited through a phone call, determination is made between the study staff and participant regarding an appropriate process to review/sign the consent documents.

13.2 Compensation for Participation:

There is no compensation.

13.3 Please describe the pre-screening process. If no pre-screening, enter Not Applicable in the text editor

Note that all women attending OB orientation have had their pregnancies verified. Additional screening for pregnancy is not required. Determination of the Inclusion/Exclusion criteria will be made with the screening form (see attachment form).

13.4 Consent Process:

Revised Common Rule, Section 219.116: General requirements for informed consent, whether written or oral, are set forth in this paragraph and apply to consent obtained in accordance with the requirements set forth in paragraphs (b) through (d) of this section. Broad consent may be obtained in lieu of informed consent obtained in accordance with paragraphs (b) and (c) of this section only with respect to the storage, maintenance, and secondary research uses of identifiable private information and identifiable biospecimens.

Are you requesting a waiver or alteration of informed consent?

○ Yes ◉ No

Please explain the consent process:

Consenting will occur in the following ways:
1. Potential participants will be consented by a research team member in a private area located in the OB /Gyn clinic. The participant will be provided the study information and if the patient is interested in participating in the study, they will be given the Informed Consent Document and HIPAA Authorization Documents (mother and infant) for review and signature. The ICD's (mother and infant) will be signed authorizing participation in the study. The research team member will review the forms with the patient. Both forms must be signed before any study-related data collection occurs. The participant will be given copies of the signed documents. Potential participants have the option to discuss with family members prior to signing consent documents.

2. Potential participants will also be given the option to receive their ICD and HIPAA documents via email form which will be signed by wet signature or electronically signed and returned to a research team member who will review the ICD and the HIPPA forms.
a) Each electronic signature will have a date stamp incorporated with it at the time of signature. [NOTE: without a date stamp, the electronic signature is not valid]
b) The investigator will have some means to personally verify the subject's identification, e.g., compare with a State-issued or government-issued ID; a driver's license; use of personal security questions; etc. This must be done BEFORE the investigator sanctions the acceptance of an individual's electronic signature. [The investigator must state the means of verification in their research files for audit purposes.]
c) After the research team member administering the consent, signs and dates the ICD, a copy of the signed ICD will be given to the subject. The ICD and HIPPA form will be signed prior to any data collection.

Once consented, the participant will then be asked to complete the first study booklet. If the participant chooses to review/complete the study documents at home rather than at their appointment they can complete them, through the web-based, Qualtrics system, specifically designed for collecting anonymous research content.

13.5 DoDI 3216.02 requires an ombudsman to be present during recruitment briefings when research involves greater than minimal risk and recruitment of Service members occurs in a group setting. If applicable, you may nominate an individual to serve as the ombudsman.

◉ N/A
○ Propose ombudsman

13.6 Withdrawal from Study Participation:

Explain the process for withdrawal and specify whether or not the subjects will be given the opportunity to withdraw their data their data/specimens in the event they wish to withdraw from the study

Participants are free to withdraw at any time. The decision to withdraw will not affect subjects' ability to receive medical care and will not be penalized or lose any benefits to which the subject would otherwise be entitled. If a subject withdraws from the study, all the associated data and tissue samples will be destroyed.

14.0 Risks and Benefits

14.1 Risks of Harm:

Identify all research-related risks of harm to which the subject will be exposed for each research procedure or intervention as a result of participation in this study. Consider the risks of breach of confidentiality, psychological, legal, social, and economic risks as well as physical risks. Do not describe risks from standard care procedures; only describe risks from procedures done for research purposes

There is minimal risk for participating in this study. Personal information (the questionnaire booklets and demographic data) are securely maintained on a password protected computer with a secured study folder. The paper documents will be locked in a file cabinet in the private secured office of the nurse researcher. Only members of the research team, listed on the research protocol, will have any access to the information. The demographic information will be entered into an electronic spreadsheet that is password protected. The data from the questionnaire booklets and the placental tissue data are entered into a computer data program with no identifiable information. This program is also password protected. At the conclusion of the study, all documents will be shredded. The slides and tissue biopsies will be destroyed unless the research subject consents to maintain the placenta tissue and research data in a data/tissue repository.

Participants could experience emotional distress or anxiety that may affect their well-being or their unborn infant. There may be feelings of isolation, particularly if one's husband/partner is away. No inducements, monetary or otherwise, will be offered to terminate a pregnancy [45 CFR 46.204(h)]. Individuals engaged in the research will have no part in any decisions as to the timing, method, or procedures used to terminate a pregnancy [45 CFR 46.204(i)]. Individuals engaged in the research will have no part in determining the viability of a neonate [45 CFR 46.204(j)].

The Principal Investigator and members of the research team are not the research participant's healthcare provider. Participants will be informed to always notify their provider or nurse in the obstetrical clinic immediately if they need help.

14.2 Measures to Minimize Risks of Harm (Precautions, safeguards):

For each research procedure or intervention, describe all measures to minimize and/or eliminate risk of harms to subjects and study personnel

A copy of the consent will be stored by the investigator in a locked file cabinet in a locked office, as part of the research record. All information about the participant, collected on this study, will be kept in an electronic database, which will be double password-protected, firewall-protected, and access-restricted to people involved in this study. Dr. Weis, the co-investigator will not have access to this dataset until which time the CRADA is signed and in place. As soon as possible, any link between identity and the research information will be destroyed which means research information about the participant will be permanently de-identified. Personal identifying information and identifiable bio-specimens will be destroyed no later than at the closure

of the study. The research information and bio-specimens collected will not be used for any additional research activity beyond what has been described in the consent.

All bio-specimens kept at the *59 MDW, BAMC or University of the Incarnate Word* will be handled and disposed of in accordance with Federal regulations and local laboratory procedures. In accordance with the authorization within the patient consent, once the tissue has been analyzed and run, one of three things will occur, 1) tissue will be destroyed, 2) the tissue will be maintained as a coded specimen within a tissue repository at CAMD, and/or 3) coded slides will be maintained for medical student use at UIW, School of Medicine.

Histology slides for review by Dr. Blandine Bustamante will be packed by the PI and/or pathology resident on the study in accordance with bio-specimen requirements. The histology slide samples will be coded and transported from BAMC pathology to UIW School of Medicine at Brooks-City-Base, TX.

All coded stored as a part of the CAMD Data/Tissue Repository for the purposes of this study - (FWH20130092H) at the *Center for Advance Molecular Detection (CAMD) at Joint Base San Antonio Lackland* and will be handled and disposed of in accordance with Federal regulations upon termination of the repository. No individual, investigator, institution or agency will have access to this database without permission of the Pl or Repository Manager, *Dr. Tony Yuan,* and where applicable, the 59 MDW Institutional Review Board (IRB).

The Repository Manager, Dr. Tony Yuan, is responsible for all PHI and PII data stored in the repository. Only de-identified data (data that meets the HIPAA standards for removing direct identifiers or information) will be released to investigators with an IRB-approved research study, so tracing specific information back to the donor of the data should not be possible. Recipient investigators may only receive limited data sets under a Data Use Agreement (DUA) including the information necessary to conduct their IRB-approved research. All recipient investigators requesting data from the repository must have approval from the Repository Manager and must have a research study approved through a DoD Institutional Review Board (IRB) and/or the 59 MDW IRB.

Once the data is collected, an individual's data will not be released or shared with anyone outside the project. Pregnancy and birth outcomes data will be collected from the participant's prenatal record via the electronic medical record. All participants will complete a HIPAA form in addition to the consent form indicating their knowledge of the study personnel obtaining this outcome data from their medical records. Research coordinators, PIs, AIs will complete CITI training and HIPAA training. The procedures outlined above will be covered during team orientation and included within the study binders. Participant questionnaire booklets will be shredded once all the data has been entered into the database twice, cleaned and verified for correctness. Participant log (key) will be needed until all final reports are completed and then shredded.

14.3 Confidentiality Protections (for research records, data and/or specimens):

Describe in detail the plan to maintain confidentiality of the research data, specimens, and records throughout the study and at its conclusion (e.g., destruction, long term storage, or banking). Explain the plan for securing the data (e.g., use of passwords, encryption, secure servers, firewalls, and other appropriate methods). If data will be shared electronically with other team members/collaborators outside the institution, describe the method of transmission and safeguards to maintain confidentiality. Explain whether this study may collect information that State or Federal law requires to be reported to other officials or ethically requires action, e.g., child or spouse abuse

There is the potential risk for loss of confidentiality, although every effort will be made to protect participant confidentiality.

The research team will secure the ICD, HIPAA and any study related documents as indicated above. When recruiting, no items will be left unattended, and all consent items will be maintained in envelopes. Participant consents will be scanned into a separate password-protected file from all other study data and submitted to the IRB annually. The paper copies of all the participant consents will be scanned at the study site into a password-protected file, separate from all other study data, and paper copies of participant consents will be shredded. Personal information are securely maintained on a password protected computer with a secured study folder. Documents will be locked in a file cabinet in the private secured office of the nurse program coordinator. Only members of the research team, listed on the research protocol, will have any access to the information. The demographic information will be entered into an electronic spreadsheet that is password protected. The data from the questionnaire booklets and the placental tissue data are entered into a computer data program with no identifiable information. This program is also password protected. At the conclusion of the study, all documents will be shredded.

Participants could experience emotional distress or anxiety that may affect the well-being of themselves or their unborn infant. There may be feelings of isolation, particularly if one's husband/partner is away. It is expected that some participants will have unexpected terminations of their pregnancy. All events that are life-threatening or fatal will be reported within 24 hours. Any other possible adverse events (expected or unexpected) will be reported to the local IRB as well as the governing IRB at BAMC and at the WHASC according to their guidelines.

See section 14.2 for additional information.

14.4 Potential Benefits:

Describe any real and potential benefits of the research to the subject and any potential benefits to a specific community or society

If the individuals in the research are considered experimental subjects (per 10 USC 980), and they cannot provide their own consent, the protocol must describe the intent to directly benefit all subjects

As the number of women serving in the military increases, it is particularly important to understand the relationship between mental health during pregnancy and its complications leading to poor birth outcomes. There is no guarantee or promise that the subjects will receive any personal benefit from this study. We hope the information learned from this study may help future patients. All the women in the study will be aware that the findings as applicable will be used to update the VA/DoD Clinical Practice Guideline for Pregnancy Management and as norming data for assessment tools used within the DoD.

14.5 Privacy for Subjects:

Describe the measures to protect subject's privacy during recruitment, the consent process, and all research activities, etc.

Potential participants will be consented by a research team member in a private area or if emailed for electronic signature, a) Each electronic signature will have a date stamp incorporated with it at the time of signature. [NOTE: without a date stamp, the electronic signature is not valid] b) The investigator will have some means to personally verify the subject's identification, e.g., compare with a State-issued or government-issued ID; a driver's license; use of personal security questions; etc. This must be done BEFORE the investigator sanctions the acceptance of an individual's electronic signature. [The investigator must state the means of verification in their research files for audit purposes.] c) After the research team member administering the consent, signs and dates the ICD, a copy of the signed ICD will be given to the subject. The ICD and HIPPA form will be signed prior to any data collection.

The research team will review the ICD and HIPAA Authorization forms with the patient. Both forms must be signed before any study-related data collection occurs, including pre-screening procedures to verify inclusion/exclusion criteria for study participation. The participant will be given copies of both documents (ICD for placental study, and the HIPPA form). Additionally, the mother will be given a Parental ICD and a Parental HIPAA Authorization for signature, authorizing her infant to be part of the study and to permit the investigators to access the infant's medical record for birth and infant outcome data. Once consented, the participant will then be asked to complete the first study booklet. If the participant chooses to review/complete the study documents at home rather than at their appointment they can complete them, through the web-based, Qualtrics system, specifically designed for collecting anonymous research content.

The research team will secure the ICD, HIPAA and any study related documents in an envelope that is kept with the research team at all times; no items will be left unattended. Participant consents will be scanned into a separate password-protected file from all other study data and submitted to the IRB annually. The paper copies of all the participant consents will be scanned at the study site into a password-protected file, separate from all other study data, and paper copies of participant consents will be shredded.

Personal information are securely maintained on a password protected computer with a secured study folder. Documents will be locked in a file cabinet in the private secured office of the nurse researcher. Only members of the research team, listed on the research protocol, will have any access to

the information. The demographic information will be entered into an electronic spreadsheet that is password protected. The data from the questionnaire booklets and the placental tissue data are entered into a computer data program with no identifiable information. This program is also password protected. At the conclusion of the study, all documents will be shredded. The slides and tissue biopsies will be destroyed unless the participant consents to maintaining the placenta tissue and research data in a data/tissue repository.

14.6 Incidental or Unexpected Findings:

Describe the plan to address incidental findings and unexpected findings about individuals from screening to the end of the subject's participation in the research. In cases where the subject could possibly benefit medically or otherwise from the information, state whether or not the results of screening, research participation, research tests, etc., will be shared with subjects or their primary care provider. State whether the researcher is obligated or mandated to report results to appropriate military or civilian authorities and explain the potential impact on the subject

Complete confidentiality cannot be promised, particularly for active duty personnel, because information regarding potential UCMJ violations or concerns regarding fitness for duty may be reported to appropriate medical, law enforcement, or command authorities. If an investigator feels at any time that a participant is at risk of self-harm, the participant will be given a direct referral to a provider within the Mental Health Services and their provider notified. Complete confidentiality for active duty pregnant women cannot be guaranteed. Importantly, Mental Health Service appointments do not have any connection with the study or participation in the study.

No blood will be taken as a part of this study. The afterbirth (the placenta) will be sent to pathology for examination as part of this study. If the subject is wanting to obtain the placenta following birth, it can still be requested and obtained through pathology, in the same manner it would be obtained if they were not in this study. If a placental examination is requested by the physician following delivery, this can and will take place regardless of whether participating in the study.

15.0 Study Monitoring

15.1 Your study requires either Data and Safety Monitoring Plan (DSMP) or a Data and Safety Monitoring Board (DSMB).

- ○ DSMP
- ○ DSMB
- ○ Both
- ◉ Not Applicable

16.0 Reportable Events

16.1 Reportable Events: Consult with the research office at your institution to ensure requirements are met. Describe plans for reporting unexpected adverse events and unanticipated problems. Address how unexpected adverse events will be identified, who will report, how often adverse events and unanticipated problems will be reviewed to determine if any changes to the protocol or consent form are needed and the scale that will be used to grade the severity of the adverse event.

Consult with the research office at your institution to ensure requirements are met

• Describe plans for reporting expected adverse events. Identify what the expected adverse events will be for this study, describe the likelihood (frequency, severity, reversibility, short-term management and any long-term implications of each expected event)

• Describe plans for reporting unexpected adverse events and unanticipated problems. Address how unexpected adverse events will be identified, who will report, how often adverse events and unanticipated problems will be reviewed to determine if any changes to the research protocol or consent form are needed and the scale that will be used to grade the severity of the adverse event

There is the potential risk for loss of confidentiality, although every effort will be made to protect participant confidentiality. The research team will secure the ICD, HIPAA and any study related documents as indicated above. When recruiting, no items will be left unattended, and all consent items will be maintained in envelopes. Participant consents will be scanned into a separate password-protected file from all other study data and submitted to the IRB annually. The paper copies of all the participant consents will be scanned at the study site into a password-protected file, separate from all other study data, and paper copies of participant consents will be shredded. Personal information are securely maintained on a password protected computer with a secured study folder. Documents will be locked in a file cabinet in the private secured office of the nurse program coordinator. Only members of the research team, listed on the research protocol, will have any access to the information. The demographic information will be entered into an electronic spreadsheet that is password protected. The data from the questionnaire booklets and the placental tissue data are entered into a computer data program with no identifiable information. This program is also password protected. At the conclusion of the study, all documents will be shredded.

Participants could experience emotional distress or anxiety that may affect the well-being of themselves or their unborn infant. There may be feelings of isolation, particularly if one's husband/partner is away. It is expected that some participants will have unexpected terminations of their pregnancy. All events that are life-threatening or fatal will be reported within 24 hours. Any other possible adverse events (expected or unexpected) will be reported to the local IRB as well as the governing IRB at BAMC and at the WHASC according to their guidelines.

17.0 Equipment/non-FDA Regulated Devices

17.1 Does the study involve the use of any unique non-medical devices/equipment?

○ Yes ◉ No

18.0 FDA-Regulated Products

18.1 Will any drugs, dietary supplements, biologics, or devices be utilized in this study?

☐ Drugs
☐ Dietary Supplements
☐ Biologics
☐ Devices
☑ N/A

18.5 Sponsor (organization/institution/company):

☑ N/A

If applicable, provide sponsor contact information:

19.0 Research Registration Requirements

19.1 ClinicalTrials.gov Registration:

- ○ Registration is not required
- ◉ Registration pending
- ○ Registration complete

19.2 Defense Technical Information Center Registration (Optional):

- ◉ Registration is not required
- ○ Registration pending
- ○ Registration complete

20.0 References and Glossary

20.1 References:

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20.2 Abbreviations and Acronyms:

AD Active Duty
BAMC Brooke Army Medical Center
CAMD Center of Advanced Molecular Detection
CPA College of American Pathologists
CRH corticotropin-releasing hormone
HIPPA Health Insurance Portability and Accountability
HPA hypothalamic-pituitary adrenal
LBW Low Birthweight
M-O-M-S Mentors Offering Maternal Support
Non-AD Non-Active Duty
PTB Preterm Birth
SAMMC San Antonio Military Medical Center
Th1 T Helper Cells Type 1 Cytokines
Th2 T Helper Cells Type 2 Cytokines
Th17 T Helper Cells Type 17 (IL-17 or Interleukin 17) Cytokines
TNF-α Tumor Necrosis Factor - alpha