Feasibility of oral lactoferrin to prevent iron deficiency anemia in obese pregnancy

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LIST OF ABBREVIATIONS

ABR	Auditory Brainstem Response
Lf	Oral Lactoferrin
BMI	Body Mass Index
COI	Conflict of Interest
CRP	C-reactive Protein
CV	Coefficient of Variation
DHA	Docosahexaenoic Acid
DHHS	Department of Health and Human Services
DMC	Data Monitoring Committee
DSMB	Data and Safety Monitoring Board
DSMP	Data and Safety Monitoring Plan
EPO	Erythropoietin
EMR	Electronic Medical Record
Fe	Iron
FERPA	Family Educational Rights and Privacy Act
FDA	Food and Drug Administration
GCP	Good Clinical Practice
Hg	Hemoglobin
HIPAA	Health Insurance Portability and Accountability Act
IBC	Institutional Biosafety Committee
ICD	Informed Consent Document
ICH	International Conference of Harmonization
ID	Iron Deficiency
IDA	Iron Deficiency Anemia
IDE	Investigational Device Exemption
IDS	Investigational Drug Service
IL-6	Interleukin-6
IND	Investigational New Drug
IRB	Institutional Review Board
LAR	Legally Authorized Representative
LD	Labor and Delivery
OHRP	Office of Human Research Protections
OPRS	Office for the Protection of Research Subjects
PHI	Protected Health Information
PI	Principal Investigator
PNV	Prenatal Vitamin
PPRA	Protection of Pupil Rights Amendment
QA/QI	Quality Assurance/Quality Improvement
SAE	Serious Adverse Event
SOP	Standard Operating Procedure
UIC	University of Illinois at Chicago
WG	Weeks Gestation

1.0 Project Summary/Abstract

Maternal iron deficiency anemia (IDA) increases the risk of adverse pregnancy outcomes and can negatively impact the iron endowment of the neonate that may cause irreversible deleterious effects on neurodevelopment. To avert IDA in pregnancy, women are universally prescribed a daily oral supplement containing ~27 mg of iron. However, there is growing concern regarding the efficacy of this one-size-fits-all approach for women with systemic inflammation, including women with obesity. Obese women experience disruption to the hepcidin-ferroportin complex deeming iron from diet, including supplemental iron, and body stores less bioavailable. This indicates an urgent need to develop and test new methods to prevent maternal IDA among women with obesity. In this planning grant application, we will develop the clinical and research infrastructure to test the preliminary efficacy of oral lactoferrin (Lf) supplementation for the prevention of maternal IDA among obese women. Lactoferrin is a natural compound found in secretions from mammals, including milk, and is an important regulator of body iron balance given its effects on inflammation and the hepcidin-ferroportin complex. Daily oral Lf has been shown to be superior to oral iron supplementation for treating IDA in pregnant women with inflammation but has not been studied for its ability to prevent IDA in pregnancy. The specific aims of this clinical trial planning grant include: 1) Conduct provider facing one-on-one interviews to inform and optimize a clinical research intervention into the clinical infrastructure at the Center for Women's Health at UIC and, 2) Pilot test a randomized controlled trial of oral Lf supplementation from early second trimester up through delivery among 40 pregnant women with obesity at risk of IDA. In this aim we seek to understand recruitment, adherence, and retention rates and intervention acceptability to determine feasibility and preliminary efficacy on maternal inflammation and maternal and neonatal iron and hematological-related markers. This study will provide first of its kind data, necessary and sufficient to inform and develop a large-scale efficacy trial of oral Lf as a safe and scalable natural compound for the prevention of IDA among obese women. Ultimately if successful, this project is an important first step toward improving prenatal care for obese women who represent more than a third of reproductive-age women in the United States.

2.0 Background/Scientific Rationale

Iron is essential for all cells and is particularly vital in pregnancy for the growth and maintenance of the placenta, expansion of the maternal red blood cell mass, fetal growth and neurocognitive development, and the neonatal iron endowment that supports ex utero brain development. [1] *Conversely, decreases in maternal iron status that lead to IDA increase the risk of maternal and infant mortality, spontaneous preterm birth, and enduring neurocognitive defects in infants.* [2] *Recent animal and human data indicate that maternal IDA and reduced maternal iron bioavailability is associated with poor infant iron status at birth,*[3,4] *posing a significant threat to neurocognitive development. In an effort to optimize iron status and avert IDA in pregnancy, U.S. women are universally prescribed a daily oral prenatal supplement containing ~27 mg of iron as a ferric or ferrous iron salt.* [5] *However, there is growing concern regarding the efficacy of this one-size-fits-all approach given the discovery of the hepcidin-ferroportin complex and its role in systemic iron regulation.*[5,6]

In pregnancy, a woman's dietary iron absorption efficiency is enhanced, a compensatory mechanism to fulfill maternal iron needs and increased placental iron uptake and iron flux to the fetus as the pregnancy progresses[1]*. It is believed this mechanism is largely supported by the suppression of maternal hepcidin, a liver-derived peptide hormone and master regulator of systemic iron homeostasis.*[7,8] *Hepcidin controls systemic iron metabolism through its regulation of the ferroportin iron exporter.*[9] *Hepcidin promotes ferroportin's degradation thereby reducing the efflux of iron from intestinal enterocytes, reticuloendothelial macrophages, and hepatocytes. Hepcidin is simultaneously regulated by iron stores, erythropoiesis and systemic inflammation.*[10] *In persons with systemic inflammation, hepcidin production is enhanced, resulting in diminished ferroportin expression and reduced iron export into circulation from diet and stores.*[11] *This phenotype, commonly referred to as the anemia of inflammation, is associated with iron delocalization specifically normal to elevated ferritin (i.e., iron trapping), low hematological parameters [e.g., hemoglobin (Hb)], and systemic inflammation [e.g., elevated interleukin-6 (IL-6)].*[12] *Importantly, supplementing with oral iron salts (e.g., ferrous sulfate) in persons with underlying systemic inflammation has limited efficacy for preventing or treating IDA because hepcidin is a negative regulator of dietary iron absorption.*[13]

There is increasing evidence that hepcidin can be overexpressed in pregnant women with obesity. Pre-pregnancy obesity, a condition affecting 36% of reproductive age women in the U.S.,[14] *is often accompanied by chronic systemic inflammation and is a risk factor for neonatal ID.*[15,16] *In 2014, a small case control study reported that women with pre-pregnancy obesity (n = 20) had higher systemic inflammation and hepcidin in the second trimester and at the time of delivery compared to lean women (n = 20, BMI 18.5 - 24.9 kg/m2).*[17] *Moreover, the neonates birthed by the obese women had lower cord iron status compared to the neonates born to the lean women. In a separate case-control study, maternal obesity was associated with impaired neonatal cord iron status at delivery compared to neonates birthed by lean women.*[15] *Specifically, neonatal tissue iron stores were compromised to support Hb, a partitioning that may have a profound*

effect on growth and neurodevelopment. Several other research teams have examined links between maternal obesity and maternal-fetal iron regulation in large observational cohorts, with findings garnering mixed results.[16,18–20] *The lack of consistency among the various studies may be related to the degree of adiposity of the women investigated.*[19] *For example, one study stratified by extreme obesity (BMI ≥ 35.0 kg/m2) found that serum hepcidin was 50% higher in the 2nd trimester and at the time of delivery among severely obese women (but not among overweight or class I obese women) compared to women that were lean, indicating that a certain BMI threshold may be necessary to skew iron homeostasis in pregnancy.*[19] *In our preliminary data, described in detail below, pregnant women with pre-pregnancy obesity (mean BMI 35.3 ± 5.0 kg/m2) had greater systemic inflammation, overexpressed hepcidin, lower dietary iron uptake and less iron transferred to their fetus. Our findings are important given we used a stable iron isotope to objectively trace iron from maternal uptake to neonatal cord blood – no other studies to date have used this rigorous approach to examine iron homeostasis in obese pregnancy. Our findings indicate that the flow of iron from mother to fetus is impaired in women with pre-pregnancy obesity. Moreover, our preliminary data suggest that the current universal approach to prevent IDA in pregnant women with obesity, a daily oral prenatal supplement containing iron, is suboptimal given their underlying inflammatory status. This is a call to action given that disturbances to maternal iron homeostasis can result in irreversible, lifelong adverse health effects in infants,*[21] *coupled with the high rate of obesity among reproductive age women in the U.S.*[14] *Clearly, alternatives to preventing maternal IDA are needed for pregnancies complicated by obesity.*

Lactoferrin (Lf) is an iron binding glycoprotein secreted by exocrine glands and neutrophils that is present in mammalian biological fluids including milk, saliva, tears, and cervical mucus.[22] *Lf is involved in both iron homeostasis and inflammation and is closely related to the iron transport protein transferrin although it can retain iron at a lower pH*[23]*. Of relevance to the proposed trial is evidence that Lf can suppress the production and release of pro-inflammatory cytokines including IL-6.*[24] *This mechanism of action is important given IL-6 modulates hepcidin (i.e., promotes its upregulation) which leads to reduced ferroportin activity.*[9] *In a trial conducted in pregnant women with concurrent hereditary thrombophilia, IDA, and underlying systemic inflammation, led by our team member Valenti,*[22] *oral supplementation with bovine-derived Lf (Lf) (which has similar structure to human Lf and can bind to human Lf receptors to exert biological activity)*[23] *led to large reductions in circulating IL-6 and clinically significant improvement in iron and hematological parameters, compared to standard oral iron salt supplementation (330mg ferrous sulfate). Importantly this effect was observed in the absence of additional oral supplemental iron. Oral Lf is thought to improve body iron balance by enhancing ferroportin-mediated iron export by limiting inflammation and decreasing blood levels of both IL-6 and hepcidin — ultimately increasing iron bioavailability in the absence of additional direct iron supplementation.*[22] *Moreover, in pregnant women with IDA, daily oral Lf when tested against standard oral ferrous sulfate and ferrous fumarate supplementation was superior for improving Hb and was associated with significantly fewer adverse side-effects (i.e., gastrointestinal disturbances) and enhanced acceptability.*[25] *To our knowledge, there exists no clinical*

data to show the effectiveness of oral Lf supplementation for preventing IDA among pregnant women with obesity, despite a biological pretense for why oral Lf may be beneficial in this context. In summary, oral Lf is a safe compound to counteract inflammation, regulate the hepcidin-ferroportin axis and rebalance iron homeostasis in the absence of additional oral iron in a way that may be uniquely beneficial for preventing IDA among pregnant women with obesity given their underlying inflammatory status.

Effects of oral Lf vs. oral iron on iron homeostasis among women with hematologic disorders and complicated pregnancies. Our team member, professor Piera Valenti recently published a study examining the efficacy of twice daily (100mg/dose) oral Lf vs. once daily oral iron supplementation (330mg ferrous sulfate) for the treatment of concurrent anemia of inflammation and IDA in pregnant women with the hereditary hematologic disorder thrombophilia.[22] *Women with anemia of inflammation/IDA have a similar phenotype, albeit more extreme, to what is observed in obesity, i.e., systemic inflammation, overexpressed hepcidin, elevated body iron stores (ferritin) and decreased Hb. Valenti found that after 30 days of supplementation and at the time of delivery, oral Lf was associated with a significant reduction in IL-6 and hepcidin and statistically and clinically significant improvements in iron and hematological parameters (Table 1). Notably there were no changes in the women receiving oral ferrous sulfate. Also noteworthy is the fact that there were no adverse effects on the fetus/neonate (e.g., amniotic fluid index or birth outcomes) with either intervention. An exploratory analysis examining the impact of daily oral Lf on iron and hematological parameters in pregnant women with insulin resistance (n = 3) showed that after 30 days of supplementation, there was an increase from baseline in Hb (10.4 ± 0.3 to 11.4 ± 0.3 g/dL) and serum iron (33 ± 2 to 56 ± 11 μg/dL). This data is particularly intriguing given pregnant women with insulin resistance most closely represent the metabolic phenotype observed in obesity. Together, the data suggest that oral Lf supplementation safely elicits an anti-inflammatory effect that modulates the hepcidin-ferroportin complex to improve maternal iron homeostasis. This trial provides strong evidence that oral Lf supplementation, in the absence of additional oral iron, could be used to safely optimize iron homeostasis and prevent IDA in women with obesity.*

Table 1. Hematological, iron, IL-6 and hepcidin mean values ± SD of pregnant women with hereditary thrombophilia and anemia of inflammation treated with oral bLf or ferrous sulfate baseline, after 30 days of treatment, and at delivery

	Oral bLf (n=40)			Oral ferrous sulfate (n=25)		
	Baseline	**After 30 days**	**Delivery**	**Baseline**	**After 30 days**	**Delivery**
Red blood cells x 10^3	3,750 ± 127	4,354 ± 198*	4,482 ± 147*	3,650 ± 163	3,960 ± 230	4,029 ± 287
Hb, g/dL	10.6 ± 0.4	12.2 ± 0.4*	12.7 ± 0.5*	10.8 ± 0.8	11.3 ± 1.1	11.2 ± 0.9
Serum iron, µg/dL	36 ± 11	63 ± 9*	85 ± 10*	40 ± 15	40 ± 6	38 ± 11
Serum ferritin, ng/mL	11 ± 6	18 ± 2	31 ± 3*	17 ± 14	14 ± 9	12 ± 10
Serum IL-6, pg/mL	89 ± 8	58 ± 6*	50 ± 5*	85 ± 12	108 ± 7	113 ± 15
Serum hepcidin, ng/mL	115 ± 23	65 ± 10*	54 ± 13*	107 ± 29	112 ± 32	116 ± 26
*significantly different from baseline, $p < 0.001$						

3.0 Objectives/Aims

Aim 1: Conduct provider interviews to gauge best practices for integrating clinical research and patient recruitment into the Center for Women's Health clinical workflow. *We will recruit 9-12 providers from the Physician attending/Physician resident, nurse-midwife and nurse positions to complete a 30-60-minute one-on-one interview and 5-minute electronic demographics survey.*

Aim 2: Pilot test a randomized controlled trial of oral Lf supplementation among pregnant women with pre-pregnancy obesity at risk of IDA. *We will randomize 40 women to once-daily oral Lf (250mg) or usual care from early second trimester through delivery to:* ***a)*** *Understand recruitment, adherence, and retention rates and intervention acceptability to determine feasibility; and* ***b)*** *Determine preliminary efficacy on maternal inflammation and maternal and neonatal iron and hematological markers.*

4.0 Eligibility

Aim 1: We will recruit physician attendings, physician residents, nurse-midwives and nurses from the Center for Women's Health at UIC (9-12 individuals in total). The only eligibility is to be a current provider at the Center for Women's Health.
Aim 2: We will recruit 60 women to enroll 40 women and obtain a final sample of 32 women (16/arm). Women will be referred by their provider to the study team and recruited following their initial new OB visit either in-person at an upcoming clinical visit or by phone to determine interest and confirm eligibility. Women will also be identified and pre-screened by study staff through provider scheduling records and patient EMR to assess provider referral rates. We have found in our previous studies that waiting

until after the new OB visit is completed streamlines the recruitment process substantially, since the initial visit yields a detailed History and Physical, estimated dating of gestational age, and new OB clinical labs. Interested and eligible women will be invited to schedule a baseline research visit.

4.1 Inclusion Criteria

Aim 1:

- *current physician, nurse-midwife or nurse in the Center for Women's Health at UIC*

Aim 2:

- *single*
- *naturally conceived pregnancy*
- *at risk of IDA [Hb 11.0 – 12.9 g/dL (first trimester)/10.5 – 12.5 g/dL (second trimester)] based on new OB complete blood count (CBC) results obtained from the EMR*
- *18 – 45 years old*
- *pre-conception BMI ≥ 28.0 kg/m2 [based on measured height in EMR and recent pre-conception weight (within 3 months of pregnancy) from EMR if available or self-reported]; < 24 WG*
- *fluency in English to provide consent and complete study procedures*
- *ability to provide consent*
- *and ownership of a smartphone (currently more than 90% of our patient population at the Center for Women's Health)*
- *For ABR testing, the infant must have a normal hearing screen recorded in the electronic medical record (EMR). This is a requirement for normal ABR testing protocol.*

4.2 Exclusion Criteria

Aim 1:

- None

Aim 2

- *IDA requiring high dose supplemental iron*
- *allergy to milk proteins or wheat*
- *vegan (due to content of the supplements)*
- *recent blood transfusion*
- *previously diagnosed type 1 or type 2 diabetes*
- *autoimmune disorder (e.g., rheumatoid arthritis)*
- *inflammatory bowel disease*

- *current bacterial or viral infection*
- *history of bariatric surgery*
- *malabsorptive disease*
- *current hyperemesis*
- *current eating disorder*
- *hematologic disorder or trait carrier (e.g., hemochromatosis, β-thalassemia)*
- *current tobacco, alcohol or illicit drug use (not including marijuana)*
- *and regular use of medications that may interfere with nutrient absorption*
- *We have also found in our previous studies a recent traumatic event (e.g., death of a significant other or parent) may make it difficult to comply with the interventions, hence these women will also be excluded.*
- *First trimester Patient Health Questionnaire depression screener (PHQ-8) >10 is indicative of moderate to severe depressive symptoms and in our previous studies women with a score PHQ-8 >10 had difficulty complying with study measures. Women with a score >10 from the initial prenatal visit in the EMR, or if one is not available in the EMR, at screening will be excluded for study participation. The participant's provider will be notified via EPIC in basket message flagged as high priority and a member of the research team will monitor whether the provider read the message from the study team. If the participant is found to have a score >10 in the preliminary screening of the EMR, they will not be approached for recruitment.*

4.3 Excluded or Vulnerable Populations

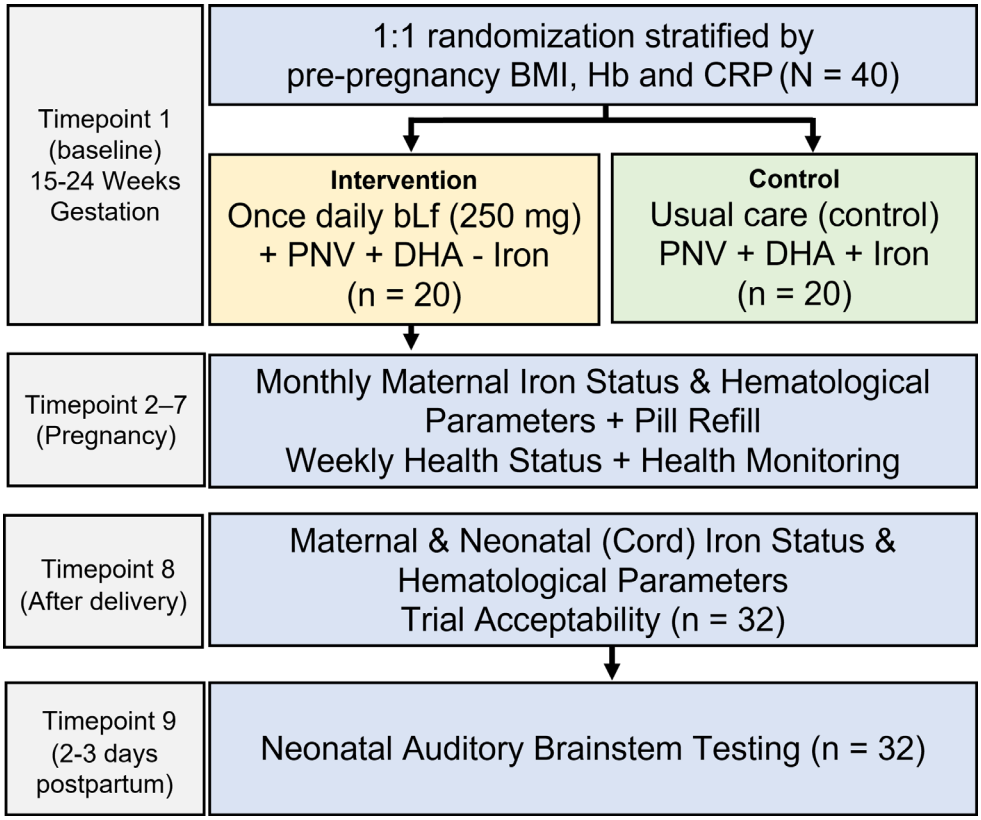
Sufficient fluency in English is required to complete consent, study forms, and questionnaires. During screening, a member of the research team will abstract from the medical record (if available) whether the participant is English speaking. A member of the research team will ask the participant whether they have sufficient fluency in English to complete the consent study forms and questionnaires.
Pregnant women will be enrolled and are considered a vulnerable population.

5.0 Subject Enrollment

Aim 1: We will recruit 9-12 (3-4 from each group) physician attending/resident, nurse-midwife and nurse in the Center for Women's Health at UIC.

Aim 2: We will recruit 60 pregnant women to enroll 40 and obtain a final sample of 32 women (16/arm). Women will be referred by their provider to the study team and recruited following their initial new OB visit either in-person at an upcoming clinical visit, by phone, by text or via MyChart messaging to determine interest and confirm eligibility. Only one MyChart message will be sent to gauge individual interest. Potential participants can decline participation in the study through MyChart messaging. A member of the research staff will indicate through MyChart that the participant's response will be directed to a member of the research staff only and not to the practitioner. Women will also be identified and pre-screened by study staff through provider scheduling records and patient EMR to assess provider referral rates. We have found in our previous studies that waiting until after the new OB visit is completed streamlines the recruitment process substantially, since the initial visit yields a detailed

History and Physical, estimated dating of gestational age, and new OB clinical labs. Interested and eligible women will be invited to schedule a baseline research visit.



6.0Study Design and Procedures

Aim 1.
Center for Women's Health providers agreeing to participate will engage in a one-on-one interview with study staff. The interview consists of prepared questions as well as organic dialogue and subsequent questions stemming from the interview responses. The interview will take place in person at UIC in a private office in the College of Nursing or College of Medicine (with COVID distancing and masking in place) or via phone or UIC hosted video conferencing platform, depending on provider preference. The one-time conversation will be audio recorded and will last approximately 30-60 minutes. The provider/participant will also complete a brief 5-minute demographics questions on a study provided tablet via REDCap. Provider/subjects will be compensated with a $25 Starbuck's gift card upon completion of the interview.

Aim 2.
Study Design. The feasibility and acceptability of daily oral Lf supplementation taken from early second trimester (15 – 24 WG) up through delivery will be assessed in a parallel 2-arm randomized controlled pilot trial conducted at the University of Illinois Hospital and Health Sciences System (i.e., Center for Women's Health and

Department of Family Medicine at University Village and Pilsen).

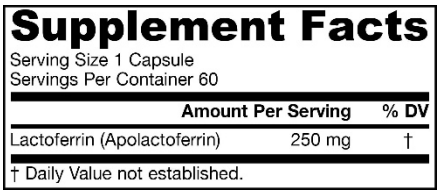
among 40 pregnant women with pre-pregnancy obesity at risk of IDA (Fig. 1). Women will be asked to complete the PHQ-8 to determine eligibility. This will only be completed if there is not an available score in the EMR from the patient's initial prenatal visit. If the woman has a PHQ-8 score <10 she will be eligible to participate. Women will be randomized (3:1) to one of two arms in the early second trimester (15 - 24 WG): once daily oral Lf + prenatal vitamin/mineral supplement without iron or once daily standard prenatal vitamin/mineral supplement with iron (usual care). We will examine feasibility, acceptability, and preliminary efficacy as main study outcomes.

Randomization. Dr. Steffen, the study biostatistician, will develop the treatment arm allocation schedule and ensure it is concealed from staff responsible for recruitment and enrollment until after written informed consent via electronic signature is obtained. We will use stratified, permuted block randomization considering pre-pregnancy BMI (28-34.9 kg/m2 and 35+ kg/m2), Hb (10.5 – 11.4 g/dL and 11.5 – 12.5 g/dL) and CRP (3.0 – 6.49 mg/L and 6.5 – 9.99 mg/L) to ensure balance across treatment arms. Forty women will be randomized to either once daily oral Lf + prenatal vitamin/mineral supplement without iron or daily prenatal vitamin/mineral supplement with iron (usual care) using the REDCap® randomization module developed to obtain a final sample of 32 women (16/arm assuming 20% attrition).

Study Arms. Women will be randomly assigned (3:1) to one of the two study arms described below. Women will not be blinded to their treatment assignment. Women will receive a month's worth of supplements at a time. Refills will occur at monthly research visits that are coordinated with clinical care. Based on our current probiotic supplementation trial, women receive between 5-6 refills over the course of their pregnancy depending on the timing of their study entry. We have opted to test once daily Lf (250mg) versus a lower dose (100mg) taken twice daily (as was used by our team member Valenti in her previous trial)[22] due to the common availability of Lf formulations in the U.S. commercial market, as well as the logistics around once vs. twice daily supplementation in our patient population. The supplements will be dispensed in bottles by the UIC Investigational Drug Service.

*Once daily Oral Lf (250mg). Women assigned to this group will be instructed to consume an oral Lf capsule one hour prior to their afternoon meal and two prenatal vitamin/mineral supplement gummies <u>without</u> iron with omega-3 fatty acids before bed from early second trimester (15 - 24 WG) up through delivery. Women are advised to consume the Lf prior to meals, given our team member Valenti's unpublished work shows its superior efficacy for improving iron and hematological parameters among pregnant women with hereditary thrombophilia versus when consumed with meals. The 250mg Lf capsule will be sourced from Jarrow Formulas (Los Angeles, CA. The supplement (**Fig. 2**), available commercially, has been previously tested in pregnancy[25] and includes 250mg Lf in addition to cellulose, magnesium stearate (vegetable source), silicon dioxide, and gelatin capsule material. Jarrow sources their Lf from Glanbia Nutritionals (Chicago, Illinois). Per the manufacturer, the Lf is approximately 14.3% saturated with iron or contains 17.6 mg/100g; we will confirm this with our own purity testing as described below. The prenatal vitamin/mineral gummies will be a commercially available product (OLLY The Essential Prenatal Multi). The nutritional content of the prenatal gummies is presented in **Fig. 3**. Women in both groups will be advised to consume an iron-rich diet and provided a handout detailing foods rich in heme and non-heme iron.*

Figure 2. Jarrow Formulas Oral Lactoferrin supplement Facts Label



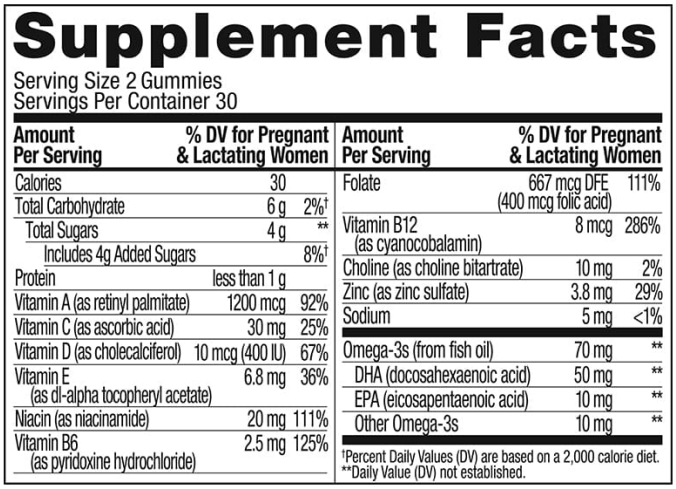
Supplement Facts

Serving Size 1 Capsule
Servings Per Container 60

	Amount Per Serving	% DV
Lactoferrin (Apolactoferrin)	250 mg	†

† Daily Value not established.

Figure 3. OLLY The Essential Prenatal Multi, Supplement Facts



Supplement Facts

Serving Size 2 Gummies
Servings Per Container 30

Amount Per Serving	% DV for Pregnant & Lactating Women	Amount Per Serving	% DV for Pregnant & Lactating Women
Calories	30	Folate	667 mcg DFE (400 mcg folic acid) 111%
Total Carbohydrate	6 g 2%†	Vitamin B12 (as cyanocobalamin)	8 mcg 286%
Total Sugars	4 g **	Choline (as choline bitartrate)	10 mg 2%
Includes 4g Added Sugars	8%†	Zinc (as zinc sulfate)	3.8 mg 29%
Protein	less than 1 g	Sodium	5 mg <1%
Vitamin A (as retinyl palmitate)	1200 mcg 92%	Omega-3s (from fish oil)	70 mg **
Vitamin C (as ascorbic acid)	30 mg 25%	DHA (docosahexaenoic acid)	50 mg **
Vitamin D (as cholecalciferol)	10 mcg (400 IU) 67%	EPA (eicosapentaenoic acid)	10 mg **
Vitamin E (as dl-alpha tocopheryl acetate)	6.8 mg 36%	Other Omega-3s	10 mg **
Niacin (as niacinamide)	20 mg 111%		
Vitamin B6 (as pyridoxine hydrochloride)	2.5 mg 125%		

†Percent Daily Values (DV) are based on a 2,000 calorie diet.
**Daily Value (DV) not established.

Usual Care (Control). Women assigned to this group will be instructed to consume a commercially available prenatal vitamin/mineral supplement with iron and omega-3 fatty acids (Prenatal 1, Bayer Healthcare, Whippany, NJ) before bed from early second trimester (15-24 WG) through delivery. To minimize variability in prenatal vitamin/supplement use across the participants, we have opted to standardize the prenatal vitamin/mineral supplement by providing women in the usual care arm a supplement that is nutritionally like what is prescribed by the Center for Women's Health providers. The nutritional content of the study-provided prenatal vitamin/mineral supplement is presented in ***Fig. 4****. Women will be advised to consume an iron-rich diet and provided a handout describing foods rich in heme and non-heme iron.*

Figure 4. Prenatal-1, Bayer Healthcare, Supplement Facts

Supplement Facts
Serving Size: One softgel

	Amount Per Serving	% Daily Value for Pregnant and Lactating Women
Calories	5	
Calories From Fat	5	
Total Fat	0.5 g	*
Polyunsaturated Fat	0.5 g	*
Cholesterol	25 mg	*
Total Carbohydrate	0 g	*
Sugars	0 g	*
Vitamin A (50% as beta-carotene)	4000 IU	50%
Vitamin C	60 mg	100%
Vitamin D	400 IU	100%
Vitamin E	30 IU	100%
Thiamin (B_1)	1.7 mg	100%
Riboflavin (B_2)	2 mg	100%
Niacin	20 mg	100%
Vitamin B_6	2.5 mg	100%
Folic Acid	800 mcg	100%
Vitamin B_{12}	8 mcg	100%
Biotin	300 mcg	100%
Pantothenic Acid	10 mg	100%
Calcium	200 mg	15%
Iron	28 mg	156%
Iodine	150 mcg	100%
Magnesium	50 mg	11%
Zinc	15 mg	100%
Copper	2 mg	100%
Omega-3 Fatty Acids (from fish oil)	235 mg	*
DHA (docosahexaenoic acid)	200 mg	*
EPA (eicosapentaenoic acid)	35 mg	*

Purity Testing of Oral Lf Capsules. Purity testing of the Lf capsules will be conducted using SDS-PAGE and silver nitrate staining, concentration via UV spectroscopy, iron saturation via spectroscopy and lipopolysaccharide contamination by Limulus Amebocyte Lysate assay (Sigma Aldrich, St. Louis, MO). Lf purchased from Sigma Aldrich will serve as the reference standard (St. Louis, MO). All purity testing will be conducted by Avomeen Analytical Services (Ann Arbor, MI).

Study Feasibility. We will collect detailed records of the number of women eligible and then referred by Center for Women's Health clinical staff to the research team. We will also track the number of referred women approached (by phone & in person) by our research team for enrollment and the number of women who decline and their reasons for non-enrollment. Once a woman is enrolled in the study, attendance at study visits, completeness of data, and overall and treatment specific loss to follow-up/withdrawal will be closely monitored. To track progress of subjects through the trial, we will adhere

to and update weekly the Consolidated Standards of Reporting Trials guidelines subject flow diagram. Women who voluntarily withdraw will be asked to provide reasons for revoking their enrollment. To further supplement these process measures, the study coordinator will take detailed notes during weekly research clinic meetings to document barriers to and facilitators of subject enrollment and retainment in the study, which will be used in our implementation analysis. Rates of enrollment and retainment are key feasibility outcomes. To facilitate recruitment and retention, transportation and participation monetary incentives will be provided. Together these activities will allow for the assessment of success and failure points to guide future work. Expected results: The study will be deemed feasible if clinicians refer ≥ 75% of women that are eligible, we recruit ≥ 50% of those referred at a minimum rate of 3-4 women/month, women complete ≥ 80% of planned study visits, and we retain ≥ 80% of women in both treatment arms through delivery.

Optimizing Adherence. . Hand pill counts will be performed by UIC Investigational Drug Service. ***Expected result:*** *Women will be deemed adherent to the interventions if ≥ 80% of the supplements provided are consumed.*

Health Status Monitoring. Health status will be assessed weekly through text messaging via texting service SimpleTexting (subject will be contacted by phone if additional information is needed beyond what is captured by text) and at monthly research visits. SimpleTexting is a HIPPA compliant online texting service. Subjects will receive this message on their smartphone. The SimpleTexting account is password protected and only accessible by key research personnel. Reported health changes will be reviewed and coded using a standardized approach.[26] *This information will be stored in REDCap. Other maternal (e.g., preeclampsia, gestational diabetes mellitus, infections), pregnancy (e.g., spontaneous pre-term birth), fetal (e.g., amniotic fluid index measured clinically in the second and third trimesters) and neonatal (e.g., birth weight, hypoglycemia) health outcomes will be collected from the subject EMR. We anticipate the most frequently reported health changes will be gastrointestinal (GI) in nature (e.g., constipation, loose stools). Therefore, a GI symptoms survey*[27] *will be administered at each monthly in-person research encounter.* ***Expected result:*** *We expect that the type and rate of health events will be similar between study arms.*

The research participants will complete 7-9 study visits depending on their gestational age when they enter the study. T1 (Screening/Baseline) will occur at 15-24 weeks gestation (WG), T2 will occur at 19-24 WG,T3 will occur at 23-28 WG, T4 will occur at 27-32 WG, T5 will occur at 31-36WG, T6 will occur at 35-40 WG, T7 will occur at 39-41WG, T8 will occur at delivery, and T9 will occur 2-3 days after delivery or at 38-42 weeks postmenstrual age for infants born preterm.

Participants will receive cash for each completed study visit. If they screen out at baseline due to their PHQ-8 score (if applicable), they will receive $10 and be withdrawn. If they do not finish the study, they will be compensated only for the visits they have completed. Participants will receive $50 at baseline, $20 at each monthly visit, and $29 at delivery. If a participant completes all parts of the study, they will receive a total of
$179-$199 in cash depending on when they entered the study, compensation for UIC parking and/or public transportation costs, and a baby-related gift with a cash value of approximately $101 (car seat, highchair or bassinet). Participants will receive another baby-related gift with a cash value of approximately $101 (car seat, highchair or bassinet) if they agree and complete ABR testing. Participants will choose a gift from the gift catalog provided by a member of the research team. Participants who screen out for PHQ-8 (if applicable) will receive $10 and compensation for UIC parking or public transportation costs.

We will monitor hemoglobin and iron status monthly at study visits and review results weekly with Dr. Gloria Elam, the medical oversight physician for the study. If needed, Dr. Gloria Elam will coordinate care with the participant's primary practitioner.

7.0 Expected Risks/Benefits

Aim 1:

- *We do not anticipate risks related to the one-on-one provider interviews. The only identified risk is related to loss of confidentiality. However, every precaution will be taken to keep information safe. A unique study ID number will be assigned to protect identity. Any electronic data will be stored in a password protected, restricted access electronic database (REDCap). The audio data will be stored on a password protected UIC Box folder. Only authorized research personnel will have access to this data.*

Aim 2:

Side effects, risks, and/or discomforts from participation in this study include:

- *Blood draws. Some people may be uncomfortable with having their blood drawn and may feel lightheaded, dizzy or even faint. Other risks and discomforts associated with drawing blood from a vein include pain or bruising at the site of the blood draw and rarely, infection at this site. Care will be taken to reduce these risks by having the phlebotomist conduct the blood draw.*
- *Loss of confidentiality. Because participants are sharing personal information, there is always the possibility that confidentiality will be violated. However, every precaution will be taken to keep information safe. A unique study ID number will be assigned to protect identity. Any electronic data will be stored in a password protected, restricted access electronic database. The blood and urine samples will be stored in a locked laboratory at UIC in a locked freezer. The survey data will be directly entered into a password protected research database. Only authorized research personnel will have access to this data.*
- *Questionnaires and interviews. Responding to study questionnaires and interview*

questions is not expected to but may provoke uncomfortable feelings or cause mild distress. If participants experience any of these symptoms, they may choose not to

answer any question that causes discomfort or may choose to withdraw from the study altogether. If their responses to the questionnaires indicate they may have depression or anxiety, it is our standard practice to refer them back to their clinical caregiver for further screening and evaluation. We will also screen using the PHQ-8, and the participant must complete this questionnaire to remain eligible.

- *Adverse effects of consuming a prenatal supplement that lacks iron. Based on existing data, it is highly unlikely to see a significant decrease in Hb or iron related parameters with oral Lf supplementation is the subject is adherent with the regimen. Nonetheless, we will monitor Hb and iron status monthly and review results with Dr. Gloria Elam who is the medical oversight for the study and medical director of the clinic where all the research subjects are receiving prenatal care.*
- *Adverse effects from oral Lf use. We will monitor daily changes to the subject's health reported through text messaging via SimpleTexting throughout the study and follow up with a phone call for additional information if needed. Additionally, the research coordinator will call participants to evaluate reported symptoms and advise administration for improved tolerability during the first week of Lf or usual care treatment. If in the unlikely situation that symptoms due to the intervention are intolerable or significant (unable to maintain intake by mouth or dehydration from diarrhea), the intervention will be immediately discontinued. To safeguard the risk of significant adverse effects, women with contraindications or history of bariatric surgery, inflammatory bowel disease, chronic diarrhea, or with hyperemesis gravidarum or eating disorder with the current pregnancy are excluded from the study. All adverse health effects will be coded and reviewed by the study team. Decision regarding continuing in the study rests solely with Drs. Koenig and Tussing-Humphreys.*
- *Anthropometric assessments. To minimize discomfort related to body measurements, assessments will be conducted by a trained and experienced research team member in a private area.*
- *ABR testing. If you agree to ABR testing on your baby, it is possible that your baby may experience minor skin irritation at the site of probe placement from the adhesive pads after testing is done, and this is expected to resolve in one day. The adhesive pads used in the testing are the same used for the clinical hearing testing performed in the university hospital. Care will be taken to ensure that the adhesive does not stay on your baby for longer than necessary for testing to reduce any possibility of irritation.*

8.0 Data Collection and Management Procedures

Aim 1: Providers will be involved in one interview and will complete one on-line survey (REDCap). The interview will be audio recorded or UIC hosted video conferencing platform.

Aim 2: Women will be involved in 7-9 research visits (depending on timing of study entry) that will take place at the Clinical Research Center and the Hospital LD unit.

Antenatal visits will be coordinated with clinical care to minimize subject burden. Measures to be obtained at each study visit are detailed in Table 2. Follow-up visit 7 will likely only be required for women entering the trial at 15-16 WG. A brief description and rationale for each measure is described below.

Table 2. Measures obtained at each timepoint (T1-T9)

Construct	Timepoint								
	1	2	3	4	5	6	7	8	9
MATERNAL									
Socio-demographics, health history & physical activity	•								
Height	•								
Weight	•	•	•	•	•	•	•	•	
Dietary intake	•		•		•				
GI symptoms	•	•	•	•	•	•	•		
Health and medication changes		•	•	•	•	•	•	•	
Iron & hematological parameters	•	•	•	•	•	•	•	•	
CRP	•	•	•	•	•	•	•	•	
Hepcidin, IL-6, & EPO	•		•		•			•	
Urine	•		•		•				
L&D outcomes								•	
Hand capsule counts		•	•	•	•	•	•	•	
Experience with intervention								•	
FETAL/NEONATAL									
Well-being		•			•			•	
Cord iron & hematological parameters								•	
Sex, gestational age, weight, and health status								•	
Neonatal Auditory brainstem testing									•

T1 = baseline [15-24 weeks gestation (WG)]; T2 = 19-24 WG; T3 = 23-28 WG; T4 = 27-32 WG; T5 = 31-36 WG; T6 = 35-40 WG; T7 = 39-41 WG; T8 = Delivery T9 = 2-3 days postpartum

Maternal Iron Status & Hematological Parameters. Maternal iron status and hematological parameters will be assessed from antecubital non-fasting blood at baseline, monthly, and at admittance for delivery. Expected results: The primary maternal outcome is Hb. Compared to the control group, we expect (based on our preliminary data in pregnant women with insulin resistance) a clinically significant improvement in Hb (+ 0.5 – 1.0 g/dL) at timepoint (T) 2 (~ 30 days after starting the supplement) for women randomized to daily oral Lf that is maintained through delivery. We also expect in the women randomized to oral Lf significantly higher serum ferritin, iron, hematocrit and red blood cells and significantly lower soluble transferrin receptor (sTFR) at T2 that is maintained through delivery compared with the control group.

- *Ferritin & Iron. Maternal ferritin and iron will be measured in serum by immunoassay and spectrophotometry by a local commercial lab (Quest Diagnostics, Wood Dale, IL).*
- *sTFR. Maternal sTFR will be measured in serum using a commercially available immunoassay (ELISA) (R&D Systems, Minneapolis, MN). Our lab's intra-assay coefficient of variation (CV) for this kit is 5.3%.*
- *Calculated Total Body Iron (TBI). Maternal TBI will be calculated from sTFR and serum ferritin using an equation developed by Cook and colleagues.*[28,29]
- *Complete Blood Count (CBC). Maternal CBC with differential will be measured in whole blood by electronic cell sizing/counting/cytometry/microscopy by a local commercial lab (Quest Diagnostics, Wood Dale, IL). Hb, obtained from the CBC, will be used to define trimester-specific IDA with a downward correction of 0.8 g/dL for Black women.*[30] *Hb is the primary clinical outcome for the trial given it is the most commonly assessed iron/hematological status marker in pregnancy.*

Correcting Maternal Ferritin, sTFR & TBI for Systemic Inflammation. The Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) project inflammation-related correction factors will be used to correct serum ferritin, sTFR and TBI values for C-reactive protein (CRP).[31] *CRP will be measured via nephelometry by a local commercial lab (Quest Diagnostics, Wood Dale, IL).*

Maternal Hepcidin. Maternal hepcidin will be measured in serum at baseline, T3, T5, and at admittance for delivery with a commercially available ELISA (Intrinsic LifeSciences, La Jolla, CA).[32] *The minimum level of detection for the assay is 2.5 ng/mL. Expected results: We expect significantly lower hepcidin at T2 that is maintained through delivery in women randomized to oral Lf compared with control.*

Maternal IL-6. Inflammation, specifically the pro-inflammatory cytokine IL-6, is a positive regulator of hepcidin.[12] *Maternal IL-6 will be measured in serum at baseline, T3, T5, and at admittance for labor and delivery with a commercially-available ELISA (R&D Systems, Minneapolis, MN). Our lab's intra-assay CV is 5.7%. Expected results: We expect significantly lower IL-6 at T2 that is maintained through delivery in women randomized to oral Lf compared with control.*

Maternal Erythropoietin. Erythropoiesis is a negative regulator of hepcidin.[9] *Maternal erythropoietin will be measured in serum at baseline, T3, T5, and at admittance for labor and delivery with a commercially available ELISA (R&D Systems, Minneapolis, MN). Our lab's intra-assay CV is 2.6%.*

Maternal Dietary Intake. Dietary macro- and micro-nutrient intake will be assessed using the web-based Diet History Questionnaire III (DHQ-III) food frequency questionnaire (baseline only).[28] *The DHQ-III will be self-administered. Recent dietary intake/supplement use will be assessed at baseline, T3, and T5, via Nutrition Data System for Research interviewer-assisted 24-hour diet recall software.*[33] *Mean total, food, and supplemental iron intake can be estimated from both dietary measures.*

Maternal Anthropometrics & Gestational Weight Gain. Maternal height will be measured using a fixed stadiometer (baseline only) and weight at baseline, monthly, and at admittance for labor and delivery with a calibrated digital scale. BMI will be calculated as kg/m2. Pre-pregnancy weight will be self-reported (confirmed by EMR, if available) and used to derive gestational weight gain using published methods.[29] *Rationale for determining gestational weight gain is the reported evidence that excessive gestational weight gain can negatively affect maternal and neonatal iron status and regulation.*[15–18]

Neonatal Cord Iron Status & Hematological Parameters. Neonatal cord iron status and hematologic parameters will be assessed from venous umbilical cord blood obtained bedside following delivery of the placenta. Even with delayed umbilical cord-clamping, we can successfully obtain 40-50 milliliters of umbilical venous cord blood. We will employ our effective 24/7 call schedule (including holidays) with two team members on call daily to cover LD collections. We have an 87% (40 of 46 women) success rate of collecting samples bedside at delivery as indicated in our preliminary studies section.

Expected results: Compared with control, we expect the neonates born to women in the oral Lf group will have superior cord markers of iron status, cord TBI and cord Hb at delivery.

- *Cord Ferritin & Iron. Cord ferritin and iron will be measured in serum by immunoassay and spectrophotometry at a local commercial lab (Quest Diagnostics, Wood Dale, IL).*
- *Cord sTFR. Cord sTFR will be measured with an immunoassay (ELISA) (R&D Systems, Minneapolis, MN). Our lab's intra-assay coefficient of variation (CV) for this kit is 5.3%.*
- *Cord TBI. Cord TBI will be calculated from cord sTFR and serum ferritin using a published equation.*[28,29]
- *Cord CBC. Cord CBC with differential, which includes Hb, will be measured by electronic cell sizing/counting/cytometry/microscopy at a local commercial lab (Quest Diagnostics, Wood Dale, IL).*

Maternal Urinary Metabolite. Urine metabolite analysis related to oxysterols will be assessed from spot urine collection at T1, T3 and T5. Pregnant individuals will provide a spot urine sample in a sterile cup. 0.5ml of urine will be aliquoted and frozen at -80C until further analysis. Urine oxysterols and chemicals, such as neonicotinoids, present in the urine will be quantified using mass spectrometry at the UIC metabolomics core.

Neonatal Characteristics. There is evidence that fetal sex (considering sex as a biological variable), neonatal weight at delivery and gestational age at delivery and can affect maternal and neonatal iron status and hematological parameters.[15,34] *We will obtain these variables from the patient EMR.*

Neonatal Auditory Brainstem Response (ABR) Testing. Cognitive development in neonates will be assessed with ABR testing at T9. ABR testing in hearing infants measures the conduction speed of neurons along different levels of the auditory pathway.[35] *Longer latencies indicate delayed myelination in the brainstem and the brain more broadly.*[36] *Maternal and infant ID have been associated with poorer ABR performance in neonates and later in infancy, which is hypothesized to due to iron's role in synthesizing lipid required for myelination.*[37,38] *An experienced audiologist will conduct the ABR testing at a target age of 2-3 days postpartum in neonates of individuals enrolled on the trial. Infants born preterm will be tested at 38-42 weeks postmenstrual age. The audiologist will be blinded to the mother's assigned treatment group, and the ABR data will be analyzed by an audiologist who is blind to iron status and treatment group. The test in noninvasive and performed while the infant is lying supine in quiet sleep. Testing will be conducted either in the hospital or in the UIC audiology clinic. ABRs will be elicited with click stimuli at 75-85 dB (Biologic version 5.70 model 317, Bio-logic Navigator Pro-Natus Medical Incorporated, San Carlos, CA). Bilateral monaural ABR testing will produce latency and amplitude measures for waves I, III and V and inter-peak latencies between I and III, III and V, and I and V in each ear. Latencies, amplitudes and inter-peak latencies for each wave will be analyzed separately in relation to treatment group and maternal and neonatal iron status measures to investigate whether, to what extent and in what location(s) brainstem myelination differs by iron status or treatment in the study participants. Recordings will be obtained from both ears. At least two recordings will be obtained for each intensity*

and data from the better ear (shorter interpeak latency I-V) will be used for the final analyses of interpeak latencies.

Additional Surveys & Questionnaires. Subjects will complete surveys pertaining to socio-demographics, reproductive/mental health, physical activity[39] and medication/supplement use. If the participant is interested in completing questionnaires remotely in advance of the subsequent monthly visits after the baseline visit, they will have the option to receive a link via REDCap to the GAD 7, PSS 10, and PPAQ questionnaires which should be completed 1-3 days before their research visit. We will also obtain data at follow-up and supplement refill visits pertaining to health or medication/supplement changes since the last research-related visit that we will verify with the EMR.

Satisfaction Survey. Subjects will complete the Treatment Satisfaction Questionnaire for Medication (TSQM)[40] to determine satisfaction with the interventions. The TSQM will assess participants' satisfaction with the effects and ease of taking the intervention supplements. There are 14 items in the TSQM that cover four domains: effectiveness (3 items), side effects (5 items), convenience (3 items), and global satisfaction (3 items). Expected results: Subject satisfaction with the oral Lf intervention will be determined by achieving a mean score ≥ 70.

Overall trial acceptability. Semi-structured interviews using questions adapted from DasMahaptra et al.[41] and Pflugeisen et al.[42] will address multiple domains of participation in the trial including satisfaction with study visits, study staff, reimbursement, and intentions for participation in the trial. The interviews will be conducted one day after delivery in the patient's hospital room. We have used this technique in our other studies with success. Responses will inform refinement and implementation procedures for the larger efficacy trial.

9.0 Data Analysis

Aim 1. Interview data will be transferred to and stored on UIC Box. The interviews will be de-identified and transcribed verbatim. Survey questions will be summarized using descriptive statistics. Interview transcripts will be analyzed using NVivo, a qualitative analysis software. The PI and co-investigators will review transcripts to sort the discussion topics into codes and categories. An initial code list will be created. Data will be analyzed using the constant comparative method to identify recurrent themes until theoretical saturation is achieved. The research team will discuss the codes, categories, and themes generated to resolve discrepancies through interpretive discussions, consensus building, and refinement as needed. Results will be presented as quotes and vignettes as supportive evidence. Measures to maintain trustworthiness will include audit trail, peer debriefing, and reflexivity notes. Data will be used to inform and refine Aim 2.

Aim 2. We will summarize feasibility metrics and preliminary efficacy using descriptive statistics such as frequencies, percentages, means, standard deviations, and effect sizes, and corresponding confidence intervals, to estimate a plausible range of values for planning a larger efficacy trial. We will examine both intent-to-treat and as-treated effects and seek to understand possible differences. Missing data and attrition will be monitored continuously and investigated to refine our methods to prevent their occurrence. We will seek to identify predictors of missingness to support the missing at random assumption for future analyses of a fully powered efficacy trial.[43,44] *These pilot data will also be valuable for identifying possible subgroups for whom oral Lf may be more or less effective (e.g., pre-pregnancy BMI ≥ 35.0 kg/m*^{2}*). We will examine the trend plot of each participant and summarize trends by treatment arm to understand the pattern of change (linear, quadratic) in inflammation, maternal iron status, and hematologic parameters collected monthly over the course of gestation. Mixed effect linear regression will be used to explore the relationships among these intensive longitudinal measures.*[45] *We will examine sex of the fetus as a biological variable that may influence our intervention effects or the relationships among inflammation and hematologic parameters.*

10.0 Quality Control and Quality Assurance

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. Co-I Liese will evaluate the qualitative interview data for quality. For Aim 2, 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.

11.0 Data and Safety Monitoring

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. Any instances of adverse events or unanticipated problems will be reported according to the standard forms and/or procedures that have been established by the IRB.

12.0 Statistical Considerations

This clinical trial pilot study is not powered to detect significant effects for improvement in maternal inflammation or maternal/cord Hb/iron status. This sample size will be sufficient for calculating confidence intervals for feasibility and effect size components.[46]

13.0 Regulatory Requirements

13.1 Informed Consent

- *Trained key personnel will explain the purpose of the study risks and benefits and answer any questions that arise from participants in-person or via zoom. Prior to engaging participants in any part of this research, an electronic informed consent document that contains appropriate human subject protection information relevant to the study in plain language will be presented to the participants via REDCap. The electronic informed consent document will be presented in-person at the baseline visit or prior to the baseline visit remotely over Zoom. If consented over Zoom, the consent form will still be signed electronically by the research participant and key research personnel but be presented remotely. If consented remotely, participants will be consented by key research personnel, and it will be documented in REDCap with the date and time, how it occurred, the name of the witness and the participant, and confirmation that the subject received the informed consent document. The same REDCap electronic informed consent document will be presented to the participant via a survey invitation tool for the participant to sign and date. The survey invitation tool will be sent via participant email and verification of receipt will be obtained by the participant's response to the email and their ability to complete the form via Zoom. The participant and key research personnel will sign and date the form together utilizing the methods listed below. Participants will be consented by project staff who have completed human subjects training and project- specific training, whether consenting is taking place in person or remotely. The participants are advised that their participation is completely voluntary and participation/non-participation does not affect their health care at the University of Illinois Hospital and Health Sciences System. Upon agreeance, subjects will grant consent to participate in the study by clicking "Yes" in the eConsent instrument in REDCap. Both the participant and key research personnel will e-sign the consent from in REDCap. All participants will be provided with a hard copy of the informed consent form. Whether consented in-*

person or via Zoom, the hard copy of the informed consent form will be printed at the Clinical Research Center. Remotely consented participants will also receive a copy of the signed consent form via email. Participants are free to not answer any questions that they find uncomfortable and they are free to withdraw at any time. All electronically signed eConsents will be stored on the secure REDCap server. Only key research personnel will have access to these documents.

13.2 Subject Confidentiality

- *Participants will be given a unique study ID number to protect their identity. Any electronic data will be stored in a password protected, restricted access electronic database. Blood and hair samples will be stored in a locked laboratory with subject ID. Only authorized research personnel will have access to the data.*

13.3 Unanticipated Problems

- *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.*
- *First trimester Patient Health Questionnaire depression screener (PHQ-8) >10 is indicative of moderate to severe depressive symptoms and in our previous studies women with a score PHQ-8 >10 had difficulty complying with study measures. Women with a score >10 at screening will be excluded for study participation. If the participant has a score >10 at baseline screening, their provider will be notified via EPIC in basket message flagged as high priority and a member of the research team will monitor whether the provider read the message from the study team.*

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