Diagnostic accuracy of a novel machine learning algorithm to estimate gestational age

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Protocol Signature Page

Protocol Title:	Diagnostic Accuracy of a Novel Machine Learning Algorithm to Estimate Gestational Age
Short Title:	FAMLI Diagnostic Accuracy
Protocol Version:	1.3
Version Date:	25 May 2023

I, the Investigator of Record, agree to conduct this study in full accordance with the provisions of this protocol. I will comply with the provisions of this protocol, all requirements regarding the obligations of clinical investigators as fully outlined in the International Conference on Harmonization (Section E6(R1) Good Clinical Practice), local regulatory requirements, and the Investigator's Agreement, which I have also signed.

I have read and understand the information in the Investigator's Brochure(s), including the potential risks and side effects of the product under investigation, and will ensure that all staff assisting in the conduct of the study are informed about the obligations incurred by their contribution to the study.

Investigator of Record Name: _____

Investigator of Record Signature: _____

Date: _____

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ABBREVIATIONS AND ACRONYMS

AC	Abdominal circumference
ACOG	American College of Obstetricians and Gynecologists
AI	Artificial intelligence
ANC	Antenatal care
BMGF	Bill and Melinda Gates Foundation
BOE	Best obstetric estimate
CNN	Convolutional Neural Network
DICOM	Digital Imaging and Communications in Medicine
EDD	Estimated date of delivery
FAMLI	Fetal Age Machine Learning Initiative
FH	Fundal Height
FWA	Federal wide assurance
GA	Gestational age
hCG	Human chorionic gonadotropin hormone
IVF	In vitro fertilization
LH	Luteinizing hormone
LMIC	Low- and middle-income countries
LMP	Last menstrual period
MAE	Mean absolute error
ML	Machine Learning
MUAC	Mid-upper arm circumference
OHRP	US Office for Human Research Protections
PHI	Protected Health Information
PI	Principal investigator
SAE	Serious adverse event
SOP	Standard operating procedure
UNC	University of North Carolina
UNZA	University of Zambia
US	Ultrasound
UTH	University Teaching Hospital
WHO	World Health Organization

PROTOCOL SUMMARY

Background: Most of the world's obstetrical providers do not have access to the diagnostic benefit of ultrasound because of the historically high cost of equipment and need for trained sonographers. We have developed a machine learning tool ("FAMLI Technology") that can estimate fetal gestational age from a series of ultrasound blind sweeps collected by an untrained operator using a low-cost, battery-powered device.

Objectives: The overall aim of this study is to assess the diagnostic accuracy of a machine learning algorithm in estimating gestational age in the second and third trimesters of pregnancy. The primary study outcome is diagnostic accuracy, measured as the difference in mean absolute error (MAE) of the FAMLI Technology and standard fetal biometry in the primary evaluation window.

Design: This is a prospective cohort study of women enrolled early in pregnancy, with randomization to determine the timing of three follow-up visits in the second and third trimester**.** At each of these follow-up visits, we will assess gestational age with the FAMLI technology and compare that estimate to the known gestational age established early in pregnancy. We will also conduct standard ultrasound biometry measurements likewise assessing the accuracy of this approach. Our study design will allow precise characterization of the technology's accuracy (against the previously established ground truth) and also allow head-to-head comparison of the FAMLI Technology to standard fetal biometry.

Population: 400 pregnant women aged 18 or older enrolled before 14 weeks of gestation as documented by a baseline ultrasound scan and followed through delivery. All participants must have a viable, singleton, intrauterine pregnancy.

Study Sites: This study will be conducted in the antenatal clinics of the University Teaching Hospital (UTH) and the Kamwala District Health Centre in Lusaka, Zambia and the prenatal clinics of the University of North Carolina at Chapel Hill.

Duration/Follow up: Participants will be enrolled over approximately one year and followed through delivery. The study is expected to take two years to complete.

Relevance: Among the most critical benefits of obstetric sonography is its ability to measure anatomical structures within the fetus and from this, make an estimate of fetal gestational age. Obstetric care providers offer a well-established set of screening and preventative activities as pregnancy proceeds, and the timing of many of these relies upon accurate estimation of the GA. Given the importance of GA to guide appropriate clinical care, accurate measurement is critically necessary to improve pregnancy outcomes worldwide.

1.0 Introduction

A lynchpin of modern pregnancy care, obstetrical sonography has been in routine use in North America and Europe since the 1970s. Ever-advancing technology makes it now possible to fit into a pocket-sized unit the imaging technology that once would have required a 200-pound machine on casters. Yet, because of the historically high cost of equipment and need for trained sonographers, most of the world's obstetrical providers do not have access to the diagnostic benefit of ultrasound. Without it, they are armed only with centuries-old physical exam techniques that do not provide sufficient diagnostic discrimination to identify and appropriately refer most high-risk cases.

In 2018, our team received funding from the Bill and Melinda Gates Foundation to generate data, engineering, and analytical solutions to contribute to the development of robust, inexpensive, ultrasound technologies that could be deployed in low-resource settings to improve obstetric diagnostics. The first phase of that project – the Fetal Age Machine Learning Initiative (FAMLI) protocol (UNZA BREC Ref 005-08-18, UNC IRB #18-1848) – has enrolled nearly 5,000 women in Zambia and North Carolina into a prospective data collection protocol. We have analyzed data from these participants and built a tool that can estimate gestational age (GA) from blindly obtained ultrasound sweeps of the gravid abdomen. This innovation takes advantage of two new technologies. The first is the miniaturization of medical ultrasound technology. Over the last decade, several low-cost, handheld, ultrasound devices have become available – many of which have obtained U.S. Food and Drug Administration (FDA) 510(k) clearance and CE conformity marking in the European Union.[1,2] The second new technology is the application of machine learning (ML) computer vision models for automated medical image interpretation.[3,4]

This protocol describes a diagnostic accuracy study to assess the performance of our new tool (hereinafter "FAMLI Technology") in assessing gestational age. We will enroll 400 pregnant volunteers prior to 14 completed gestational weeks and obtain accurate "ground truth" GA dating with standard ultrasound biometry, using the crown-rump length. These participants will then be asked to return for three follow-up visits, which will include a routine sonogram performed by a trained sonographer and the collection of a set of blind sweep cineloop videos using a low-cost, battery-operated device. The research will be conducted in Chapel Hill, North Carolina (at the University of North Carolina Hospital and/or sites associated with UNC OBGYN) and in Lusaka, Zambia (at the University Teaching Hospital or Kamwala District Health Centre). Approximately equal numbers of participants will be enrolled from each country.

NB: The primary purpose of this research is to assess the diagnostic accuracy of the FAMLI Technology, a novel machine learning-based tool for gestational age assessment that can run on a smart phone or tablet. As such, we are not evaluating the performance of any ultrasound device and this protocol will only use ultrasound scanners that are approved for use in the participating countries.

2.0 Statement of the Problem

Approximately 830 women die each day worldwide from preventable causes related to pregnancy and childbirth, and almost all of these deaths occur in low-resource settings.[5,6] Approximately 2.7 million neonates die per year,[7] and an additional 2.6 million are stillborn.[8] Early ascertainment of fetal gestational age is critical to good clinical management, as is detection of clinical obstetric conditions that put both mothers and fetuses at increased risk of morbidity and mortality. Ultrasound improves GA estimation and can provide detailed

information on fetal wellbeing and maternal conditions, thus identifying high-risk pregnancies and enabling informed decisions around patient management (Table 1). Despite these well-described benefits, obstetric sonography is not available to the majority of the world's pregnant women.[9]

Table 1: Obstetrical diagnoses facilitated by sonography

Diagnosis	Proportion of pregnancies affected	Adverse maternal outcomes	Adverse fetal or neonatal outcomes
Ectopic pregnancy	0.015[10]	Hemorrhage, death	-
Twin or triplet gestation	0.013[11]	Preeclampsia, hemorrhage, death	Prematurity, perinatal death
Placenta previa, accreta, vasa previa	0.005[12]	Hemorrhage, death	Anoxic brain injury, perinatal death
Fetal malpresentation	0.038[13]	Obstructed labor, fistula, pelvic trauma, sepsis, hemorrhage, death	Anoxic brain injury, perinatal death
Fetal growth restriction	0.10[14]	-	Anoxic brain injury, perinatal death
Macrosomia ($\geq$ 4,000g)	0.08[15]	Obstructed labor, fistula, pelvic trauma, sepsis, hemorrhage, death	Birth trauma, peripheral nerve injury, anoxic brain injury, perinatal death
Oligohydramnios	0.11[16]	-	Perinatal death
Polyhydramnios	0.01[17]	Respiratory compromise, hemorrhage, death	Prematurity, perinatal death
Pre-term birth	0.111[18]	-	Prematurity, neonatal death
Post-term pregnancy	0.004-0.07[19]	Obstructed labor, fistula, pelvic trauma, sepsis	Anoxic brain injury, meconium aspiration syndrome, perinatal death
Intrauterine fetal death	0.0184[20]	Coagulopathy, sepsis, hemorrhage, death	-
Retained placenta	0.02[21]	Sepsis, hemorrhage, death	-

3.0 Rationale

Among the most critical benefits of obstetric sonography is its ability to measure anatomical structures within the fetus and from this, make an estimate of fetal gestational age. Obstetric care providers offer a well-established set of screening and preventative activities as pregnancy proceeds,[22] and the timing of many of these relies upon accurate estimation of the GA. Similarly, if a complication arises during routine care, an accurate gestational age estimate is an essential piece of information required to make appropriate decisions around the clinical management of mother and fetus. Providers may make drastically different decisions, for instance, in the care of a woman who develops a pregnancy complication prior to fetal viability compared to that same complication developing at term. Given the importance of GA to guide appropriate clinical care, accurate measurement is critically necessary to improve pregnancy outcomes worldwide.

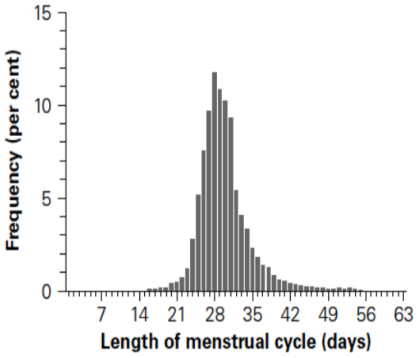
4.0 Background and Literature Review

By convention, pregnancies are dated from the first day of the last normal menstrual period (LMP). Conception typically happens 14 days later, but this period can vary substantially among individuals (Figure 1).[23] Thus, we rarely know the exact date of conception. A notable exception includes *in vitro* fertilization (IVF), where we know the exact date of fertilization

and thus the age of the embryo on the date of transfer to the uterus. Among women who monitor ovulation with luteinizing hormone (LH) test strips, we can estimate the date of conception within 1-3 days. A urine pregnancy test, which detects human chorionic gonadotropin hormone (hCG) in the urine, typically becomes positive 2 weeks after conception (i.e., 4 weeks GA by convention).

Gestational age dating in settings where obstetric ultrasound is not routinely available relies primarily upon the LMP. This is typically ascertained through patient interview at the first antenatal visit. An estimated date of delivery (EDD) is assigned by adding 280 days (40 weeks) to the first day of the recalled LMP (Naegele's rule).[24] While this approach is simple and cost-effective, it is well known that LMP recall call be inaccurate,[25] especially in settings where first presentation to antenatal care is later in gestation.[26] Indeed, data on over 100,000 pregnancies in Lusaka, Zambia indicate a median GA at presentation of 23 weeks.[27] Further, data from our research site in Zambia suggests that participant recall is not only imprecise, but also may introduce bias (Figure 2).[26]

Figure 1: Variability in menstrual cycle length (18,084 cycles recorded by 701 women in Japan, 1958-59)[1]



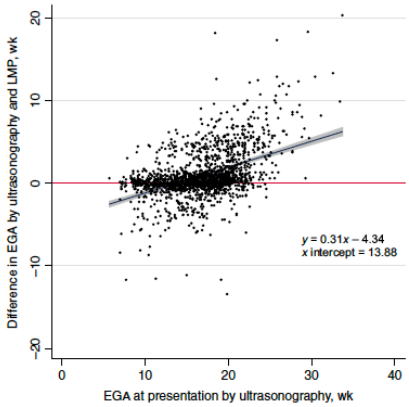
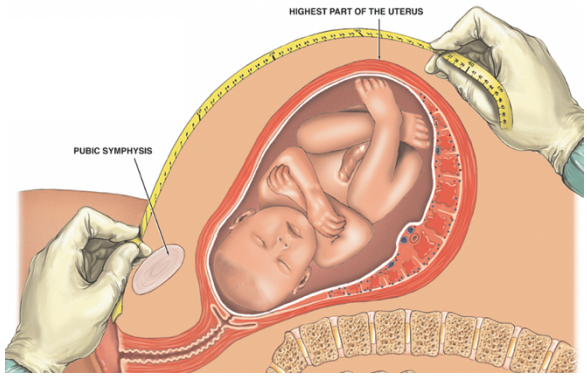


FIGURE 2 Discrepancy between ultrasonography- and LMP-based EGA by ultrasonography-based gestational age at presentation (n=1785). Abbreviations: EGA, estimate gestational age; LMP, last menstrual period.

Gestational age can also be estimated by physical exam and in many settings, this approach is used to supplement – and occasionally overrule – estimate made by LMP. The height of the uterine fundus ("fundal height") is ascertained with a simple flexible tape measure (Figure 3) and is defined as the distance between the pubic symphysis and the uterine fundus. In the absence of uterine pathology (e.g., large leiomyoma) or fetal conditions that might cause the uterus to be unusually large (e.g., polyhydramnios, multiple gestation), the fundal height (in centimeters) roughly corresponds to the current gestational age (in weeks) once the uterus is palpable above the pubic bone.[28]

Figure 3: Measurement of symphysis-fundal height

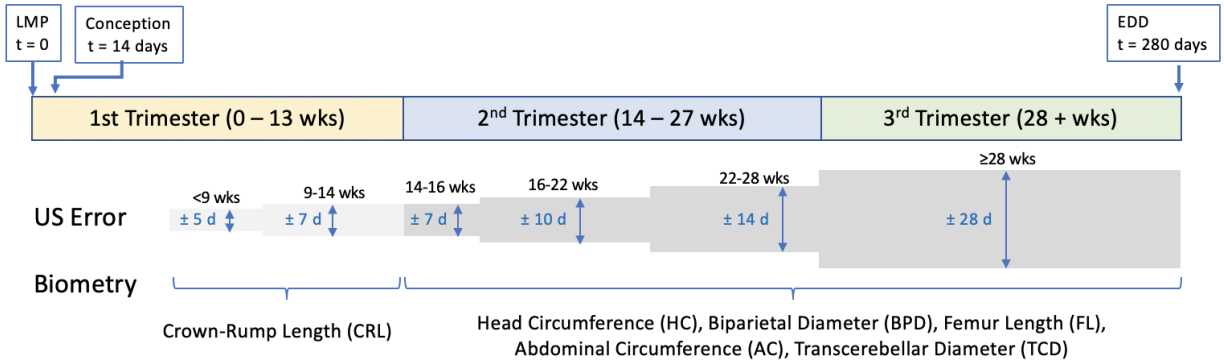


The standard of care for GA estimation in settings where antenatal ultrasound (US) is available instead uses an algorithm known as the best obstetric estimate (BOE).[24] Typically, an ultrasound is performed at the first antenatal visit or soon thereafter. The sonographer measures known fetal structures ("biometry") which are used to estimate the fetal GA on the day of the ultrasound. The error associated with current biometry formulae (of which there are several[29-32]) increases with advancing GA.[24]

The BOE is calculated by comparing the GA according to LMP to the GA according to ultrasound. The reported LMP is used to estimate GA unless the ultrasound measurement

differs by a predefined amount (Figure 4), in which case the US estimate is used. For women whose LMP is uncertain or unknown, the ultrasound estimate alone is used. Obstetrical providers generally do not change the estimated due date once it is set by the BOE, since a subsequent ultrasound estimate is typically less accurate than an earlier one.

Figure 4: Gestational age dependent ultrasound measurement error[24]



4.1 Preliminary Data

The FAMLI Technology being evaluated in this protocol is derived from the original FAMLI study, which opened to enrollment in September 2018. Between that time and June 2021, we collected prospective data from 4,695 participants in Zambia and North Carolina. Women were eligible to enroll at any time during pregnancy and could return for repeat scans no more frequently than monthly in Zambia and bi-weekly in North Carolina. After provision of written informed consent, we collected background clinical and obstetrical information on each participant through interview and medical record review. We also documented their LMP (if known) and EDD from prior ultrasound (if performed).

Ultrasound procedures included documentation of fetal viability, pregnancy location, number of fetuses, fetal heart rate, fetal lie, and assessment of amniotic fluid volume. Measurements of gestational age-appropriate structures for fetal biometry (crown-rump length, abdominal circumference, head circumference, bi-parietal diameter, femur length, transcerebellar diameter) were each performed twice.

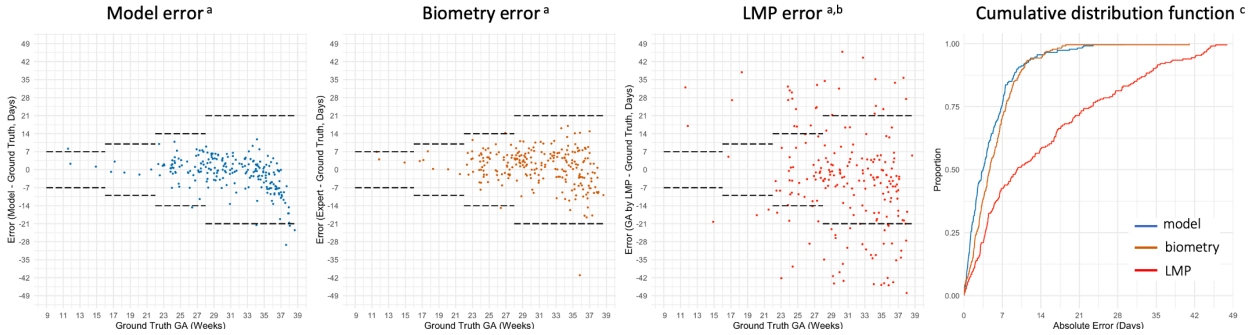
During the same examination, we also collected a series of blind sweep cineloop videos. These were free-hand sweeps, 5 to 10 seconds in length, across the gravid abdomen in multiple directions and probe configurations. Cranio-caudal sweeps started at the pubis and ended at the level of uterine fundus with the probe indicator facing toward the maternal right either perpendicular (90 degrees) or angled (15 and 45 degrees) to the line of probe movement. Lateral sweeps were performed with the probe indicator facing superiorly, starting just above the pubis and sweeping from the left to the right lateral uterine borders and moving cephalad to the uterine fundus. Complete sets of blind sweeps were collected by the study sonographer on both the commercial ultrasound machine and a low-cost, battery-powered device (Butterfly iQ; Guilford, CT, USA).

4.1.1 Performance of the deep learning model

Preliminary results from the novice test set informed the development of the FAMLI Technology being assessed in the present protocol. This test set contains 249 participant exams in which blind sweeps were obtained by an untrained user on a low-cost, battery-powered device (Butterfly iQ). We compared the deep learning model estimates to biometry obtained by a trained sonographer on a commercial ultrasound (Figure 5). We also compared

model estimates to the gestational age that would have been calculated had only the LMP been available (as is overwhelmingly the case in Zambia and other similar settings where this technology would be used). We found that the model and biometry performed similarly: overall model mean absolute error (MAE) 4.9 days (standard error [SE] 0.29) versus biometry MAE 5.4 days (SE 0.28); difference -0.6 days (95% CI: 1.3, 0.1); p=0.11). However, when compared to LMP, the model was clearly superior: model MAE 4.9 days (SE 0.29) versus LMP MAE 17.4 days (SE 1.17); difference -12.7 days (95% CI: -15.0, -10.3); p<0.001). Based on the empirical cumulative distribution function of the model error, the proportion of study scans that were correctly classified within 7 days was substantially higher for the model than for LMP (71.9% vs 40.1%; difference 36.1% [95% CI 28.0%, 44.2%]; p<0.001). The model similarly outperformed LMP using a 14-day classification window (94.8% vs 55.1%; difference 40.5% [95% CI 33.9%, 47.1%]; p<0.001).

Figure 5: Gestational age estimation of deep learning model compared to trained sonographer and last menstrual period (LMP) in the novice test set



[a] dashed horizontal lines represent expected accuracy of ultrasound biometry[4]
[b] data missing from 22 participants who could not recall their LMP
[c] 13 studies from GA by LMP excluded from the plot because the absolute error is truncated at 49 days

5.0 Research Aims

The FAMLI Technology is a machine learning tool that uses a novel deep convolutional neural network to estimate fetal gestational age from a series of ultrasound blind sweeps. We intend to assess the diagnostic accuracy of the technology by enrolling a cohort of women in early pregnancy, establishing their gestational age by crown-rump length measurement, and then assigning follow-up visits at random times during three gestational age windows. At each of these visits, we will assess gestational age with the FAMLI technology and compare that estimate to the known gestational age established at the baseline ultrasound. Alongside this assessment, we will also conduct standard ultrasound biometry measurements likewise assessing the accuracy of this approach. Our study design will allow precise characterization of the technology's accuracy (against the previously established ground truth) and also allow head-to-head comparison of the FAMLI technology to standard fetal biometry (which is the current gold standard).

Terms:

- **Ground truth** – the gestational age and corresponding estimated date of delivery (EDD) established prior to 14 weeks of gestation and assumed in this protocol to represent a gold standard measurement.
- **Blind sweep** – freehand ultrasound sweeps of the gravid abdomen collected as cineloop video.

- **FAMLI Technology**– the trained deep learning model when deployed on an Android or iOS device and receiving blind sweep ultrasound data from a low-cost probe (e.g., Butterfly iQ+).
- **Untrained operator** – a clinician (e.g., nurse or midwife) with general obstetrics knowledge but with no prior training in sonography.
- **Index test** – a term of art in diagnostic accuracy studies that refers to the test being assessed, in this case the FAMLI Technology.
- **Clinical reference standard** – a term of art in diagnostic accuracy studies that refers to the test against which the index test is evaluated, in this case standard fetal biometry performed by a trained sonographer on a commercial ultrasound machine.
- **Primary evaluation window** – the GA window corresponding to the second trimester of pregnancy (14 $^{0}/_{7}$ through 27 $^{6}/_{7}$ gestational weeks, inclusive). At enrollment each participant will be assigned a random date within this window to return for a study ultrasound evaluation.
- **Secondary evaluation windows** – the GA windows corresponding to the preterm third trimester (28 $^{0}/_{7}$ through 36 $^{6}/_{7}$ weeks, inclusive) and term gestation (37 $^{0}/_{7}$ through 40 $^{6}/_{7}$ weeks, inclusive). At enrollment each participant will be assigned random dates within these windows to return for a study ultrasound evaluation.

5.1 Primary objective

- To assess the diagnostic accuracy of the FAMLI algorithm in estimating gestational age in the second and third trimesters of pregnancy. This objective will be met by comparing the measurement error (defined in Section 6.8.1) of the index test (i.e., the FAMLI Technology interpreting blind sweeps obtained on a low-cost probe by an untrained operator) to that of the clinical reference standard (i.e., standard fetal biometry performed by a trained sonographer on a commercial ultrasound machine).

5.2 Secondary objectives

- To assess the extent to which operator experience affects performance of the FAMLI algorithm by comparing diagnostic accuracy of blind sweeps collected by an untrained operator to blind sweeps collected by a trained sonographer.
- To assess the extent to which individual transducer technology affects performance of the FAMLI algorithm by comparing diagnostic accuracy of blind sweeps collected on a CMUT/CMOS probe to blind sweeps collected on a traditional piezoelectric ultrasound probe.
- To assess the potential of biological metabolites/biomarkers to augment the performance of the machine learning model in estimating gestational age

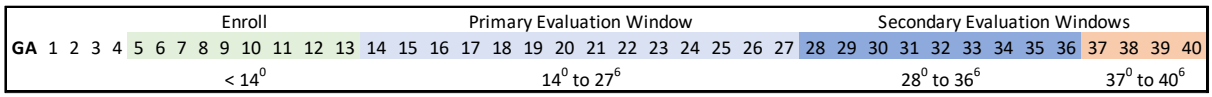
6.0 Study Methodology

6.1 Study design

We propose a prospective cohort study of 400 women enrolled before 14 weeks of gestation who will undergo 3 follow-up ultrasound evaluations during pregnancy. These follow-up visits will be assigned at random dates within 3 specific GA windows corresponding to the second trimester, the preterm third trimester, and term gestation (Figure 6). At each study visit, the participants will be evaluated with both the FAMLI Technology and standard of care ultrasound. We will also collect low-volume blood and urine specimens at each visit for

development of a gestational age estimation tool using maternal biomarkers and for future protocol-related testing.

Figure 6: Study visit windows



6.2 Study sites and study population

This study will be conducted in the antenatal clinics of the University Teaching Hospital (UTH) and the Kamwala District Health Centre in Lusaka, Zambia and the prenatal clinics of the University of North Carolina at Chapel Hill.

Inclusion criteria:

1. 18 years of age or older
2. viable intrauterine pregnancy at < 14 $^0/_7$ weeks of gestation
3. ability and willingness to provide written informed consent
4. intent to remain in current geographical area of residence for the duration of study
5. willingness to adhere to study procedures

Exclusion criteria:

1. maternal body mass index ≥ 40 kg/m^2
2. multiple gestation (i.e., twins or higher order)
3. major fetal malformation or anomaly
4. any other condition (social or medical) that, in the opinion of the study staff, would make study participation unsafe or complicate data interpretation.

6.3 Study visit procedures

6.3.1 Screening and Randomization

In Zambia, our recruitment activities will begin with community sensitization in the catchment area of the recruitment clinics to educate community members about the study and encourage early presentation for ultrasound. This community and clinic sensitization will be underway prior to initiation of this protocol as a result of other programs ongoing in the catchment areas. In North Carolina, we will advertise the study through flyers, posters, and other electronic or written materials.

Study staff will approach potentially eligible women – either in groups or individually – to inform them about the study (including eligibility criteria). Staff will provide interested women with additional information and referrals to the study clinic for eligibility assessment.

All protocol-specified ultrasound procedures will occur after 13 completed gestational weeks (i.e., 14 $^0/_7$ weeks or greater). To be eligible for study participation, women must have documentation of a baseline scan conducted by a trained sonographer prior to 14 weeks gestation to confirm pregnancy location, viability, and gestational age by crown-rump length.

After completing the study informed consent process, study staff will gather targeted information on demographics, health and risk behaviors, medical and obstetrical history, medication use, stress, and nutrition. Participants will undergo blood pressure assessment and anthropometry, including height, weight, mid-upper arm circumference (MUAC), and fundal height (FH). Blood and urine will be collected as detailed in Table 3.

Following final confirmation of eligibility, participant will be assigned a date for each of their follow-up visits in a fashion that ensures approximately equal distribution of study visits across the GA range within each designated visit window. For instance, the primary evaluation window comprises the 14-week period between 14 $^{0}/_{7}$ and 27 $^{6}/_{7}$ gestational weeks, inclusive. Thus, through the randomization procedure, a participant will have a 1 in 14 chance of being allocated to a given gestational week within the primary evaluation window (Table 2). A statistician not otherwise associated with the study will design the scheme and pre-generate the visit schedules for each participant prior to study commencement. Women will be allowed to choose their preferred day of participation within the randomized week.

Participants who are randomly allocated to a follow-up visit schedule who do not complete visit 1.0 for any reason (e.g., because they deliver prior to the target window or do not return for the visit) will count as screened but will not count toward the overall enrollment target of 400 participants.

Table 2: Random visit allocation

	Gestational Week	GA Range (days)	Number allocated
Primary Evaluation Window	14^{0} - 14^{6}	98 - 104	28
	15^{0} - 15^{6}	105 - 111	28
	16^{0} - 16^{6}	112 - 118	28
	17^{0} - 17^{6}	119 - 125	28
	18^{0} - 18^{6}	126 - 132	28
	19^{0} - 19^{6}	133 - 139	28
	20^{0} - 20^{6}	140 - 146	29
	21^{0} - 21^{6}	147 - 153	29
	22^{0} - 22^{6}	154 - 160	29
	23^{0} - 23^{6}	161 - 167	29
	24^{0} - 24^{6}	168 - 174	29
	25^{0} - 25^{6}	175 - 181	29
	26^{0} - 26^{6}	182 - 188	29
	27^{0} -27^{6}	189 - 195	29

	Gestational Week	GA Range (days)	Number allocated
Secondary Evaluation Windows	28^{0} - 28^{6}	196 - 202	44
	29^{0} - 29^{6}	203 - 209	44
	30^{0} - 30^{6}	210 - 216	44
	31^{0} - 31^{6}	217 - 223	44
	32^{0} - 32^{6}	224 - 230	44
	33^{0} - 33^{6}	231 - 237	45
	34^{0} - 34^{6}	238 - 244	45
	35^{0} - 35^{6}	245 - 251	45
	36^{0} - 36^{6}	252 - 258	45
	37^{0} - 37^{6}	259 - 265	100
	38^{0} - 38^{6}	266 - 272	100
	39^{0} - 39^{6}	273 - 279	100
	40^{0} - 40^{6}	280 - 286	100

6.3.2 Study follow-up

At each study follow-up visit, research staff will collect interval medical and obstetric history, perform anthropometry, measure blood pressure and fundal height, and collect blood and urine specimens for storage and future approved testing (Table 3). An untrained operator will conduct blind sweep procedures using both a commercial ultrasound machine and an approved portable low-cost device. More than one low-cost device may be used for data collection. Next, the sonographer will perform full standard biometry procedures and additional protocol-related ultrasound sweeps using a commercial ultrasound machine. Finally, the trained sonographer will perform biometry and blind sweeps with the low-cost device.

NB: The FAMLI Technology software will not be used to assess gestational age at the time of novice or trained sonographer assessment in an effort to avoid biasing sonographer data collection during biometry. We will also train sonographers not to ask participants their due date or last menstrual period prior to commencing the study scan.

All participants will be followed through delivery. Study staff will document gestational age, vital status, birthweight, and sex at delivery through a combination of medical record review

and participant interview as needed. This visit may occur over the phone or in person. Based on the timing of delivery, not all participants may complete all three planned study follow-up visits.

TABLE 3: Schedule of Evaluations

Visit Number	**0.0**	**1.0**	**2.0**	**3.0**	**4.0**
Gestational Age (weeks)	<14	14-27[6]	28-36[6]	37-40[6]	Delivery
ADMINISTRATIVE / REGULATORY PROCEDURES					
Informed consent	•				
Confirmation of eligibility	•				
Collection / review of locator info	•	•	•	•	•
Randomization of visit timing	•				
CLINICAL / BEHAVIORAL PROCEDURES					
Obstetrical and medical history	•	•	•	•	•
Anthropometry	•	•	•	•	
Blood pressure measurement	•	•	•	•	
Fundal height measurement	•	•	•	•	
Physical exam	•				
Untrained operator scan		•	•	•	
Trained sonographer scan		•	•	•	
LABORATORY PROCEDURES					
Urine storage	•	•	•	•	
Blood storage	•	•	•	•	

6.4 Retention

Once a participant is enrolled in the study, the study team will make all reasonable efforts to retain her in follow-up to minimize bias associated with loss to follow-up. The study team will track retention rates and address any issues related to retention. Strategies will include:

- Thorough explanation of the study visit schedule and procedures during informed consent, and re-emphasis at each study visit.
- Encouragement of participants to discuss potential study participation with their husbands/partners and other influential family members before agreeing to enrol in the study.
- Collection of detailed locator information at screening, and review and updating of this information at each study visit.
- Use of appropriate and timely visit reminder mechanisms (including phone calls and text messages).
- Mobilization of trained outreach workers to complete in-person contact with participants at their homes and/or other locations.

6.5 Safety monitoring

At each study visit, study staff will evaluate participants for social harms and adverse events (AEs). A social harm will be defined as a non-medical untoward consequence of study participation, including: difficulties in personal relationships, stigma, or discrimination from family or community. An AE will be defined as any untoward medical occurrence in a study participant including any abnormal sign (e.g., abnormal physical exam or laboratory finding),

symptom, or disease, temporally associated with the individual's participation in the research, whether considered related to participation in the research or not. In addition to events related to study procedures, which are expected to be very rare, we can expect that this population of pregnant women will experience adverse events unrelated to study procedures, including adverse obstetrical outcomes, infections, side effects from routine medications, hospitalization, and death.

We expect events to be minimal, but all will be documented, assessed for seriousness / severity and relatedness, and carefully monitored. The severity of study-related adverse events and social harms will be graded using the National Institute of Health's Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events; this is standard of care for all studies conducted at our sites. We will also record information on all serious adverse events (SAEs) occurring in maternal participants whether or not they are related to study participation, including AEs that:

1. Result in hospital admission (unless hospitalization is preplanned, i.e., for delivery) or prolongation of existing hospitalization,
2. Are immediately life-threatening,
3. Cause significant, persistent, or permanent harm or disability, either physical or psychological,
4. Result in death, including fetal demise after 20 weeks of gestation, or
5. Are congenital anomalies/birth defects.

Information on adverse events or social harms that are related to the study and all SAEs will be documented on study data forms and routinely reported to the Principal Investigator (PI) or designee. If the PI, co-investigators, or their designees determine that study-related AEs are occurring at an unexpected rate, they will assess the need for and facilitate staff re-training, protocol amendment, or study cessation. Serious study-related AEs will be reported to all regulatory bodies and the study sponsors within 72 hours of site awareness. Other events will be reported according to each ethics committees' individual guidelines.

6.5.1 Identification and management of clinical complications of pregnancy

When collecting data or performing physical exams, study staff may identify medical or other issues requiring follow-up, treatment, or other clinical care. Similarly, during the course of performing ultrasound procedures on study participants, sonographers may identify medical issues requiring follow-up, treatment, or other clinical care. All team members will be trained on the recognition of adverse events and social harms and what to do if encountered. Safety issues requiring follow-up will be brought to the attention of the senior clinical staff, who will make referrals if the issue is beyond staff knowledge or skills to be able to adequately address.

Potential reasons for clinical referral include, but are not limited to:

- Draining of fluid or concern for ruptured membranes
- Vaginal bleeding or concern for threatened abortion or antepartum hemorrhage
- Abnormal vaginal discharge
- Hypertension
- Fetal anomalies
- Abnormalities of fetal growth
- Oligo- or polyhydramnios
- Intrauterine embryonic or fetal demise

- Malpresentation at or near term
- Suspected extrauterine pregnancy (ectopic)

When possible, the participant will be invited to remain in the study. Additional ultrasound follow-up procedures as determined by obstetrical management recommendations will be provided by study sonographers as requested and free of charge.

6.6 Biological specimen collection

All samples will be obtained from study participants by trained clinical staff according to approved standard operating procedures. Biological samples (i.e., blood and urine) will be used for untargeted metabolomics and other multi-omic analyses to identify metabolites, proteins, transcripts, and host genetic factors associated with fetal maturation, gestational age, parturition timing, and adverse outcomes. Each study site will house its own biological samples and make plans for off-site redundancy in case of power failures or freezer malfunction. Lab testing will be conducted in parallel with the primary analysis and will take advantage of the excellent gestational age dating available for all participants and the even distribution of sample collection across the gestational age spectrum.

Use of stored specimens for testing that is not specifically designated in this protocol as described above will require additional regulatory approval. Separate written informed consent for specimen storage and future use will be specifically obtained during the informed consent process.

6.6.1 Quality control and quality assurance procedures

Standard Operating Procedures (SOPs) following manufacturer's protocols and detailing technical procedures involved (e.g., sample collection, processing, and storage) will be developed and used by the study team. Site coordinators will complete annual recertification. The certification process is an opportunity to ensure the highest specimen quality and standardize collection techniques.

6.7 Data security and management

Research data will be captured in a dedicated study database. Study data management (e.g., data transmission, query resolution, etc.) will follow procedures outlined in study SOPs. Study identification numbers will be used on all forms and communications related to the study. A separate confidential register will link study identification numbers and participants' names. All data instruments and registers will be securely stored. All study computers and electronic devices will be password protected and their access restricted to authorized study personnel. Backups of the data will be made routinely. Data may be transmitted electronically to the study investigators through secure servers. Study information will not be released without written permission of the participant, except when necessary for monitoring by the relevant ethical committees or their designees.

Identifiable data will be maintained for at least ten years after completion of participant follow-up. Biological specimens will be incinerated after ten years from collection or as dictated by in-country guidelines. Study records will be disposed of following sponsor guidelines after being stored in-country for at least three years following the end of study follow-up. Paper records will be destroyed (e.g., shredded or incinerated) prior to disposal.

6.8 Statistical considerations

6.8.1 Study outcomes

The primary study outcome is diagnostic accuracy, measured as difference in mean absolute error (MAE) of the index test and clinical reference standard in the primary evaluation window. MAEs will be calculated as the average of the absolute differences between the GA estimated by the index test (i.e., the FAMLI Technology interpreting blind sweep data generated by an untrained operator with a low-cost probe) or clinical reference standard (i.e., standard fetal biometry performed by a trained sonographer on a commercial ultrasound machine) and previously established ground truth GA.

Secondary study outcomes will be assessed for both the index test and clinical reference standard and include (1) MAE in the secondary evaluation windows; (2) root mean squared error (RMSE) in all three evaluation windows; and (3) proportion of studies correctly classified within 7 days of the ground truth.

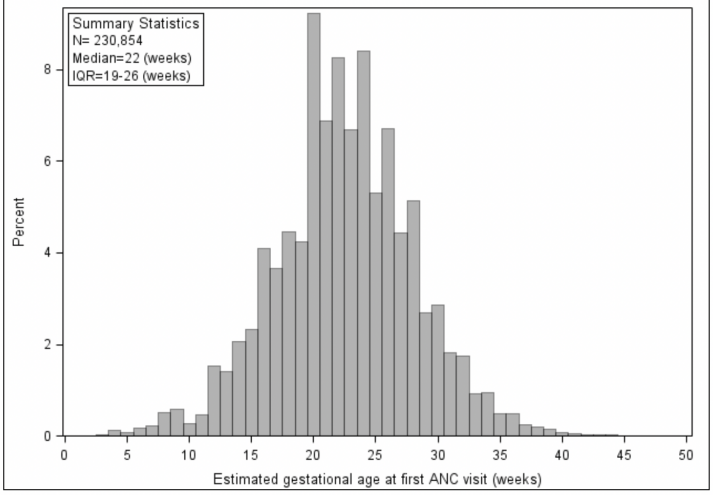
Secondary lab outcomes will be assessed for all participants. Untargeted metabolomics will investigate organic acids, organoheterocyclic compounds, lipids and lipid-like molecules, benzenoids, organic oxygen compounds, and other minor chemical classes potentially associated with gestational age. Other multi-omics markers including proteins, transcripts, and host genetic factors may also be analyzed.

6.8.2 Sample size calculations

We designed this diagnostic accuracy study to test a non-inferiority hypothesis, such that if we observe a 95% confidence interval (CI) to be *completely* contained in the interval (-*c, c*) then we declare non-inferiority (or equivalence to bound *c*), with type 1 error 2.5%. We chose a sample size such that the statistical power for the non-inferiority hypothesis test is 95%. One implication of choosing the above type 1 error and statistical power for this non-inferiority test is that, if we observe the 95% CI to *not* be contained in the interval (-*c, c*), i.e., the lower end of the observed 95% CI is smaller than -*c* or the upper end is larger than *c*, then we may declare superiority, with type 1 error 5%, because the null hypothesis for superiority (i.e., a 0-difference) would be excluded from this observed 95% CI.

We used Monte Carlo simulation to find the sample size that yields a statistical power of at least 95% for the non-inferiority test with type 1 error of 2.5%, and non-inferiority bound of *c* = 2 day error in estimated GA. The statistical test is based on a difference in mean absolute error (MAE), comparing the FAMLI Technology to standard biometry, with both approaches measured in the primary evaluation window, which is between 14 $^{0}/_{7}$ and 27 $^{6}/_{7}$ weeks of gestation, inclusive (Figure 6). We chose this window for our primary evaluation because it corresponds to the second trimester of pregnancy, when most women

Figure 7: Gestational Age at Presentation for 230,854 women in Lusaka, Zambia (2006 – 2012)



first present in low-resource settings (Figure 7). Both the FAMLI Technology-estimated GA and standard biometry calculate absolute error with respect to the enrollment "ground truth" estimated GA, which is assigned by first trimester ultrasound or, in the case of *in vitro* fertilization, a known embryo age and transfer date.

We used women enrolled in the ZAPPS cohort with estimated GA < 13 weeks (n = 105) to inform the distribution of enrollment GAs, and another ZAPPS estimated GA measured in the 14 to 27-week window to inform the distribution of primary evaluation EGAs, by the standard approach. The enrollment estimated GA by standard biometry (intergrowth formula) was 74.5 ± 10.5 days. The follow up estimated GA in the primary evaluation window, also obtained by standard biometry (Intergrowth formula), was 155.4 ± 6.1 days, and after adjustment (subtracting the number of days between enrollment and primary evaluation) was 75.6 ± 12.3 days. The observed regression function for the adjusted estimated GA during the primary evaluation window was -7 + 1.1 (enrollment estimated GA) ± 4 days. Estimates below are based on 2000 simulations, which yields simulation error less than 2% for statistical power.

We assume the GA estimate produced by the FAMLI Technology has properties (i.e., intercept and slope) similar to standard biometry, but reduces the random error (τ). In a sensitivity analysis, we assumed the FAMLI Technology estimate also removed bias (i.e., intercept 0, slope 1), which yields a slight increase in power (results not shown). We do not anticipate that the FAMLI Technology will induce bias over the standard approach. We vary the random error τ for the FAMLI Technology from τ = 1 to 5, where τ = 4 days is the superiority null hypothesis of no difference between approaches.

Figure 8 shows the statistical power for non-inferiority as a function of the difference in absolute error between approaches (in days) and sample size. A horizontal reference line is drawn at 95% power. Under our assumptions, at a 0 difference in absolute errors between approaches, sample sizes of 200 or more have power in excess of 95%.

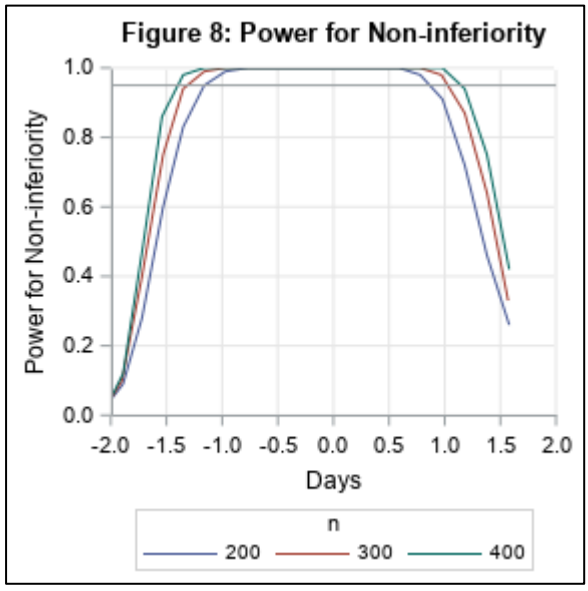
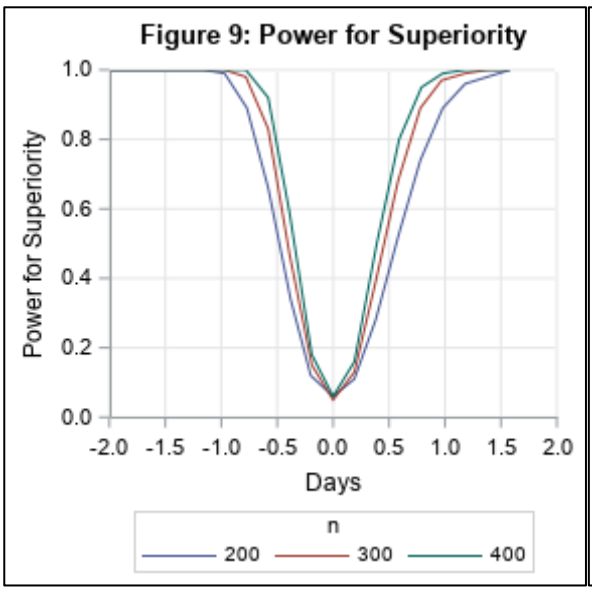
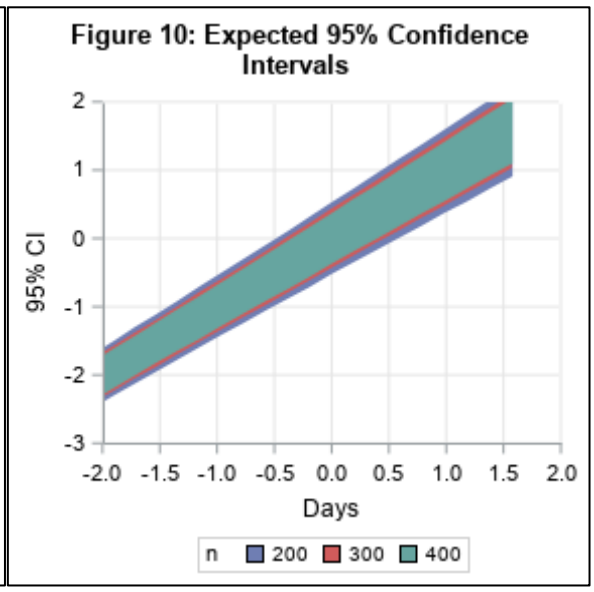


Figure 9 shows the statistical power for superiority as a function of the difference in absolute error between approaches (in days) and sample size. At the null hypothesis of 0 difference, the statistical power is approximately 5%, regardless of sample size (i.e., this is a valid statistical test). At a -0.5-day difference in absolute error (i.e., the FAMLI Technology estimated GA reduces random error by 1/2 day over standard approach), a sample size of 200 has less than 60% power, while a sample size of 400 has 80% power. Figure 10 shows the expected 95% CI as a function of the difference in absolute error between approaches (in days) and sample size.





In conclusion, we settle on a sample size of 400 women for the FAMLI study. Select numeric results are also shown in Table 4.

Table 4: Statistical power for non-inferiority and superiority, by difference in absolute error, and sample size *

		Statistical Power, %					
T	Days	Non-inferiority			Superiority		
		N=200	N=300	N=400	N=200	N=300	N=400
1.00	-2.22	0	0	0	100	100	100
1.25	-2.06	2	1	1	100	100	100
1.50	-1.89	9	10	12	100	100	100
1.75	-1.72	28	40	49	100	100	100
2.00	-1.53	60	75	86	100	100	100
2.25	-1.35	83	94	98	100	100	100
2.50	-1.16	95	99	100	100	100	100
2.75	-0.97	99	100	100	99	100	100
3.00	-0.77	100	100	100	89	98	100
3.25	-0.58	100	100	100	66	83	92
3.50	-0.38	100	100	100	34	47	59
3.75	-0.20	100	100	100	12	15	18
4.00	0.00	100	100	100	6	5	6
4.25	0.19	100	100	100	11	13	16
4.50	0.38	100	100	100	28	39	50
4.75	0.59	100	100	100	53	68	80
5.00	0.78	98	100	100	74	89	95
5.25	0.98	91	98	100	89	97	99
5.50	1.18	72	87	94	96	99	100
5.75	1.38	46	64	75	98	100	100
6.00	1.58	26	33	42	100	100	100

* Monte Carlo simulation error < 2%

6.8.3 Analysis plan

For each woman, we will determine the estimated GA based on the initial first trimester visit, which will be our reference value. Then, for each approach, we will calculate the estimated GA and the difference between this estimate and the reference value. In a primary analysis, we will compare the mean absolute errors for the different approaches. The approach with smaller mean absolute error is preferred. In secondary analyses, we will compare the root mean squared errors for the different approaches.

Because approaches are conducted on all women, comparisons of approaches are naturally controlled for all possible time fixed confounding factors. To account for differential loss to follow up, if loss to follow up exceeds 9%, and possible resultant selection bias, we will employ inverse probability of censoring weights.[3] Censoring weights will be estimated using a pooled logistic regression model fit by maximum likelihood.[4] Continuous covariates will be included using restricted quadratic splines.[5]

6.9 Dissemination of findings

Study findings will be disseminated through appropriate local channels, including academic and public health research symposia. One or more publications will also be submitted to a peer-reviewed journal. The study participants' privacy and confidentiality will be strictly maintained in all results dissemination or publication activities.

Sharing of data generated by this project is an essential part of our proposed activities and will be carried out in several different ways. The findings of this study will be made available through appropriate local channels, including academic and public health research symposia. We will make results available to the community of scientists interested in maternal and child health to avoid unintentional duplication of research.

Presentations at national scientific meetings. It is expected that several national meeting audiences would be interested in the findings generated by proposal completion. We plan to present findings at the annual meeting of the Society for Maternal Fetal Medicine, the annual meeting of Pediatric Academic Societies, and the International Conference on Maternal and Child Healthcare.

Access to data. This research will enroll 400 participants, each of whom will attend 3 follow-up visits in pregnancy (i.e., up to 1,200 research ultrasound scans performed). In addition to ultrasound data, the final study dataset will include a limited amount of demographic and behavioral data, medical history, pregnancy outcome, and physical exam findings. We will make de-identified data and associated documentation available to users only under a data-sharing agreement that binds the user to: (1) using the data for research purposes; (2) securing the data with appropriate technology; (3) destroying or returning the data after analyses are completed; (4) making appropriate attribution to data provenance; and (5) ensuring the Gates Foundation Global Access requirements are met.

7.0 Ethical Considerations

The protocol, informed consent documents, and any subsequent modifications will be reviewed and approved by all relevant ethics committees responsible for oversight of the study and maintaining Federal Wide Assurances (FWA) with the Office for Human Research Protections (OHRP) approval. For this study, relevant ethics committees include the

University of Zambia Biomedical Research Ethics Committee and the University of North Carolina at Chapel Hill Institutional Review Board.

Participation in this study will be voluntary. All participants involved in prospective collection of research data will provide written, informed consent prior to study enrolment. All care and procedures will be conducted according to local standards of routine clinical care. All staff who have contact with participants will receive training on the protection of human research participants prior to conducting any study activities and routinely thereafter. Key staff will also complete Good Clinical Practices training.

7.1 Informed consent

Discussions with prospective participants and informed consent procedures will be conducted in private to protect patient confidentiality. Where possible, a private room will be used to discuss the study and potential participant's eligibility. If a private room is not available, a designated area far enough away from other participants such that they cannot hear the conversation will be used. Study staff will obtain written informed consent from all participants. The study procedures, risks, and benefits will be discussed, and the responsible staff member will answer all questions prior to obtaining consent. The consent forms will be translated into local languages and back-translated into English to ensure accurate translation. All versions of the consent forms will be approved by the relevant ethics committees prior to study initiation. For illiterate participants, a literate impartial witness will be present during the entire consent process to ensure that all of the relevant information has been provided and the participant voluntarily gives consent.

Eligible women who do not wish to participate in this study will continue to receive antenatal care and treatment according to local clinical standards.

7.2 Potential risks to participants

Investigators will make efforts to minimize risks to participants. This study involves minimal risk to participants, no greater than at routine antenatal or clinical examinations. Participants will be asked to have additional ultrasound procedures beyond what they would normally receive for clinical purposes, but because ultrasound uses sound waves instead of radiation, we do not expect this additional exposure to offer any significant risk. Providers have used ultrasound for more than 30 years, and they have not found any dangerous risks.

Physical risks also include the risk of discomfort, bruising or swelling from venipuncture. The risks that are associated with venipuncture are infrequent and minimized with the use of proper technique. Such risks include (1) bleeding, (2) bruising, or (3) rarely infection at the site of needle insertion. Individuals may also rarely become faint, in which case symptoms abate after several minutes in a recumbent position. Blood volumes for the study have been calculated to ensure safety and participants will be reassured that such feelings are transient.

Participation in clinical research includes the risks of loss of confidentiality and discomfort with the personal nature of questions, particularly when discussing HIV infection or sexual behaviors. At each step in the study, we will protect participant privacy and confidentiality to reduce these risks (e.g., consenting participants in a private setting, not including names on case report forms, etc.). Although investigators make every effort to protect participant privacy and confidentiality, it is possible that participant involvement in the study could become known to others, and that social harms may result.

The confidentiality of all study records will be safeguarded to the extent legally possible. To maintain participant confidentiality, all laboratory specimens, reports, study data and administrative forms will be identified by a coded number only. All databases will be secured with password-protected access systems, and computer entries will be identified by coded number only. Forms, lists, logbooks, appointment books, and any other listings or data forms that link participant ID numbers to other identifying information will be stored in a separate, locked cabinet. All data analysis will be performed using datasets which have only the study number as a unique identifier. Clinical information with individual identifiers will not be released without the written permission of the participant. We expect these procedures to adequately protect participant confidentiality.

7.3 Potential benefits to participants and others

Individual participants may benefit from clinical monitoring through routine ultrasounds and health assessments, with referrals provided as needed. They may also receive no direct benefit. Women may appreciate the opportunity to see their fetus during pregnancy and will be provided with printed or electronic copies of ultrasound images.

Knowledge generated from this study has the potential to contribute to the development of widely deployable ultrasound technologies that can assess gestational age and other important obstetric conditions while requiring minimal user expertise, which has the potential to improve maternal and infant outcomes in low-resource settings worldwide.

In summary, risks to participants in this study are minimal and do not differ significantly from the risks inherent in the local standard of care for pregnant women. These risks are reasonable in relation to the anticipated benefits of the project.

7.4 Inclusion of children, sub-populations, and vulnerable populations

This study focuses specifically on pregnant women and, as such, they must be included in our study population. Prisoners will be excluded as they receive care at separate facilities.

8.0 Timeline

We have allocated 3 months to start-up activities and 21 months to study implementation.

Month	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8
Protocol and CRF development								
Ethical submission and review								
Site preparation								
Site training								
Enrollment								
Data entry and management								
Follow-up								
Statistical analysis								
Publication and dissemination								

9.0 Budget – Zambia field costs

The following costs are projected over the two-year study period.

ITEM	ZMW TOTAL	USD TOTAL
Personnel	3,750,000	$250,000
Equipment	750,000	$50,000

Supplies & materials	457,500	$30,500
Participant reimbursement	180,000	$12,000
Other – communication, data, translation, regulatory, community outreach, shipping, and training costs	937,500	$62,500
TOTAL	6.075,000	$405,000

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