TITLE: **"Pro-Moms" - Mitigating the effects of structural violence on maternal iron status: a randomized controlled pilot study of probiotic supplementation in at-risk pregnant women**

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1. Background and specific aims: In the United States (U.S.), Black women are three times more likely to experience iron deficiency (ID) in pregnancy compared to women from other racial/ethnic groups, for reasons that remain largely unknown[1]. Women who are iron deficient during pregnancy experience higher rates of maternal and infant mortality[2], preterm birth[3], low infant birth weight[4], and irreversible neurocognitive defects in their children[2,3]. Indeed, the highest rates of adverse maternal-infant health outcomes occur in Black women, a phenomenon that may be driven by their sub-optimal iron status[1,3]. Specific to Chicago, in some low income predominately Black neighborhoods (e.g. Englewood, Austin), preterm birth rates range between 18.5% and 34.8% far exceeding the national average of 9.6% and the state of Illinois at 10.3%. *Exposure to **structural violence** may compromise maternal iron status that contributes to adverse maternal-infant health outcomes in Black women in at-risk communities.*

Black women nationally and in Chicago are more likely to live in urban areas that are fraught with ***structural violence***. Structural violence refers to the multiple ways in which social, economic, and political systems disproportionately expose particular populations to risks and vulnerabilities including poverty, crime, violence, and limited access to healthcare and healthy foods that contribute to excess morbidity and mortality.[5,6] These socio-environmental exposures likely interact with biology to drive maternal-infant health disparities in Black women including maternal ID[7]. Specifically, chronic exposure to ***structural violence*** can dysregulate the stress response (i.e., hypothalamic pituitary adrenal (HPA) axis)[8] eliciting altered production of glucorticoids (i.e., cortisol) and a propagated pro-inflammatory immune cascade[5]. Such stress-induced changes can adversely affect the health of pregnant women and their offspring. In fact, exposure to chronic stress is associated with ID[6] and decreased dietary iron absorption[9]. In pregnant women, exposure to chronic stress is also associated with lower infant iron status at birth which is concerning given iron is essential to postnatal neurodevelopment[10]. Stress-induced inflammation alters iron metabolism by promoting production of the iron-regulatory hormone hepcidin[11-13]. Elevated hepcidin reduces dietary iron absorption and access to body iron stores that leads to ID[14]. In our preliminary study, pregnant Black women exposed to ***structural violence*** (i.e., discrimination) had elevated hepcidin compared to those not exposed (Koenig, unpublished). Importantly, providing supplemental dietary iron to a stressed and iron deficient woman in pregnancy will not correct the underlying ID due to the negative effects of elevated hepcidin on dietary iron absorption[15,16]. Thus, interventions that can safely and effectively mitigate the physiologic effect of stress (i.e., inflammation) have the potential to improve maternal and infant iron status that reduces suboptimal pregnancy and birth outcomes.

There is growing evidence that probiotic bacteria can reduce stress and its adverse physiologic effects. For instance, daily probiotic consumption is associated with reduced cortisol output and decreased self-reported stress in healthy volunteers[17]. The gut, gut bacteria, and mental health are intimately linked through the gut-microbiota-brain axis[18], a system that mediates the molecular interactions between intestinal bacterial communities and host neurological, immunological and endocrinological processes[19,20]. Therefore, the effect of probiotics on stress is likely mediated through modulation and recolonization of the gut microbiota with commensal microbes, such as lactobacilli, that promote positive effects on the HPA axis (i.e., cortisol output) and the pro-inflammatory immune response[21]. Presumably, such effects would also normalize iron metabolism. Notably, supplementation with Lactobacillus Plantarum 299v (Lp299v) has been linked to reduced cortisol in stressed patients[22], decreased systemic inflammation[23,24], and enhanced dietary iron absorption[25]. Probiotics are safe for use in pregnancy[26, 27, 28], thus, Lp299v may be a safe and inexpensive daily probiotic therapy to improve iron status of women burdened by exposure to chronic stress from structural violence in pregnancy. Additionally, we recognize that health-related resources for pregnant women are limited in many of the marginalized neighborhoods of Chicago. Thus, along with pilot testing a potentially innovative therapy aimed at reducing the physiologic effects of chronic stress from structural violence, we strive to build a sustainable university-community partnership to disseminate research findings and promote lifestyle and dietary approaches to mitigate the effects of chronic stress from structural violence for at-risk pregnant Black women in Chicago.

Our specific aims are as follows:

Specific Aim 1: To determine the impact of consuming daily Lp299v + standard PNVI vs. standard PNVI + placebo beginning between 15-20 weeks gestation through delivery on maternal iron status and metabolism. To fully characterize maternal iron metabolism and regulators or maternal iron metabolism we will measure several blood-based markers including hemoglobin, hematocrit, ferritin, transferrin receptor, transferrin saturation, serum iron, erythropoietin, systemic inflammation and hepcidin at baseline, 24-28 and 34 - 36 weeks gestation, and at

delivery. *Hypothesis: Women consuming Lp229v will have superior iron status at –24-28 and 34 - 36 weeks gestation and at delivery compared to control women.*

Specific Aim 2: To determine colonization and propagation of the gut microbial community with Lp299v from rectal swabs collected at baseline,24-28 weeks and 34 - 36 weeks gestation in women consuming Lp299v + standard PNVI vs. standard PNVI + placebo. *Hypothesis: Women consuming LP299v will have greater colonization and propagation of Lp299v in their rectal swabs at –24-28 week and 34 - 36 weeks gestation compared to women consuming PNVI + placebo.*

Specific Aim 3: *To explore the effect of daily oral Lp299v vs. placebo on infant iron status at delivery. Infant iron status will be measured (via cord blood at delivery) (i.e., hemoglobin, ferritin, transferrin, serum iron, and transferrin receptor). Hypothesis: Infant of women consuming LP299v will have higher iron status at delivery.*

Specific Aim 4: *To determine the feasibility and tolerability of daily oral Lp299v supplementation (15-20 weeks gestation-delivery. Hypothesis: Feasibility and tolerability will be high in both treatment groups of the study for the duration of the trial.*

Exploratory Aim 1: There is some evidence that probiotic use can positively influence maternal health outcomes including mood. We will explore differences in the development of gestational hypertension, pre-eclampsia, and gestational diabetes using data extracted from the electronic health record (EHR). We will also examine changes to maternal stress response (i.e., serum 17-hydroxycorticosterone and hair cortisol via immunoassay) and self-reported stress, depression, anxiety, resilience, experiences of discrimination, childhood trauma, social support, post-traumatic stress, and spirituality via surveys in women consuming Lp299v + standard PNVI vs. standard PNVI + placebo.

Exploratory Aim 2: To build a sustainable university-community partnership to disseminate research and promote lifestyle and dietary approaches to mitigate the physiologic effects of chronic stress from structural violence for at-risk pregnant Black women in Chicago. We will conduct the Maternal Stress Reduction Project, “Happy Moms”, which entails focus groups and a demographic questionnaire to inform future programming and curriculum.

2. Methods.

I. **Research participants.** We will recruit and enroll 40 women early in pregnancy (< 20 weeks gestation) from the University of Illinois at Chicago (UIC) Obstetrics (OB) clinics located at the University of Illinois Hospital and Health Sciences System (UIHHSS) Outpatient Care Center, Center for Women’s Health. Women will be between the ages of 18 – 45 years old and at risk for maternal ID based on hemoglobin of 10.0 – 11.9 g/dl assessed as part of standard clinical care during the first prenatal OB visit.

II. **Eligibility, recruitment, and informed consent:** After obtaining a waiver of consent for recruitment purposes, we will complete a pre-screen eligibility checklist of women < 20 weeks gestation who are seeking prenatal care at the UIC Obstetrics Clinics at the UIHHSS Center for Women’s Health. Potentially eligible women will be identified through clinic schedules and eligibility assessed via Cerner/EHR (only if data is available, data will not be available for new UIC patients). Women identified as potentially eligible through the EHR data or new UIC patients attending their first OB prenatal appointment will be approached in clinic. A research team member will introduce the study, assess interest, and further determine eligibility using a screening checklist. Interested and eligible women will be asked to provide contact information. As part of routine care, maternal hemoglobin will be assessed following the first OB visit. For women that express interest in the study and provide contact information, hemoglobin results will be followed up in Cerner. Women will be called regardless of their results to report on continued study eligibility. If hemoglobin is within the study defined range (10.0 – 11.9 g/dl) women will be invited to participate. Those who continue to express interest will be asked to schedule a baseline appointment at the UIC Clinical Research Center (CRC)s. Women will be consented at the baseline visit. Research personnel will explain to every eligible woman that their choice to participate will in no way affect their standard of care at UIHHS.

III. **Inclusion/exclusion criteria**.

a. Inclusion criteria: obtaining prenatal care at UIC; initial prenatal hemoglobin between 10.0 – 11.9 g/dl; 18 - 45 years of age; spontaneous/natural conception; singleton pregnancy; < 20 weeks gestation; and willing to: a) fast for at least 2 hours prior to research data collection visits (3 occasions), b) agree to randomization; c) ingest Lp299v + standard PNVI or standard PNVI + placebo daily through delivery; c) refrain from **ALL** other supplements including other PNVs unless medically indicated (e.g., folate); d) attend 3 research data collection visits at UIC; e) undergo 3 venipunctures; and f) come to UIC on 4-5 occasions (separate from research visit but will be coordinated around standard clinical care visits) to pick up supplements and have study compliance monitored/checked. Women will also be able to read and write English, and have access to a smart phone. Also, women must agree to refrain from **ALL** other pre and probiotic supplements (unless medically indicated) including Activia yogurt, Kefir, Tropicana Probiotic, and Good Belly products while in the study.

b. Exclusion criteria: Autoimmune disorder; current or previous premature rupture of membranes or clinical chorioamnionitis; current bacterial or viral infection; oral antibiotic use in the last 3 months; receiving steroid or anti-inflammatory treatment; previous bariatric surgery; malabsorptive condition; current hyperemesis; hematologic disorder (i.e., sickle cell disease or hemochromatosis); current tobacco use or within the past 1 month; current alcohol consumption; current drug use; or type 1 or 2 diabetes given these factors may influence the biological markers.

IV. Randomization. Women will be randomized to the Lp229V + standard PNVI or standard PNVI + placebo following the baseline visit.

V. Treatment groups. Women will be assigned to one of two treatment groups as described below.

a. Lp299v + standard PNVI. Starting at 15-20 weeks gestation, women assigned to this group will consume Lp299v + standard PNVI capsules each, once daily, through delivery. Women will be asked to consume both pills with dinner along with a cold or room temperature beverage. The Lp299v supplement will be provided by NatureMade, Pharmavite. This probiotic formulation has a bacterial density of 10^{10} colony forming units (CFUs) per capsule. The PNVI is from NatureMade, Pharmavite and the nutrient content is comparable to a standard prenatal plus vitamin prescribed by clinicians at UIC. The PNVI contains 27 mg of iron as ferrous fumarate. The pills will be provided in separate Pillsy containers and labeled as Vitamin and just generically as "supplement". Women will receive 30-40 days of pills at the baseline visit and asked to start taking the pills on the 1st day of the 15^{th}-20^{th} gestational week. Dr. Gloria Elam, Associate Professor of Clinical Obstetrics & Gynecology at UIC and Director of the Center for Women's Health, will write the prescription for both the Lp299v and standard PNVI. The UIC Pharmacy will fill the prescription and deliver to the CRC. Women will pick up new pills at UIC every 28 days during a scheduled standard care prenatal visit or during a separate pill pick up or research data collection visit. If necessary, research staff will hand deliver supplements to the participant home if requested.

***b. Standard PNVI + placebo*.** Starting at 15-20 weeks gestation, women assigned to this group will consume standard PNVI + placebo once daily, through delivery. Taste and packing of the placebo will be identical to the Lp299v, minus the Lp299v, and will be produced by the UIC Pharmacy. Women will be asked to consume both pills with dinner along with a cold or room temperature beverage. The PNVI is from NatureMade, Pharmavite. The PNVI nutrient content is comparable to a standard prenatal plus vitamin prescribed by UIC clinicians. The PNVI contains 27 mg of iron as ferrous fumarate. The pills will be provided in separate Pillsy containers and labeled as Prenatal Vitamin Tablets and Probiotic or Placebo Capsules. Women will receive 30 days of pills at the baseline and asked to start taking the pills on the 1st day of the 15^{th}-20^{th} gestational week. Women will pick up new pills at UIC every 28 days and whenever possible during a scheduled standard

care prenatal visit or during a separate pill pick up or research data collection visit. If necessary, research staff will hand deliver supplements to the participant home if requested.

Supplement Facts
Serving Size 1 Tablet

Amount Per Tablet	% Daily Value for Pregnant and Lactating Women
Vitamin A (as Beta Carotene) 2567 IU	32%
Vitamin C (as Ascorbic Acid) 85 mg	142%
Vitamin D_3 (as Cholecalciferol) 1000 IU	250%
Vitamin E (as dl-Alpha Tocopheryl Acetate) 33 IU	110%
Vitamin K (as Phytonadione) 90 mcg	*
Thiamin (as Thiamin Mononitrate) 1.4 mg	82%
Riboflavin 1.4 mg	70%
Niacin (as Niacinamide) 18 mg	90%
Vitamin B_6 (as Pyridoxine Hydrochloride) 1.9 mg	76%
Folic Acid 800 mcg	100%
Vitamin B_{12} (as Cyanocobalamin) 5.2 mcg	65%
Biotin 30 mcg	10%
Pantothenic Acid (as d-Calcium Pantothenate) 6 mg	60%
Calcium (as Calcium Carbonate) 250 mg	19%
Iron (as Ferrous Fumarate) 27 mg	150%
Iodine (as Potassium Iodide) 150 mcg	100%
Magnesium (as Magnesium Oxide) 45 mg	10%
Zinc (as Zinc Oxide) 11 mg	73%

*Daily value not established

No Color Added • No Artificial Flavors • No Yeast • Gluten Free
Nature Made Multi Prenatal provides key vitamins and minerals for nutritional support before and during pregnancy.†
SUGGESTED USE: Take one tablet daily with a meal. For easier swallowing, take with water before and during ingestion.
Keep bottle tightly closed. Store in a cool, dry place.
Do not use if imprinted seal under cap is broken or missing.

WARNING: Accidental overdose of iron-containing products is a leading cause of fatal poisoning in children under 6. Keep this product out of reach of children. In case of accidental overdose, call a doctor or poison control center immediately.

OTHER INGREDIENTS: Cellulose Gel, Corn Starch, Maltodextrin, Hydrogenated Soybean Oil, Dibasic Calcium Phosphate, Croscarmellose Sodium, Hypromellose, Silicon Dioxide, Gelatin, Magnesium Stearate, Polyethylene Glycol.

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†This statement has not been evaluated by the Food and Drug Administration. This product is not intended to diagnose, treat, cure or prevent any disease.

VI. Research-related visits. Data will be collected at baseline (<20 weeks gestation), 24-28 and 34-36 weeks gestation and at delivery. Tolerability [potential adverse events (AEs) (e.g., GI symptoms)] will be assessed via phone/email/text weekly for the first 4 weeks and bi-weekly thereafter. Compliance data will be collected at pill pick-up visits (scheduled around usual care prenatal visits whenever possible) and during research visits. Women will be called or texted and emailed 2-3 days prior to each research-related visit and reminded of their appointment time, location and instructions how to prepare (e.g., fast for 2 hours, bring their pill containers). The visits will take place at the Clinical Research Center 912 S. Wood Street 2nd floor Chicago, IL. The research-related visits and data collected at each visit are described below:

a. ***Baseline (<20 weeks gestation)****.*

Reminder Phone Call or Text and Email: Women will be called or texted and emailed 2-3 days before the baseline visit. Women will be reminded of their appointment time and location, asked to not eat for at least 2 hours before the appointment, and reminded about the visit procedures.

Obtaining Informed Consent: Consent will be obtained using IRB approved materials by trained research staff at the baseline study visit before any study procedures take place.

Data collection procedures: Women will be weighed with an electronic scale; height measured (baseline only); a venous blood sample (30 ml) obtained by research staff; and surveys (described in detail below) will be interviewer administered by trained staff. Women will be asked to download the Pillsy app to their smart phones and instructions on how to use the Pillsy bottle and Pillsy app will be

given. If there is extra blood available from the venous blood sample and the patient consents, it may be used for future research to learn more about how to prevent, detect, or treat other health problems in pregnancy.

Surveys: Women will be asked to complete the following surveys at baseline:

1. Maternal Health History Questionnaire. This questionnaire was developed by the research team, and asks about maternal health history, maternal obstetric history, current and past medications, personal history, and demographics.

2. Food Frequency Questionnaire (FFQ). This questionnaire is a 125-item dietary assessment tool used to determine habitual food consumption over the past year.

3. 24-hour diet recall. The 24-hour diet recall will be collected using Nutrition Data System for Research (NDSR) software, a computer-based software application developed at the University of Minnesota that facilitates the collection of dietary recalls in a standardized fashion. Dietary intake data gathered by interview is governed by a multiple-pass interview approach. Dietary supplement use will be assessed in conjunction with collection of 24-hour dietary recalls using the Dietary Supplement Assessment Module included in NDSR. Use of all types of dietary supplements and non-prescription antacids are queried in the module. The RAs are NDSR certified through the University of Illinois Cancer Center Diet and Behavior Shared Resource.

4. GI symptoms checklist. This short survey probes for recent GI problems including abdominal pain, nausea, constipation, and diarrhea.

5. Mood and Anxiety Symptom Questionnaire – Anhedonia subscale (MASQ-AD). This is a 22-item subscale of the MASQ, a measure of depressive disorders.

6. Patient Health Questionnaire (PHQ-9). This is a 9-item questionnaire assessing depressive symptoms.

7. Edinburgh Postnatal Depression Scale (EPDS). This is a 10-item scale intended to measure depressive symptoms during the perinatal period.

8. Childhood Trauma Questionnaire (CTQ). This is a 28-item psychological screening for traumatic experiences during childhood.

9. Perceived stress scale. This is a 10-item scale intended to measure perceived stress over the last month.

10. Post-traumatic stress symptoms National Stressful Events Survey PTSD Short Scale (NSESSS). The National Stressful Events Survey PTSD Short Scale (NSESSS) is a 9-item measure that assesses the severity of posttraumatic stress disorder in individuals age 18 and older following an extremely stressful event or experience.

11. Adult violence exposure. This is a 29-item survey used to identify exposure to violence of the past 7 days. The survey is used to classify low- and high-violence exposure that has demonstrated good construct validity, internal consistency, test-retest reliability, and validity.

12. Major experiences of Discrimination. This is a 6-item measure to assess experience of major discrimination over the past year.

13. Social support – MOS social support survey. This survey was designed to be comprehensive in terms of recent thinking about the various dimensions of social support.

14. Spiritual Experience Index-Revised. A 23-item scale that measures faith and spiritual journey, aiming to not impose any particular faith as part of the questions.
15. Residential History. Residential history over the previous 10 years.

16. Healthy Pregnancy Stress Scale. This is an 18-item scale that measures pregnancy stressors women have experienced during their pregnancy.

17. Adverse Childhood Experiences. This is a 10-item scale that measures adverse childhood experiences.

18. Hair collection survey. This survey was developed by the research team and asks about hair care practices.

19. Short Scale Anxiety Sensitivity Index (SSASI). This is 5-item scale that measures anxiety.

20. Behavioral Inhibition System & Behavioral Activation System Scales. This is a 24-item scale that measures behavioral inhibition and activation.

21. Brief Irritability Test (BITe). This is a 5-item scale that measures irritability.

22. Generalized Anxiety Disorder 7-item (GAD-7) Scale. This is a 7-item scale that measures anxiety.

23. IDAS-II III Temper (Anger) Scale. This is a 5-item scale that measures anger.

24. Intolerance of Uncertainty Scale – Short Form. This is a 12-item scale that measures intolerance of uncertainty.

25. Pittsburgh Sleep Quality Index. This is a 24-item scale that measures sleep.

Hair collection: If the woman agrees, a sample of hair (70-100 strands) will be collected from the crown of her head (about 70-100 strands) by trained research staff.

Rectal Swab: If the woman agrees, using the kit provided, she will collect two rectal swabs.

b. Research follow-up visits (24–28 and 34–36 weeks gestation)

Reminder Phone Call, Text, and Email: Women will be called or texted and emailed 2-3 days before the visit. Women will be reminded of their appointment time and location, asked to not eat for at least 2 hours before the appointment, and reminded about the visit procedures.

Data collection procedures: Women will be weighed with an electronic scale; a venous blood sample (30 ml) obtained by research staff; 2 rectal swab samples collected, optional; and surveys (described in detail below) will be interviewer administered by trained staff. If there is extra blood available from the venous blood sample and the patient consents, it may be used for future research to learn more about how to prevent, detect, or treat other health problems in pregnancy.

Surveys: Women will be asked to complete the following surveys:

1. 24 hour diet recall. The 24-hour diet recall will be collected using Nutrition Data System for Research (NDSR) software, a computer-based software application developed at the University of Minnesota that facilitates the collection of dietary recalls in a standardized fashion. Dietary intake data gathered by interview is governed by a multiple-pass interview approach. Dietary supplement use will be assessed in conjunction with collection of 24-hour dietary recalls using

the Dietary Supplement Assessment Module included in NDSR. Use of all types of dietary supplements and non-prescription antacids are queried in the module. The RAs are NDSR certified through the University of Illinois Cancer Center Diet and Behavior Shared Resource.

2. GI symptoms checklist. This short survey probes for recent GI problems including abdominal pain, nausea, constipation, and diarrhea.

3. Mood and Anxiety Symptom Questionnaire – Anhedonia subscale (MASQ-AD). This is a 22-item subscale of the MASQ, is a measure of depressive disorders.
4. Patient Health Questionnaire (PHQ-9). This is a 9-item questionnaire assessing depressive symptoms.
5. Edinburgh Postnatal Depression Scale (EPDS). This is a 10-item scale intended to measure depressive symptoms during the perinatal period.

6. Perceived stress scale. This is a 10-item scale intended to measure perceived stress over the last month.

7. Adult violence exposure. This is a 29-item survey used to identify exposure to violence of the past 7 days. The survey is used to classify low- and high-violence exposure that has demonstrated good construct validity, internal consistency, test-retest reliability, and validity.

8. Social support – MOS social support survey. This survey was designed to be comprehensive in terms of recent thinking about the various dimensions of social support.

9. Healthy Pregnancy Stress Scale. This is an 18-item scale that measures pregnancy stressors women have experienced during their pregnancy.

10. Hair collection survey. This survey was developed by the research team and asks about hair care practices.
11. Short Scale Anxiety Sensitivity Index (SSASI). This is 5-item scale that measures anxiety.

12. Behavioral Inhibition System & Behavioral Activation System Scales. This is a 24-item scale that measures behavioral inhibition and activation.

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14. Generalized Anxiety Disorder 7-item (GAD-7) Scale. This is a 7-item scale that measures anxiety.

15. IDAS-II III Temper (Anger) Scale. This is a 5-item scale that measures anger.

16. Intolerance of Uncertainty Scale – Short Form. This is a 12-item scale that measures intolerance of uncertainty.

17. Pittsburgh Sleep Quality Index. This is a 24-item scale that measures sleep.

<u>Hair collection (34-36 weeks only):</u> If the woman agrees, a sample of hair will be collected from the crown of her head (70-100 strands) by trained research staff.

<u>Rectal Swab (baseline, 24-28 and 34-36 weeks):</u> If the woman agrees, using the kit provided, she will collect two rectal swabs.

Maternal health data: We will abstract from the EHR data pertaining to recent illness or infection, new medications, and change in health status (i.e., development of a pregnancy related condition like gestational diabetes).

Pill pick up and adherence monitoring (24-28 weeks and 34-36 weeks gestation):
We will monitor adherence using electronic pill monitoring technology Pillsy and standard pill counts. Women will get a new 30-40-day allotment of pills. During these visits, we will download data from the Pillsy app on their smart phones and conduct a physical pill count. If a woman is found to be non-compliant (i.e., consuming <75% of pills) with her permission, she will receive automated daily text messages around dinner time to help increase compliance with the supplement interventions.

Pill pick up and adherence monitoring visits (approximately @ 18-19 weeks, 22-23 weeks, 30-31 weeks, 38-39 weeks). We will monitor adherence using electronic pill monitoring technology Pillsy and standard pill counts. Women will visit UIC approximately every 28 days to get a new 30-40-day allotment of pills and for compliance to be assessed. If possible, these encounters will be coordinated around standard clinical prenatal care appointments. If not, separate pill pick up visits and under certain conditions home pill drop-off will be coordinated. During these visits, we will download data from the Pillsy app on their smart phones and conduct a physical pill count. If a woman is found to be non-compliant (i.e., consuming < 75% of pills) with her permission, she will receive automated daily text messages around dinner time to help increase compliance with the supplement interventions. We will administer the GI symptoms checklist at each visit.

c. Feasibility and Tolerability. Feasibility will be based on accrual and withdrawal/loss to follow-up rates at the end of the study and adherence determined using the Pillsy smart bottles and standard pill counts. The intervention will be considered feasible if 50% of women approached for participation enroll, overall withdrawal rate is ≤ 15% and adherence to the supplement regimen is ≥85%. Tolerability [(potential adverse events (AEs) (e.g., GI symptoms)] will be assessed via phone/email/text using the Pillsy app weekly for the first 4 weeks and bi-weekly thereafter. The intervention will be deemed tolerable if no serious AEs (highly unlikely) are documented, and the rate of non-serious AEs is similar between study arms.

e. Labor and delivery.
*Reminders for contacting research staff and participant tracking**.*** During the 34–36 gestational week research visit and/or at the final pill pick, women will be provided a laminated card with the study number and staff personal contact information. Women will be instructed to contact the research team when they are admitted to UIC labor and delivery. In addition, we will follow the progress of these women closely via phone call, text message, email and through the patient EHR until they are admitted to UIC labor and delivery. UIC labor and delivery staff will also be in-serviced regarding the study procedures.

1) Maternal venous blood draw: Following admission, as part of routine care, women will undergo a venous blood collection by labor and delivery staff. As part of this routine care, we will request an additional two tubes of blood (30 ml) for research purposes. If there is extra blood available from the venous blood sample and the patient consents, it may be used for future research to learn more about how to prevent, detect, or treat other health problems in pregnancy.

2) Umbilical venous cord blood collection (placental cord blood): Immediately following delivery, the research team will collect a maximum of three tubes of umbilical venous cord blood (~30 mL). If a research participant chooses to bank their umbilical cord blood, and therefore the research team cannot obtain 30 mL of umbilical cord blood, the research team will draw 30 mL of placental blood for analysis. If there is extra cord blood available from the cord blood sample and the patient consents, it may be used for future research to learn more about how to prevent, detect, or treat other health problems in pregnancy.

3) Placenta (20 grams): After the cord blood has been obtained, the placenta will be placed in a basin with lid and transferred to the Tussing-Humphreys lab space in COMRB. Following placental

characteristics will be examined (i.e., weight, length) and processed for analysis and stored at -80C until later analysis.

4) Maternal and infant labor and delivery data: Following participant discharge, a research team member will abstract pertinent maternal and infant L&D data (i.e., route of delivery, infant weight, and infant length) from the EHR.

VII. Compensation. Women will receive $70 at baseline, $50 at 24-28 and 34-36 weeks and $29 and gifts of 1 pack each of diapers and wipes at labor and delivery for completing each data collection visit.
Participant incentives for pill pick-ups: Subjects will require supplementation refills 4 times outside of the research visits during the study. Participants will receive 1 pack each of diapers and wipes for each pill pick up. Women completing all aspects of the study will receive up to $199 dollars.

VIII. Blood processing and analyses. All maternal blood samples will be processed at the CON research space and the cord blood samples processed at Dr. Tussing-Humphreys 3060/3028 COMRB lab space. All samples will be stored at -80ºC until analysis. The following assays will be conducted:

1) Hemoglobin and hematocrit will be assessed from whole maternal and cord blood in house or at Quest Diagnostics (Chicago, IL).

2) Serum iron, transferrin saturation, and ferritin will be quantified from maternal and cord serum at Quest Diagnostics (Chicago, IL).

3) Soluble transferrin receptor (sTfR) will be measured from maternal and cord serum via ELISA (R & D systems, Minneapolis, MN).

4) Hepcidin will be measured from maternal and cord serum via competitive ELISA at Intrinsic LifeSciences (La Jolla, CA).

5) Erythropoietin. Erythropoietin will be measured from maternal and cord serum via ELISA (R & D systems, Minneapolis, MN).

6) high sensitivity C-Reactive Protein (hs-CRP) will be measured from maternal and cord serum at Quest Diagnostics (Chicago, IL).

7). IL-6 will be measured from maternal and cord serum via ELISA (R & D systems, Minneapolis, MN).

8) Circulating stress-related metabolite, serum 17-hydroxycorticosterone will be measured by UIC RRC metabolomics core.

CHANGES DUE TO COVID-19 PANDEMIC, 3.17.2020:

To allow for the provision of mailed supplements during the coronavirus-19 pandemic and alterations to our in person data collection methods. This is an effort to reduce subject and researcher contact while maintaining study integrity for those already enrolled.

1) Supplement refills: Supplement refills will be mailed. Subjects are being informed by phone regarding the change in procedure. UIC Investigational Drug Service will continue to dispense the supplements. UIC IDS will provide a labeled study bottle that fits with the Smart Caps being used to monitor adherence (already at home with the participant) and generic capped study bottles containing the newly dispensed supplements (probiotic/placebo or supplement). The supplements will be mailed using an overnight courier (e.g., UPS). When the pills are received, study staff will instruct over the phone/FaceTime how to refill their supplement bottles as

well as conduct a hand pill count. Subjects will be asked to save their remaining pills in the generic pill bottles provided and to return to the research staff at a later date.
2) Surveys. All surveys will be conducted by phone or via a RECap link (subject preference) that coincide with study and pill refill visits.
3) Blood draws. Subjects will be sent labeled blood tubes to have their blood drawn at their 24-28 week and third trimester clinically indicated blood draw at UIC Clinical Phlebotomy. Subjects will then bring their blood samples to a locked specimen box located outside of the UIC CRC. The subject will alert the study staff and blood tubes will be picked up by the PI processed and stored or sent to Quest Diagnostics.
4) Anthropometrics. Gestational weight at a date the coincides with our defined study visits (24-28 weeks, & 34-36 weeks) will be obtained from the EHR vs. physically measured.
5) Labor and delivery samples. Nurse mid-wives (Kylea Liese and Katherine Erbe) working clinically at the time of admittance for delivery, will assist with obtaining maternal blood and venous cord blood. The PI will be alerted by phone call or text message and will retrieve the samples for processing.

IX. Rectal swabs processing and analyses. All maternal rectal swabs will be processed at Dr. Tussing-Humphreys 3060/3028 COMRB lab space and stored at -80ºC until analysis. Bacterial DNA will be extracted from the rectal swab using a commercial kit following standard procedures. The microbial community will be assessed via 16s rRNA analysis, whole genome shotgun sequencing, and qPCR.

X. **Placenta analysis.** Placenta will be used to assess gene expression of iron regulatory, inflammatory proteins, and tissue iron content. Placenta will be weighed, measured, and systematically biopsied and frozen at -80C for gene and protein analysis.

i. Nucleic acid extraction: total RNA will be extracted from homogenized placental samples by the UIC DNA services lab and shipped to UCLA for the gene expression analyses.

ii. Gene (mRNA) expression: 8 representative biopsy samples will be taken, with the fetal and maternal membranes removed for later mRNA expression analyses (qPCR). The samples will be placed in RNAlater and stored at -80C until analyses.

*XI. **Hair analysis.** Exposure to chronic stress will be assessed by measuring cortisol from a small hair sample (70-100 strands) using a commercial ELISA kit (Alpco Diagnostics, Salem, NH)[29]. Hair cortisol has been validated against 24-hour urine cortisol as a relevant biomarker of long-term cortisol exposure.*

XII. Data management.

Research staff will enter all data directly into a Research Electronic Data Capture (RedCap) (Vanderbilt University, Nashville, TN) data structure. Standard checks for outliers, duplicates, and other errors associated with data entry will be conducted. Dietary data will be collected using the online Diet History Questionnaire-II (NCI, Bethesda, MD).

XIII. Statistical analysis. Statistical analysis will be performed using SAS (v. 9.4., Cary, NC). Variables not normally distributed will be transformed and if normality not achieved, non-parametric tests will be utilized. Data that are left censored due to undetectable levels will be analyzed using imputation or tobit regression to avoid biased estimates. Descriptive statistics including means, medians and standard deviations for continuous variables and frequency for categorical variables will be used to describe the women, serum, and rectal swabs outcomes at baseline. Differences between treatment groups (Lp299v + standard PNVI vs. standard PNVI + placebo) for the baseline variables will be continuous variables will be compared by t-test or non-parametric equivalent with Bonferroni correction and categorical data via chi-square or Fisher exact text. The correlation between the continuous variables at each time-point, baseline, 20 – 24, 34 – 36 and delivery overall and within each group will be assessed by Spearman or Pearson correlation coefficients. A two-way analysis of variance, using a general linear model for repeated measures between treatment groups and time-points will be examined. If significant effects are observed over time, single different pregnancy time-points (i.e., 24 - 28, 34 - 36 gestational week) will be tested. All *P* values were based on a two-sided test of statistical significance accepted at the level of $P<0.05$.

The rectal swabs microbial 16s rRNA sequencing data will be analyzed with QIIME and PICRUSt. Baseline microbial abundance, diversity, and predicted microbial metabolisms will be assessed. For feasibility, # of charts screened, prescreening eligible, approached, and enrolled will be calculated. The # of visits completed at each time point and end of study attrition will be determined and differences between groups calculated. Adherence (# of pills consumed) and # of SAEs/AEs will be calculated and compared between groups. Maternal stress response, maternal iron-related parameters, and inflammation at baseline, 24-28 weeks, 34-36 weeks and labor and delivery, will be evaluated using repeated measures ANOVA and ANCOVA to adjust for baseline differences. Infant iron status at delivery will be compared between groups (Student's t-test). Microbial abundance, diversity and microbial metabolisms will be compared between groups at baseline, 24-28 weeks, 34-36 weeks (Student's t-test/Mann Whitney U) and within group changes at 24-28 weeks and 34-36 weeks will be determined. The mediating effects of maternal rectal swabs microbiome, systemic inflammation and iron regulatory responses on the relationship between Lp299v on maternal stress- and iron-related outcomes will be explored using generalized linear models. Multiple comparisons and false discovery rate adjustments will be used accordingly.

XIV. Data and safety monitoring plan.

The PI will establish a data safety monitoring plan and follow all procedures for the protection of human subjects as required by UIC. The purpose of this data and safety monitoring plan is: (1) to ensure the safety of study subjects and the validity of data; and (2) to produce high quality research while considering both risks and benefits. The PI and research assistants will meet weekly to monitor the components of the research study.

Assessment of study risk. The probiotic proposed for use in the study is not contraindicated during pregnancy and therefore has not been shown to pose any adverse risk to the pregnant woman or her fetus. All other study procedures, based on UIC guidelines should be determined as minimal risk.

Data monitoring plan**.** The PI will monitor the following items: data quality, completeness, and timeliness; adequacy of compliance with goals for recruitment and retention, including those related to participation of minorities; adherence to the protocol; and adverse events reported by subjects at study and adherence visits. If an adverse event is reported during the study, subjects will be requested to report the event to their physician and the PI. All adverse events will be documented and reported to the UIC IRB in a timely manner based on the type of adverse event (adverse event vs. serious adverse event). We will also record and track reasons for dropout. All authorized study personnel will be required to notify the PI of any unanticipated problems/adverse events immediately upon discovery. The PIs will immediately notify the UIC IRB. The study will be subject to annual review and recertification by the OPRS.

Experimental Aim 2: The Maternal Stress Reduction Project, "Happy Moms" Focus Groups
Information collected from the focus groups will help inform the development of a curriculum focusing on lifestyle and dietary approaches to reduce chronic stress from structural violence. The curriculum will be developed into a series of classes that will be offered at the New Moms Austin Chicago location. The classes will be open to focus group participants and women currently participating in Family Support programs offered by New Moms.

The Maternal Stress Reduction Project, "Happy Moms": Recruitment, Eligibility, Informed Consent and Data Collection

Recruitment: will be conducted via informational handout by members of the New Moms staff or members of the research team. New Moms offers both in-home and onsite programing for their clients. During these programs, members of the New Moms staff or a member of the research team will inform their clients about the opportunity to participate in a focus group centered around healthy diet and lifestyle choices. Interested individuals will be provided with a copy of the "Informational Handout" and instructed to contact the study number on the handout for more information or to enroll. We will recruit up to 24 women, between the age of 18-45 that are currently attending New Moms Family Support programing. Recruitment of participants will take place during New Moms Family Support programming via Informational Handout by a member of the

research study staff or a New Moms staff member. During the class sessions, a study staff member or New Moms staff member will distribute the Informational Handout informing Family Support programming participants about the opportunity to participate in a research study. Interested individuals will be directed to contact the study number on the Informational Handout for more information or to enroll. Interested individuals that contact the study number will receive a call from a member of the research staff in a semi-private space in the Westside Research Office Building to determine eligibility and explain focus group activities. Only members of the research staff and focus group participants for that session will be aware of a participant's status as a research participant.

Eligibility: A member of the study staff will return the call of a potential participant that leaves a message on the study number voicemail. When returning the call, the study staff member will follow the "Phone Script" to explain that the project is a research study and to describe the study purpose and activities to the potential participant. During the call, eligibility will be determined by asking the potential participant if they currently attend New Moms Family Support programs. If yes, they will be invited to participate in a focus group session and offered one of two focus group dates. The potential participant will choose the date that best fits their schedule. Once a date is selected, the potential participant will be provided with a location and time. No additional information will be collected from the participant at this time. All focus groups will be conducted at New Moms Austin location in Chicago.

Informed Consent: will be conducted at the beginning of the focus group session. A member of the study staff will review each section of the consent form with the focus group members as a group. The focus group attendees will be given the opportunity to ask questions and be reminded that their participation in the study is completely voluntary and that they can discontinue participation at any time. Individuals that choose to participate in the focus group will be asked to sign and date two copies of the consent form (one to keep and one to be given to study staff). Study staff will also sign and date both copies of the consent form at that time. Those that choose not to participate will be thanked for their time and asked to leave the focus group session before it begins.

Data Collection: Once all participants are consented, the brief demographic questionnaire will be distributed. When all questionnaires are completed, a member of the study staff will collect the questionnaire and begin the focus group session. Participants will be asked about their barriers and challenges to a healthy diet and lifestyle. The focus group sessions will be audio recorded to allow research staff to capture the discussion in full. During the focus group session participants will use first names only. All name tags will be collected at the end of the focus group session. For the Maternal Stress Reduction Project, "Happy Moms", a 1-2-hour focus group session. We are audio recording the focus group sessions for immediate download onto a laptop with TruCrypt software. The focus group session audio recordings will not be stored on the audio recording device. The audio recordings will be transferred to the laptop with TruCrypt immediately following the focus group session and deleted from the audio recording device. All focus group participants will be asked not to discuss the focus group session or its participants with anyone not in the focus group session. During the focus group, participants will be issued name tags with only their first names that will be collected at the end of the session.

Data Analysis: All qualitative data will be analyzed using qualitative analysis software (ATLAS.ti). We will identify emergent themes about experiences using the team consensus codes. Codes will be developed and discussed in team meetings to enhance consistency in application. Final codes will be compiled in the master codebook and applied to the coding of all qualitative date. The data will be double-coded; after coding separately, the 2 coders will consult with the team to review discrepancies, refine code definitions and recode until intercoder reliability exceeds 85%. The most common themes will be used to inform the intervention. For example, if a top common theme is fast-food consumption as a means to reduce stress, this would be targeted in the future intervention/curriculum.

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