Enhancing Nutrition and Antenatal Infection Treatment for Maternal and Child Health (ENAT) Study

A Randomized Pragmatic Effectiveness Study in Amhara Region, Ethiopia

STATISTICAL ANALYSIS PLAN

Version 3.0

Date: Feb 2023

Sponsor: Bill and Melinda Gates Foundation

Implementing Partners: Addis Continental Institute of Public Health, Johns Hopkins University School of Public Health, Brigham and Women's Hospital

Study PIs: Anne CC Lee, Yemane Berhane

Study Statisticians: Luke Mullany, Alemayehu Worku

https://www.isrctn.com/ISRCTN15116516

ENAT STUDY
SAP Table of Contents

1. INTRODUCTION **3**

1A. Primary Aims 3

1B. Secondary Aims 3

2. BACKGROUND AND RATIONALE **3**

2A. Maternal Nutrition and Birth Outcomes 4

2B. Maternal Infections and Birth Outcomes 4

2C. The interaction between maternal nutrition and infections 5

3. METHODS **6**

3A. Participant Selection 6

3B. Study Procedures 9

3C. Interventions 11

3D. Randomization 18

3E. Statistical Analysis 20

3E1. Study Outcomes/Endpoints 20

3E2. Sample Size Estimates 21

3E3. Data Analysis 21

3E4. Additional Statistical Considerations 25

3E5. Data Management 26

3E6. Routine Study Monitoring 26

REFERENCES 28

APPENDIX 35

- Template Consort Diagram 35
- Template Tables 36
- SAP Versions/Revisions 46

1. INTRODUCTION

Our overall study objective is to determine the impact of a combined package of antenatal care interventions to enhance maternal nutritional status and manage maternal pregnancy infections on newborn size at birth.

1A. Primary aims:

To determine the effectiveness of a multi-pronged approach of packages of WHO-recommended antenatal interventions to optimize maternal nutrition and manage maternal pregnancy infections to increase newborn birth size in rural communities in Amhara region, Ethiopia.

1. To determine if a package of interventions to enhance antenatal screening and treatment of infections during pregnancy will increase birth weight by at least 58 grams and birth length by at least 3.0 mm, compared to newborns of pregnant women receiving routine care.
2. To determine if a package of interventions to enhance maternal nutritional status (nutritional counseling, iron-folate and iodized salt, balanced energy protein supplement to women with MUAC <23 cm) will increase birth weight by at least 80.3 gm and birth length by 7.8 mm.
3. To determine if A package of interventions to enhance both maternal nutrition AND antenatal infection management will increase birth weight by at least 78.9 grams and birth length by at least 4.2 mm, compared to newborns of women receiving routine care (neither of these packages).

1B. Secondary Aims:

1. To determine if provision of a package of interventions to enhance maternal nutrition and manage maternal pregnancy infections can:
   1.1. Increase mean length of gestation
   1.2. Reduce rates of low birth weight, small-for-gestational age, preterm birth, preterm delivery, or stillbirth
   1.3. Increase Z-scores for infant length-for-age, weight for length, weight-for-age at 6 months of age
   1.4. Increase maternal weight gain
   1.5. Reduce maternal anemia

2. To determine the association of maternal and neonatal inflammation with preterm birth and low birth weight
3. To identify additional risk factors for preterm birth and low birthweight in rural Amhara, with a focus on nutrition (micronutrient deficiencies), maternal and neonatal inflammation, psycho-social factors, and other pregnancy infections (TORCH)
4. To estimate the cost of ENAT interventions

To conduct implementation research to identify and address barriers to increasing coverage of FMOH-WHO recommended nutrition and infection interventions in pregnancy.

2. BACKGROUND AND RATIONALE

The World Health Organization Third Global Nutrition Target aims to reduce the proportion of babies born low birthweight (LBW, <2500 grams) by 30% by the year 2025 (eLENA 2017). The main etiologies of low birthweight are preterm birth and fetal growth restriction, commonly classified by birth small-for-gestational-age (SGA).

In 2012 in low and middle-income countries, an estimated 23.3 million infants, or one in five births, were born small for gestational age, defined as <10% birth weight for gestational age and sex using the Intergrowth 21st standard (Lee et al. 2017). 14 million babies (12%) were born preterm (<37 weeks), and available trend data show that rates of preterm birth are rising globally (Blencowe et al. 2012). The Child Health Epidemiology Reference group estimated that in Ethiopia in 2012, 635,000 (20%) babies were born with low birth weight, 640,000 small-for-gestational-age (20.1%), and 320,000 (10%) preterm, defined as <37 weeks' gestation (Lee et al. 2017; Blencowe et al. 2012).

SGA and preterm infants carry increased risk of mortality in the neonatal period and throughout childhood. An estimated 80% of newborn deaths are in infants who are either SGA and/or preterm (Hawn et al. 2014). These infants also carry higher risk of childhood stunting, neurodevelopmental impairment, and later adult chronic disease (Katz et al. 2013; Christian et al. 2013; Blenowe et al. 2013; Murray et al. 2015; Danaei et al. 2016). Prevention of preterm birth and SGA is a key public health strategy to improve child survival and health in LMIC.

2A. Maternal Nutrition and Low Birth Weight

In LMIC, maternal undernutrition is highly prevalent and a major cause of LBW. In Ethiopia, according to DHS data (2016), in Amhara region an estimated 23% of women of reproductive age had a BMI <18.5 kg/m^2 (Ethiopia DHS). Maternal underweight (low BMI <18.5 kg/m2) carries greater risk of spontaneous preterm birth (aRR 1.32, 95% CI 1.10–1.57) and LBW (aRR 1.48, 95% CI 1.29–1.68) (Han et al. 2011). Chronic maternal undernutrition, as measured by maternal short stature, is associated with increased risk of preterm birth and SGA (aRR 2.03, 95% CI: 1.76, 2.35; preterm AGA: aRR 1.45, 95% CI: 1.26, 1.66) (Kozuki et al. 2015). An estimated 6.5 million SGA and/or preterm births are attributed to maternal stunting (Kozuki et al. 2015). Furthermore, SGA and preterm birth increase risk of childhood stunting, resulting in an intergenerational cycle of sub-optimal growth.

Interventions to improve maternal nutritional status in pregnancy including iron-folate (Pena-Rosas et al. 2015), multiple-micronutrients (Haider and Bhutta 2017) and balanced protein-energy supplementation (Imdad 2012; Ota et al. 2015) have been individually tested and found to be somewhat effective in increasing mean birthweight (Pena-Rosas et al. 2015; Haider and Bhutta 2017; Imdad 2012; Ota et al. 2015), although the evidence for an impact on the two causes of LBW (SGA and preterm birth) is weak (Pena-Rosas et al. 2015; Ota et al. 2015; Stevens et al. 2015; Thorne-Lyman and Fawzi 2012; McCauley et al. 2015). A lower than expected benefit has been observed with the individual nutritional interventions, with each resulting in an increase mean birthweight of ~40-60 grams (Pena-Rosas et al. 2015; Ota et al. 2015; Buppasiri et al. 2011). ***Identification and targeting of additional factors influencing fetal growth and health are, therefore, needed to accelerate efforts to reduce low birth weight.***

2B. Maternal Infections and Low Birth Weight

Maternal infections in pregnancy, and systemic inflammation, are also common, yet under-recognized risk factors for preterm birth and poor fetal growth in LMIC. Genital tract infections may ascend the reproductive tract and lead to infection and inflammation in the amniotic fluid, predisposing to preterm birth (Goldenberg et al. 2000). Antenatal infections may also lead to systemic infection and inflammation, placental insufficiency, anemia, or epigenetic modifications, resulting in poor fetal growth and preterm birth. Thus, improving the screening and treatment of infections in pregnancy may play a crucial role in the prevention of fetal growth restriction, preterm birth, and other adverse pregnancy and birth outcomes.

In LMIC, such as Ethiopia, the burden of infectious diseases is high, yet there is limited epidemiologic data. Health systems are weak and the access to infection screening in antenatal care is limited. The prevalence of syphilis in Ethiopia ranges from 1.2% (Report on the 2014 Round Antenatal Care 2015) to as high as 9.8% in HIV+ mothers (Eticha et al. 2013); the national coverage of screening and treatment for syphilis during ANC is 34% (Global Health Observatory data repository 2015). Rates of urinary tract infection (UTI) or asymptomatic bacteriuria (ASB) in sub-Saharan Africa range from 9-80% (Gilbert et al. 2013). Screening for bacteriuria is typically done by urine dipstick, however, urine screening is done in only 2/3rd of ANC presenters, and stockouts are frequent. Geohelminths are highly prevalent, ranging from 21.1-43.5% (Shiferaw et al. 2017; Belyhun et al. 2010), with hookworm, *Ascaris lumbricoides*, and *Trichuris trichiura* as the primary pathogens. National coverage of de-worming drugs given during pregnancy is around 6% (National Nutrition Program 2016-2020 2016). There are limited estimates of rates of STI infections of gonorrhea and chlamydia, given that screening is not standard of care.

Maternal infections in pregnancy have been significantly associated with preterm birth and fetal growth restriction; and there is evidence that interventions to control certain maternal infections in pregnancy may improve fetal growth and increase the duration of pregnancy. In malaria endemic regions, intermittent prevention and treatment of malaria (IPTp-SP) increases mean birthweight by 100 grams, reduces the risk of low birthweight by 19% (Radeva-Petrova et al. 2014), and increases infant linear growth (Hallamaa et al. 2018). In a randomized trial in Uganda, presumptive treatment of sexually transmitted infections (STI) resulted in lower rates of STI infection (gonorrhea, chlamydia, trichomonas) as well as lower risk of low birthweight (0.68; 95% CI, 0.53-0.86), and preterm delivery (0.77; 95% CI, 0.56-1.05) (Gray et al. 2001). In a meta-analysis of 5 studies, treatment of syphilis may reduce the risk of stillbirth by 82% (95% CI 67-90) and preterm birth by 64% (95% CI 68-87%) (Blencowe et al. 2011). Observational data from Nepal demonstrated reductions in low birthweight and infant mortality among offspring of women who received albendazole once or twice during pregnancy for treating geohelminths in a dose-response manner (Christian et al. 2004). Prior to routine screening and treatment of asymptomatic bacteriuria in high income countries, a few studies from the 1960-70's demonstrated that treatment of bacteriuria reduced rates of low birthweight and preterm birth (Smaill and Vazquez 2015), although a recently completed RCT in rural Bangladesh showed no effect (Lee et al. 2015).

Poly-microbial infections, including bacterial vaginosis and periodontal disease, have also been significantly associated with preterm birth, although the evidence for the impact of screening and treatment at the population level is inconclusive. Bacterial vaginosis (BV) has been consistently associated with preterm birth, late miscarriage, and stillbirth (Leitich et al. 2003; Leitich and Kiss 2007; Ugwumadu et al. 2003). Treatment of symptomatic BV in pregnancy is standard of care in high-income countries (Force UPST 2014) and included in the WHO's syndromic approach (National Guidelines for the Management of Sexually Transmitted Infections Using Syndromic Approach 2015). However, in the multicenter NICHD trial (Carey et al. 2000) and PREMEVA trial in France (Subtil et al. 2018), treatment of asymptomatic BV did not reduce rates of preterm birth in low-risk pregnancies. Periodontal disease has been consistently associated with preterm birth, with a 2.30 (95% CI 1.21-4.38, meta-analysis of 5 studies) fold increased risk of a preterm or low birthweight birth (Khader and Ta'ani 2005). However, studies of treatment of periodontal disease have been mixed (Iheozor-Ejiofor et al. 2017). These conditions require further investigation in LMIC settings, to determine their potential.

2C. The interaction between maternal nutrition and infections

In addition to their independent role, maternal infections may interact with maternal nutritional status, modifying the impact of nutritional interventions in promoting fetal growth and term birth (Christensen et al. 2011; Krawinkel 2012; Vohr et al. 2017; Kim et al. 2011). However, these relationships between maternal nutrition and infection in pregnancy are complex and inadequately characterized and have been identified by a NIH Global Health Expert Convention as a major research gap (Kutlesic et al. 2017).

Maternal undernutrition may predispose mothers to maternal infections and vice versa, leading to a cycle of pathology resulting in low birthweight. Figure 1 depicts several of the pathways/mechanisms linking maternal nutrition and infections in pregnancy, that provide basis for the hypothesis that targeting both risk factors in pregnancy may lead to more substantial, and potentially synergistic, improvements in fetal growth and pregnancy length (Collin et al. 2007).

A) *Undernutrition leading to infection/inflammation:* Chronic protein energy malnutrition impairs immune function (i.e. antigen-presenting cell and cell mediated T-cell function), thereby increasing infection risk (Raiten et al. 2015; Ahmad et al. 2009; Hughes et al. 2009). Deficits in specific immuno-modulatory and/or antioxidant nutrients (i.e. long-chain polyunsaturated fatty acids, folate, vitamin B12) may also place the fetus at risk of inflammation. Furthermore, chronic undernutrition may also activate the hypothalamic-pituitary-adrenal axis and result in immune dysregulation (Shanks and Lightman 2001).
B) *Infection leading to undernutrition:* Chronic infections may lead to undernutrition due to reduced dietary intake, malabsorption/increased nutrient losses, and/or increased metabolic requirements.
C) *Pathways to preterm birth and fetal growth restriction:* Maternal undernutrition and infections may lead to fetal growth restriction and/or prematurity via several common, and potentially synergistic, pathways.

Both undernutrition and infections may result in systemic or fetal inflammation, placental insufficiency, and/or anemia. Furthermore, both factors may be epigenetic regulators of immune function, controlling fetal inflammatory cytokine and chemokine responses (Claycombe et al. 2015).

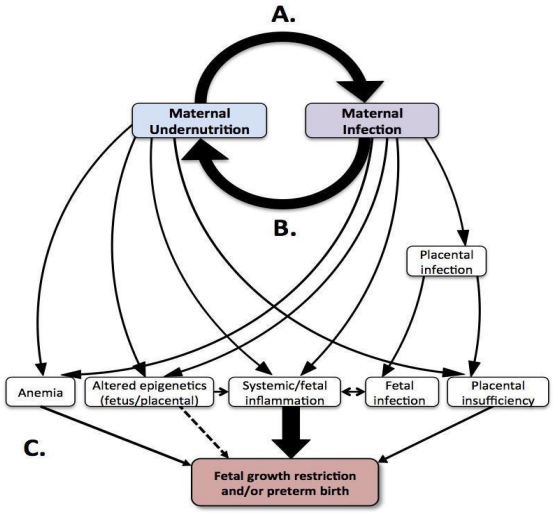

Figure 1. **Conceptual diagram showing the pathways that link maternal undernutrition, maternal infection, and infant outcomes** (A, B, and C represent mechanisms, which are described in detail in the associated sections above)

Overall, there is strong evidence supporting the role of undernutrition and infections in adverse pregnancy outcomes. While interventions have individually been tested and resulted in small gains in fetal growth, we hypothesize that an integrative approach addressing both maternal infection control with nutritional may result in more substantial and possibly synergistic impacts on pregnancy health and birth outcomes. The goal of the ENAT study is to rigorously test this hypothesis in a high burden region in rural Ethiopia.

3. METHODS

3A. Participant Selection

We will conduct a pragmatic effectiveness study to determine the impact of strengthening the delivery of interventions to optimize maternal nutritional status and infection control on maternal health, birth and infant outcomes in rural Ethiopia.

The study will be a 2x2 factorial design with cluster randomization of the enhanced nutrition package (ENP) (n=12 health centers), and individual level randomization of an enhanced infection management package (EIMP).

In all ENAT health centers (n=12, each serving ~20-25,000 population), our study partner Jhpiego, a non-profit affiliate of Johns Hopkins University, will support the Ethiopian Federal Ministry of Health (FMOH) to strengthen delivery of routine ANC care as per current FMOH guidelines. The **Jhpiego ANC Strengthening Interventions** will include community mobilization, and strengthening of health center capacity to provide quality ANC.

In the ENAT study, antenatal intervention packages will be randomized at two levels (Figure 1).

1) First, **health centers** will be randomized to receive a) ***ENAT Nutrition Package (ENP)*** (n=6 health centers) or b) *routine care* (n=6 health centers). The randomization approach (details below) will be restricted to ensure overall balance across the groups in health center characteristics such as annual/monthly volume of ANC, number and proportion of facility-birth rate, and distances to Bahir Dar, the capital of the Amhara region.

2) Secondly, **individual pregnant women** presenting for antenatal care at all 12 (i.e. ENP and non-ENP) health centers, will be randomized into one of two groups for antenatal infection management: a) ***ENAT Enhanced Infection Management Package (EIMP)*** or b) routine care.

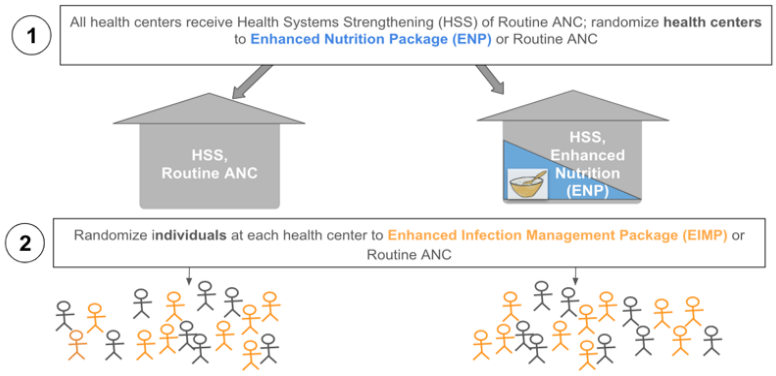

Figure 2: ENAT study design

3A1. Study Sites

The ENAT Study will be conducted in two zones of the Amhara regional state --West Gojjam Zone (South and North Achefer districts [woredas]) and in the South Gondar Zone (Dera and Libokemem districts). 12 health centers in these districts have been selected as ENAT study centers. The study health centers were chosen based on accessibility, ANC volume (min 250 ANC presenters/year), and distance to hospital (>5 km from closest hospital).

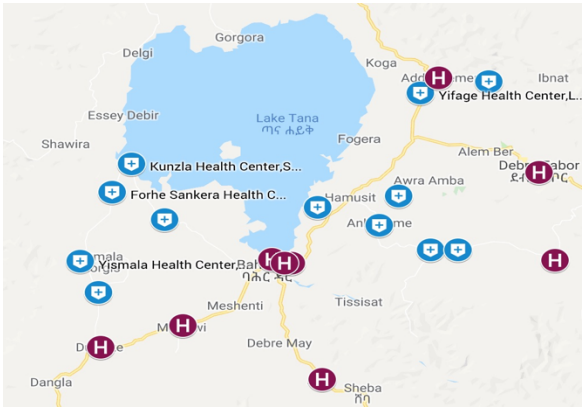

Figure 3: ENAT study sites

3A2. Study Participants

Pregnant women will be recruited from antenatal care visits in the designated ENAT study health centers. Women will be followed up through until 6 months post-partum, and their infants will be enrolled and followed until 6 months of age. We plan to enroll 2,400 pregnant mothers in 12 health centers (2 00/per health center) over a 12-month period.

3A3. Recruitment

Community mobilization efforts will be led by the Amhara Regional Health Bureau in collaboration with Jhpiego to increase community members' awareness of the benefits of early antenatal care in pregnancy. Community cadres, including health extension workers (HEWs) and the women development armies (WDAs), may be recruited to help disseminate information about the ENAT study. Radio campaigns may be undertaken to sensitize the community to the importance of nutrition and ANC in pregnancy. Campaigns

may be held on market days and religious holidays to increase awareness. Additionally, recruitment flyers may be placed in health centers and district health offices.

3A4. Inclusion Criteria

- Pregnant women presenting at ENAT study health centers for their first ANC visit ≤24 weeks gestation based on a best clinical algorithm (LMP and/or symphysis fundal height) who have a viable pregnancy
- Infants of enrolled pregnancies will be followed up regardless of where they are born (health center, referral hospital or home)

3A5. Exclusion Criteria

The following women will be excluded from the study:

- Pregnant women who live >2 hours walking distance from ENAT health centers
- Pregnant women presenting for first ANC >24 weeks
- Pregnant women presenting at first ANC with fetus that is non-viable (without a heartbeat on enrollment ultrasound)
- Pregnant women planning to deliver outside of the study's geographic catchment area (selected study woreda)
- Pregnant women who do not consent to be contacted by study staff for follow up

3B. Study Procedures

	STUDY PERIOD						
		Post-allocation					
	Allocation	Prenatal				Postnatal	
TIMEPOINT	Enrolment <24 weeks	*ANC1*	*ANC2*	*ANC3*	*etc.*	*Birth*	*1 month*
ENROLMENT:							
Eligibility screen	X						
Informed consent	X						
Allocation	X						
INTERVENTIONS:							
ENP	X	X	X	X	X		
EIMP	X	X	X	X	X		
ENP + EIMP	X	X	X	X	X		
ASSESSMENTS:							
MOTHERS							
US (fetal growth & GA determination)	X			X			
Basic medical & obstetric history	X						
Socioeconomic status	X						
Health care costs	X		X				
Food insecurity and Dietary Intake	X	X		X			
Maternal stress and depression		X		X			X
Maternal anthropometrics	X	X	X	X	etc.	X	X
Maternal morbidity	X	X	X	X	etc.	X	X
Labor and Delivery characteristics						X	
Assessment of home environment						X	
INFANTS							
Anthropometrics						X	X
Breast feeding practices						X	X
Morbidity and mortality						X	X

Figure 4: ENAT Interventions

3B1. Screening and Consent

Nurses or midwives employed by the health system who are providing routine ANC services at ENAT study health centers will screen mothers for participation in the ENAT study. During routine ANC visits, they will ask eligible women if they have interest in speaking with an ENAT study nurse to learn more about the study. If the pregnant woman expresses interest, she will be referred to an ENAT study nurse. The ENAT

study nurse will be located in a separate study room in the health center and explain the study procedures to the potential study participant and answer any questions. The anticipated benefits and risks or discomforts will be described to the potential participant. The woman will have as much time as needed to decide upon her participation. Women will be asked to sign a consent form. If she is unable to read and write, the study will be described to her and we will have an impartial witness attest to her consent and mark the form with her thumbprint.

3B2. Study Enrollment Visit
At the enrollment visit, data will be collected on the participant's socio-economic status, basic medical and obstetric history, pregnancy history, maternal morbidity, anxiety/depression, food security, and dietary intake. A dietary diversity questionnaire or food frequency questionnaires will be administered, which have been used in the Ethiopian context (Workicho et al. 2016).

3B3. Ultrasonography
A basic obstetric ultrasound will be performed in all study participants at the enrollment visit (target <24 weeks), or at the earliest follow-up date, for the primary purpose of pregnancy dating. A trained research nurse/staff will conduct an abdominal obstetric ultrasound. Training will be provided for ENAT study nurses, recruited specifically for ultrasound provision. There will be one ultrasonography trained research staff in each of the 12 ENAT study health centers. The ultrasonography training will be provided for approximately one month with ongoing refresher training and support. General Electric (GE) has developed a training program for basic obstetric sonography in collaboration with the Ethiopian Radiography Association (ERA) and Ethiopian Society of Obstetrics and Gynecology (ESOG). A two-week training will be provided by a radiographer from ERA, which will be arranged by GE. The sonographers will receive ongoing training and technical support from the GE Vscan program. Following this training, an intensive 1-week training and standardization for the purposes of pregnancy dating will also be provided for the same research nurses by two obstetricians from Harvard University. Standard bio-metric parameters will be measured in triplicate for the purposes of pregnancy dating. Sonographers will measure crown-rump length, bi-parietal diameter (outer-outer and outer to inner), head circumference, femoral length, abdominal circumference.

The following algorithm will be used to assign gold standard gestational age. For CRL measuring <95mm, the Intergrowth formula for CRL will be used to assign GA (Papageorghiou et al. 2014). If CRL is >=95mm, the WHO tables (Kiseurd et al. 2017) will be used for assigning gestational age based on the average gestational age using the biparietal diameter and femoral length. The sonographer will also assess for major complications (fetal loss, placenta previa, multiple pregnancy). Any major pregnancy complication will be referred to the primary referral hospital for a repeat scan and further evaluation and management within the existing health system. The research team will facilitate referral and help the family to cover costs of treatment.

3B4. Maternal Anthropometrics:
Weight and height will be measured by research staff trained in anthropometry. Maternal weight will be measured with a high-quality digital scale once (ADE M317600, Germany; precision 100 gm). Height will be measured using a high-quality adult stadiometer (Shorr Productions HeightLite).

3B5. Maternal Blood:
On a sub-set of up to 1200 women in selected health centers (recruited equally in each of the study arms), during the blood collection for routine first ANC screening (HIV, RPR), we will collect an additional 7.5 ml aliquot of maternal blood from the same blood draw, that will be processed to store serum, whole blood, plasma, and buffy coat and preserved for future testing for micronutrient concentrations (including iron, zinc, and others such as calcium, selenium, DHA, choline if available) and other infectious exposures (TORCH infection titers). From the same sample, two-three drops of whole blood will also be collected on a Whatman card to test for maternal inflammation biomarkers as described in the section *Inflammation Proteins* below. These will be air-dried and stored in Ziplock bags with silicone gel packs. Samples will be stored for up to 5 years in a deep freezer at -80 C at APHI.

3B6. Routine ANC services and group ANC

Currently, there are gaps in coverage of recommended ANC screening. For example, the national coverage of screening and treatment for syphilis during ANC is 34% (Global Health Observatory data repository 2015), urine testing ~60%, and de-worming during pregnancy ~6% (Ethiopia National Nutrition Program 2016-2020, 2016). Lack of laboratory personnel and stockouts are frequent. While rates of first ANC are high, rates of 4 ANC are 32% nationally (Ethiopia DHS 2016).

Our study partners Jhpiego, a non-profit affiliate of Johns Hopkins University, will support the Amhara Regional Health Bureau (RHB) to strengthen ANC service delivery of routine ANC services in the ENAT health centers. These activities are separate from the current study for which we are seeking approval. Jhpiego activities will target increasing coverage and quality of delivery of routine FMOH-recommended ANC services. This will include iron-folate, tetanus toxoid, blood pressure and anemia monitoring, as well as screening and treatment of certain infections that are already recommended by the FMOH (including HIV, syphilis, malaria, and tuberculosis). Daily elemental iron (60mg) and folate (0.40 mg) is provided to all mothers at routine facility-based ANC visits by the FMOH. Tetanus toxoid vaccination is typically provided on the first facility-based ANC encounter (and at the subsequent visit if required). Jhpiego will train staff in current ANC standards and guidelines of the FMOH, ensure health facilities are equipped and stocked with equipment/medications, diagnostic tests, and augment laboratory capacity (gram staining, microscopy) and training providers in existing infection management protocols.

3B7. Maternal Nutrition Screening in Routine ANC

According to current FMOH guidelines, all pregnant mothers have nutritional counseling during monthly pregnant woman conferences that includes education of the pregnant mother regarding nutritious foods in pregnancy and cooking demonstrations with local foods. Across all study areas, including the routine-care arm, strengthening of the standard nutrition screening, counseling and management within the health center will be reinforced by Jhpiego and ARHB.

As part of the ANC strengthening activities, health center providers will be trained to accurately measure and record middle upper arm circumference (MUAC) at each ANC visit in all ENAT health facilities. A MUAC cutoff of <23 cm is used in Ethiopia to classify a woman as malnourished (Ververs et al 2013). All women will receive nutritional counseling. If the health center is in a woreda that has been classified as food insecure by the national food supplementation program, women with MUAC <23 cm may receive food supplementation consistent with FMOH guidelines.

3C. Interventions

3C1. Enhanced Nutrition Package (ENP)

Pregnant women who present for ANC at the 6 health centers randomized to the ENAT Enhanced Nutrition Package arm (ENP) will receive the following **additional nutritional activities**:

1) **MUAC measurement/screening:** In addition to measurements performed by health center staff, ENAT trained data collectors will measure maternal MUAC in the left arm in all mothers in the ENP health centers in triplicate using a plastic insertion MUAC tape (Shorr productions, Maryland, USA).

2) **Nutritional Education/Counseling on Adequate Pregnancy Nutrition and Weight Gain:** ENAT nurses will provide customized nutrition counseling to pregnant women in conjunction with frequent weight monitoring (at least 4 times in pregnancy). At enrollment, all pregnant women will be counselled on BMI-based recommended weight gain. Women be counselled to have an adequate number of meals, increasing consumption of protein and energy rich foods in pregnancy, and at least four food groups daily with information about relevant micronutrient rich food sources (iron, vitamin A, vitamin C, and iodization), and iron folate supplementation. For women with BMI >25.0 kg/m^2, the risk of pre-eclampsia or hypertension, excessive weight gain will be reviewed. Various

nutrition education materials such as booklets, posters, bulletin board, role play, video/animation, and/or tablet-based education materials will be equipped at health centers to promote women's behavior change and maximize their exposure to various but consistent nutrition messages. Nutritional counselling will continue in the postnatal period. Husbands or mother-in-laws, who can be important facilitators/barriers in dietary practices, will be encouraged to co-attend visits.

3) **Iron Folate for all Pregnant Women:** The Ethiopian FMOH recommends 60 mg Fe/400 µg folate orally once daily in pregnancy. In the pilot period, coverage of iron-folate was 46.3% in the ENAT health centers. In Fall 2019, Jhpiego conducted provider training and addressed supply chain issues for Fe-Folate. In our formative work, barriers to Fe-folate included local beliefs about "big babies" and side effects. In the ENP health centers, study nurses will conduct frequent counseling and utilize video/media to address common cultural beliefs. Women will be followed up at (ANC visit or monthly phone call) to assess adherence, counsel and addressing side effects (ex. constipation).

4) **Balanced Energy Protein Supplement for Malnourished Women (MUAC <23 cm).** The Ethiopian FMOH recommends a corn soya supplement for pregnant women with MUAC <23 cm. In the ENP health centers, women who are identified to have MUAC <23 cm will be provided with a monthly supply of locally produced corn soya flour blend (Faffa Food Share Company) at every ANC visit from the health center pharmacy. The daily corn soya blend (CSB) supplement (200gm) will be in addition to meals and contain 28 g of protein and 784 kcal. This protein composition falls within the ranges of the Gates foundation guidance (BMGF 2016), which stipulates that a BEP supplement provide approximately 50% of the additional protein requirement in the 3rd trimester (range of 28-36g for malnourished populations).

   35 sachets will be distributed to women monthly to allow for additional doses in case she is delayed in returning for ANC and a small amount for potential family sharing practices.

   Currently according to FMOH guidelines, the follow up for ANC visits and for malnourished women (MUAC <23cm) is monthly. At each ANC visit, malnourished women will be provided an adequate amount of BEP supplement to last for 1 month. Women will be encouraged to attend ANC monthly in order monitor adherence and distribute another month's supply of BEP supplement. At any ANC visit, if a mother has a MUAC <23 identified she will receive BEP until she delivers.

5) **Provision of Adequately Iodized Salt to Pregnant Women:** Maternal iodine deficiency during pregnancy has been recognized as a cause of impairment in the neurological, psychological and intellectual development of infants and children (Glinoer 2007). In the Amhara region, the prevalence of iodine deficiency amongst women of reproductive age is 72.3%, the highest of any region in Ethiopia (Ethiopia MNS 2015). Although there has been great progress made to make iodized salt available nationally, only 15% percent of households are utilizing adequately iodized salt (Ethiopia MNS 2015). Pregnant women are recommended to consume at least 250 mcg of iodine a day (WHO Iodine Guidelines 2007).

   The Ethiopian Quality and Standards Authority set the iodine level in salt in the range of potassium iodate 60-80 PPM, to account for losses during distribution and storage. The recommended minimum level for adequate iodization is >=15 PPM. Inadequate iodization at the site of production and losses during storage and distribution affect the levels of iodine in salt at the household level. A study done in Mekelle, Ethiopia found that the concentration of iodine in the sampled iodized salts decreased by 57% from production site to the consumers at household level, finding that 90% of pregnant women in the region were at risk of insufficient iodine intake (Shawel et al 2010). Additional factors that affect the iodine content in salt at the point of use include humidity, light, heat, pH, storage conditions, and denaturing after prolonged cooking.

We will provide all pregnant women in ENP health centers with a supply of locally produced, adequately iodized salt at enrollment and follow up ANC visits (500 gm-1kg supply). Salt will be procured directly from the producer and tested for iodine levels using the iodometric titration method (APHI) to ensure levels within the recommended range (60-80PPM). We will mitigate losses by direct procurement and storage under proper conditions until distribution (sealed, dry container with minimal light exposure). Assuming a 20% loss during distribution to household level and 20% loss during cooking preparation, a salt with 60 PPM would result in a 35 PPM to the pregnant woman. A pregnant woman would be recommended to use approximately 7grams per day of salt to achieve a 250mcg daily dosage of iodine as recommended by the WHO/IOM. We will provide iodine counseling and education regarding the importance of intake, proper storage conditions, and use after meal preparation. We will create visuals aids and video and monitor usage and intake of iodized salt on a monthly basis.

A sub-sample (n= 350, 175 ENP arm, 175 non-ENP) of women in the third trimester will have their urine tested for iodine using urinary iodine concentration (UIC) testing. We will test 350 households (half ENP, half non-ENP) for iodized salt adequacy using a rapid test kit, such as the MBI Kit (MBI Kits International, Tamilnadu, India).

3C2. Enhanced Infection Management Package (EIMP)

Pregnant women within a health center will be randomized to one of two infection management arms:

A) Enhanced Infection Management Package (EIMP)
B) Routine care

Research staff will not be blinded women who are assigned to EIMP study arm, as they will be performing screening and treatment. If the pregnant woman presents for her first ANC visit in the first trimester, she will be asked to return >12 weeks so that the infection management interventions can be provided during her first 2nd trimester ANC visit.

Enhanced Infection Management Package (EIMP)

One half of women in each health center will randomized to a set of procedures to strengthen the FMOH's current program of screening and treatment of infections in pregnancy. At the first ANC visit (≥12 weeks), women randomized to the EIMP intervention, will receive the following additional interventions:

Table 1: EIMP Components

Infection	**ENAT Enhanced Infection Management Package (EIMP) Activity**
Urinary Tract Infection/Asymptomatic Bacteriuria	*Screen:* Urine culture and antibiotic sensitivity. *Treat:* Initially per clinical protocol, with targeted antibiotic treatment based on antibiotic resistance patterns.
Sexually Transmitted/Reproductive Tract Infections	*Screen:* 1) **ALL pregnant women** for gonorrhea, chlamydia using accurate rapid diagnostic testing for chlamydia and gonorrhea 2) Pregnant women ***with symptoms*** will be screened for trichomonas and bacterial vaginosis *Treat:* All positive cases and partners
Geo-helminths	Presumptive deworming with mebendazole 500 mg or albendazole 400 mg as per MOH guideline; in 3rd trimester a test of cure stool specimen will be taken to screen for persistent helminth infection. If positive, treatment per MOH guidelines

Urinary Tract Infection/Asymptomatic Bacteriuria:

- **Specimen Collection:** A clean catch midstream urine specimen will be obtained for urine culture. The research staff will instruct the woman to spread the labia widely, wipe the external labia with an antiseptic cleansing wipe from front to back, and collect 20-30mL of the midstream urine into a sterile wide-mouthed container.

- **Specimen transport and Lab testing:**
  Urine specimens will be collected and aliquoted into a vacutainer with boric acid preservative using sterile technique. This will allow preservation at room temperature until transportation to the APHI laboratory, and inoculation within 48 hours.

  Urine specimens will be inoculated on standard MacConky and Blood agar plates incubated for 48 hours. Bacterial growth will be quantified, isolated and speciated using standard microbial techniques. Isolates considered significant uropathogens will be inoculated into barium chloride 0.5 McFarland density standard solution before plating on Mueller-Hinton agar. Standard laboratory methods will be used to assess antibiotic susceptibility, including the Vitek method (bioMerieux, Marcy l'Etoile, France), Kirby Bauer Disk Diffusion, and E-strip test method.

- **Classification of UTI:**
  The following definitions (Table 2) will be used to classify UTI:

UTI Terminology	Definition
High-burden growth	bacteriuria of $>10^5$ colony forming units (CFU) per 1mL of urine of a single uropathogen [49],
Intermediate growth	bacteriuria with $>10^3$ -10^5 CFU/mL of a single uropathogen,
Contamination	bacterial growth of $\geq$3 micro-organism OR growth of a non-urinary tract pathogen.
UTI symptoms	dysuria, urinary frequency, urinary urgency, hematuria, abdominal pain, fever, OR flank pain
Symptomatic intermediate growth	women with intermediate burden growth and UTI symptoms (as above)
Asymptomatic bacteriuria	women with high burden bacterial growth without UTI symptoms
Cystitis	women with positive urine culture (high burden or intermediate growth) and symptoms of dysuria, urinary frequency, hematuria, urinary urgency or suprapubic tenderness, without upper urinary tract symptoms (fever, chills, flank or back pain) [49]
Pyelonephritis	women with positive urine culture and systemic symptoms (fever, chills, flank pain or back pain) [49].

- **Results Notification:** Women will be requested to return to the health center within 7 days to receive the laboratory results. We will also consent mothers if they agree to be contacted and be visited at home, to remind them to receive the results and facilitate the treatment at the health center as needed. All mothers with positive test results and a random selection of mothers with negative test results would be followed up if they do not show up for their results at the health center.

  Culture Contamination: If urine results are contaminated, the nurse will request a repeat specimen when the woman returns to the health center or contact the woman to provide a repeat specimen.

  Treatment: At the enrollment ANC visit when urine specimens are first collected, women will initially be managed by health center staff according to FMOH guidelines since the urine culture results may take approximately 3-4 days to process. Women will receive antibiotic treatment if they have high-burden growth or intermediate growth with UTI symptoms. The treatment will be

individually tailored based on the antibiotic sensitivity patterns. Recommendations will be made to health center staff with the appropriate antibiotic choice based on antibiotic resistance patterns. If antibiotic is not available in the regular health center formulary, the research team will procure and provide an alternative EFDA approved oral antibiotic to the pregnant woman at no cost. A seven-day antibiotic course of oral antibiotics will be prescribed. The first dose will be directly observed. For difficult to treat infections, cases will be discussed with and referred to the obstetrics department at the primary hospital, where IV antibiotics are available.

Test of Cure: Women will be requested to return to the health center at next ANC visit to obtain a repeat urine culture to document test of cure.
If repeat cultures are positive, the woman will be retreated based on the antibiotic sensitivity patterns of the second urine culture. If urine cultures remain positive after the 2nd treatment, the women will be referred to the primary level hospital for further management. The research team will cover the costs of transportation and treatment at the referral facility.

- **Specimen Preservation**: The remaining urine specimen after aliquoting the culture specimen will be saved in -80C in cryo-freezers at APHI, for testing to determine the prevalence of other infections (viruses including cytomegalovirus infections) and environmental toxin exposures.

Sexually Transmitted/Reproductive Tract Infections

ALL PREGNANT WOMEN in the EIMP arm (symptomatic or asymptomatic), will self-collect vaginal specimens for testing for **gonorrhea and chlamydia**.

- **Specimen Collection:** Self-administered vaginal swabs have been used in diverse populations including in South Asia and Africa, with high acceptability and quality, providing cost-savings and cost effectiveness for STI testing (Christian et al. 2011; Knox et al. 2002, Blake et al 2008; Anemona et al. 2002). The acceptability and feasibility of self-collection of vaginal swabs were assessed during formative research in the ENAT study site in October 2018. This was considered acceptable in the study population with a low refusal rate (<4%). Women will be instructed by the study nurse to insert a single vaginal swab (one Xpert vaginal swab) ~4-5 cm into the vagina, allow the swab to stand for 15 seconds, and rub the lateral walls of the vagina for 4-5 seconds prior to withdrawal (Ugwumadu et al. 2004).

- **Specimen Transport:**
  Samples collected by Xpert vaginal swabs for gonorrhea and chlamydia testing will be kept at room temperature (Xpert kit stability at 2-30C for up to 60 days) and transported to the APHI laboratory within 1 week of collection.

- **Diagnostic Testing:**
  - **Chlamydia and Gonorrhea** will be tested using the *Xpert® CT/NG assay* (*Cepheid*, Sunnyvale, CA) at the APHI laboratory. The Xpert test will be performed by a laboratory technician at APHI using a modular cartridge-based platform for testing each specimen by nucleic acid amplification. Vaginal swabs will be manually transferred from transportation tubes to individual Xpert CT/NG test cartridges, and then inserted into the GeneXpert® instrument for analysis by the Dx system computer software. The test takes approximately 90 minutes and yields a positive or negative result separately for chlamydia and gonorrhea, or an indeterminate result. Specimens generating an indeterminate result will be retested.

- **Results Notification:** Given that the Gonorrhea and Chlamydia testing will be done at the APHI, there will be a delay of up to 1 week until the test results are available. Symptomatic women will have been already treated according to the FMOH standard of care (i.e. syndromic management) during her ANC visit at the health center.

All women in the EIMP arm will be requested to return to the health center within 7 days to receive the laboratory results. We will also consent mothers, if they agree to be visited at home, to remind them to receive the results and facilitate the treatment at the health center as needed. All mothers with positive test results and a random selection of mothers with negative test results would be followed up if they do not show up for their results at the health center. Women who do not consent to be contacted by study staff will be treated at the next ANC visit.

- **Treatment** (CDC Sexually Transmitted Diseases 2015)**:** Symptomatic women will have been already treated according to the FMOH standard of care (i.e. syndromic management) during her initial ANC visit at the health center. If mothers were NOT previously treated, then she will receive directly observed treatment as below:
    - Chlamydia will be treated with Azithromycin 1 gm orally once
    - Gonorrhea will be treated with Ceftriaxone 250 mg IM once + Azithromycin 1gm orally once

  Partner Treatment (CDC 2015)**:**
  All women diagnosed with Gonorrhea and Chlamydia infections will be counseled about the importance of urgent partner evaluation and treatment. The benefits of partner treatment will be explained in detail for the mother that the partner needs to be treated so that she will not be re-infected. The mother will also be counseled to bring their partners into the health center for treatment and counseled on using condoms to prevent STI transmission. As stated in the National STI management guidelines, the process of partner treatment will be on a voluntary basis and non-coercive (Ethiopian National Guidelines for STI Management, 2015). The partner will be treated in the health center with:
    - Chlamydia: Azithromycin 1gm orally once for partner
    - Gonorrhea: Ceftriaxone 250 mg IM once AND Azithromycin 1gm orally once for partner
- **Test of Cure Sample** (CDC 2015)
  Test of cure at next ANC visit (target ~4 weeks after treatment)

For symptomatic women in the EIMP arm:

For pregnant women enrolled in the EIMP arm **WHO REPORT SYMPTOMS** during their study visit at the health clinic defined as below, an additional self-collected vaginal swab will be collected for testing for trichomonas and bacterial vaginosis for immediate diagnosis and treatment at the health clinic.

STI symptoms will be defined based on the Ethiopian FMOH guidelines (National Guidelines for the Management of Sexually Transmitted Infections 2015) as:

- Abnormal Vaginal discharge
- Vulvar itching, burning, erythema, or pain
- Lower abdominal tenderness

- **Point of Care Diagnostic Testing at the Health Clinic**

    - **Trichomonas** will be tested using the OSOM® trichomonas rapid test (Sekisui Diagnostics, Lexington, MA). Using the supplier dropper top, 0.5 ml of sample buffer will be added to flexible plastic test tubes. Vaginal swabs will be placed in the tubes containing sample buffer, mixed vigorously, and allowed to sit for approximately 1 min. Excess liquid will be removed from the swabs by squeezing the sides of the test tubes, and the swabs will be removed. An OSOM® trichomonas rapid test stick will then be placed in each tube containing the buffer-sample mixture, and results will be read at 10 min. The presence of a blue test line along with a red control line will indicate a positive result, whereas only visible red control line will indicate a negative result. The test will be repeated if the result is invalid (no visible red control line).
    - **Bacterial vaginosis** will be tested a rapid POC diagnostic (such as OSOM® BVBLUE® test (Sekisui Diagnostics, Lexington, MA), or Diagnosit® BVBLUE® test (Gryphus Diagnostics,

Knoxville, TN). A vaginal swab will be placed in the BVBlue test vessel containing the chromogenic substrate of bacterial sialidase and the mixture will be gently swirled. The BV test vessel containing the swab will be left standing for 10 minutes. One drop of developer solution will be added to the BV test vessel and the mixture will be swirled gently again. Results will be read immediately; a blue or green color in the BV test vessel or on the head of the swab will be considered positive, while a yellow color in the BV test vessel will be considered negative. The test will be repeated if the result is not blue/green or yellow.

- **Treatment** (CDC Sexually Transmitted Diseases 2015)**:** Women with positive test results on point of care diagnostic testing positive will treated.
    - Trichomonas will be treated with Metronidazole 2 grams orally once
    - Bacterial Vaginosis will be treated with metronidazole 500 mg twice daily x 7 days

- **Partner Treatment:** Trichomonas will be treated with Metronidazole 2 grams orally once for partner.

- **Adherence:** Single dose therapy will be directly observed. Treatment adherence for Bacterial Vaginosis will be measured by a medication diary and pill count at the next ANC visit.

Geo-helminths/Deworming:

Ethiopia is considered endemic for geo-helminths (baseline prevalence of hookworm and/or T. trichiura infection is $\geq$20% (WHO 2016)

- **Presumptive Treatment:** For all pregnant mothers, mebendazole (500 mg) or albendazole (400 mg) will be provided in once in the 2nd and 3rd trimesters, as per the 2016 WHO Guidelines (preventive chemotherapy to control soil-transmitted helminth infections in high-risk groups).

  The first dose will be provided at health center by health center staff at the enrollment visit, and the 2nd dose at an ANC at least 4 weeks after the initial dose. Treatment will be directly observed therapy (DOT) at ANC visits. If a woman does not return for ANC at the health center by the 3rd trimester, she will be contacted by study staff to reminder her to return for her 2nd dose.

3C3. Follow Up Antenatal Care Visits

Additional data and measurements will be performed at all subsequent ANC visits in the health center. We anticipate data collection may occur on up to 3 further contact points, recommended by the FMOH (2nd trimester: 20-<30 weeks, early 3rd trimester: 30-36 weeks, and later 3rd trimester 36-40 weeks), but up to monthly depending on the timing of uptake of new WHO recommendations for monthly ANC visits. During the follow up ANC visits, research staff will interview women about their health status, morbidity, pregnancy history/complications, counselling/services received, maternal mental health screen, and dietary intake. Data will be collected from ANC records, including blood pressure, lab testing results, and management. Maternal weight and mid-upper arm circumference (in ENP facilities) will also be measured. In a subset of 200 women, a semi-quantitative food frequency questionnaire will be administered at least one ANC visit each visit. As per FMOH recommendations, hemoglobin will be measured during a 2nd and early 3rd trimester ANC visit.

During both 2nd and 3rd trimester recommended ANC hemoglobin screenings; additional blood will be collected on filter paper (Whatman card) to preserve for later inflammation biomarker testing. 2-3 drops of blood will be placed on filter paper and allowed to dry at room temperature, stored in a Ziplock bag with desiccant (silica gel) until they are able to be transported to the APHI lab for storage at -20 C or -80C. The samples will be preserved for up to 5 years. A repeat ultrasound will be performed in 3rd trimester to monitor fetal growth and assess the position of the baby. If the fetus is determined to be in non-vertex positioning, the nurse will recommend that the women deliver in a primary hospital with Cesarean section capacity.

3C4. Birth Notification
A community-based notification system will be developed by the study team during the pilot phase. Family members of pregnant women will be requested to call/notify study staff immediately after delivery. The occurrence of home births will also be identified and reported by community health worker cadres, including health development armies and health extension workers, or by community focal persons/health promoters prearranged for the purpose of this study.

3C5. Intrapartum Visit
For women who deliver in the health center or hospital, data will be gathered from women (via-self report) and from chart review about the delivery and immediate postpartum period. Medical records will be reviewed for intrapartum history (e.g. vital signs, duration of labor), delivery complications, and maternal/neonatal morbidity.

For up to 1200 infants who are delivered in a study health center or hospital, we will collect 2-3 drops of umbilical cord blood on a Whatman card at birth. Cord blood will only be collected at delivery from health center births. The Whatman card will be allowed to air dry at room temperature and then stored in Ziplock bags with silicone desiccant . These samples will be used for inflammation biomarker testing as described below. Samples will be preserved for up to 5 years.

3C6. Postpartum Visit
First Visit After Birth (<72 hours of life): Participants who deliver in health facilities will be assessed by research staff based in health centers or hospitals as soon as possible after birth, but within 72 hours of life. For deliveries that occur at home, a home visit will be made by research staff (data collectors) as soon as possible upon birth notification (within 72 hours). Maternal history will be obtained for pregnancy health, delivery history/complications, and postpartum maternal/neonatal morbidity.

Infant anthropometrics: ***Weight*** of the unclothed infant will be measured using a high quality digital infant scale (ADE M112600, Germany; precision 5 gm). ***Infant length*** will be measured using a portable infantometer (Perspective Enterprises PE-RILB-LTWT, Michigan USA, precision 1 mm); which has a fixed headboard and movable footboard. Recumbent length will be recorded to the last completed (not the nearest) mm. ***Head, chest, and mid upper arm circumferences (MUAC)*** will be measured to the nearest millimeter (mm) using insertion tapes (Shorr productions, Maryland USA). Infant length and circumferences will be measured twice. Regular calibration checks will be made before each use of weighing scales, and length boards to ensure accuracy of measurement (WHO child growth standards 2007; de Onis et al. 2004).

3C7. Maternal/Infant Follow Up Visits
Mothers and infants will be visited at 1, 3, 6 months after birth to assess vital signs, measure infant/maternal anthropometrics, and obtain a history of feeding, dietary intake, morbidity/illness, maternal mental health, signs of neonatal/maternal infection and history of hospitalization. At each post-partum visit, we will also obtain a maternal food frequency questionnaire (FFQ), 24-hour dietary history, a 24-hour breast-feeding diary, infant morbidity and feeding history. A brief infant neurodevelopmental screening will be done using a tool such as the World Health Organization GSED or the Caregiver Reported Early Childhood Development Instrument (CREDI), which has been used in LMIC settings (McCoy 2017). An assessment of the home environment will be done at 1 month by a brief questionnaire, the Family Care Indicators from UNICEF's MICs survey.

3D. Randomization
At the **first level of randomization**, we plan to randomize **clusters (i.e. health centers)** into one of two nutrition interventions:

a) the Enhanced Nutrition Package (ENP)
b) Non-ENP/routine care

Cluster randomization of the health center will reduce the likelihood of unintended crossovers, limit opportunities for community members to exchange experiences, and increase the ease of distribution of the nutrition package. We will seek to conduct a constrained randomization to ensure that some key indicators are balanced across the two arms of the study; such indicators would include an estimate of number of pregnancies (ANC volume) or crude birth rate (to ensure balance in size of clusters, thereby improving statistical efficiency).

In general, our approach will be to 1) set reasonable tolerance levels for the restriction variables, 2) create a large number of random sequences, where each sequence allocated 6 health centers to the ENAT Nutrition Package and 6 health centers to routine care, 3) assess each sequence as to whether or not it meets these restriction criteria, and 4) ultimately choose randomly from the subset of all such allocation sequence that meet the criteria. Randomization procedures will be conducted in R, a statistical software package.

At the **second level of randomization,** we will randomize **individual pregnant women** presenting for ANC at each health center to receive:

a) ***ENAT Enhanced Infection Management Package (EIMP)***, or
b) routine care

Each health center will receive a pre-generated randomization list with an equal allocation to each of the two arms. Each allocation sequence will be constructed using randomly permuted blocks of size 4, 6, 8, 10, or 12.

We constructed all possible randomization sequences (n=924), and selected the subset of sequences where the following ratios were within 1.4 or 1/1.4 of unity:

- ratio of the sum of total populations in each group (nutrition vs. non-nutrition)
- ratio of the sum of total anc4 in past 6 months (nutrition facilities vs non-nutrition facilities)
- ratio of the sum of total births in the past 6 months (nutrition facilities vs non-nutrition facilities)
- average minutes to Bahir Dar (nutrition facilities vs non-nutrition facilities)

Among the 438 sequences meeting these criteria, we selected on sequence at random to be the allocation for the health centers.

3E. Statistical Analysis

3E1. Study Outcomes/Endpoints

The **primary outcomes** are

P1. Newborn weight measured within 72 hours of birth
P2. Newborn length measured within 72 hours of birth

The **secondary outcomes** include:

S1. Length of gestation, with gestational age determined by <24-week pregnancy ultrasonography
S2a. Proportion of pregnancies resulting in spontaneous preterm delivery
S2b. Proportion of live births born <37 weeks gestation
S3. Proportion of newborns born of low birthweight (<2500 grams), as measured within 72 hours of life
S4. Proportion of newborns born small-for-gestational age, as defined by the INTERGROWTH 21st neonatal birth weight standard.
S5. Stillbirth rate
S6. Newborn head circumference within 72 hours of birth
S7. Infant Z-scores for weight-for-age, length-for-age, head circumference-for-age within 72 hours of birth
S8. Maternal gestational weight gain
S9. Maternal anemia (3rd trimester)

Table 3: ENAT Study Outcomes

Primary outcomes	
P1. Newborn weight <72 hours of birth	Weight of the unclothed infant measured at <72 hours of life
P2. Newborn length measured at birth	Infant Length measured at <72 hours of life
Secondary outcomes	
S1. Gestational age	Gestational age determined by enrollment ultrasound, CRL used if <95 mm (Intergrowth 21st), then BPD/FL (WHO Kiserud) used if CRL>95 mm or missing
S2a. Proportion of pregnancies resulting in preterm delivery	Numerator: number of pregnancies resulting in spontaneous termination of pregnancy from 24 to <37 weeks (including preterm live birth or fetal loss (spontaneous pregnancy loss 24 to <37 weeks, not due to induced abortion)). Denominator: All pregnancy outcomes ≥24 weeks
S2b. Preterm live birth	Numerator: Live births <37 weeks of gestation Denominator: All Livebirths
S3. Small for Gestational Age[a] (Intergrowth)	<10% birthweight for GA by sex compared to Intergrowth reference[2]
S4. Low birthweight rate	Low birthweight is defined as birthweight (measured within the first 72 h of life) of <2500 g. We will also assess the outcome of birthweight <2000 g.
S5. Stillbirth rate	Numerator: Stillbirth (≥28 weeks gestation)- fetal death with no signs of life *A preterm stillbirth is defined as an infant born without signs of life (no spontaneous crying, breathing, and/or movement) at 28 to <37 weeks gestation.* *A term stillbirth is defined as an infant born without signs of life at ≥37 wks* Denominator: All live births and stillbirths ≥28 weeks

S6. Newborn head circumference	Head circumference of the infant measured at <72 hours of age
S7. Newborn weight, length, and head circumference for age Z-scores	Infant weight, length, and head circumference for age z-scores measured at <72 hours of life, calculated using the Intergrowth reference for size at birth.
S8. Rate of weight gain in pregnancy	Maternal weight gain (kg) per week gestation in the 2nd and 3rd trimester
S9. Maternal anemia	Mean hemoglobin concentration in 3rd trimester (Mission hemoglobinometer)

3E2. Sample Size Estimates

We have estimated the effect size detectable with 80% power under a cluster-randomised design, with six health centers per study arm. Fixing recruitment of pregnant women to 18 months, we estimated that within this time period the average health center in the proposed study site would enroll around 200 women into ANC at ≤24 weeks gestation and would yield 112 live born infants weighed within 72 hours of life (assuming~70% of enrolled pregnancies result in a live birth and ~80%are followed-up and weighed within 72 hours). Beyond the above determination of average cluster size, we have additionally made the following assumptions in order to estimate effect sizes detectable with 80% power: (1) mean birth weight and SD as per prior studies in Gondor (mean birth weight of 2900g, SD 450g) and (2) variation in distribution of weight between clusters as reflected through an coefficient of variation (k=0.01) (West KP 2014). In total this includes 2400 pregnant mothers enrolled in 12 health centres, resulting in 1440 live births with a birth weight within 72 hours. This sample size provides 80% power to detect a 66g difference in birth weight between the ENAT EIMP or routine care group in a marginal analysis (ie, irrespective of whether mothers did or did not receive the ENP), and a 90g difference between ENP versus routine care (marginal analysis).

With the assumptions of clusters and enrolment above, we assumed mean infant length of 49.5cm (SD 2.4) (based on data from Malawi (Luntamo 2010) and coefficient of variation k=0.008 (sector level variation in JiVitA study) (West 2014). For the EIMP versus routine care comparison, we would have 80% power to detect a 3.0mm difference in mean infant length. For the ENP versus non-ENP comparison (marginal analysis) we would have 80% power to detect a 7.8 mm difference in infant length between the women receiving the package of enhanced nutrition-infection compared with standard nutrition care.

Given the highly correlated nature of the outcomes, birthweight and length, we did not adjust for multiple comparisons in our power calculations (Schulz KF 2005).

3E3. Data Analysis

Data collected in this study will enable us to conduct a comprehensive analysis of the impact of interventions randomized at the health center and individual level. The primary outcomes are mean infant weight measured within 72 hours of birth and mean infant length measured within 72 hours of birth. Our statistical approach will have multiple steps, and will include a description of the health centers and pregnant women enrolled in the study, a descriptive quantitative analysis of variables at multiple levels to assess the degree to which our randomization scheme resulted in similar sub-populations of pregnant women, an assessment of receipt and adherence/compliance with interventions offered, descriptive analyses of the primary and secondary outcomes, comparison of the outcomes between intervention groups, assessment of potential effect modifiers, and pre-specified sub-group analysis. We will estimate marginal effects, and the combined effects of both interventions compared to the group receiving neither intervention.

Participant Flow
First, we will fully describe participation of health centers, approached/recruited/enrolled pregnant women, and their infants in the study, through a series of flowcharts. These participant flow diagrams will form the base analyses for the eventual Consort Flow Diagram that will be utilized in formal reporting of the main result(s) of the study. The flow diagrams will provide details at both the cluster and individual level, with the former focusing on means and range of cluster size included in each stage, and the latter depicting numbers of women retained at each step, allowing for assessment of follow-up / completion of study participation. In the primary analysis we will include all women who were enrolled and randomized as intention to treat. In sensitivity analysis, we will also conduct analysis among women who were enrolled at <24 weeks confirmed by ultrasound-based dating.

Baseline comparability of health centers and participants
Next, we will conduct a complete descriptive analysis of the health centers and participants in the study, stratified by intervention group, in order to assess comparability of groups. These descriptive analyses are done at multiple levels, including health center, household, maternal/paternal, and infant. Tables 1-3 show planned baseline cluster and participant characteristic tables. Variables that appear imbalanced across arms will be noted for possible incorporation as potential confounders in models estimating the impact of the intervention(s). This description of the comparability of groups is done among the entire set of women enrolled in the study, and also among the subset of women that ultimately contribute an infant(s) in the primary outcome analysis.

Analysis Population
For this pragmatic effectiveness study, the primary analysis will be intention to treat (ITT), following the randomized allocation study arms. Study intervention groups will include all eligible, randomized participants, who will be analyzed according to the study arm in which they were randomized, irrespective of the actual interventions or treatment received.

We will additionally conduct secondary analysis using per protocol analysis. In this analysis the study intervention population will only include those who received a minimum threshold of nutrition intervention components for the ENP arm (ie. TBD, >50% or >75% threshold of BEP adherence), or who received a threshold (TBD) of recommended EIMP interventions (screening-treatment for UTI, STI/RTI or deworming).

Study Intervention Coverage
1) Coverage of routine ANC components
First, at the health center level, health systems strengthening will be assessed across a broad range of antenatal care services and interventions, including 1) basic measurements and monitoring (for example weight, height, blood pressure, etc.), 2) counseling across a range of domains including nutrition, birth preparedness, normal progression of pregnancy, care-seeking for maternal, and subsequently, neonatal danger signs, 3) provision of commodities, and 4) recommended routine lab services (for example, urine and blood tests). While this effort of health systems strengthening is applied uniformly across the 12 health centers engaged in the ENAT study, differences between the ENP and non-ENP centers may certainly arise, due to inter-center variability in programmatic inputs, pre-implementation differences in service provision not adequately accounted for in the restricted randomization process, and/or variability in catchment population characteristics across the health centers.

2) Coverage of Nutrition Interventions (ENP Arm)
Second, we will examine the individual-level receipt and adherence to ENAT-specific ENP activities: iron folate, iodized salt, nutrition counselling and BEP supplement (Table 4). All women enrolled in ENP clusters should receive IFA and iodized salt, regardless of the MUAC measurement at enrollment, while those with a screening MUAC <23 will also receive the BEP supplement. For both types of interventions, we will report the proportion of eligible women receiving the initial supplement, the mean/median gestational age at first receipt, and the adherence to the supplements, expressed in a number of ways. Adherence will be expressed in absolute terms (number of doses consumed) for each intervention, an adherence index (proportion of possible/expected doses consumed based on difference between GA at receipt and GA at

delivery), and adherence over the pregnancy gestation (trimesters) (within women and across participants). Adherence will also be expressed as proportion exceeding various thresholds of compliance (for example, % exceeding 70% adherence, etc.) We will describe the distribution of adherence indicators overall, and by clusters (i.e., between health centers). Percentage of women self-reporting receipt of nutrition counseling/messages from health providers will be examined by intervention arm .

3) Coverage of Infection Management Interventions (EIMP Arm)
Third, we will describe the receipt and adherence to individual-level randomized EIMP interventions (Table 5). These comprise the two groups of women within each health center: enhanced infection management package (EIMP) and routine care.

The one-half of women in each health center that are randomized to the EIMP package will receive screening (and treatment, if necessary) for bacteriuria, screening (and treatment, if necessary) for sexual and reproductive infections, and deworming. For UTI/bacteriuria screening, we will estimate the proportion of women providing a urine sample, quantitative indicators related to the collection, handling and processing of the sample (for example, time between collection and laboratory receipt, time to results or treatment etc), and the proportion (overall, and among those providing a sample) for whom classification across the range of UTI definitions (web appendix 1) are available. Measures of intervention delivery and compliance in terms of treatment are assessed by describing the proportion of women who initiate treatment (among those requiring such), and the number of antibiotic doses taken. We will follow a similar analytic approach to describe the completeness of the screen and treat protocol for sexually transmitted infections. For the third EIMP component, deworming, we will estimate the proportion of women receiving both, one, or none of the deworming doses, the average and distributional characteristics of gestational age at receipt, and the time between doses. For the women enrolled after May 2021 when the stool screen-treat intervention was introduced, data will be summarized similar to the UTI screen-treat approach.

Impact of Interventions on Primary Study Outcomes
After fully describing the clusters, participants, and the interventions received, the next stage of analysis will focus on outcomes and the impact of the interventions on those outcomes (Table 6). The primary outcomes are birth weight (grams) and infant length (cm). In general, we will estimate marginal effects of EIMP (vs non-EIMP) and ENP (vs non-ENP) (i.e., ignoring interactive effects between the infection-oriented interventions and the nutrition-oriented interventions) on the primary outcomes. We will also estimate the combined effects of both interventions (EIMP+ ENP) vs routine care receiving neither intervention . Given inadequate power, we will not test for interaction. In all cases we will follow an intention-to-treat approach as the primary analysis. As recommended by CONSORT and FDA, we will *a priori* select prognostic factors that are established to be associated with outcomes to adjust for in the primary and secondary analysis. These will include: maternal age, parity, BMI, height, education, and occupation (Holmberg MJ, Andersen LW., 2022). We will also adjust for any imbalanced baseline covariates. Implausible birth anthropometrics will be defined as values with z-score <-5 or >5 per Intergrowth newborn anthropometric standards (Intergrowth 2014), as recommended by the Child Health Epidemiology Reference Group/Small Vulnerable Newborn analysis technical working groups.

EIMP effect: Below, we provide a description of our statistical approach using the primary outcome **(P1: weight)** and the comparison of EIMP vs non-EIMP, as an illustrative example; the approach is similar for the other continuous outcomes **(P2: length)**. Among all participating women, we will describe the proportion whose pregnancy outcome is known and whose pregnancy ends in one or more live births (i.e., see description of patient flow diagrams above). The mean (SD), median, and IQR of first weight after birth is described overall, and among those where the weight was recorded within 72 hours. Our comparative contrast of interest is to compare the mean first weight after birth between the women receiving the EIMP package and the routine care, irrespective of ENP status. We will estimate the difference in mean weight along with a 95% confidence interval with multivariate linear regression model with robust variance accounting for clustering at the health center level. When conducting these analyses, we will incorporate as necessary covariates that might confound the true relationship between intervention group and birthweight using multivariate regression.

ENP Effect: To estimate the impact of the ENP package , the initial approach is to compare the birthweight [and length] of babies born to the half of women allocated to routine care in the ENP health centers with the birthweight [length] of the babies born to half of women allocated to routine care in in the non ENP centers. As this intervention is allocated at the center level, our estimate of the standard error in the difference in mean birthweight [or length] is underestimated unless we account for the correlation within clusters (or equivalently, the difference between clusters). Given the small number of clusters (6 per group), we will conduct cluster-level analysis and estimate the mean birthweight separately for each cluster and compare the distribution of cluster-specific birthweights between study arms using a t-test. The mean cluster specific birthweights will be adjusted for the a priori identified prognostic factors.

EIMP+ENP Effect: Finally, we will examine the effect of the combined EIMP + ENP intervention on primary outcome compared to the routine care group receiving neither intervention. This will be limited to pregnancies randomized to those study arms and be calculated using generalized estimating equations accounting for clustering by health center, and adjusting for a priori identified prognostic factors.

Analysis of Secondary Outcomes

For secondary outcomes, we will take a similar approach to the analysis as for the primary analysis. **S1 (Mean length of gestation):** this analysis is conducted on all enrolled women for whom we have a date of delivery/pregnancy termination, excluding early miscarriages. Our first approach is to use gestational age as determined by ultrasound; we will follow by conducting parallel analyses where, i) missing GA by ultrasound is supplemented by GA estimated by LMP, and then ii) all GA measures are replaced by GA estimated by LMP. In each case, the mean GA is compared across intervention groups and 95% confidence intervals constructed (with adjustment for cluster randomization, when necessary, i.e., when the contrast under study includes the cluster-level randomization at the health center level). ENP effects will be determined with cluster level analysis, EIMP with multiple linear regression and robust variance to account for clustering at the health center level. EIMP+ENP effects will be determine with GEE equations accounting for clustering at the health center level. **S2 (preterm):** We will utilize these estimates of GA to construct binary variables such as % women delivering <37 weeks, or <34 weeks, estimate the proportion across groups. For EIMP effects we will construct risk ratios (and accompanying 95% confidence intervals) using binomial regression models with log link functions with robust variance to account for clustering at health center. To estimate ENP effects, we will compile cluster level summaries and estimate risk differences using t-tests. For the combined ENP-EIMP, we will use GEE to account for clustering by health center. This same approach will also be used for all dichotomous outcomes. **S3 (% low birthweight)** and **S4 (% SGA)**, with the exception that these outcomes are defined only for the subset of pregnancies that end in live birth and are measured within 72 hours of birth. Low birthweight (S3) is defined as those <2500 grams. SGA (S4) is defined using the sex, weight, and gestational age estimate and adhere to the Intergrowth 21st standard for SGA). **S5 (Stillbirth):** We will define stillbirth as fetal deaths occurring after 28 weeks' gestation; to maximize the power for estimating stillbirth rates and to estimate impact of interventions on this outcome, we will utilize GA estimated by ultrasound complemented with GA estimated by LMP to define the subset of pregnancy/fetus dyads contributing to this analysis. The number of stillbirths per 1000 births is estimated overall and by intervention group, and rate ratios for contrasts (along with 95% confidence intervals) are constructed using binomial regression models with log link functions. Again, where necessary, we will utilize either generalized estimating equations to account for clustered randomization, or compile cluster-level summaries and estimate risk differences using t-tests. **S6 (Newborn Head circumference):** Head circumference will be analyzed in a similar manner to P1 and P2. **S7 (Infant Z scores for WAZ, LAZ, HCAZ)** Z-scores at birth will be calculated using the Intergrowth 21st newborn standard; the analytic approach will be similar to other continuous measures described above.

S8 (Maternal weight gain): First, the difference between pregnancy weight at enrollment and weight at last ANC visit prior to delivery is estimated for all women. We will describe the mean, median, and other distributional characteristics of this measure overall, and across the 6 intervention-exposure groups. This simple approach is unlikely to adequately describe the trajectory of weight gain/loss during pregnancy as women's initial and last weight is measured at different times (i.e., gestational ages) during pregnancy.

Further, we will have additional measures of weight collected between these initial and last weights. These repeated measures of weight across different time points are leveraged to estimate using longitudinal models, overall and separately by group, the weight trajectory during pregnancy. We will use generalized linear mixed effects models to estimate the impact of the interventions on these weight patterns. For **S9 (anemia)**, we will estimate the mean hemoglobin levels by group and the proportion below cutoffs for anemia (defined as hemoglobin concentration < 110 g/L), following approaches for continuous and binary outcomes, respectively, described above.

3E4. Additional Statistical Considerations

Sub-Group Analysis

The following p re-specified sub-group analysis will be performed:

- MUAC: <23 cm vs >23 cm)
- Maternal BMI <18.5, 18.5-<25, >=25 kg/m2
- Maternal height
- Nulliparous vs multiparous
- Maternal age <20 vs >20

Consideration of Intervention Intensity and Supplement Adherence

Given the pragmatic nature of the ENAT study in the Ethiopian health system, we anticipate that some participants in the intervention arms may not receive all intervention package components, adhere fully to the nutrition interventions or medications. Similarly, we anticipate that some participants in the standard care arms may receive some study interventions or screenings. We will conduct the following sensitivity analyses.

A) We will also explore the associations of receipt and intensity of different intervention components (deworming, nutrition counseling, nutrition supplements) and study outcomes, controlling for potential confounders. This will include dose-response analysis with consideration of adherence to nutritional supplements (BEP, IFA) [categoried by high vs low adherence, by quartile or tertile]. We will examine a composite score to indicate receipt of the number of interventions in the ENAT packages (nutrition counseling, IFA, iodized salt, BEP, deworming).

B) Given that intention to treat analysis may underestimate intervention efficacy with non-adherence, in sensitivity analysis, we may estimate the complier average causal effect (CACE) parameter to estimate treatment efficacy accounting for imperfect adherence.

Missing Data

A primary reason for missing data will be losses of participants to follow-up. We will describe the patterns and participant characteristics of those who are lost to follow up. We will enumerate loss to follow-up at the cluster and individual levels and missing values among key variables, and report reasons for missingness to assess plausibility of missing at random (MAR) assumptions. If participants are not seen at a study visit, we will attempt to make telephone contact to obtain basic pregnancy outcome information, including the date of the pregnancy outcome and intervention adherence. In the primary analysis, we will not impute outcome data.

If it is required to provide adjusted estimates of effect sizes for the primary or secondary study outcomes, if there is missing data of baseline SES characteristics of >5% and the data is assumed to be missing at random, we may use multiple imputation to replace missing covariate data. If missingness is substantial, we may impute outcome data as sensitivity analyses.

3E5. Data Management

The core of the data collection system is the Survey Solutions platform (World Bank, V.20.08, 2021). Study nurses enter data directly into electronic tablets with programmed validity checks during study visits. Paper forms are used if tablets are temporarily unavailable. The tablets are regularly synchronised to the server on the ACIPH campus. A web-based dashboard supports data collectors, supervisors and investigators in real time management and monitoring of study activities.

Device and data security will be overseen by the Data Supervisor at Addis Continental Institute of Public Health. The tablet for point-of-contact data collection (and for subsequent entry of information collected on paper forms) will be secured by encryption, and access to both the device/operating system and the data collection application level will be password protected using strong passwords. User accounts and permissions will be set up and managed by the Data Supervisor; each user will log in to the study device and the data collection application(s) with their own individual username and password, logging out upon completion of their work (devices will also auto-off with required re-entry of password to re-open applications).

Data Encryption and Transfer
After each data form is finalized on the device, Survey Solutions tools encrypt each form with its own unique 256-bit encryption key prior to transfer. In addition, our server will be configured with a TSL/SSL certificate, ensuring that both the data being transferred from the device to the server, and the transfer process itself are encrypted.

3E6. Routine Study Monitoring
In the field site, data collectors will be supervised by ENAT study supervisors at the health center and woreda level, who will routinely (bi-weekly) check the accuracy and completeness of informed consent forms, data modules/forms, and timely reporting of adverse events.

The data-coordinating center at ACIPH will collect and summarize data routinely for reporting and quality assurance. This report will include the following:

- Number of subjects recruited for the study, and a summary of reasons for exclusion
- Summary of missing data, particularly outcome data and subjects lost to follow-up
- Proportion of subjects' adherent to the intervention
- Summary of timeliness of data entry and data transfer to ACIPH coordinating center
- Baseline characteristics by intervention group to assess randomization
- Intervention coverage
- Laboratory results, treatment and follow up
- Number of births followed up and weighed

The reports will be generated and reviewed routinely for the study team.

Study monitoring committee
An external Study Monitoring Committee (SMC) is established to monitor the progress of the study, including enrollment , progress indicators and adverse events. The SMC includes an independent Ethiopian obstetrician (Dr Delayehu Bekele) and an epidemiologist (Professor Simon Cousens). The committee met before study initiation, at mid-enrolment and every 6 months to review study progress. Interim analysis will not be performed.

Table 4: Data forms

Module Name	Timing	Content
BEP Diary	Monthly follow-up	Assessment of when and how often woman is taking BEP
Healthcare Access	Enrollment visit	Access to healthcare, distance to facility, average amount of time waiting for care, affordability of services, health insurance coverage
Identification	Every visit	Participant ID number, name, date of visit, staff ID, MRN, zone, district/woreda, health center, kebele, village/gote, house number

Infant Anthros and Vitals	Intrapartum visit, postpartum visit (1, 3, 6)	Length, weight, head circumference, MUAC, chest circumference, respiratory rate, temperature
Infant Birth Assessment	Intrapartum visit	Infant vital status, infant vitals, infant crying/breathing behavior, hygiene around infant caretaking/handling events, umbilical cord behavior, infant skincare, initiation of breastfeeding/colostrum, birth anthros, neonatal adverse events, treatments
Infant Neurodevelopment	Postpartum visit (6)	Adapted Caregiver Reported Early Childhood Development Instrument (CREDI) or GSED
Lab Result	As needed	Laboratory results for UTI (APHI), RTI (POC), and STI (APHI)
Maternal Morbidity and Care Seeking Behavior	Every visit	In the last 7 days and throughout pregnancy: general symptoms (fever, chills, headache, swelling of feet/hands/legs, vision changes, abdominal pain, fetal movement, rupture of membranes, convulsions, skin infection, diarrhea, jaundice), vaginal symptoms (bleeding, discharge, redness/itching, pain during intercourse), UTI symptoms, respiratory (chest pain, shortness of breath, cough), GI (abdominal pain, nausea, vomiting, diarrhea, constipation) Illness, treatment sought for illness (name, dose and duration), where treatment took place, how long was mother hospitalized, follow up appointments
Maternal Anthros and Vital Signs	Every visit	Maternal weight, height (only enrollment), blood pressure, temperature, pulse, respiratory rate, MUAC
Maternal Birth	Intrapartum visit	Date and time of admission, place and time of delivery, mode of delivery, method of delivery, home vs. facility delivery, hygiene around delivery events, assistance during delivery, medicines given during childbirth, length of labor, length of delivery, what was the outcome, EGA
Maternal Diet	Every visit	FFQ (consumption of major food groups in the past 7 days, how many days food was consumed, consumption of food within the past 24 hours)
Maternal Environment	Enrollment visit	Measuring physical environment, physical workload, tobacco/chaat/alcohol use, primary fuel source, access to clean water, general questions about occupation and farming, access to main road
Maternal Stress, Anxiety/Depression, IPV	Enrollment visit	PHQ-9, Cohen's Perceived Stress Scale (PSS)
Nutrition Distribution and Adherence	Monthly follow-up	IFA, BEP, and iodine intake info (distributed, consumed, missed, sold, shared, returned), reasons for missed nutrition, side effects
OB and FGC History	Enrollment visit	OB History: LMP, past contraceptive use, number of pregnancies, outcomes of past pregnancies (liveborn, stillborn, miscarriage, abortion), past preterm birth and low birthweight, mode and place of recent delivery, and number and sex of alive children FGC History: (Questions adapted from Ethiopia DHS); Type of circumcision; who performed circumcision
Partner Treatment	As needed	Partner disclosure, partner status, treatment status
Postnatal Maternal and Infant Followup	Postpartum visit (1, 3, 6)	Infant vital status and hospitalizations, childbirth care practices, infant danger signs, infant feeding, infant complications, services and counseling received at the postpartum visits
Respectful Maternity Care	Postpartum visit (1)	Adapted from the Respectful Maternity Care (RMC) Tool
Routine ANC Services	Enrollment visit, follow-up ANC visit(s)	Counseling received, date and type of vaccination/supplements received, infection screening and results (HIV, syphilis, tuberculosis, malaria others), hemoglobin screening/results, urine dipstick, treatments given
Sociodemographic	Enrollment visit	Education, occupation (participant and partner), household income, religion, marriage status, household item assessment
Specimen Collection and Storage	Refer to biospecimen matrix for timing	Sample collection order, reason for collecting, date and time collected, date sent for lab analysis
Treatment Adherence- UTI	For UTI: 1-week therapy For BV: 5-day therapy	Treatment adherence (date started regimen, date completed regimen, missed tablets, reasons for missing) side effects
Treatment Initiation DOT	For UTI/STI: As needed For deworming: 1st and 3rd ANC visits	Diagnosis, treatment started/given, direct observation, side effects

Ultrasound	Enrollment visit, 3rd trimester visit	Crown-rump length, bi-parietal diameter (outer-outer and outer to inner), head circumference, femoral length, abdominal circumference, transcerebellar diameter (if EGA >24 weeks)
Verbal Autopsy	As needed	Information on the deceased, assessment of stillbirth and injuries/accidents, medical history associated with final illness, maternal pregnancy and health, signs and symptoms associated with final illness, health service utilization

Data Sharing
The ENAT study aims to expedite advances in maternal-infant health. The ENAT investigative team will support the sharing and pooling of ENAT study data with similar maternal nutrition trials to facilitate this mission. Specific Data from the ENAT study will be made available to the Gates Foundation and/or individuals affiliated with the Foundation upon request and approval by the study PIs (Dr. CC Lee and Professor Yemane Berhane) and regulatory bodies. The likely file format will be STATA, R and/or CSV.

Data will be de-identified, with no personal identifiers, and shared with accompanying meta-data. Meta-data may include tables of contents, data dictionary/codebooks, brief summary of methods related to data collection and important features of the dataset. Data will not be transferable to a third party and will not be shared, published, or otherwise released to unapproved individuals or organization without prior notice and request for approval from the study PIs and regulatory bodies.

Quality Assurance
We will adhere to certain quality assurance guidelines to ensure that our interventions and measurements are meeting a minimal standard.

For ultrasound dating, sonographers will be trained and standardized by a maternal fetal medicine ultrasonographer supervised by Dr. Wylie prior to the start of the study, ensuring that all individual biometric parameters are measured with adequate quality and agreement compared to the gold standard. A random subset of 10% of the scans will be independently reviewed monthly by the sonography expert and scored for quality based on the WHO AMANHI Late Pregnancy Ultrasound QC checklist. Feedback will be provided directly to sonographers on an ongoing basis for quality improvement.

Testing for adherence to nutritional interventions has been described in earlier sections. For the infection screening, a random 5% of laboratory specimens (stool, malaria, urine) will be reviewed by a supervising lab technician on the research team for quality control. On-going monitoring of the coverage of the infection interventions as part of the health system will be measured by the ARHB and local partners and identify coverage gaps that need to be addressed to ensure high coverage of the interventions.

For all anthropometrics, anthropometrists will be trained and standardized at the beginning of the study and every 3 months thereafter using methods modified from procedures used in the WHO multi-country growth reference study. An estimated 10% of anthropometrics will have an independent 2nd measure by a field supervisor/expert anthropometrist. Birth weight scales will be calibrated daily with standardized weights, and length boards with standardized length rods.

References

1. Ahmad, S. M., M. J. Haskell, R. Raqib, and C. B. Stephensen. 2009. 'Markers of innate immune function are associated with vitamin A stores in men', *J Nutr*, 139: 377-85.
2. Anemona, A., M. Plummer, J. Chilongani, J. Wamoyi, D. Everett, J. Changalucha, and et al. 2002. "The acceptability and quality of self-administered vaginal swabs among adolescents in rural Tanzania." In *the XIV International AIDS Conference*, MoPeB3116. Barcelona.
3. "Bangladesh Demographic and Health Survey 2014." In. 2016. Dhaka, Bangladesh and Rockville, Maryland: National Institute of Population Research and Training (NIPORT), Mitra and Associates, and ICF International.

4. Belyhun, Y., G. Medhin, A. Amberbir, B. Erko, C. Hanlon, A. Alem, A. Venn, J. Britton, and G. Davey. 2010. 'Prevalence and risk factors for soil-transmitted helminth infection in mothers and their infants in Butajira, Ethiopia: a population-based study', *BMC Public Health*, 10: 21.
5. Blake, D. R., N. Maldeis, M. R. Barnes, A. Hardick, T. C. Quinn, and C. A. Gaydos. 2008. 'Cost-effectiveness of screening strategies for Chlamydia trachomatis using cervical swabs, urine, and self-obtained vaginal swabs in a sexually transmitted disease clinic setting', *Sex Transm Dis*, 35: 649-55.
6. Blencowe, H., S. Cousens, M. Kamb, S. Berman, and J. E. Lawn. 2011. 'Lives Saved Tool supplement detection and treatment of syphilis in pregnancy to reduce syphilis related stillbirths and neonatal mortality', *BMC Public Health*, 11 Suppl 3: S9.
7. Blencowe, H., S. Cousens, M. Z. Oestergaard, D. Chou, A. B. Moller, R. Narwal, A. Adler, C. Vera Garcia, S. Rohde, L. Say, and J. E. Lawn. 2012. 'National, regional, and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: a systematic analysis and implications', *Lancet*, 379: 2162-72.
8. Blencowe, H., A. C. Lee, S. Cousens, A. Bahalim, R. Narwal, N. Zhong, D. Chou, L. Say, N. Modi, J. Katz, T. Vos, N. Marlow, and J. E. Lawn. 2013. 'Preterm birth-associated neurodevelopmental impairment estimates at regional and global levels for 2010', *Pediatr Res*, 74 Suppl 1: 17-34.
9. Buntin, M. B., and A. M. Zaslavsky. 2004. 'Too much ado about two-part models and transformation? Comparing methods of modeling Medicare expenditures', *J Health Econ*, 23: 525-42.
10. Buppasiri, P., P. Lumbiganon, J. Thinkhamrop, C. Ngamjarus, and M. Laopaiboon. 2011. 'Calcium supplementation (other than for preventing or treating hypertension) for improving pregnancy and infant outcomes', *Cochrane Database Syst Rev*: CD007079.
11. Carey, J. C., M. A. Klebanoff, J. C. Hauth, S. L. Hillier, E. A. Thom, J. M. Ernest, R. P. Heine, R. P. Nugent, M. L. Fischer, K. J. Leveno, R. Wapner, and M. Varner. 2000. 'Metronidazole to prevent preterm delivery in pregnant women with asymptomatic bacterial vaginosis. National Institute of Child Health and Human Development Network of Maternal-Fetal Medicine Units', *N Engl J Med*, 342: 534-40.
12. Christensen, D. L., A. Kapur, and I. C. Bygbjerg. 2011. 'Physiological adaption to maternal malaria and other adverse exposure: low birth weight, functional capacity, and possible metabolic disease in adult life', *Int J Gynaecol Obstet*, 115 Suppl 1: S16-9.
13. Christian, P., S. K. Khatry, and K. P. West, Jr. 2004. 'Antenatal anthelmintic treatment, birthweight, and infant survival in rural Nepal', *Lancet*, 364: 981-3.
14. Christian, P., A. B. Labrique, H. Ali, M. J. Richman, L. Wu, M. Rashid, and K. P. West, Jr. 2011. 'Maternal vitamin A and beta-carotene supplementation and risk of bacterial vaginosis: a randomized controlled trial in rural Bangladesh', *Am J Clin Nutr*, 94: 1643-9.
15. Christian, P., S. E. Lee, M. Donahue Angel, L. S. Adair, S. E. Arifeen, P. Ashorn, F. C. Barros, C. H. Fall, W. W. Fawzi, W. Hao, G. Hu, J. H. Humphrey, L. Huybregts, C. V. Joglekar, S. K. Kariuki, P. Kolsteren, G. V. Krishnaveni, E. Liu, R. Martorell, D. Osrin, L. A. Persson, U. Ramakrishnan, L. Richter, D. Roberfroid, A. Sania, F. O. Ter Kuile, J. Tielsch, C. G. Victora, C. S. Yajnik, H. Yan, L. Zeng, and R. E. Black. 2013. 'Risk of childhood undernutrition related to small-for-gestational age and preterm birth in low- and middle-income countries', *Int J Epidemiol*, 42: 1340-55.
16. Claycombe, K. J., C. A. Brissette, and O. Ghribi. 2015. 'Epigenetics of inflammation, maternal infection, and nutrition', *J Nutr*, 145: 1109S-15S.
17. Collin, S. M., R. F. Baggaley, R. Pittrof, and V. Filippi. 2007. 'Could a simple antenatal package combining micronutritional supplementation with presumptive treatment of infection prevent maternal deaths in sub-Saharan Africa?', *BMC Pregnancy Childbirth*, 7: 6.
18. "The control of soil-transmitted helminths in high-risk groups: preventive chemotherapy in preschool children, school-age children and women of childbearing age." In. 2016. *WHO guideline development group – deworming meeting*. WHO Headquarters, Geneva, Switzerland: World Health Organization (WHO).
19. Danaei, G., K. G. Andrews, C. R. Sudfeld, G. Fink, D. C. McCoy, E. Peet, A. Sania, M. C. Smith Fawzi, M. Ezzati, and W. W. Fawzi. 2016. 'Risk Factors for Childhood Stunting in 137 Developing

Countries: A Comparative Risk Assessment Analysis at Global, Regional, and Country Levels', *PLoS Med*, 13: e1002164.
20. de Onis M., Van den Broeck J., Chumlea W.C., Martorell R., for the WHO Multicentre Growth Reference Study Group. 2004. 'Measurement and standardization protocols for anthropometry used in the construction of a new international growth reference', *Food Nutr Bull*, 25: S27-S36.
21. "e-Library of Evidence for Nutrition Actions (eLENA)." In. Department of Nutrition for Health and Development: World Health Organization (WHO).
22. Efron, B, and R Tibshirani. 1993. *An introduction to the bootstrap* (Chapman and Hall: London).
23. Eticha, B. T., Z. Sisay, A. Alemayehu, and T. Shimelis. 2013. 'Seroprevalence of syphilis among HIV-infected individuals in Addis Ababa, Ethiopia: a hospital-based cross-sectional study', *BMJ Open*, 3.
24. "Expedited partner therapy in the management of sexually transmitted diseases." In. 2006. Atlanta, GA: Centers for Disease Control and Prevention, US Department of Health and Human Services.
25. "Ethiopian National Drug Formulary: 1st Edition". 2008. Ethiopia: Drug Administration and Control Authority of Ethiopia.
26. "Ethiopian National Micronutrient Survey Report." September 2016. Ethiopia Public Health Institute.
27. Force, US Preventative Services Task. 2008. 'Screening for Bacterial Vaginosis in Pregnancy to Prevent Preterm Delivery ', Accessed March 14, 2014. http://www.uspreventiveservicestaskforce.org/uspstf/uspsbvag.htm.
28. Gilbert, N. M., V. P. O'Brien, S. Hultgren, G. Macones, W. G. Lewis, and A. L. Lewis. 2013. 'Urinary tract infection as a preventable cause of pregnancy complications: opportunities, challenges, and a global call to action', *Glob Adv Health Med*, 2: 59-69.
29. Glinoer D. 2007. 'The importance of iodine nutrition during pregnancy'. *Public health nutrition,* 10(12A):1542-6.
30. 'Global Health Observatory data repository: Antenatal care (ANC) attendees tested for syphilis at first ANC visit (Data by country)'. 2015. WHO, Accessed Oct 1.
31. Goldenberg, R. L., J. C. Hauth, and W. W. Andrews. 2000. 'Intrauterine infection and preterm delivery', *N Engl J Med*, 342: 1500-7.
32. Goldstein, H. 2003. *Multilevel statistical models, 3rd edition* (Arnold: London).
33. Gray, R. H., F. Wabwire-Mangen, G. Kigozi, N. K. Sewankambo, D. Serwadda, L. H. Moulton, T. C. Quinn, K. L. O'Brien, M. Meehan, C. Abramowsky, M. Robb, and M. J. Wawer. 2001. 'Randomized trial of presumptive sexually transmitted disease therapy during pregnancy in Rakai, Uganda', *Am J Obstet Gynecol*, 185: 1209-17.
34. "Guideline: preventive chemotherapy to control soil-transmitted helminth infections in high-risk groups " In. in press. Geneva, Switzerland: World Health Organization (WHO).
35. Haider, B. A., and Z. A. Bhutta. 2017. 'Multiple-micronutrient supplementation for women during pregnancy', *Cochrane Database Syst Rev*, 4: CD004905.
36. Hallamaa, L., Y. B. Cheung, K. Maleta, M. Luntamo, U. Ashorn, M. Gladstone, T. Kulmala, C. Mangani, and P. Ashorn. 2018. 'Child Health Outcomes After Presumptive Infection Treatment in Pregnant Women: A Randomized Trial', *Pediatrics*.
37. Han, Z., S. Mulla, J. Beyene, G. Liao, S. D. McDonald, and Group Knowledge Synthesis. 2011. 'Maternal underweight and the risk of preterm birth and low birth weight: a systematic review and meta-analyses', *Int J Epidemiol*, 40: 65-101.
38. Holmberg MJ, Andersen LW. Adjustment for Baseline Characteristics in Randomized Clinical Trials. *JAMA.* 2022;328(21):2155–2156. doi:10.1001/jama.2022.21506
39. Hughes, S. M., B. Amadi, M. Mwiya, H. Nkamba, A. Tomkins, and D. Goldblatt. 2009. 'Dendritic cell anergy results from endotoxemia in severe malnutrition', *J Immunol*, 183: 2818-26.
40. Iheozor-Ejiofor, Z., P. Middleton, M. Esposito, and A. M. Glenny. 2017. 'Treating periodontal disease for preventing adverse birth outcomes in pregnant women', *Cochrane Database Syst Rev*, 6: CD005297.
41. Imdad A, Bhutta ZA. 2012. 'Maternal nutrition and birth outcomes: effect of balanced protein-energy supplementation.', *Paediatr Perinat Epidemiol.*, 26: 178-90.
42. Katz, J., A. C. Lee, N. Kozuki, J. E. Lawn, S. Cousens, H. Blencowe, M. Ezzati, Z. A. Bhutta, T. Marchant, B. A. Willey, L. Adair, F. Barros, A. H. Baqui, P. Christian, W. Fawzi, R. Gonzalez, J.

Humphrey, L. Huybregts, P. Kolsteren, A. Mongkolchati, L. C. Mullany, R. Ndyomugyenyi, J. K. Nien, D. Osrin, D. Roberfroid, A. Sania, C. Schmiegelow, M. F. Silveira, J. Tielsch, A. Vaidya, S. C. Velaphi, C. G. Victora, D. Watson-Jones, R. E. Black, and Cherg Small-for-Gestational-Age-Preterm Birth Working Group. 2013. 'Mortality risk in preterm and small-for-gestational-age infants in low-income and middle-income countries: a pooled country analysis', *Lancet*, 382: 417-25.
43. Khader, Y. S., and Q. Ta'ani. 2005. 'Periodontal diseases and the risk of preterm birth and low birth weight: a meta-analysis', *J Periodontol*, 76: 161-5.
44. Kim, H., J. Y. Hwang, E. H. Ha, H. Park, M. Ha, S. J. Lee, Y. C. Hong, and N. Chang. 2011. 'Association of maternal folate nutrition and serum C-reactive protein concentrations with gestational age at delivery', *Eur J Clin Nutr*, 65: 350-6.
45. Kiserud, T., G. Piaggio, G. Carroli, M. Widmer, J. Carvalho, L. Neerup Jensen, D. Giordano, J. G. Cecatti, H. Abdel Aleem, S. A. Talegawkar, A. Benachi, A. Diemert, A. Tshefu Kitoto, J. Thinkhamrop, P. Lumbiganon, A. Tabor, A. Kriplani, R. Gonzalez Perez, K. Hecher, M. A. Hanson, A. M. Gulmezoglu, and L. D. Platt. 2017. 'The World Health Organization Fetal Growth Charts: A Multinational Longitudinal Study of Ultrasound Biometric Measurements and Estimated Fetal Weight', *PLoS Med*, 14: e1002220.
46. Knox, J., S. N. Tabrizi, P. Miller, K. Petoumenos, M. Law, S. Chen, and S. M. Garland. 2002. 'Evaluation of self-collected samples in contrast to practitioner-collected samples for detection of Chlamydia trachomatis, Neisseria gonorrhoeae, and Trichomonas vaginalis by polymerase chain reaction among women living in remote areas', *Sex Transm Dis*, 29: 647-54.
47. Kozuki, N., J. Katz, A. C. Lee, J. P. Vogel, M. F. Silveira, A. Sania, G. A. Stevens, S. Cousens, L. E. Caulfield, P. Christian, L. Huybregts, D. Roberfroid, C. Schmiegelow, L. S. Adair, F. C. Barros, M. Cowan, W. Fawzi, P. Kolsteren, M. Merialdi, A. Mongkolchati, N. Saville, C. G. Victora, Z. A. Bhutta, H. Blencowe, M. Ezzati, J. E. Lawn, R. E. Black, and Group Child Health Epidemiology Reference Group Small-for-Gestational-Age/Preterm Birth Working. 2015. 'Short Maternal Stature Increases Risk of Small-for-Gestational-Age and Preterm Births in Low- and Middle-Income Countries: Individual Participant Data Meta-Analysis and Population Attributable Fraction', *J Nutr*, 145: 2542-50.
48. Krawinkel, M. B. 2012. 'Interaction of nutrition and infections globally: an overview', *Ann Nutr Metab*, 61 Suppl 1: 39-45.
49. Kutlesic, V., M. Brewinski Isaacs, L. S. Freund, R. Hazra, and D. J. Raiten. 2017. 'Executive Summary: Research Gaps at the Intersection of Pediatric Neurodevelopment, Nutrition, and Inflammation in Low-Resource Settings', *Pediatrics*, 139: S1-S11.
50. Lawn, J. E., H. Blencowe, S. Oza, Y. Danzhen, A. C. Lee, P. Waiswa, M. Lalli, Z. Bhutta, A. J. D. Barros, P. Christian, C. Mathers, S. N. Cousens, and The Lancet Every Newborn Study Group. 2014. 'Every Newborn: progress, priorities, and potential beyond survival', *The Lancet*, 384: 189-205.
51. Lee, A. C., N. Kozuki, S. Cousens, G. A. Stevens, H. Blencowe, M. F. Silveira, A. Sania, H. E. Rosen, C. Schmiegelow, L. S. Adair, A. H. Baqui, F. C. Barros, Z. A. Bhutta, L. E. Caulfield, P. Christian, S. E. Clarke, W. Fawzi, R. Gonzalez, J. Humphrey, L. Hybraets, S. Kariuki, P. Kolsteren, J. Lusingu, D. Manandhar, A. Mongkolchati, L. C. Mullany, R. Ndyomugyenyi, J. K. Nien, D. Roberfroid, N. Saville, D. J. Terlouw, J. M. Tielsch, C. G. Victora, S. C. Velaphi, D. Watson-Jones, B. A. Willey, M. Ezzati, J. E. Lawn, R. E. Black, J. Katz, and CHERG Small-for-Gestational-Age-Preterm Birth Working Group. 2017. 'Estimates of burden and consequences of infants born small for gestational age in low- and middle-income countries with INTERGROWTH-21st standard: analysis of CHERG datasets', *Bmj*, 17: j3677.
52. Lee, A. C., M. A. Quaiyum, L. C. Mullany, D. K. Mitra, A. Labrique, P. Ahmed, J. Uddin, I. Rafiqullah, S. DasGupta, A. Mahmud, E. H. Koumans, P. Christian, S. Saha, A. H. Baqui, and Group Projahnmo Study. 2015. 'Screening and treatment of maternal genitourinary tract infections in early pregnancy to prevent preterm birth in rural Sylhet, Bangladesh: a cluster randomized trial', *BMC Pregnancy Childbirth*, 15: 326.
53. Lee AC, Abate FW, Mullany LC*, et al* Enhancing Nutrition and Antenatal Infection Treatment (ENAT) study: protocol of a pragmatic clinical effectiveness study to improve birth outcomes in Ethiopia *BMJ Paediatrics Open* 2022;**6:**e001327. doi: 10.1136/bmjpo-

54. Leitich, H., B. Bodner-Adler, M. Brunbauer, A. Kaider, C. Egarter, and P. Husslein. 2003. 'Bacterial vaginosis as a risk factor for preterm delivery: a meta-analysis', *Am J Obstet Gynecol*, 189: 139-47.
55. Leitich, H., and H. Kiss. 2007. 'Asymptomatic bacterial vaginosis and intermediate flora as risk factors for adverse pregnancy outcome', *Best Pract Res Clin Obstet Gynaecol*, 21: 375-90.
56. Lu, C., S. Tsai, J. Ruhumuriza, G. Umugiraneza, and S. Kandamutsa. 2013. "Obtaining financing data for local health facilities in two districts of Rwanda: General guidelines." In. Boston, MA: Program in Global Helath Economics and Social Change, Harvard Medical School.
57. Lu, C., S. Tsai, J. Ruhumuriza, G. Umugiraneza, S. Kandamutsa, P. P. Salvatore, Z. Zhang, A. Binagwaho, and F. Ngabo. 2014. 'Tracking rural health facility financial data in resource-limited settings: a case study from Rwanda', *PLoS Med*, 11: e1001763.
58. Luntamo, M., T. Kulmala, Y. B. Cheung, K. Maleta, and P. Ashorn. 2013. 'The effect of antenatal monthly sulphadoxine-pyrimethamine, alone or with azithromycin, on foetal and neonatal growth faltering in Malawi: a randomised controlled trial', *Trop Med Int Health*, 18: 386-97.
59. Luntamo, M., T. Kulmala, B. Mbewe, Y. B. Cheung, K. Maleta, and P. Ashorn. 2010. 'Effect of repeated treatment of pregnant women with sulfadoxine-pyrimethamine and azithromycin on preterm delivery in Malawi: a randomized controlled trial', *Am J Trop Med Hyg*, 83: 1212-20.
60. Manning, W. G. 1998. 'The logged dependent variable, heteroscedasticity, and the retransformation problem', *J Health Econ*, 17: 283-95.
61. Manning, W. G., and J. Mullahy. 2001. 'Estimating log models: to transform or not to transform?', *J Health Econ*, 20: 461-94.
62. McCauley, M. E., N. van den Broek, L. Dou, and M. Othman. 2015. 'Vitamin A supplementation during pregnancy for maternal and newborn outcomes', *Cochrane Database Syst Rev*: CD008666.
63. McCoy DC, Sudfeld CR, Bellinger DC, et al. 2017. Development and validation of an early childhood development scale for use in low-resourced settings. *Popul Health Metr*. 2017;15(1):3.
64. Mobeen, N., I. Jehan, N. Banday, J. Moore, E. M. McClure, O. Pasha, L. L. Wright, and R. L. Goldenberg. 2008. 'Periodontal disease and adverse birth outcomes: a study from Pakistan', *Am J Obstet Gynecol*, 198: 514 e1-8.
65. Moulton, L. , and S. Zeger. 1989. 'Analyzing repeated measures on generalized linear models via the bootstrap', *Biometrics*, 45: 381-94.
66. Murray, E., M. Fernandes, M. Fazel, S. H. Kennedy, J. Villar, and A. Stein. 2015. 'Differential effect of intrauterine growth restriction on childhood neurodevelopment: a systematic review', *Bjog*, 122: 1062-72.
67. "National Guidelines for the Management of Sexually Transmitted Infections Using Syndromic Approach." In. 2015. Addis Ababa, Ethiopia: Federal Democratic Republic of Ethiopia Ministry of Health.
68. "National Nutrition Program 2016-2020." In 2016. Ethiopia: Government of the Federal Democratic Republic of Ethiopia.
69. 'Nutrient Recommendations: Dietrary Reference Intakes (DRI)'. Food and Nutrition Board, Institute of Medicine, National Academy of Sciences. https://ods.od.nih.gov/Health_Information/Dietary_Reference_Intakes.aspx.
70. Ota, E., H. Hori, R. Mori, R. Tobe-Gai, and D. Farrar. 2015. 'Antenatal dietary education and supplementation to increase energy and protein intake', *Cochrane Database Syst Rev*: CD000032.
71. Papageorghiou, A.T., S. H. Kennedy, L.J. Salomon, E.O. Ohuma, L. Cheikh Ismail, F. C. Barros, A. Lambert, M. Carvalho, Y.A. Jaffer, E. Bertino, M.G. Gravett, D.G. Altman, M. Purwar, J.A. Noble, R. Pang, C. G. Victora, Z. A. Bhutta, J. Villar, and International Fetal and Newborn Growth Consortium for the 21st Century (INTERGROWTH-21st). 2014. 'International standards for early fetal size and pregnancy dating based on ultrasound measurement of crown-rump length in the first trimester of pregnancy', *Ultrasound Obstet Gynecol*, 44: 641-8.
72. Pena-Rosas, J. P., L. M. De-Regil, M. N. Garcia-Casal, and T. Dowswell. 2015. 'Daily oral iron supplementation during pregnancy', *Cochrane Database Syst Rev*: CD004736.
73. 'Periodontal Examinations and Treatments'. Field Family Dentistry, Accessed October 10. http://www.feildfamilydentistry.com/periodontal-care.html.

74. Radeva-Petrova, D., K. Kayentao, F. O. ter Kuile, D. Sinclair, and P. Garner. 2014. 'Drugs for preventing malaria in pregnant women in endemic areas: any drug regimen versus placebo or no treatment', *Cochrane Database Syst Rev*: CD000169.
75. Raiten, D. J., F. A. Sakr Ashour, A. C. Ross, S. N. Meydani, H. D. Dawson, C. B. Stephensen, B. J. Brabin, P. S. Suchdev, B. van Ommen, and Inspire Consultative Group. 2015. 'Inflammation and Nutritional Science for Programs/Policies and Interpretation of Research Evidence (INSPIRE)', *J Nutr*, 145: 1039S-108S.
76. "Report on the 2014 Round Antenatal Care based Sentinel HIV Surveillance in Ethiopia." In. 2015. Addis Ababa, Ethiopia: Ethiopian Public Health Institute.
77. Schulz KF, Grimes DA. Multiplicity in randomised trials II: subgroup and interim analyses. Lancet. 2005 May 7-13;365(9471):1657-61. doi: 10.1016/S0140-6736(05)66516-6. PMID: 15885299.
78. 'Screening Recommendations and Considerations Referenced in Treatment Guidelines and Original Sources'. 2015. Centers for Disease Control and Prevention (CDC). https://www.cdc.gov/std/tg2015/screening-recommendations.htm.
79. "The Selection and Use of Essential Medicines: Report of the WHO Expert Committee, 2017 (including the 20th WHO Model List of Essential Medicines and the 6th WHO Model List of Essential Medicines for Children)." In. 2017. *WHO Technical Report Series; no. 1006*. Geneva, Switzerland: World Health Organization.
80. "Sexually Transmitted Diseases: Summary of 2015 CDC Treatment Guidelines." In. 2015. Atlanta, GA: Centers for Disease Control and Prevention, US Department of Health and Human Services.
81. Shanks, N., and S. L. Lightman. 2001. 'The maternal-neonatal neuro-immune interface: are there long-term implications for inflammatory or stress-related disease?', *J Clin Invest*, 108: 1567-73.
82. Shawel, D., Hagos, S., Lachat, C.K., Kimanya, M.E. and Kolsteren, P., 2010. Post-production losses in iodine concentration of salt hamper the control of iodine deficiency disorders: a case study in northern Ethiopia. Journal of health, population, and nutrition, 28(3), p.238.
83. Shiferaw, M. B., A. M. Zegeye, and A. D. Mengistu. 2017. 'Helminth infections and practice of prevention and control measures among pregnant women attending antenatal care at Anbesame health center, Northwest Ethiopia', *BMC Res Notes*, 10: 274.
84. Smaill, F. M., and J. C. Vazquez. 2015. 'Antibiotics for asymptomatic bacteriuria in pregnancy', *Cochrane Database Syst Rev*: CD000490.
85. Solensky R. Hypersensitivity reactions to macrolides, aminoglycosides, tetracyclines, clindamycin, and metronidazole. https://www.uptodate.com/contents/hypersensitivity-reactions-to-macrolides-aminoglycosides-tetracyclines-clindamycin-and-metronidazole?search=anaphylaxis%20azithromycin&source=search_result&selectedTitle=2~150&usage_type=default&display_rank=2. Accessed 1/23/2019.
86. Stevens, B., P. Buettner, K. Watt, A. Clough, J. Brimblecombe, and J. Judd. 2015. 'The effect of balanced protein energy supplementation in undernourished pregnant women and child physical growth in low- and middle-income countries: a systematic review and meta-analysis', *Matern Child Nutr*, 11: 415-32.
87. Subtil, D., G. Brabant, E. Tilloy, P. Devos, F. Canis, A. Fruchart, M. C. Bissinger, J. C. Dugimont, C. Nolf, C. Hacot, S. Gautier, J. Chantrel, M. Jousse, D. Desseauve, J. L. Plennevaux, C. Delaeter, S. Deghilage, A. Personne, E. Joyez, E. Guinard, E. Kipnis, K. Faure, B. Grandbastien, P. Y. Ancel, F. Goffinet, and R. Dessein. 2018. 'Early clindamycin for bacterial vaginosis in pregnancy (PREMEVA): a multicentre, double-blind, randomised controlled trial', *Lancet*.
88. Thorne-Lyman, A. L., and W. W. Fawzi. 2012. 'Vitamin A and carotenoids during pregnancy and maternal, neonatal and infant health outcomes: a systematic review and meta-analysis', *Paediatr Perinat Epidemiol*, 26 Suppl 1: 36-54.
89. Tsai, S., G. Umugiraneza, J. Ruhumuriza, S. Kandamutsa, and L. Lu. 2013. "Training manual on tracking financial data in health facilities in Kayonza and Kirehe." In. Boston, MA: Program in Global Health Economics and Social Change, Harvard Medical School.

90. Ugwumadu, A., I. Manyonda, F. Reid, and P. Hay. 2003. 'Effect of early oral clindamycin on late miscarriage and preterm delivery in asymptomatic women with abnormal vaginal flora and bacterial vaginosis: a randomised controlled trial', *Lancet*, 361: 983-8.
91. Ugwumadu, A., F. Reid, P. Hay, and I. Manyonda. 2004. 'Natural history of bacterial vaginosis and intermediate flora in pregnancy and effect of oral clindamycin', *Obstet Gynecol*, 104: 114-9.
92. van der Linden PD, van der Lei J, Vlug AE, Stricker BH. Skin reactions to antibacterial agents in general practice. J Clin Epidemiol. 1998 Aug;51(8):703-8.
93. Ververs, M. T., A. Antierens, A. Sackl, N. Staderini, and V. Captier. 2013. 'Which anthropometric indicators identify a pregnant woman as acutely malnourished and predict adverse birth outcomes in the humanitarian context?', *PLoS Curr*, 5.
94. Vohr, B. R., E. Poggi Davis, C. A. Wanke, and N. F. Krebs. 2017. 'Neurodevelopment: The Impact of Nutrition and Inflammation During Preconception and Pregnancy in Low-Resource Settings', *Pediatrics*, 139: S38-S49.
95. West, K. P., Jr., A. A. Shamim, S. Mehra, A. B. Labrique, H. Ali, S. Shaikh, R. D. Klemm, L. S. Wu, M. Mitra, R. Haque, A. A. Hanif, A. B. Massie, R. D. Merrill, K. J. Schulze, and P. Christian. 2014. 'Effect of maternal multiple micronutrient vs iron-folic acid supplementation on infant mortality and adverse birth outcomes in rural Bangladesh: the JiVitA-3 randomized trial', *JAMA*, 312: 2649-58.
96. WHO. 2007. "WHO child growth standards: head circumference-for-age, arm circumference-for-age, triceps skinfold-for-age and supscapular skinfold-for-age: methods and development." In.
97. WHO. 2007. "Assessment of iodine deficiency disorders and monitoring their elimination: third edition."
98. World Health Organization. 2016. "WHO recommendations on antental care for a positive pregnancy experience."
99. "WHO Guide to Cost-Effectiveness Analysis." In. 2003. edited by T. Tan-Torres Edejer, R. Baltussen, T. Adam, R. Hutubessy, A. Acharya, D.B. Evans and C.J.L. Murray. Geneva, Switzerland: World Health Organization.
100. Workicho, A., T. Belachew, G. T. Feyissa, B. Wondafrash, C. Lachat, R. Verstraeten, and P. Kolsteren. 2016. 'Household dietary diversity and Animal Source Food consumption in Ethiopia: evidence from the 2011 Welfare Monitoring Survey', *BMC Public Health*, 16: 1192.
101. "The world health report 2000 - Health systems: improving performance." In. 2000. Geneva, Switzerland: World Health Organization.
~~**102.**~~

I. Appendix

○ CONSORT FLOW DIAGRAM OF STUDY PARTICIPANTS

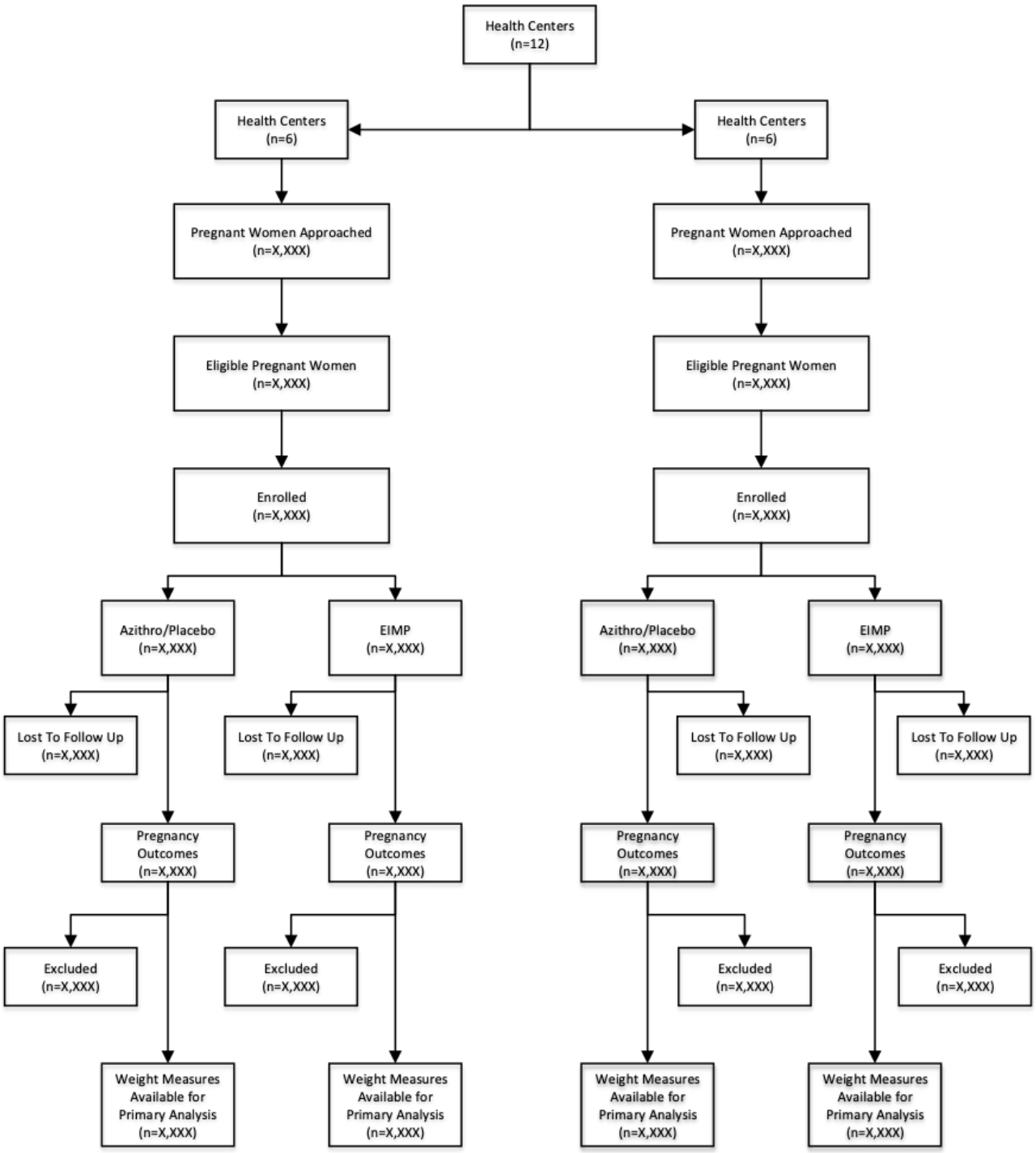

Table 1: Cluster (Health Center) Level Randomization Balance

	Nutrition (cl = 6, n=X,XXX)		Routine (cl = 6, n=X,XXX)	
	Mean	**SD**	**Mean**	**SD**
Mean Distance to Bahir Dar				
Mean cluster size (enrolled women per health center)				
Mean loss of follow up rate				
Mean GA at presentation				
Mean number of ANC visits				
Mean number of births				

Table 2: Pregnancy Level Randomization Balance: Nutrition Intervention

	Nutrition (n=X,XXX)	Routine (n=X,XXX)
	m/sd or n/%	m/sd or n/%
Household level variables		
Land ownership		
Monthly expenditures		
Animal ownership		
Wealth index		
Fuel access/use		
Toilet/water access		
Food insecurity		
Secure		
Mildly insecure		
Moderately insecure		
Severely insecure		
Woman Enrollment variables		
Marital Status		
Religion		
Education / Literacy		
Occupation		
Agriculture-related		
Waged occupation		
No formal occupation		
Age		
Preg Enrollment Indicators		
Mean GA at enrollment		
Maternal parity		
Maternal MUAC MUAC <23 cm		
Maternal Weight		
Maternal BMI BMI <18.5		
Prior history of Preterm Birth		
Morbidity measures at enrollment		
Fever		
ARI		
Diarrhea		
STI		
UTI		
Pregnancy Care-seeking		
ANC contact		
1 contact		
2-3 contact		
≥ 4 contact		
Facility delivery		
Health center		
Hospital		
Home/Other		

Table 3: Pregnancy Level Randomization Balance: Infection Interventions

	EIMP (n=X,XXX)	Routine (n=X,XXX)
	m/sd or n/%	m/sd or n/%
Household level variables		
Land ownership		
Monthly expenditures		
Animal ownership		
Fuel access/use		
Wealth index		
Toilet/water access		
Food insecurity		
Secure		
Mildly insecure		
Moderately insecure		
Severely insecure		
Woman Enrollment variables		
Marital Status		
Religion		
Education / Literacy		
Occupation		
Agriculture-related		
Waged occupation		
No formal occupation		
Age		
Preg Enrollment Indicators		
Mean GA at enrollment		
Maternal parity		
Maternal MUAC		
Maternal Weight		
Maternal BMI		
Prior history of Preterm Birth		
Morbidity measures at enrollment		
Fever		
ARI		
Diarrhea		
STI		
UTI		
Pregnancy Care-seeking		
ANC contact		
1 contact		
2-3 contact		
≥ 4 contact		
Facility delivery		
Health center		
Hospital		
Home/Other		
- % with HIV test		
- % with Syphilis testing		

Table 4: Nutrition Intervention Delivery and Compliance

Number of women enrolled	ENP	Non-ENP
BEP		
# (%) Women screened with MUAC <23 cm identified on any visit		
# (%) Women ever distributed BEP on any occasion		
# (%) of days BEP consumed/ # of days should have been on BEP (upon measurement<23 cm until delivery)		
Mean number of days on BEP (upon measurement<23 cm until delivery)		
# (%) that consumed more than 50% of BEP		
# (%) that consumed more than 75% of BEP		
Mean kcals consumed per day from supplement (Sub-study only)		
Mean protein (g) consumed per day from supplement (Sub-study only)		
IFA-pregnancy		
# (%) of days supplement consumed/ # of days should have been on IFA (upon enrollment until delivery)		
Mean # days of taking IFA (upon enrollment until delivery)		
# (%) that consumed more than 50% of IFA in pregnancy		
# (%) that consumed more than 75% of IFA in pregnancy		
IFA-postpartum		
# (%) of days supplement consumed/ # of days should have been on IFA (upon delivery until 6 mo postpartum)		
Mean # days of taking IFA (upon delivery until 6 mo postpartum)		
# (%) that consumed more than 50% of IFA postpartum		
# (%) that consumed more than 75% of IFA postpartum		
Iodized salt		
# (%) of days iodized consumed in pregnancy/ # of days should have been on iodized salt in pregnancy		

Mean # days of taking iodized salt between enrollment and delivery		
# (%) of days iodized consumed in postpartum/ # of days should have been on iodized salt postpartum		
Mean # (%) of days iodized consumed postpartum		
Nutrition counseling		
# (%) that ever received nutrition counseling		
# (%) that received at least 2 times of nutrition counseling		

Table 5: EIMP Group Compliance/Coverage

Number of women (pregnancies) enrolled	EIMP	Non-EIMP
Deworming		
Number (%) that received one dose		
Refused		
Mean GA first dose		
Number (%) received 2 doses		
Refused		
Mean GA 2nd dose		
Number (%) that had stool screening for ova/parasites		
Ova/parasite identified		
O/P treated		
STI/RTI		
Number (%) assessed for vaginal symptoms		
Number (%) with vaginal symptoms		
GC/Chlamydia screening done (specimen obtained)		
Number (%) Gonorrhea test positive		
Number treated		
Number partner treated		
TOC obtained		
TOC positive-referral		
Number (%) Chlamydia test positive		
Number treated		
Number partner treated		
TOC obtained		
TOC positive-referral		
POC testing done for BV or Trich		
Number Trich positive		
Number treated for Trich		
Number BV positive		
Number initiated treatment		
Number completed treatment		
UTI		
First urine culture screen completed		
Adequate screening urine specimen collected		
Positive/require treatment for UTI		
Number who initiated treated for UTI		
Treated by health system by syndromic approach		
Treated based on urine culture results		
Antibiotic Treatment completed		
Second urine culture screen		
Adequate urine collected		
Positive/require treatment for UTI		
Number who initiated treated for UTI		
Treated by health system by syndromic approach		
Treated based on urine culture results		
Antibiotic Treatment completed		
Treatment Completed		
Overall UTI resolution		

Table 6: Primary and Secondary ENAT study outcomes: by Nutrition Arms

	Nutrition	Routine
	N(%) or mean(sd)	N (%) or mean(sd)
Pregnant women with outcome data		
Birth outcomes ≥12 wks		
Live births		
Fetal deaths		
Fetal loss <22 weeks		
Fetal loss 22-<28 weeks		
Fetal loss (≥28 weeks) [ie. stillbirth WHO definition]		
GA at pregnancy outcomes (mean, sd)		
Pregnancy-level Outcomes		
Birth outcomes >22 wks		
Pregnancies resulting in spontaneous preterm delivery		
Maternal Weight Gain (monthly rate; mean, sd)		
Maternal Anemia		
Diet		
Dietary Diversity Score (mean, sd)		
% Minimum Dietary Diversity		
Hypertensive Disorders		
% Gestational hypertension		
% Preeclampsia		
Late pregnancy/postpartum clinical UTI		
Maternal puerperal sepsis		
Live born Infants		
Live Births measured <72 hours		
Infant weight (mean, sd)		
Infant length (mean, sd)		
Infant head circumference (mean, sd)		
Number (%) of newborns born preterm (<37 wks)		
Number (%) of newborns of low birth weight		
Number (%) of newborns born SGA		
LAZ <72 hours (mean, sd)		
WLZ<72 hours (mean, sd)		
WAZ <72 hours (mean, sd)		

- As prespecified exploratory analysis we will also determine the outcomes stratified by maternal nutrition status (ie. MUAC <23 cm vs ≥ 23, or BMI <18.5 kg/m^2 vs ≥ 18.5)

Table 7: Primary and Secondary ENAT study outcomes: by Infection Arms

	EIMP	Non-EIMP
	N(%) or mean(sd)	N (%) or mean(sd)
Pregnant women with outcome data		
Birth outcomes ≥12 wks		
Live births		
Fetal deaths		
Fetal loss <22 weeks		
Fetal loss 22-<28 weeks		
Fetal loss (≥28 weeks) [ie. stillbirth WHO definition]		
GA at pregnancy outcomes (mean, sd)		
Pregnancy-level Outcomes		
Birth outcomes >22 wks		
Pregnancies resulting in spontaneous preterm delivery		
Maternal Weight Gain (monthly rate; mean, sd)		
Maternal Anemia		
Diet		
Dietary Diversity Score (mean, sd)		
% Minimum Dietary Diversity		
Hypertensive Disorders		
% Gestational hypertension		
% Preeclampsia		
Late pregnancy/postpartum clinical UTI		
Maternal puerperal sepsis		
Live born Infants		
Live Births measured <72 hours		
Infant weight (mean, sd)		
Infant length (mean, sd)		
Infant head circumference (mean, sd)		
Number (%) of newborns born preterm (<37 wks)		
Number (%) of newborns of low birth weight		
Number (%) of newborns born SGA		
LAZ <72 hours (mean, sd)		
WLZ<72 hours (mean, sd)		
WAZ <72 hours (mean, sd)		

Table 8: Primary and Secondary ENAT study outcomes: by Nutrition +Infection vs Neither

	Nutrition+Infection ENP+EIMP	Neither Nutrition or Infection
	N(%) or mean(sd)	N (%) or mean(sd)
Pregnant women with outcome data		
Birth outcomes ≥12 wks		
Live births		
Fetal deaths		
Fetal loss <22 weeks		
Fetal loss 22-<28 weeks		
Fetal loss (≥28 weeks) [ie. stillbirth WHO definition]		
GA at pregnancy outcomes (mean, sd)		
Pregnancy-level Outcomes		
Birth outcomes >22 wks		
Pregnancies resulting in spontaneous preterm delivery		
Maternal Weight Gain (monthly rate; mean, sd)		
Maternal Anemia		
Diet		
Dietary Diversity Score (mean, sd)		
% Minimum Dietary Diversity		
Hypertensive Disorders		
% Gestational hypertension		
% Preeclampsia		
Late pregnancy/postpartum clinical UTI		
Maternal puerperal sepsis		
Live born Infants		
Live Births measured <72 hours		
Infant weight (mean, sd)		
Infant length (mean, sd)		
Infant head circumference (mean, sd)		
Number (%) of newborns born preterm (<37 wks)		
Number (%) of newborns of low birth weight		
Number (%) of newborns born SGA		
LAZ <72 hours (mean, sd)		
WLZ<72 hours (mean, sd)		
WAZ <72 hours (mean, sd)		

Table 9: Adverse Events

	Nutrition		Infection Management Arms	
	Nutrition	**Routine**	**EIMP**	**Routine**
Pregnancies Enrolled				
Live births				
Serious adverse events				
Stillbirth				
Abortion				
Maternal death				
Neonatal death				
Hospitalization				
Congenital anomaly				
Anaphylaxis				
Severe AE (aggregate)				
Moderate AE (aggregate)				
Mild AE (aggregate)				
Obstructed labor (proxy)				
Cephalo-pelvic disproportion (CPD)				

- **SAP Versions/Revisions**

Version	Date	Comments
V2	June 2022	• Change of cost-effectiveness analysis to costing analysis • Added analysis population • Update to deworming procedures to include test-and-treat in the 3rd trimester
V3	Feb 2023	• Adjustment of intervention effects by prespecified prognostic factors per FDA/CONSORT guidance • Removal of secondary outcome- periodontal disease and maternal morbidity • Added statistical methods of cluster analysis • Reduced sample size and adjustment of sample size calculation given covid • Given reduced power, removed interaction testing of interventions, removed the calculation of simple intervention effects • Clarification of analysis of combined ENP+EIMP effects vs neither intervention arm • Additional pre-specified sub-group analysis added