Gestational Age Estimation in Resource Poor Settings

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For the women and children of Kilifi.
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Statement of Originality

I declare that the work presented in this thesis is my own and has not been submitted for any other degree in this or any other university or institute of learning.
Abstract

Background and objectives: The incidence of preterm birth (PTB), and the extent to which it results in perinatal mortality in sub-Saharan Africa (sSA) is unclear, partly because reliable estimates of gestational age (GA) at birth are lacking. This research: 1) Describes how clinical and ultrasound (US) estimates of gestational age (GA) influence PTB rates and perinatal mortality amongst a population in Kilifi, Kenya; 2) Implements a novel PTB classification system as proof of concept that such systems are feasible in low-income settings, and 3) Presents two novel approaches for estimating GA for women presenting >24 weeks' gestation.

Methods: Objectives 1) and 2) used a perinatal surveillance platform developed at the KEMRI/Wellcome Trust Research Programme in Kilifi, Kenya. Ultrasound (US) was offered for GA estimation in women ≤24 weeks' gestation clinically. To achieve objective 3), two candidate US dating equations were derived by combining a machine learning algorithm with polynomial regression analyses. Lastly, an entirely automated model with the capacity to estimate GA using computational image analysis of the fetal cerebral cortex was developed and tested.

Results: 1) Between November 2011 and July 2013, 3630 women presented for antenatal care, 1107 women had US and data were available for 950 (86%) of these. The PTB rate by US (US-GA) was 10.0% compared to 17.1% by a best clinical estimate of GA (C-GA), although the number of perinatal deaths that were preterm by US and C-GA were similar; 2) Implementation of a novel PTB classification system is feasible, and 3) New dating equations and an automated model provide estimates in the 3rd trimester with a prediction error at 34 weeks of 12.4 and 14.2 days, respectively.

Conclusion: Clinical estimates of GA significantly overestimate the rate of PTBs. Despite this, the proportion of perinatal deaths in those identified as preterm by clinical and US methods was similar, suggesting that US may be a better predictor of PTB and its associated mortality. Novel dating methods can estimate GA at 34 weeks' gestation with an error equivalent to that provided by routine clinical methods at 22 weeks’. This has important implications and may extend capacity to provide GA estimates amongst a large group of women whose birth phenotypes remain poorly described.
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List of Abbreviations

95% CIs 95% Confidence Interval
AC Abdominal Circumference
ADM Autoamted dating model
AGA Appropriate for Gestational Age
ANC Antenatal Care
APH Antepartum Heamorrhage
ART Assisted Reproduction Technologies
BPD Biparietal Diameter
C-GA Clinical Estimate of GA
CAST team Community Advisory and Support Team
CRF Care Record Form
CRL Crown-Rump Length
EDD Expected Date of Delivery
FH Fetal Heart
FHR Fetal heartrate
FL Femur Length
GA Gestational age
GA\text{model} Predicted Gestational Age
GA\text{truth} True Gestational Age
GAPPS Global Alliance to Prevent Prematurity and Stillbirth
GPS Global Positioning System
HC Head Circumference
HIV Human Immunodeficiency Virus
IUFD Intrauterine Fetal Death
KDH Kilifi District Hospital
KEMRI Kenya Medical Research Institute
KHDSS Kilifi Health and Demographic Surveillance System
KIN - Number Kilifi Ultrasound Study Specific Identifier
KIPMAT Kilifi Perinatal and Maternal Health Surveillance
KWTRP KEMRI-Wellcome Trust Research Programme
LBW Low Birthweight
LMP Last Menstrual Period
LMP-GA Gestational Age by Last Menstrual Period
MAR Maternity Admission Record
MCH Maternal and Child Health
MOH Ministry of Health
NICU Neonatal Intensive Care Unit
PersonID Person Specific Identifier - UID in KHDSS
PMR Perinatal Mortality Rate
PND Perinatal Death
PregID Pregnancy Specific Identifier - UID in KHDSS
PTB Preterm Birth (<37 weeks' gestation)
RDT Rapid Diagnostic Test
RFC Regression Forest Classifiers
RMSE Root Mean Squared Error
SD Standard Deviation
SFH Symphyseal Fundal Height
SGA Small for Gestational Age
sSA Sub-Saharan Africa
STATA STATA statistical analysis software
UID Unique Identification Number
US Ultrasound
WHO World Health Organisation
\( t\)-SNE \( t\)-Stochastic Neighbor Embedding
Chapter 1

Introduction

Worldwide, seven million neonates are stillborn or die within the first week of life each year. Sub-Saharan Africa (sSA) has the highest stillbirth (36/1000) and neonatal mortality (44/1000) rates, four times those of resource rich nations [1, 2]. Whilst data reporting causes of adverse birth outcomes in low-income settings are limited, at least 60% of the four million neonatal deaths each year are associated with low birthweight (LBW: birthweight <2500g) [3].

Birthweight is simple to measure, widely available [4] and, as a standalone predictor of birth outcome, identifies groups of high-risk newborns [5]. However, when used selectively to target interventions designed to improve birth outcomes, birthweight becomes less effective [4, 6]. The explanation for this lies in the fact that any LBW cohort will comprise a heterogeneous group of newborns including some, but not all, preterm births (PTB <37 weeks), as well as some, but again not all, newborns that are small for gestational age (SGA: birthweight <10th centile for gestational age (GA)). Whilst sharing LBW as a common characteristic, preterm and SGA newborns represent two distinct birth phenotypes that are themselves, products of different pathological processes. As such, single interventions targeting one subgroup of newborns within the LBW group are deemed ineffective by analyses using LBW per se as the denominator.

Supplementing birthweight with GA information produces four distinct phenotypes: preterm and weight appropriate for GA (AGA); preterm SGA; term SGA, and term
AGA. This distinction is important for a range of clinical and research purposes, particularly since the risk of death for a preterm, SGA fetus is almost five times that of a term, SGA fetus [7]. Increasingly persuasive data conclude that PTB is the single the largest determinant of perinatal mortality across the globe and, also, that being born preterm carries a major, long-term burden of impairment amongst communities [8].

Whilst data on birthweight are available for over half of all births globally [9], GA data are not collected in many parts of the world where newborns are at greatest risk. In fact, GA data for as many as 54% of all births worldwide are not collected, with estimates of PTB rates dependent upon statistical models [10]. This represents a major problem for global health research, with an urgent need for data from sSA to understand better: 1) The epidemiological links between PTB and perinatal mortality, and 2) The feasibility of using different methods for estimating GA in these logistically challenging settings. Only once such data are available will it be possible to begin to make the informed decisions needed to develop an international pregnancy dating policy [11, 12].

The questions underlying this thesis were originally conceived whilst providing clinical leadership in a district hospital in Kilifi - a rural town on the coast of Kenya. Patients frequently presented in early labour with no information available for dating purposes. The adverse outcomes that were the direct consequence of those births where GA could not be determined were evident on a daily basis. Words can not convey the effects that exposure to such outcomes on a daily basis have upon a health system, and the extent to which individual clinicians are affected by being forced to manage such cases ‘blindly’.

During the four year period in which this thesis has evolved, there has been substantial research output examining the consequences of PTB. In particular, a consensus has emerged that the links between GA and birth outcomes are poorly understood and that this presents a significant ‘roadblock’ to research targeting improvements in birth outcomes. Thus, numerous calls have been made for data examining GA esti-
mates from low-income settings, including sSA, in order to inform the development of an international pregnancy dating policy. This thesis contributes directly to this evidence base by providing a large dataset with detailed analyses of the relationship between GA, its estimation and associations with PTB. Moreover, the work has provided a basis for multi-disciplinary collaborations examining questions relevant to the field.
Chapter 2

Literature review

Gestational age measures the time interval between the first day of a woman’s last menstrual period (LMP) and any given time point in pregnancy; enables the expected date of delivery (EDD) for a pregnancy to be estimated, and forms the legal basis for important, time critical management decisions throughout pregnancy. The purpose of this literature review is to summarise current understanding of the most commonly used methods available for estimating GA. The mechanism underlying each dating method is discussed and the evidence examined with respect to their associated effects on population estimates of pregnancy outcomes. A review of the current literature on GA assessment from sSA and other low-income settings is then presented along with the available data summarising the use of ultrasound (US) in such settings. Lastly, the hypothesis for the thesis is formulated along with the specific aim and objectives.

2.1 Background

The first historical accounts relating the duration of pregnancy to the menstrual cycle date back to Aristotle (384-322 BC), who wrote: “The human fetus is expelled both in the 7th and 10th month, and at any period between these” [13]. Whilst Nägele (1778-1851) is widely credited for describing the first rule linking LMP to the EDD,
2.2 Menstrual dating

this rule was, in fact, first proposed by Herman Boerhaave and used later by Nägele in his extensive work on pregnancy. Although best practice assumes that Nägele’s rule refers to the first day of the LMP, there is no evidence upon which to substantiate this claim [14].

For spontaneously conceived pregnancies, it is not possible to measure the time of conception precisely. Consequently, a range of surrogate markers, such as the LMP have been adopted as proxy indicators for this process. Alternative methods are also described, including the height of the uterine fundus, as well as methods for estimating the timing of ovulation, based upon changes in basal body temperature and alteration in sex hormone profiles. In many settings, ultrasound (US) measurement of fetal biometry now forms the basis for GA estimation in clinical practice and is used alone, or in combination with the LMP, to estimate the EDD for pregnancies. Each method is subject to a range of limitations and there is, as yet, no international consensus on the ‘gold standard’ for estimating ‘true gestational age’. Consequently, there is considerable variation in the selection of methods used for both clinical practice and research. Comparisons of data between studies must therefore consider the possibility of methodological heterogeneity at all times.

Given the diversity of clinical and research applications of GA data, it is essential that any review examining the performance of such methods considers the purpose for which GA is used. Whilst the ability to estimate GA with a precision of 2.7 days is essential for the interpretation of prenatal diagnostic testing [15], and is thus standard practice in high-income settings, such precision is unlikely to be required, in the first instance, by the healthcare systems of low-income settings - where a different spectrum of GA related problems are faced.

2.2 Menstrual dating

Nägele’s rule assumes that the mean length of human pregnancy is 280 days [15]. By implication, the rule also assumes that the endocrine links regulating the
hypothalamic-pituitary-ovarian axis are fixed in such a way that women’s menstrual cycles are regular and 28 days in length; that ovulation occurs on day 14, and, importantly, that conception occurs within a predictable time window thereafter. Although simple to conceptualise and thus easy to measure, the assumptions underlying LMP as a predictor or GA have been questioned repeatedly.

### 2.2.1 Mechanism: menstrual dates to gestational age

The most obvious criticism is the absolute dependence of LMP on a woman’s ability to recall her dates. Accurate recall varies from as high as 79% from a dataset from Aberdeen, UK, based on a sample of 11,602 women [16] to as little as 32% amongst women in Cameroon, sSA [17]. Not surprisingly, therefore, when examined as a standalone test of GA, the performance of LMP varies considerably [16]. Hall and colleagues were the first to examine the association between uncertain dates and a range of health and socio-demographic characteristics, and concluded that dates were less reliable in women of lower socioeconomic status, younger age, lower educational attainment and higher parity [16]. Although these claims were first published without statistical validation, subsequent multivariate analyses have confirmed these findings as significant [18, 19, 20]. Recall of LMP is further biased by digit preference [19] and by variation in the recording of dates when these are based on the Lunar, Mayan or other calendar variants in use throughout the world [21, 22, 23].

The biological assumptions underlying use of the time of ovulation as a basis for approximating conception are questionable. Ovulation, as the cellular endpoint of a process of follicular maturation can be monitored biochemically to detect the LH surge associated with subsequent follicle rupture, ovulation and formation of the corpus luteum. There is evidence that variation during the follicular phase of the ovarian cycle results in more than 30% of women reporting that their ‘typical’ menstrual cycle length is 30 days or more. On this basis, it was suggested that the length
of human pregnancy should be considered as 283 days rather than the widely held 280 days [24]. However, since the post-ovulatory, luteal phase of the menstrual cycle is relatively constant [25], women with longer cycles will ovulate later. Thus, delayed ovulation is the most likely explanation of the additional time to delivery, rather than a longer gestation per se.

As well as inconsistent recall of LMP, vaginal bleeding in early, but viable pregnancies [26, 27], inhibition of ovulation by hormonal contraception, breast feeding, infection and nutritional status have all been proposed as sources of variation that influence the regularity of the menstrual cycle and, therefore, question the overall accuracy of LMP as a predictor of GA [28, 29, 30].

### 2.2.2 Population effects

The consequences at a population level of the variants discussed above were investigated by two large, population based, epidemiological studies. The California Statewide Expanded Alpha-fetoprotein Screening Program (n = 165,908 births) and the Medical Register of Norway (n = 1.5 million births) examined how phenotypic differences related to birthweight might be used to explain the relationship between variants of the menstrual cycle and their relative effects upon estimates of GA [27, 31].

The California dataset examined the birthweight distribution by LMP using US as a 'gold standard'. When GA was <32 weeks' gestation, the distribution of birthweight by LMP was bimodal, compared to a single mode when GA was examined by US in the same group [31]. The major component of these data-points grouped around a first modal peak at 1500g, with a second, less common mode at a birthweight more than double this (approximately 3200g). Given that such birth weights are biologically implausible for their associated GA range (>97th centile for age [32]), this observation most likely represents inaccurate reporting of LMP dates. Therefore, whilst some suggest that PTB is more likely amongst women who are uncertain of
dates, or who report LMP with error [16, 19], it is conceivable that such pregnancies are misclassified on the basis of inaccurate recall of LMP.

Gjessing and colleagues also stratified GA by birthweight in their analysis of the Norwegian birth registry dataset [27]. This group hypothesised that within sufficiently narrow birthweight strata (100g was proposed arbitrarily), that the range of true GA within such groups is likely to be small and thus, that any observed variation in GA at birth most likely represents variation in dating accuracy, rather than the consequence of heterogeneous birth phenotypes (i.e. dating error and not a group comprising preterm and term births). Using this approach the authors applied a methodology to examine for the presence of multimodal distributions. They suggested that the multimodal nature of their data represents a composite dataset, which in fact comprises multiple, normally distributed sub-groups, whose individual mode averages correspond to the relevant peaks seen when the data are examined as a single composite dataset. By applying a series of model fitting criteria, Gjessing and colleagues claim that each composite distribution optimally separates into three component distributions, each with a separation interval of four gestational weeks [27]. On these grounds, they further hypothesised that this observed difference in time was introduced as a consequence of error associated with inaccurate recall of LMP dates, i.e. that some pregnancies are 4 weeks more advanced than reported, with the pregnancy being conceived during a normal cycle and followed by early pregnancy bleeding that is erroneously reported as the LMP [27].

Whilst providing an elegant mathematical description of the potential biological correlates of the problems associated with dating by LMP, the description of the methodology applied for the analyses can be questioned. In particular, it is unclear on what grounds the decision to apply a 3 component model was made, i.e. whether this was an arbitrary decision or whether the choice was informed by the actual data itself. An alternative approach might have been to use a Gaussian Mixture model selection algorithm, which is an approach that has been widely used to examine mixed composite distributions in biological datasets [33, 34]. In combination with
Akaike’s Information Criterion, it may be possible to derive a purely mathematical estimate for the optimal number of component distributions within their larger, multimodal dataset. Such an approach would provide an unbiased, best mathematical fit to examine the components of their observed poly-modal dataset [35, 36]. At present, since the justification for selecting 3 models is unclear, the fact that this results in a plausible explanation for GA by LMP may be a mathematical coincidence that does not support the biological mechanisms proposed.

2.3 Ultrasound dating

Robinson and colleagues published the first study using US to predict GA in 1973 [29]. Using a static US source linked to a graticule and polaroid imaging film, US images of the fetal crown-rump length (CRL) were measured in women with certain menstrual histories. A regression function was subsequently derived and forms the basis of current ‘best practice’ in first trimester US dating [15]. Whilst also subject to biological and observer variation in measurements, CRL, when measured between 8 and 14 weeks’ gestation, estimates the mean GA with a 95% CI of 2.7 days based upon three independent measurements [29]. On the basis of such findings, US is now widely accepted as a more accurate predictor of GA than LMP and is recommended as the standard for dating pregnancies throughout the developed world [37, 38, 39].

As a fundamental principle, US measured GA assumes that fetal growth in early pregnancy is sufficiently uniform within a population that size can be used as a proxy to represent GA [40]. The extent to which this assumption is justified depends on a range of factors: 1) The GA at which biometry is measured; 2) The choice of structure measured, and 3) A variety of technical factors related to image acquisition, operator skill, regression equation and other factors that were examined in a recent systematic review [41]. In many situations, the bases for the observed differences in practice are entirely arbitrary, often determined by software specifi-
2.3 Ultrasound dating

cations shipped with commercial machines, and thus have no scientific basis. It is, therefore, important to acknowledge that there may be considerable variation in the methods used to obtain apparently similar findings.

It is not sufficient to refer to GA measured by US alone - as is frequently the case; rather that the specific biometric characteristics that were analysed should be stated, along with the GA ranges in pregnancy at which measurements were obtained. For the purposes of this thesis, US will refer to CRL based estimates (widely accepted as the most accurate estimate) before 14 weeks’ gestation unless otherwise stated.

2.3.1 First trimester

Robinson’s formula has been validated by subsequent studies in a range of settings [42] using high resolution US technology, including data acquired by the transvaginal route [39]. Most recently, a large, multinational study examined fetal growth in an optimally healthy population and revisited regression analyses of CRL to estimate GA. Based on data from eight geographically diverse populations, collected using a highly standardised approach, regression analyses derived a formula similar to Robinson’s original equation [43].

Whilst CRL is considered to be the ‘most accurate’ predictor of GA, it must be acknowledged that CRL measurements are not uniform amongst fetuses. Biological variation within pregnancies of the same GA has been reported since the first studies using US [44]. Subsequently, it has been shown that abnormal growth in the first trimester, resulting in lower than expected CRL measurements, is associated with an increased risk of miscarriage [45, 46, 47, 48].

Abnormal pregnancies, particularly those with chromosomal abnormalities also demonstrate clear evidence of abnormal growth in the first trimester [49, 50]. Maternal BMI and first trimester growth was examined by Sarris and colleagues, who showed that there is no association with first trimester CRL measurements [51].
Overall, whilst differences of a few days are evident amongst data reporting GA assessment by CRL, the clinical consequences of this are unclear and it is generally accepted that any error associated with US is less than that with LMP or other dating methods.

### 2.3.2 Second trimester and beyond

Beyond 14 weeks’ gestation, fetal neck movements and increased biological variation limit the precision of CRL measurements for dating purposes [15]. Consequently, it is recommended that GA estimates are based on measurement of the head circumference (HC), biparietal diameter (BPD), abdominal circumference (AC) or femur length (FL). Using the same principle to approximate GA as a function of size, a large number of regression formulae have been published based on single and multivariate combinations. Early models were based on certain LMP as their reference standard [52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62], although these have been revisited and validated using pregnancies conceived by assisted reproduction technologies (ART) [59, 63, 64] and later in pregnancies where LMP and CRL estimates of GA were concordant in the first trimester [63, 65].

Chervenak and colleagues examined the performance of 38 published formulae using a sample of ART pregnancies and reported the systematic and 95 percent confidence intervals (95% CIs) as indicators of clinical performance of the models [63]. Their modelling confirms that HC is the best single predictor of GA (14-22 weeks’ gestation) with a random error (=1 SD) of 3.77 days, consistent with data elsewhere [39, 60]. Chervenak also demonstrated that the performance of single parameter models could be statistically improved by the addition of one or two additional parameters (AC and FL) to HC, although the gains afforded (<1 day) with respect to GA were unlikely to be clinically useful [63, 66].

Beyond 22 weeks’ gestation, the biological variation associated with estimates of each parameter increases, such that 95% CIs increase beyond clinically useful lev-
HC, for example, after 24 weeks’ gestation has a 95% CI ± 2.3 weeks [67], which increases to >3.5 weeks beyond 30 weeks’ gestation. Similar trends are seen with BPD, FL and AC [66, 68, 69, 70]. Multivariate analyses were attempted by a number of groups to reduce this variance, but were unable to achieve models with clinically useful error margins (±2.6 weeks using a combination of HC, BPD, AC and FL, with a limited sample size (n=177) and a poor model fit ($r^2=0.89$ [55]). Consequently, US biometry is not recommended for dating purposes beyond 22-24 weeks’ gestation.

2.4 Clinical methods for dating

2.4.1 Symphyseal fundal height

In many settings, GA estimates for women unable to recall their LMP are based on clinical estimates using the height of the uterine fundus relative to the maternal pelvis - the symphyseal fundal height (SFH). Measured as the greatest distance in centimetres from the superior border of the symphysis pubis to the fundal pole of the uterus, the SFH is available throughout the world and provides a simple, ‘low-tech’ method of assigning GA in those women who are unable to recall a menstrual history [71].

The value of SFH as a predictor of GA is unclear [72] and its accuracy as a standalone test of GA has been questioned since the first studies reporting its use were published [73, 74]. The overall consensus is that GA by SFH varies by ethnic grouping [75, 76], is significantly affected by fetal growth trajectories [76], and therefore offers limited predictive power as a proxy for GA [77].

A study from 1995 examined SFH as a dating tool in the Central African Republic where fundal height measurements were recorded by paramedical staff. Instead of predicting the GA per se as the time since conception, their methodology used the SFH to predict the number of weeks remaining for measured pregnancies. Clini-
2.4 Clinical methods for dating

cal outcomes, including perinatal mortality and PTB rates, were measured and the extent to which observed delivery dates varied according to the predicted delivery dates was measured as a primary outcome. Amongst 604 births, 34/50 perinatal deaths were considered preterm by SFH estimates. There was no attempt to compare findings with alternative estimates of GA and no explanation of how an initial sample size based on 9,599 records translated into only 604 reported outcomes. Whilst the study demonstrates the use of SFH to identify high risk cases, incomplete reporting of methodology and outcomes limits the extent to which results can be generalised to other settings [78].

In South Africa, SFH is used as a clinical screening test to identify SGA fetuses. SFH charts customised according to maternal characteristics within their respective population were developed to identify pregnancies at high risk of complications related to being SGA [79]. Although the charts were developed primarily to assess growth, they have been adapted and form the basis of reference charts for estimating GA by SFH. It is, however, acknowledged that SFH when used for these purposes demonstrates poor reproducibility with significant variation of measurements amongst individuals at any given GA [71, 80, 81]. Despite these important limitations, the technical ease with which measurements can be used to predict GA continues to encourage its use for this purpose in settings where dating by other methods is not possible. In fact, in many settings, SFH may represent the only information available to clinicians for dating purposes [71], and it is widely used to guide the initiation of antiretroviral therapy in HIV positive women [82].

2.4.2 Newborn functional estimates of GA

A range of newborn functional assessments of GA were described by Dubowitz and Ballard [83, 84, 85]. On the basis that birthweight alone was insufficient for identifying and managing high risk birth phenotypes in settings where prenatal GA estimates were seldom available, neonatal estimates of GA determined by functional assessments at births have been widely studied in a variety of settings [86, 87].
Maturity is assessed using a standardised examination of the fetal skin, hair, plantar surface, breast, eye, ear and genitalia with scores being correlated to GA of the newborn using mathematical equations [83, 84, 85]. In general, neonatal approaches tend to overestimate the GA of infants born <40 weeks’ gestation and to underestimate when GA ≥40 weeks [88].

In Bangladesh, Rosenberg and colleagues compared newborn estimates to LMP amongst a group with GA <33 weeks’ gestation by US and hypothesised that functional GA estimates would have a similar clinical performance to LMP as a predictor of GA. Concordance analysis for LMP, Dubowitz and Ballard GA were 0.878, 0.914 and 0.886 respectively. The paper used an accepted analytical approach for method comparison studies as described by Bland and Altman [89, 90]. US, LMP, Ballard and Dubowitz were reported with mean GAs of 30.7, 30.3, 30.3 and 31.3 days respectively [91]. Whilst the observed difference is statistically significant, the authors claim that such differences are unlikely to be clinically important, which may be true. However, the paper made no reference to the characteristics of the newborn group studied and, therefore, it is impossible to assess how the findings of this study may be relevant to other settings.

A more recent study comparing Dubowitz and Ballard estimates to US across the entire range of pregnancy found significant misclassification of infants born at the extremes of gestation - as many as 78.5% of newborns that were preterm by US were classified as term using Ballard [92], which is consistent with data from elsewhere [86, 87]. A consensus has emerged that the 'stressful' events in utero, that themselves are associated with preterm and SGA phenotypes, result in accelerated neurological development and, subsequently, the misclassification of GA by functional estimates determined at the extremes of GA [93].
2.5 Comparing dating methods: US and LMP

Having discussed the origins of individual dating methods, it is important that their relative effects on the distributions of GA at delivery are examined and compared. Such data underpin policy decisions about pregnancy dating. A clear examination of the nature of this relationship will provide an important basis for the hypothesis of this thesis, since dating curves themselves will vary according to both the method used to estimate GA and by the population phenotypes within which estimates are determined. The latter association is less commonly examined by research. In particular, the extent to which variation in birth phenotypes among different populations augments the shape of GA distributions, affecting how GA is estimated by LMP and US, are not known.

In the first instance, it is important to consider the role of descriptive parameters when describing GA data in populations. According to Nägele, the mean duration of human pregnancy is 280 days [94]. It is fascinating that the accuracy of this finding, made almost 200 years ago, has been validated many times. In 1997, Gardosi and colleagues estimated a mean duration of 279.9 days on a sample of 25,000 women with certain menstrual dates [95].

However, reference to any curve of GA at birth reveals a negatively skewed distribution [96]. Consequently, estimates of the mean will be influenced by the relative excess of smaller values in the lower tail of distribution and so may not be the most appropriate statistic to describe the ‘typical’ length of pregnancy. In such cases, the median is often used, but with respect to GA, is likely to be influenced by the effect of induction of labour policies targeting the excess in perinatal mortality associated with prolonged gestation [97]. As such, the mode average is suggested to provide the most representative estimate of the ‘typical’ duration of pregnancy [96]. Obtaining estimates of the mode can be problematic when sample sizes are small, or grouped frequency distributions wide, since curves of their distribution tend towards ‘flattened’ peaks that limit the accuracy to which the mode is estimated. As a con-
sequence, estimates of the mode depend upon large sample sizes and are often unreported. Gardosi and colleagues in Nottingham, UK as well as Bergsjo from Bergen, Norway, both discussed the importance of the mode for the stated purpose and each reported an estimate of 283 days LMP. Gardosi, however, also used second trimester US (BPD regressed using Campbell’s formula [53]) and found the mode by US to be 281 days, the same as other groups [98].

These data allow a logical comparison of the relative effects of dating within populations by LMP and US to be made, which can be considered in terms of the factors that affect the shape of the distribution of GA by respective dating methods. The systematic difference between LMP and US, resulting from delayed ovulation, produces a left-shift of the US curve compared to that for LMP. In addition, the greater precision of US compared to LMP based estimates of GA results in smaller measures of variance amongst US dated samples [96]. The combined effect of such differences produces a curve for GA by US that is shifted to the left by 2-3 days [27, 31, 95, 96, 98, 99], as well as being taller and narrower than the curve representing GA by LMP. A graphical illustration of this relationship between the curves is provided in Figure 2.1.

For individual women, the consequences of this relationship between dating curves postpones the EDD by 2-3 days in as many as 45% of cases if US is used over LMP for dating their pregnancies [95, 98].

The relationship between US and LMP dating can seem counterintuitive. For example, how valid is the assumption that US dating formulae, that themselves are based upon research using pregnancies dated by ‘certain’ LMP, are more accurate than the LMP dates that form the standard on which they are derived? This question has been debated by clinician scientists, who provide evidence that the selection of women for the LMP based standards was sufficiently rigorous to provide an ‘optimal’ cohort of women who were likely to ovulate and conceive according to the assumed biological rules. Furthermore, data from pregnancies conceived using ART, within which the date of conception, and therefore GA, is certain, have been used to vali-
date US dating formulae derived from datasets selected according to LMP [15, 63]. Whilst a consensus has emerged that supports the use of pregnancies conceived by ART to validate US dating practice amongst the wider population, such studies use small sample sizes and inconsistent research methodologies [66, 100]. Overall, in the absence of an accepted ‘gold standard’ for GA, the evidence favouring one method over another can be conceptually difficult to resolve and should always be considered in the context of associated clinical endpoints.

### 2.5.1 Combined dating policy: LMP and US

Despite clear evidence of the superior accuracy of US estimates of GA over LMP and other methods of dating, a somewhat conservative approach to dating policy has been adopted in many clinical settings. One of the earliest studies comparing US and LMP estimates of GA was published in 1989, where the authors concluded...
that there was no indication to re-date pregnancies amongst women with ‘fairly regular’ menstrual cycles unless dates by LMP and US were discrepant by more than 14 days [101]. Although based on a sample size of <100 women, this study provided a reference standard against which new evidence was compared. Thus, despite clear evidence that US is more accurate than LMP when thresholds of difference between 4 and 14 days have been tested [102], the anchor of LMP for dating pregnancies persists to this day in many clinical settings.

As suggested by the model in Figure 2.1, the area under the curve for post-term pregnancies extending beyond 42 weeks’ gestation is considerably lower by US than that by LMP. Clinically, this translates into a reduction in the need for interventions to manage the excess risks associated with such pregnancies. This hypothesis has been tested repeatedly [37, 38, 39, 95, 103]. An observational study involving >1.2 million participants showed that US dating reduced the incidence of post-term pregnancy from 13.3 to 7.4% [42]. Eik-Nes and colleagues went on to examine this hypothesis in the setting of a randomised trial and demonstrated a 70% reduction the rates of induction of labour for presumed post-term pregnancies in Norway [104]. Such results form the basis on which national guidelines recommending first trimester US dating in the UK are based [15].

Intervention studies have proceeded to examine hypotheses assessing whether US has any beneficial effects on perinatal mortality. A systematic review with meta-analysis of nine trials of US based interventions during pregnancy in 1998 showed clearly that US in early pregnancy was not associated with significant improvements in perinatal mortality (OR 0.31, 95% CIs 0.37-1.12) [105]. Providers are, therefore, left to determine whether benefits, such as the reduced need for intervention for prolonged pregnancy justify widespread use of US in their respective settings.
2.5 Comparing dating methods: US and LMP

2.5.2 Preterm birth - US versus LMP?

The observations relating to prolonged pregnancy by US compared to LMP do not apply conversely to PTBs. Asymmetry introduced by the negative skew at the opposite tail of the GA distribution prevents a simple comparison based on the spread of GA by US compared to LMP alone. In fact, the data examining this relationship for PTB are contradictory. Yang and colleagues reported a relative increase in PTBs when GA was assigned using BPD measured ≤20 weeks’ gestation from 7.6% (by certain LMP) to 9.2% (p < 0.001) from a large hospital based sample (n = 44,623) in Canada [106]. Conversely, the California dataset comprising a statewide sample (n = 165,908) reported a PTB rate of 8.7% by LMP and 7.9% by US [31]. Neither study reported perinatal outcomes. Potentially important methodological differences include the use of a tertiary referral centre for sampling by the Canadian study compared to a statewide population based sample for the California dataset. Whilst this suggests that the differences between the two groups may be explained by variants in practice between the centres, i.e their use of interventions to augment the duration of pregnancy, such details are not reported in the methodologies. Nevertheless, the studies are sufficiently large that the reported findings are unlikely to represent a type I or II statistical error.

To date, analyses comparing the methodologies for GA estimation have considered PTB groups as a single, homogenous phenotype. However, increasingly persuasive evidence suggests that being born early (GA<259 days) may, in fact, represent an easily measured characteristic amongst a group of conditions, whose origins are non-specific, but likely to include maternal sepsis, malnutrition and a range of fetal and placental abnormalities [107, 108, 109]. As a consequence of this phenotypic heterogeneity, it is possible that the distributions of GA amongst PTB groups between settings varies and, consequently, that the shape of curves for GA will differ. Given the complex mathematical relationship that exists between the skewed tail of dating curves, it is therefore feasible that differences in phenotype composition be-
between groups may explain the observed differences in PTB rates that are currently attributed to methodological differences when estimating GA. Such interactions may explain the contradictory findings between the studies of Yang and Dietz [31, 106] i.e. that fundamental differences in composition of the PTB groups in the two studies explains the contradiction in findings, rather than any methodological factors associated with estimating GA itself.

2.6 Preterm birth: a novel classification system

As the largest determinant of mortality amongst the under-5s, PTB is the focus of a vast array of research, intervention and policy implementation programmes throughout the world. On the basis that PTB rates themselves can vary between settings as the result of differences in underlying phenotype composition, it is essential that comparisons based on GA by US and LMP alone should be interpreted with caution. Instead, estimates of GA should be considered in parallel with independent clinical outcome data, such as perinatal and neonatal mortality indices, or locally relevant morbidity indicators. Until a more specific case definition of PTB (and its sub-phenotypes) becomes available, such data must be used to assess the clinical relevance associated with different PTB rates and whether these may an artefact of heterogeneity between the groups studied.

In 2009, the International Conference on Prematurity and Stillbirth convened by the Global Alliance to Prevent Prematurity and Stillbirth (GAPPS) concluded that a new approach was needed to understand better the aetiology of PTB. It was suggested that PTB should be considered as a syndrome encompassing a range of maternal and fetal phenotypes. A prototype classification system was developed consisting of five components: maternal conditions present before presentation for delivery; fetal conditions present before presentation for delivery; placental pathological conditions; signs of initiation of parturition, and the pathway to delivery.

A series of articles review the concepts underlying the proposed classification sys-
A particularly important difference between the new system and previous analyses is the definition of PTB used. Defined arbitrarily by WHO as birth before 37 completed weeks of pregnancy, this cutoff has been used as the case definition for PTB for both clinical and research purposes. However, the new classification system suggests that on the basis of clear evidence that the risk factors, causes and recurrence risks for pregnancies ending spontaneously between 16-19 weeks’ gestation are almost identical to those occurring after 24 weeks’ gestation, that a lower boundary of 16 weeks’ gestation should be adopted. Similarly, births between 37-38 weeks’ gestation are at increased risk of morbidity related to immaturity compared to those after 39 weeks and therefore the upper boundary should be shifted to 38+6 weeks. The prototype classification system is shown in Figure 2.2 with case definitions provided by Villar et al in the third article of the series [108].
2.7 Ultrasound in sSA and other low-income settings

In recent years, US services have become increasingly available in low-income settings. Their introduction is endorsed by WHO and leading clinicians in maternal health on the basis that the technology has the potential to deliver a range of health benefits [110, 111, 112]. In many cases, these claims remain untested. However, as more robust, portable US machines enter the market at increasingly lower prices, it is likely that the technology will spread across sSA. In fact, the potential role for US is recognised more and more in such settings by respective Ministries of Health, Non-Governmental Aid and charitable organisations [113].

Access to US technology in most low-income settings remains unregulated [114] and so providers are left to determine care pathways according to the needs of their individual settings. On the background of past experience from high-income settings, where services were often configured according to the availability of commercial products rather than any evidence base per se [41], research must be undertaken to address the extent to which US can deliver benefits, if any, and whether such benefits represent value for money.

In recognition that the evidence base to inform this debate is lacking, leading clinical academics from South Africa and elsewhere across the region initiated a narrative discussion on the potential applications of US for maternal and perinatal health services. In particular, this focused upon the possibility that ‘non-traditional’ applications of US technology should be explored as a means to improve public health outcomes [115, 116].

2.7.1 A systematic review of methodology

Given the huge variation in practice with respect to the use of US in sSA and low-income settings, the methodology of published studies evaluating its use as an intervention in obstetric care was systematically reviewed. The aim was to provide
a framework of data upon which the US work for this thesis would be structured. A narrative review of high quality studies was then undertaken to describe: 1) The provision of US services in low-income settings, and 2) The use of US to assess GA in such settings.

### 2.7.2 Methods

Medline (PudMed), EMBASE, African Index Medicus and African Journals Online were used to perform two broad searches of the dataset, which are henceforth referred to as Search A and Search B.

#### 2.7.2.1 Search A

Search strings were derived from combinations of terms from each column in Table 2.1. For example, “Technology” and “Setting” and “Application” would be queried as “Antenatal care” AND “ultrasonography [MeSH]” AND “low-income country”. Truncated terms for plural and alternative spellings were used, for example “Emerging Econom∗”. The search was limited to English language articles between 1966 and April 2011.

Intervention studies were grouped according to whether or not they involved randomisation of participants. The methodological quality of randomised trials was assessed using a modified CONSORT checklist. The Newcastle Ottawa Scale (NOS)
was adapted to assess non-randomised intervention studies - see Appendix A.1 on page 193 [117, 118]. Individual studies were scrutinised by myself and Dr Angela Koech, a Medical Officer in Kilifi. A third person (Dr Aris Papageorghiou) was approached where agreement was not reached by consensus.

2.7.2.2 Search B

A subsequent search was performed in 2013 on a background of mounting literature examining the relationship between US and clinical estimates of GA in low-income settings. This used the following search term:

(Gestational age[MeSH Terms] OR Pregnancy dating OR Preterm birth) AND ((resource poor) OR low income setting) OR poverty[MeSH Terms] OR sub-Saharan Africa) AND Ultrasound[MeSH Terms]).

2.7.3 Results

2.7.3.1 Search A

A total of 794 references were retrieved and 19 articles were included in the final review. Full details of the stepwise screening process used to select the final articles are illustrated in Figure 2.3. The results of the review are summarised in Table 2.3. A data extraction pro-forma was used to examine studies, which were then assess according to the criteria from Appendix A.1. See pages 193 to 199 for details summarising the data extraction process and individual studies examined. The methodology of studies was considered to be of high quality if all relevant details were provided. Where one or two features were not reported, studies were deemed to be of medium quality, whilst those not reporting three or more features were deemed to be of low methodological quality.
Figure 2.3: Systematic review: identification of sources and the selection of articles for review.
No evidence of selective reporting

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Lamina et al; 2004 (Sagamu)

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Maharan et al; 1988 (Egypt)

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Neufeld et al; 2009 (Bangladesh)

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Nzeh et al; 1992 (Nigeria)

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Rijken et al; 2009 (Thailand)

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Seffah et al; 1999 (Ghana)

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Shah et al; 2008 (Rawanda)

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Shittu et al; 2007 (Nigeria)

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+

?

Stein et al; 2008 (Tanzania)

-

-

?

Steinmetz et al; 1999 (Cameroon)

+

+

P

-

Theron et al; 1992 (South Africa)

+

+

P

+

Clear study aim

Outcome data complete

+

Bias and confounding discussed

+

Primary outcome clear

+

Comparison group described

?
P

Intervention group described

+

-

Dissanayke et al; 1993 (Kenya)

+

Geerts et al; 2004 (South Africa)

+

Kimberleyet al; 2010 (Zambia)

+

Overall quality of evidence

Incomplete data addressed

Low

Intervention described

+

Prospective/ retrospective sample

+

Defined hypothesis

Low

-

Continued overleaf

26

?

P

+

2.7 Ultrasound in sSA and other low-income settings

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Busman et al; 2001 (Botswana)

A - Non-randomised intervention studies


2.7 Ultrasound in SSA and other low-income settings

Table 2.3: Assessment of the methodological quality of intervention studies using US in low-income settings. A - Non-randomised intervention studies examined using the Newcastle Ottawa Criteria. B - Randomized controlled trials examined using a modified CONSORT checklist. Symbols: ⬤ = detail adequately described; ⬧ = inadequate description of detail; ⬦ = no description of detail provided. Studies were deemed to be of high methodological quality if all required features were discussed. Where one feature was missing, studies were classified as medium quality, and where two or more features were missing, studies were deemed to be of low methodological quality [119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135].

<table>
<thead>
<tr>
<th>Study</th>
<th>Clear study aim</th>
<th>Defined hypothesis</th>
<th>Trial design</th>
<th>Eligibility criteria described</th>
<th>Intervention clearly described</th>
<th>Sample sequence clear</th>
<th>Primary outcome clear</th>
<th>Power calculation</th>
<th>Outcome data complete</th>
<th>Incomplete data addressed</th>
<th>No evidence of selective reporting</th>
<th>Overall quality of evidence</th>
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<tr>
<td>Geerts et al. 1996 (South Africa)</td>
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<td>Geerts et al. 2013 (South Africa)</td>
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</tbody>
</table>

Low

High
2.7 Ultrasound in sSA and other low-income settings

2.7.3.2 Search B

Search B identified 62 studies published in English since 1980. Of these, two studies (outside South Africa) compared ultrasound estimates of gestational age (GA) with newborn assessments of GA [87, 92] and one described preterm birth (PTB) rates in Malawi. The remaining studies identified by Search B include a mixture of case reports and descriptive accounts of US fetal biometry within different study groups.

2.7.4 Discussion

2.7.4.1 Ultrasound services in low-income settings

The quality of evidence relating to US in low-income settings is low. Amongst the non-randomised intervention studies, only 4/15 were assessed as being of medium or high quality. Of these, 2 were from South Africa, 1 from Thailand and 1 from Nigeria. All of the randomised trials were assessed as high quality and all were undertaken in South Africa. Whilst the health system in South Africa shares many of the challenges experienced elsewhere in sSA, South Africa is an upper middle income country [136], with a perinatal mortality rate that is half that of other countries in the region (33/1000 versus 62/1000) [136]. It is therefore debatable whether South Africa’s many well conducted studies are relevant to poorer settings.

Data from elsewhere in Africa demonstrate that US is certainly used and that services are feasible. However, individual studies frequently depend upon overseas staff to provide the service and there are very few data examining important clinical outcomes including maternal, perinatal or neonatal morbidity and/ or mortality. Only once such data are available will there be scope for evidence based policy discussions, including those already called for with respect to GA estimation in such settings. Failure to address this research gap risks a commercially driven dissemination of technology, with established international care pathways being integrated as ‘off-the-shelf’ packages into healthcare systems. The availability of technology
alone should not be considered a mandate for its use in the absence of evidence of benefits and risks causing harm as a result of unnecessary interventions, with evidence already emerging to support this contention [137].

To ensure that any US based care pathways are sensitive to the needs of low-income settings, it is important that clinical outcomes specifically relevant to such settings are examined. Moreover, the possibility of benefits emerging from ‘non-traditional’ applications of US should be considered in view of their theoretical ‘collateral’ gains [114, 116, 138, 139].

2.7.4.2 Gestational age estimation in low-income settings

The study from Malawi represents one of the first studies to report the high incidence rate of PTB in sSA. Van den Broek and colleagues reported a PTB rate of 20.3% amongst a sample of 512 women (GA = BPD, Chitty formula [140], GA < 24 weeks). However, the published data were part of a randomised trial of nutritional supplements for anaemic women and whilst the authors show a clear relationship between the degree of maternal anaemia and PTB, the characteristics of the US dated group are not provided in sufficient detail for comparisons with other estimates of GA or with data from other settings [141].

More recently, data from a South African prospective trial of US reported a statistically significant reduction in preterm labour when GA estimates were based on US rather than LMP [122]. This was a community based study comparing selective versus routine use of US amongst 3009 unselected women attending for standard antenatal care. The authors focused on clinical outcomes and included a comparison of a composite estimate of GA (GA = LMP if available or by customised SFH if unavailable) versus routine second trimester US (HC, AC, FL using Chitty’s equations [140]). The authors reported a preterm labour rate of 12.0% compared to 16.7% by LMP (p < 0.003). Whilst this is a significant difference, it is not clear how many cases of preterm labour ended in PTB. Similarly, the intervention and
control groups were not recruited simultaneously and so may have been exposed to different risks with respect to birth outcomes [122].

Another large study designed to compare GA by LMP, US and SFH was reported from Pakistan. The study prospectively recruited 1128 women between 20 and 26 weeks of gestation and recorded outcomes at delivery. The authors conclude that SFH estimates of GA were more accurate than LMP using US estimates as the ‘gold standard’ (HC, AC, FL and BPD using Hadlock’s formula [68]). Unfortunately, the methodological approach taken had a number of limitations. The sampling technique adopted was selective and arguably excluded participants where LMP and US would be more accurate earlier in pregnancy. Although the authors summarised their interpretation of the data, some of the data necessary to support their interpretation is not presented. For example, a simple comparison of the mean, median and standard error or interquartiles ranges of their observed outcomes by each method of dating. In the absence of these data, it is difficult to exclude a systematic bias in results that may have emerged as a consequence of the methodology adopted - such as their restrictive sampling policy.

To claim superior test performance in studies of this nature ideally requires: 1) Prospectively defined criteria describing the optimal test for estimating GA in the proposed analysis; 2) A discussion of how systematic and random bias between each dating method was either considered and controlled for in the design of the study, and during the analyses, or why such efforts may not have been possible, and 3) A justification for the analytical approach taken. The authors used US biometry as a ‘gold standard’ at a relatively advanced stage of pregnancy. Dating pregnancy beyond 22 to 24 weeks using US in many settings would be considered inappropriate and so to use such methods as a reference standard without discussing the rationale for this or acknowledging this is a limitation of their work.

The two previously described studies were selected for a more detailed discussion because they highlight problems that often occur with respect to the reporting of studies undertaken in sSA and other low-income settings. Such settings present a
unique range of logistical and methodological challenges that may be considered to 'compromise' research methodologies and, therefore, data quality. It is essential that studies describe the nature of the challenges encountered during the conduct of their research. Such details provide a realistic account of front-line research in many low-income settings and where important lessons are learned have the potential to benefit future research. It is conceivable that such limitations restricted the recruitment of the South African study to asynchronous groups or that the decision to use a reference standard based upon US later in pregnancy in Pakistan was based on a considered appraisal of alternatives. However, with minimal explanation of such decisions in manuscripts, the findings reported by the research are questionable. Limitations that are the consequence of the research environment must be clearly explained and are an integral component of the challenging nature of research in sSA and other low-income settings.

2.7.5 Ultrasound biometry and growth in low-income settings

There is a paucity of data examining the validity of US dating and growth formulae in sSA and other low-income settings. On the basis that marked differences exist between populations with respect to both 'normal' maternal characteristics and the prevalence of pathological phenotypes known to be associated with alterations in first and second trimester growth patterns, it is conceivable that the accuracy of US dating and growth assessment may differ according to setting. Salpou and colleagues examined whether ethnic tribal origin was associated with variation in measured fetal biometry within Cameroon and found that there was no difference in HC, BPD or FL by ethnic groups [17]. Although based upon LMP as the dating standard and a small sample size, this is one of only a few from sSA (outside SA) providing evidence to appraise US dating practices in this setting.

The effect of pathological phenotypes that are more common in low-income settings should also be considered in terms of their impact on birth outcomes. It is conceivable that fetal growth trajectories, and therefore the adequacy of growth as a
proxy for GA, may be affected by high levels of poor nutrition, maternal stunting and chronic disease. To this end, a randomised controlled trial of nutritional supplementation amongst chronically undernourished women in rural Gambia demonstrated a statistically significant positive effect upon birthweight and fetal biometry. Whilst the authors demonstrated a link between birthweight and both maternal postpartum weight and weight gain during pregnancy, there was no difference in GA at delivery between intervention and control groups [142].

The association between malaria infection during pregnancy and both PTB and fetal growth restriction as a consequence of placental sequestration of parasites is well described. There is some evidence that both Plasmodium falciparum and Plasmodium vivax can result in smaller than expected head biometry in the first half of pregnancy. Whilst this observed difference was small (approximately 1 mm for a BPD measured at ≤22 weeks), the effect persisted in women with only subclinical evidence of infection (i.e. positive serology, but asymptomatic) [139]. However, the consequences of such a small difference, in terms of assigning GA is negligible and falls within the accepted limits of measurement for BPD.

2.8 Gestational age estimation - a novel approach

US can provide accurate GA estimation to all women presenting for antenatal care (ANC). However, any gains afforded by such a service would, at present, be restricted to women presenting for care before 24 weeks’ gestation. Such limitations would potentially make US dating non-viable in resource-poor settings, where most women initiate ANC in the third trimester. However, as overall coverage of a single ANC visit exceeds 90% in most settings [143, 144], a dating service that was validated for use in the third trimester could potentially enhance the value of US and could improve the management of pregnancy.

To this end, a growing body of research has focused on evaluating a range of ‘non-traditional’ biometric parameters [145]. Observed changes in the morphology of the
fetal brain are of particular interest, where patterns observed during the deepening and branching of sulci from the surface of the cerebral cortex relate very closely to GA in pathological studies [146, 147, 148, 149], as well as neuro-imaging studies using magnetic resonance imaging [150, 151, 152, 153, 154] and US [151, 155, 156, 157, 158, 159, 160]. Figure 2.4 illustrates our current understanding of the temporal changes observed upon the surface of the fetal cerebral cortex.

Neuropathologists have suggested that this ‘developmental maturation’ of the fetal brain provides a more reliable estimate of GA than traditional biometric assessment [147]. Furthermore, the time of greatest observed change occurs during the third trimester when the smooth, agyric structure of the cortex becomes progressively more complex [151]. To date, the relationship between the appearance of the surface of the fetal cerebral cortex and GA has only been applied to determine GA standardised normal ranges of structural development for the purposes of identifying congenital abnormalities. It is hypothesised that the reverse of this relationship between surface structure and GA can be developed into models which are capable of estimating GA based upon the US appearance of the surface of the fetal cerebral cortex.
Figure 2.4: Graphical outline of the temporal changes visualised on the (a) dorsal, (b) medial and (c) lateral aspects of the cerebral cortex and colour coded according to the GA of their first appearance as determine by a neuropathological study [147]. (Figure used with permission from A Namburete, IBME, University of Oxford.)
2.9 Summary

Reliable GA data are not routinely collected in most regions of the world where the risks of adverse perinatal outcomes are the highest [11]. Although PTB is considered to be the greatest determinant of these adverse birth outcomes, PTB rates for over half of all births worldwide are based on modelled estimates and are of uncertain accuracy [10]. Understanding the burden of PTB is important because of its associated perinatal mortality as well as the long term burden of impairment in society [8].

Given the breadth of factors that determine the accuracy of pregnancy dating by LMP, US and other clinical methods, it is essential that research addressing the excess of PTB in those settings at highest risk is based upon a more precise understanding of the mechanisms by which GA is measured. In fact, the need to agree upon international standards for the accurate and standardised reporting of GA is highlighted as a research priority in itself [11, 12].
Chapter 3

Hypothesis, aim and objectives

3.1 Hypothesis

Gestational age estimation can be improved in low-income settings.

3.2 Aim

To redefine the epidemiology of preterm birth in a rural community in sub-Saharan Africa.

3.3 Objectives

1. To establish a perinatal surveillance system that integrates with an established population surveillance network to characterise the pregnant population of Kilifi, Kenya.

2. To determine the extent to which four different methods of GA estimation (LMP, SFH, US CRL and US HC) affect estimates of the incidence rate of preterm birth and associated clinical outcomes.

3. To implement a novel preterm birth classification system as a ‘proof of concept’
3.3 Objectives

that such systems are feasible in the most deprived populations at greatest risk of adverse birth outcomes.

4. To characterise deficiencies with current GA provision and propose and test novel methods of GA estimation based upon changes in morphology on the surface of the fetal cerebral cortex.
3.4 Thesis overview

Chapter 4 describes the research operations at the Kenya Medical Research Institute (KEMRI)/Wellcome Trust Research Programme in Kilifi, Kenya where the majority of this research was undertaken. The chapter includes full details of the additional infrastructure established to undertake this research and the evidence base underlying this.

Chapter 5 presents a prospective observational study comparing the extent to which PTB rates are affected by the method used to estimate GA.

Chapter 6 implements the novel PTB classification system described on page 20 and explores alternative methods for examining groupings within data.

Chapter 7 moves on to address GA estimation in the third trimester of pregnancy. A machine learning approach using the Genetic Algorithm, was applied to define novel equations for estimating GA using US biometry between 18 and 35 weeks’ gestation.

Chapter 8 continues with the theme of estimating GA in the third trimester and describes an automated model developed in collaboration with colleagues from the Institute of Biomedical Engineering at the University of Oxford to predict GA using data acquired from single 3D volume sweep of the fetal head.

Chapter 9 summarises the key findings of the thesis and discusses a number of important contextual issues relating to research in low-income settings and how findings from Kilifi may represent other settings in sSA and elsewhere. Lastly, plans for future work are provided with an overall conclusion for the work undertaken.
Chapter 4

Methods

This chapter provides a general account of the methodology underlying the work of this thesis. A summary of the baseline research infrastructure at the Kenya Medical Research Institute (KEMRI)/Wellcome Trust Research Programme (KWTRP) in place in August 2010 is provided before a detailed account of additional work undertaken specifically for the purposes of this research. Emphasis is placed on the evidence base used to inform the decisions underlying this process. The research outputs and experience at KWTRP in the fields of basic clinical, epidemiology and health systems research, social science, ethics and community engagement were instrumental in establishing a basis for sustainable perinatal research.

The broad strategic aim was to establish a perinatal surveillance system, comprising an antenatal US service, with the capacity to characterise the pregnant population attending for routine antenatal care at KDH for clinical and research purposes. It was clear from the outset that a significant investment in time and resources in the preparatory stages would enable research pathways to be integrated into routine ‘clinical’ pathways, such that data collection for research purposes could become standard practice amongst the Government employed clinicians.

As well as the specific ‘data requirements’ for investigating the hypotheses of this thesis, the surveillance network was designed upon a framework with long-term
sustainability at its core and in a manner sensitive to the needs of all stakeholders within the community accessing services. Overall, this enabled the system to evolve with risk management at its core. As a strategy, this was essential and ensured that a potentially difficult, and even controversial, new clinical service implemented primarily for research purposes had sufficient capacity to deal with expected and unexpected sequelae.

A more specific account of the methods adopted for each chapter of results is provided in each of the relevant chapters.

4.1 Setting and background

Kilifi District Hospital (KDH) lies 55 km north of Mombasa in a rural coastal region of Kenya and since 1989 has hosted the Centre for Geographic Medicine and Research - Coast (CGMR-C); a multidisciplinary biomedical research programme operated as a collaboration between the KEMRI and the University of Oxford that is jointly funded by the Government of Kenya and the Wellcome Trust.

KDH serves a geographic area of approximately $30 \times 60$ km$^2$ that is home to a population of 260,000 residents [161]. Local inhabitants belong to the Mijikenda ethnic group with $<20\%$ migration from other parts of Kenya. Poverty rates are high and range from 65\% to 84\% and most inhabitants are dependent upon subsistence farming for income. Literacy rates are low, with only 45\% of adults interviewed during a household survey in 2005 reporting that they were able to read a newspaper or letter [162, 163].

KWTRP has focused principally on health outcomes within the paediatric population and has published widely on the effects of malaria, HIV, malnutrition and pneumonia in neonates and infants [164, 165, 166, 167]. The detailed epidemiological surveillance undertaken by early studies at KWTRP has served as a platform from which interventional studies were undertaken, whose outputs have fed back to deliver significant reductions in mortality associated with a range of paediatric infec-
4.1 Setting and background

Figure 4.1: Causes of mortality in the under-5s age group across Kilifi District, Kenya (Unpublished data).

The effect of this has been an epidemiological shift in the causes of mortality in the under-5s groups, with an increase in the relative burden of neonatal causes of death from 2009 - See Figure 4.1. Such data are a powerful proof of concept that comprehensive epidemiological descriptions are certainly feasible within low-income settings and, more importantly, benefit the community by delivering improvements in the health of later generations.

Following a strategic review in 2009, the need to understand better the links between well described paediatric outcomes and their antenatal and intra-partum antecedents was highlighted as a research priority. However, existing researchers at KWTRP were unable to lead this due to a lack of relevant obstetric research expertise. Similarly, the unit was unable to provide the necessary obstetric support to manage a clinical service of sufficient capacity to ensure that any health needs revealed as a consequence of participating in research could be met in a locally appropriate and sustainable fashion.
4.2 Kilifi District Hospital

KDH is a level four district hospital with seven inpatient wards; an emergency department; an operating theatre, with services to provide a single general anaesthetic, and an outpatient department covering all specialities. Medical cover (excluding paediatrics) is provided by three Kenyan consultants and a team of 5-7 junior doctors. There are laboratory facilities for routine haematology (haemoglobin assay, blood group and rhesus status) and biochemistry (urea, electrolytes and liver function assays) along with parasite detection and HIV testing. A Radiology facility provides routine limb and chest Xrays. The onsite blood bank is frequently out of stock and autologous donation is discouraged because of high rates of HIV infection. The pharmacy has a limited stock of analgesia, antimicrobials and antiretroviral therapy, which are purchased by patients in advance of administration.

The maternity department has a four bedded ‘open’ delivery suite and a 36 bedded inpatient ward for antenatal and postnatal admissions. There are approximately 3500 deliveries per year, with a caesarean section rate varying between 11-16%. When this study began, intra-partum care was provided for a Government regulated fee, but a policy change effected after the 2013 general election now provides free access to maternity care. The unit is managed by a matron of nursing services and operated by a team of 26 nursing staff, who are trained to conduct vaginal deliveries independently. Medical management is supervised by a single non-resident Obstetric Consultant, who is frequently offsite for management duties. Resident medical cover for the ward is provided by three Intern clinicians (Postgraduate year 1 doctors), who have onsite supervision from one Medical Officer (Postgraduate year 2-4 doctors), who cross-covers all specialities and is responsible for all surgical interventions. There are no facilities for regional anaesthesia in labour or for intubation and ventilation of women with compromised airways. Fetal monitoring is by intermittent auscultation and there are no facilities for electronic monitoring. Oral dexamethasone is available to reduce the risks associated with preterm birth and, although
rhesus testing is available, the costs of anti-rhesus immunoglobulin are prohibitively expensive for most women.

Women requiring more acute services must be transferred to the Coast Province General Hospital in Mombasa, which is a two hour transfer by sealed road. Such transfers have unskilled escorts and frequently result in maternal death before arrival.

Antenatal clinic services are provided in a designated Maternal and Child Health (MCH) clinic, which is managed by a matron of services supported by 12 trained nursing staff using a similar framework to the maternity unit. A full care pathway based on the WHO focused antenatal care package is available [168, 169]. Laboratory stocks to provide basic investigations are frequently empty. Insecticide treated bed nets and malaria prophylaxis are routinely available. Point of care testing for HIV and haemoglobin is provided by research and charitable funding sources. However, syphilis testing is frequently omitted. Whilst uptake of a single visit for antenatal care is approximately 90%, which is consistent with the country as a whole [143, 144], less than half of women attend before the WHO target of 16 weeks’ gestation (unpublished data).

4.3 Kilifi Health and Demographic Surveillance System

KWTRP hosts a computerised health and demographic surveillance system - the Kilifi Health and Demographic Surveillance System (KHDSS), which tracks health events across the 260,000 inhabitants resident in the area. KHDSS was established in 2000 and is the largest health and demographic surveillance system in sSA. In most low-income settings where vital registration systems are missing or inadequate, data on health outcomes within populations are difficult to obtain and often inaccurate [170, 171].

KHDSS provides the capacity to overcome many of these limitations by linking health events and interventions to their outcomes across a geographically diverse
population. The system uses a series of unique identifiers (UIDs) to catalogue each inhabitant by linkage to the GPS coordinates of their homestead. PersonIDs provide a UID for each person, who is linked to their homestead location’s GPS link. Health events for each PersonID are captured during four-monthly surveillances when KHDSS fieldworkers visit the locations of homesteads to conduct household surveys of the health status of each linked PersonID. These data, which include births, deaths and in- and out-migrations, once cleaned and validated can be merged with hospital surveillance systems [161].

On the basis that a PersonID can have multiple pregnancy events, each individual pregnancy, once identified by the research system, is allocated a unique PregID at first contact with surveillance teams. PregIDs can also be allocated through the hospital database system and are automatically linked to PersonID and LocationIDs. During routine surveillances, PregIDs are assigned based on a reported missed LMP date, which may or may not have been confirmed by a positive pregnancy test. This is an accepted limitation of such systems in their respective environments, but was considered to provide unacceptable case specificity for this study, and thus an additional, study specific identifier was introduced: the ‘KIN-Number’ was integrated into all data systems at KWTRP to facilitate multi-level surveillance of research participants. Figure 4.2 illustrates the system for allocating UIDs and how these enable follow-up of participants throughout the study life-cycle.
Figure 4.2: Recruitment and follow-up of study participants: A study specific unique identifier (KIN-Number) was assigned to each patient at their first visit to MCH. These were combined with existing KHDSS identifiers and used to track participants and their newborns using automated hospital and population tracking systems. Person identifiers (PersonIDs) are linked to household location data and are combined with health outcomes that are recorded on a four-monthly basis using household surveys [161].
4.4 Kilifi Paediatric and Maternity Surveillance Study - KIPMAT

A core maternity surveillance system was established, KIPMAT\(^1\), within the maternity department at KDH in August 2010 in partnership with paediatric researchers at KWTRP and clinical staff employed at KDH by the Ministry of Health. Government nursing staff employed by the Ministry of Health were selected and recruited onto research contracts and subsequently trained over four months to undertake data collection for clinical and research purposes. The decision to adopt this mechanism for data collection represented a significant shift in strategy from previous research at KWTRP, where historically, clinical and research staff were appointed externally and employed to perform distinct research roles within the hospital. By adopting this policy of appointing Government staff, the study was able to benefit from the expertise and local knowledge of the existing nursing staff and, in return, contribute to capacity development amongst staff working within a routine clinical setting. The intention was that the overall standard of care provided by the maternity unit *per se* could be enhanced for the benefit of all users. Furthermore, by embedding research activity within existing management frameworks, the study systems were integrated very quickly into routine workflows within the departments, and were able to use established line management to implement change efficiently within the workplace, thus enabling the study to become fully operational within four months.

Evidence from extensive health systems research within Kenya informed the data collection strategy. A number of studies by English and colleagues have described methods for integrating data collection for research purposes into health systems using mechanisms that standardise data quality for research purposes, whilst at the same time enhancing the overall standard of care delivered [172, 173].

In particular, English and colleagues highlighted the potential benefits that can be obtained by adopting a standardised, paper form driven admission and medical record completed by Government staff. Importantly, the mechanisms were also ac-

\(^1\) [http://www.linkregistry.org/StudySummary.aspx?objectid=13]
cepted by, and considered to be a useful intervention, amongst clinical teams at the frontline of patient services. On that basis, a ‘Maternity Admission Record’ (MAR) was developed in partnership with clinical and nursing teams from the maternity and paediatric departments. The package was authorised for routine use by the Medical Superintendent of KDH and discussed at stakeholder meetings with representatives from across the wider District Health Management Team - See Appendix A.4 on page 206 for full details of the MAR. Each new admission to the maternity unit was used to initiate a new MAR, which was accompanied by routine bedside testing (BP, height, weight, blood glucose, malaria rapid diagnostic testing and urine dipstick). All patients had blood taken for a full blood count (Coulter Counter - haemoglobin, white cell count and differential with platelets counts), HIV and malaria status. Additional blood, urine and surface swabs were taken for microbiological investigation if clinically indicated.

All new staff appointed since January 2011 have had a ‘role specific’ induction to the MAR process delivered by study nursing and clinical staff. Data are collected on paper and entered on to a purpose-built database, before quality control, data cleaning and analysis. The system was designed with an integrated data report generator to provide KDH and the District Health Management teams with the data required for Government healthcare performance monitoring. This served as an incentive for managers to implement the MAR into clinical pathways.

The structure of KIPMAT was designed around links with the hospital management team and local Government. These were established in advance and used the existing liaison office between KEMRI and the Ministry of Health (MOH). The MOH Liaison Officer was appointed to manage operational links between KWTRP and the District Health Management Team - See Figure 4.3.
Figure 4.3: Study organogram. Management hierarchy of research and clinical work streams and their integration within the Kilifi community. Abbreviations: C-GA - clinical estimate of GA; CRF - care record form; RDT = rapid diagnostic test, and M,C and S - microscopy, culture and sensitivity.
4.5 Ultrasound service

Prior to this study, there were no facilities for routine US during pregnancy for women at KDH. Hence, a service was established *de-novo* to meet research requirements. Based on the work of a UK trained research fellow in the late 1980’s, who introduced US at KDH to study the utero-placental blood flow in pregnancies complicated by malaria, it was clear that such a service was feasible [174]. Unfortunately, the service was suspended due to a lack of clinical leadership when the research fellow returned to the UK in the mid 1990s. Therefore, after a familiarisation period, a consultation exercise with stakeholders was undertaken to address the following strategic areas:

**Scope:** The scope for offering US as a routine service was based on prescriptive clinical service requirements determined in advance. Discussions between the clinical and research teams were held to determine: 1) The number of scans and GAs at which women would be offered US imaging; 2) The requirements for reporting fetal anomalies within the context of services available to monitor and manage such findings at KDH, and 3) Training of non-research clinical staff to manage patients with adverse outcomes within the routine clinical environment.

**Service integration:** The new US service, as well as any additional screening and recruitment activities, should not impact upon existing workflows within the MCH clinic.

**Staff selection and training:** All services should be provided by locally trained health care workers, where possible. Training and quality control should be supervised by a specialist clinician. Opportunities to educate non-research clinicians employed at KDH should be provided.

**Community sensitisation:** The new service should have a phased integration after a period of sensitisation with the local population. This should reflect local...
4.5 Ultrasound service

health policy and promote the expanded coverage of antenatal services.

**Audit standards and care pathways:** Specific care pathways to manage adverse outcomes should be developed, with dissemination and training of staff to protect the health needs of research participants.

Stakeholder ‘service-design’ meetings were then convened to address each of the above issues and a work flow determined to integrate the new US service within the existing MCH work flow. It was agreed that all women attending for their first MCH routine antenatal care visit would undergo a standardised medical and obstetric examination and have a clinical estimate of GA assigned by clinic nursing staff. A modified MAR was developed for this purpose and MCH clinic nursing staff were recruited and trained in accordance with the same policy and management pathways initiated by KIPMAT - See Appendix A.5 on page 212.

US scanning for clinical and research purposes would be offered to those women considered to be ≤24 weeks' gestation. After one scan, no further routine US imaging would be offered until after 34 weeks' gestation, beyond which it was agreed by consensus among clinicians, that facilities were available within Kilifi to manage scan findings of a compromised fetus requiring immediate delivery in a consistent manner. Integration of the US care pathway with routine services in the MCH is summarised in Figure 4.4.
**Figure 4.4:** Flow chart detailing the interaction between study activities and routine clinical services within the MCH and maternity departments at KDH. Arrows indicate flow of clinical information and transition points of patients into clinical services as a consequence of information obtained during research involvement. All pathways were developed and agreed in partnership with the Obstetric and Paediatric Consultant body and approved by the Medical Superintendent at KDH. Abbreviations: SMC - senior medical consultation; UA - umbilical artery; CS - caesarean section, and IOL - induction of labour.
4.5 Ultrasound service

4.5.1 Scanning facility and equipment

A purpose built, air-conditioned US facility was designed and constructed adjacent to the MCH clinic waiting area. A confidential area for informed consent procedures and a waiting room were placed next to the US room. All US was performed using a mid-range, commercially available machine (Philips HD-9, Philips Ultrasound, Bothell, WA, USA) with curvilinear abdominal probes (C5-2, C6-3 and V7-3). A ‘Kilifi Ultrasound System’ database and reporting system was designed using a web-based ‘front end’ database [115]. Anonymised patient data were stored using on-site secure servers that were password protected in accordance with KWTRP data governance policies. A sample US report and screenshot from the database are provided in Appendix A.6 on page 213.

Patients benefited from this system by being provided with real-time printed clinical reports that were added to handheld maternity records. As well as providing an EDD according to US and LMP, details including the fetal number, viability, placental position and any necessary follow-up scans were provided.

Clinicians benefited from the system by regulated access to US data for individual reporting and clinical audit purposes.

4.5.2 Sonographer selection and training

Three Clinical Officers (clinicians trained to diploma level) were appointed and trained to perform US. A theoretical course was provided over seven sessions: 1) Introduction to US. Aim and objectives of the service; 2) Technical US - essential physics, image acquisition and the safety of US in pregnancy; 3) Obtaining and validating informed consent for US provision; 4) US for GA estimation, identifying multiple pregnancies, placental insertion and fetal anencephaly; 5) US diagnosis of early pregnancy loss - ‘breaking bad news, communication and evidence for clinical management; 6) Dos and don’ts with US, and 7) Third trimester scanning - biometry
and umbilical artery doppler measurement. It was a mandatory requirement that all sonographers complied with KWTRP policies on good clinical practice, attended a basic life support course and completed the National Institute for Health online training course in medical ethics for research.

A supervised introduction to practical US imaging was then undertaken with each trainee. A one-to-one approach with a specialist obstetrician was used to develop competencies in accordance with WHO and INTERGROWTH-21st guidelines [175, 176, 177].

4.5.3 Nurse selection and training

All nursing staff from the MCH clinic were invited to attend training on the management of patients for research purposes. This was a mandatory requirement for the issue of temporary employment contracts. Existing line management structures were utilised by recruiting senior nursing staff as study supervisors. Training for nursing staff was undertaken over four sessions: 1) US in Kilifi - GA estimation; 2) Clinical care and research: what is the difference? - how this will affect patients and the workflow; 3) Gestational age estimation - a standardised menstrual history and SFH measurement, and 4) Study pro-forma introduction and data entry training.

Nursing staff were required to attend a weekly study meeting with the lead sonographers to discuss recruitment, screening performance and management issues relating to the US service and routine care within the MCH clinic.

4.6 Community engagement, key messages and consent

Community engagement is viewed as an essential ethical requirement for the conduct of collaborative research [162]. Furthermore, there is increasingly persuasive evidence that such activities strengthen studies by enabling the design of studies

http://phrp/nihtraining.com
to acknowledge and protect the interests of research participants [162]. This also serves to enhance both the relevance of research findings within communities and contributes to improving the quality of research undertaken [163, 178, 179, 180, 181, 182].

A formal mechanism for the community engagement activities of this study was adopted. A Community Advisory and Support Team (CAST team) for Reproductive Health Research was formed and comprised senior team members from the community liaison group at KWTRP, senior research staff, four senior fieldworkers and volunteers from the KEMRI community representatives group, who give of their time generously to facilitate the integration of research activities into the community. The design, scope and roles of this group have been discussed elsewhere [183], but, in brief, the purpose of such a team is to provide a forum in which studies can be integrated into the activities of the community within Kilifi District; a dialogue between researchers and the community established, and, as the study progresses, a mechanism to feedback study outputs to community members, whose participation in such work is critical.

The role of the CAST team was essential to determine how best to sensitisise the community about the use of US. With a paucity of published data examining acceptability and feasibility of US services in sSA for us to refer to, and in view of the range of diverse traditional health practices and beliefs with respect to pregnancy and childbirth in Kilifi, it was considered vital that ‘key messages’ and a strategy for their delivery was determined prospectively and disseminated widely to ensure clear and consistent dialogue with the community.

In particular, concerns that the introduction of US had the potential to identify adverse fetal outcomes before they were otherwise clinically apparent was discussed extensively to ensure that the content and methods of communication were appropriate and clear.

By engaging with a diverse range of subgroups within the community, it was pos-
sible to establish ‘key-players’ who were likely to be instrumental in disseminating information.

An information delivery strategy was produced as a result of the community engagement process. This was based on a published framework derived from locally conducted research and provided a formal basis for all subsequent dialogue with the community. The strategy is summarise in Table 4.1.
### 4.6 Community engagement, key messages and consent

<table>
<thead>
<tr>
<th>Information delivery goals</th>
<th>Key messages</th>
<th>Audience &amp; approach</th>
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<td><strong>A - Sensitisation Theme 1</strong></td>
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**Research aims:**
- Summarise maternal and child health research at KWTRP.
- Introduce US dating study.
- Establish current baseline knowledge of US.
- Identify and address community concerns relating to US in pregnancy.

**Study procedure:**
- US machine: used to see a picture of the baby in the womb on a computer screen.
- Procedure: Non-invasive and pain free.
- Capacity: Unable to visualise all problems; no guarantees of problem free pregnancy.
- Charges: US service is free of charge. Routine charges for ANC still apply (until July 2013).

**Community stakeholder groups:**
1. Kilifi Chief and Village Elders Network
2. KEMRI Community Representatives
3. Individual Group Session for Female Community Members

**Mechanism:**
- Study specific sensitisation meetings - 6 across Kilifi District townships.
- Information leaflet distributing using KHDSS.
- Staff training of all groups providing healthcare to women and children in Kilifi.

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<th>Information delivery goals</th>
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<td><strong>B - Sensitisation theme 2</strong></td>
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**Integration of research and routine clinical services:**
Outline the interface between study and routine clinical care.

US service to be offered in addition to routine clinical care:
1. Participation in the study is voluntary.
2. Non-participants will be provided with the same standard of clinical care.
3. Service available to all women $\leq 24$ weeks’ gestation for no charge.
4. Safety - no evidence of harm in 40 years.
5. Enhanced antenatal diagnostics offer early diagnosis of twins and placental problems.
6. Potential for diagnosing problems before clinically obvious. Rare and no causal link with US.

**Routine care provision:**
1. Core health care standards maintained.
2. Additional staff and resources already in place to meet increases in demand.
3. Research staff and MOH collaborating to enhance care standards.
4. New equipment and testing available for all mothers and newborns.
5. Funds available to support individual cases where US identifies problems that could not have been detected otherwise.

**KDH based clinical groups:**
1. Medical superintendent and senior management team.
2. Nursing team - maternity department.
3. Nursing team - MCH clinic.
4. Junior medical team.

**Mechanism:**
Reproductive health management team assembled. Hospital and district management attendance encouraged.

Study specific sensitisation meetings - staff groups.

Training opportunities for all staff.

Mandatory training, research quality control and management structure clauses agreed in contracts for research staff.

Continued overleaf
### Information delivery goals

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<th>Key messages</th>
<th>Audience &amp; approach</th>
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<td><strong>C - Sensitisation theme 3</strong></td>
<td><strong>District Medical Officer for health (and team):</strong></td>
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<tr>
<td></td>
<td>1. District Medical Officer - public health planning.</td>
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<td></td>
<td>2. District Health Management Team.</td>
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<td></td>
<td>3. Health centre and dispensary staff - satellite clinics providing pregnancy care in Kilifi.</td>
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<tr>
<td><strong>Information exchange:</strong></td>
<td><strong>Mechanism:</strong></td>
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<tr>
<td>Open invitation for dialogue with community.</td>
<td>Regular attendance to feedback during weekly management meetings.</td>
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<tr>
<td>Study representatives available for all community engagement events.</td>
<td>Study team visits to health centres and dispensaries to discuss findings.</td>
</tr>
<tr>
<td>Study telephone line available and details published on all study information.</td>
<td>Open referrals for US from the community.</td>
</tr>
</tbody>
</table>

#### Approved by:

Kenyan National Scientific and Ethical Review Committees.

#### Anticipated research outputs:

1. Description of health problems for mothers and newborns in Kilifi.
2. Preterm birth rates in Kilifi.
3. Description of fetal growth.
4. Use of US as a research/clinical tool in sSA.

---

Table 4.1: Community engagement and information dissemination strategy. Designed in accordance with output from the community engagement team [163].
4.6 Community engagement, key messages and consent

4.6.1 Sensitisation programme outcomes

Six meetings were held over a six week period between 28 June 2011 and 14 July 2011 across Kilifi. Sixty one members of the public attended, with six Chiefs and their assistants. The exercise was well received overall. There was a recognition generally that maternal and newborn health was an unmet research need within the community. Frequent comments included:

“Pregnant women are dying from losing too much blood, please act fast.”

“If the child has a defect, how can you help?”

Others questioned why US was not offered after 24 weeks’ gestation. Observations by a dedicated social scientist overseeing the meetings depicted a highly receptive and supportive outlook to the introduction of US and the associated research surveillance network (unpublished data). The need for more accurate dating of pregnancies was highlighted as a need by the community themselves. Specific details relating to the process of US imaging were questioned extensively, including whether US offered any therapeutic options or if the need for operative delivery could be predicted.

In response to the sensitisation events, a qualitative research programme was established to investigate the perceptions amongst patient and provider groups of the acceptability of US as a component of routine antenatal services at KDH. In high-income settings across Europe and North America, the attitudes and perceptions of women towards US in pregnancy have been extensively discussed, and the technology is considered to be beneficial amongst user groups by providing reassurance about individual pregnancies, whilst at the same time, promoting healthy behaviour amongst pregnant women[184, 185, 186]. A trained social sciences research assistant (RA) was appointed to lead this observational study. She was jointly supervised by the author of this thesis and a senior social scientist. Data were collected longitudinally in three phases between June 2011 and April 2012 using structured observations and individual interviews.
In total, 59 participants were interviewed immediately before first exposure to the US service, and then again immediately after the first scan to explore perceptions of the technology with respect to their pregnancy and antenatal care. Interviews in Kiswahili and Kigiriama, the two local languages in Kilifi, were transcribed verbatim and translated into English before being explored by a thematic framework technique [187]. Overall the service was viewed positively. Specifically, women were reassured by “seeing their baby” and considered the additional information provided by US to be of added value in managing their pregnancy.

**4.7 Scientific and ethical approval**

Approval to undertake the study was obtained from multiple agencies. The study protocol, communication and consent pathways were presented to the local scientific steering and consent and communication committees in Kilifi in 2010. Revisions were then forwarded to the national scientific steering and ethics review committees of the Kenya Medical Research Institute. Approval to commence operations was granted in June 2011 and renewed annually with the Kenya National Ethics Review Committee (SSC 1969) after submission of annual progress reports.

**4.8 Recruitment and informed consent**

Once operational, the study was introduced to all potential participants each morning during a routine ‘health-talk’ provided by MCH clinic nursing staff. This summarised the study objectives, background and eligibility criteria in Kiswahili and Kigiriama according to the key messages agreed with the CAST team. Women were then directed into two streams of work for each clinic session according to whether they were attending for a follow-up visit or for the first time. This former group were then screened to determine eligibility for the study.
4.8 Recruitment and informed consent

4.8.1 Eligibility criteria

All women presenting to the MCH with an estimated GA ≤24 weeks’ gestation were invited for US. This was determined by LMP if the date was certain. Otherwise, the GA was determined by SFH using dating charts standardised for use across the region [79]. Women who had breastfed, used hormonal contraception, or who reported irregular menstrual cycles within the last 2 months were also screened by SFH irrespective of LMP. In the event that both LMP and SFH were unavailable, women were offered a urine pregnancy test and invited for GA estimation by US if the test was positive.

4.8.2 Informed consent

A multi-staged, informed consent process was adopted. After the health talk and eligibility screening, all potential women were invited for US. At this point, a group of study trained fieldworkers who were fluent in Kiswahili and Kigiriama would counsel women on an individual basis using the approved communication and consent packages. All women were asked to provide written consent before undergoing US. This included information about the potential for US to identify problems with the pregnancy before they became clinically apparent. In the event that women were unable to write, a fingerprint was taken as confirmation of consent to participate in the study. Contact details with study personnel were provided for continuity and later communications. For those women attending for repeat third trimester US, written consent was reconfirmed. Consent forms and examples of the information provided during the informed consent process are provided in Appendices A.2 and A.3 on pages 200 to 203.
4.9 Ultrasound protocol

All ultrasonography staff operated in accordance with a strict US protocol and standard operating procedures. These are provided in Appendix A.7 on page 216. The US parameters measured at each visit are summarised in Table 4.2.

4.9.1 US biometry

Each US scan records the number and viability of fetuses and their presence within the uterine cavity, the placental location and amniotic fluid index. Fetal biometry was measured in accordance with Table 4.2. The US machine’s proprietary software was modified to conceal all measurements from the user until each parameter was recorded in triplicate. After this, data were revealed for clinical purposes.

4.9.2 Gestational age estimation

GA by US was estimated for CRL and HC using the Robinson [29] and Chitty [140] formulae, respectively.

4.10 Data collection and management

All clinical data were recorded on pre-printed and pre-numbered study pro-formas by clinical and fieldworker teams. Data from the MCH clinic and US service were then recorded into the Kilifi Ultrasound System. Clinical staff were required to enter and validate the data in real-time. See Appendix A.6 on page 213 for details of the database system. Clinical data from the maternity unit collected at the time of admission and delivery were entered into a separate inpatient database housed on the same KEMRI servers. All data were recorded using the PersonID, PregID and KIN number series of unique identifiers. Paper copies of all forms were archived.
Table 4.2: Summarised US biometry image acquisition protocol. All image acquisition, calliper placement and sonographer quality control procedures are previously described [175, 176, 177].

and any suspected errors in the clinical data were referred back to paper sources if necessary.

Supervision of all data checking, cleaning and correction of errors was included within the job description of a study specific data manager. All changes to the original files were recorded with names and dates for audit purposes. To ensure that the integrity of source data was maintained at all times, analyses and reporting were always performed using data extracts of the source data. Master scripts were used to extract locked copies of the dataset remotely and also included automated processes to merge, clean and validate data before any analyses were undertaken.
4.11 Summary

The methodology described in this chapter represents one year of full-time working. The author of this thesis developed the research protocol and was responsible for all aspects of its implementation. This included acting as chairperson for all meetings with external stakeholders, leading the recruitment and training a team of 30 study team members and providing all clinical training and mentorship.
Chapter 5

The effect of ultrasound based gestational age assessment on preterm births in rural Kenya

5.1 Introduction

Every year approximately 3.1 million babies die in the first seven days of life. Three-quarters of these perinatal deaths occur in low-income settings across sSA and South Asia, and the most common cause is PTB [11]. Determining the PTB rate - defined as the percentage of live births at <37 weeks (or <259 days) from the first day of the LMP - clearly depends upon estimating GA at birth accurately.

As discussed in Chapter 2, since the date of the LMP is often unknown or poorly recalled, US measurement of fetal biometry is recommended as a more accurate estimate of GA [95]. However, US is not routinely available in many low-income settings with high perinatal mortality [141] and so healthcare workers have to rely upon the LMP or other ‘dating’ methods such as measuring the SFH, despite their well-recognised poor predictive power [71].

Not surprisingly, therefore, differences in PTB rates are observed depending on the
5.2 Methods

A perinatal surveillance system, including a routine US service, was established within the MCH clinic at KDH as previously described in Chapter 4.

Since October 2011, all women attending for their first antenatal care visit to the MCH clinic have been assessed for clinical and research purposes by government nursing staff. Data collection for analysis commenced after a one month trial period in which procedures were optimised and staff training completed. The analyses presented in this chapter include data from clinic episodes between 1 November 2011 and 30 July 2013.

5.2.1 Recruitment and study sample definition

All women attending for their first clinic visit were screened to determine study eligibility. A preprinted study pro-forma with a preallocated KIN-Number (study UID) was completed during the routine assessment conducted for all women by nursing staff in the MCH clinic. Women subsequently identified as eligible for US were provided with their forms and directed to the US unit for counselling by fieldworkers, who were responsible for obtaining informed consent from participants.

An audit system was used to track the pro-formas based upon the progress of KIN-Numbers through the research pathway. ‘Active’ numbers for each session were
logged and a meeting held between the research nurse ‘in-charge’ and the sonographer to review recruitment on a daily basis. This forum provided an opportunity to discuss instances where potentially eligible cases were missed, and so served to manage recruitment problems proactively in a very difficult environment. Once firmly established, the loss of KIN-Numbers became a reflection of ‘silent refusals’ amongst women who had been invited to participate, but who had subsequently decided against this.

5.2.2 Clinical examination: gestational age estimation

Nursing staff completed the multiple choice pro-formas and recorded maternal anthropometry measures (height, weight, and mid-upper arm circumference). The study dating protocol to estimate GA according to LMP and SFH is summarised as follows:

5.2.2.1 LMP-GA

The first day of the LMP was recorded and judged to be reliable if the date was recalled with certainty, an approach consistent with other studies [122]. Cycle regularity, breast feeding status and the use of hormonal contraception in the previous two months were also documented.

5.2.2.2 SFH and C-GA

Where women were uncertain of the LMP, SFH was also measured, to the nearest centimetre, as the greatest distance between the superior border of the symphysis pubis and the fundal pole of the uterus on abdominal palpation [71].

Data from the MCH clinic were validated by study clinicians and used to estimate GA by the LMP, if available and certain (LMP-GA). Otherwise, GA was estimated by the SFH measurement using dating charts from South Africa [79]. For practical and
analytical purposes, we defined a composite ‘best clinical estimate’ of GA (C-GA) based on the LMP if certain or the SFH in the case of uncertainty, which closely reflects clinical practice across the region [81].

5.2.2.3 US-GA

Women were eligible for US if C-GA ≤ 24 weeks’ gestation. In the event that both LMP and SFH were unavailable, C-GA was recorded as unknown, a pregnancy test performed and US offered to those women with a positive test.

Four Kenyan clinicians (Clinical Officers) performed basic obstetric US in accordance with WHO and INTERGROWTH-21st guidelines - see Chapter 4 [175, 177].

If the sonographer was unable to determine fetal viability or GA, women were invited for a repeat scan four weeks later. Multiple pregnancies and those where US-GA > 24 weeks’ gestation were provided with a printed clinical report in their handheld record, but were excluded from the study.

Routine follow-up US was arranged for women presenting with a low lying placenta after 34 weeks’ gestation. Where placenta praevia was confirmed at follow-up, women were referred for senior obstetric review. A research medical officer (postgraduate year 2+) was available to support clinical decisions when the consultant was absent.

All adverse fetal outcomes that were identified as a consequence of participation in the study were managed according to a pre-defined patient care plan in Figure 4.4. Individual clinical decisions for such cases were guided by SOPs 1 to 4 - see Appendix A.7 on pages 216 to 223. Audit forms were stored and cases were discussed with the local clinical team. Whilst the study was committed to enhancing the care for all women at KDH, it was considered essential that where diagnoses were revealed as a consequence of participating in research, that sufficient capacity to manage such cases was consistently provided.
5.3 Delivery and follow-up

5.3.1 Hospital deliveries

KIPMAT recorded a comprehensive dataset, including all clinical examinations, and bedside and laboratory test results, for all deliveries at KDH, including early pregnancy losses (<24 weeks’ gestation). Data were collected using the ‘MAR’ structure, with each new admission to the maternity unit prompting a new MAR data sequence. Multiple admissions during the study were linked using PregID and date. Only admissions that were closed with a summary of the delivery were analysed as part of this chapter.

At all times, one additional government nurse was provided by the study to oversee data and research sample collection for that shift. For data quality purposes, this nurse was not allocated to clinical duties. Newborn admissions to the neonatal care unit at KDH were tracked electronically. The paediatric service operated a different clinical admissions process, but this was based upon the same principles of standardised data collection and used the sample identifications procedures.

Women admitted with early pregnancy problems were admitted on to general adult medical wards. Pregnancy losses were diagnosed clinically. It is acknowledged that an US service offers the potential to enhance the diagnosis of ectopic pregnancy and antepartum haemorrhage as well as supporting clinical decisions around the timing of elective caesarean sections for those women requiring operative delivery where GA is unclear. However, it was determined by consensus amongst all stakeholders that there was insufficient capacity within KDH to guarantee that such services would be consistently available throughout the entire study period and, therefore, that it was not appropriate to offer them as part of the clinical service provided by the study.
5.3 Delivery and follow-up

5.3.2 Non-hospital deliveries

Deliveries outside KDH were tracked using KHDSS. This restricted data collection to the date and location of delivery, birth outcome of the newborn (born alive or stillborn) and the vital status of the mother and newborn (alive or dead) at each subsequent round of KHDSS surveillance during the study follow-up. For those women choosing not to deliver at KDH, the only requirement for follow-up was that they remained resident and delivered within the KHDSS surveillance zone; all migrations out of area were recorded to enable ‘loss to follow-up’ tracking.

5.3.3 Data management

The allocation of unique identifiers and merging of datasets was designed to identify each woman using a unique combination consisting of the KIN-Number + PregID + PersonID. At birth, each newborn was allocated a Person ID within KHDSS in real time. These were automatically linked to the PersonID of the mother and their homestead location.

Using the data management processes described previously (Chapter 4), study data were held in four databases that were hosted onsite by KWTRP. A virtual, ‘single-study’ dataset was constructed for analytical purposes. Programming scripts were assembled to merge datasets, and to validate and clean the source data before presenting it for analysis. Therefore, analyses used a standardised, clean dataset for each ‘round’ and were standardised in terms of their data structure.

Previous experience from research teams within KWTRP suggested that there would be occasions during the KHDSS surveillances that prevented fieldworkers from accessing network data in real time. During network ‘downtime’, the system responsible for linking the birth details of newborns delivered outside KDH to their mothers was susceptible to failure. To conserve the integrity of data for this study, a parallel validation procedure was developed for such circumstances. This alerted the re-
search team when a newborn was registered into a household where an on-going study pregnancy was registered, but where no link had been established between the mother and newborn. These alerts prompted a referral back to the paper pro-formas to assess whether such pregnancies were eligible for analyses.

### 5.3.4 Outcome Indicators

WHO define PTB as those with a GA < 259 days' (37 weeks') gestation, but are not clear on the lower limit of GA to be included. The threshold used by analyses amongst different studies varies between 16 and 28 weeks' gestation. Many groups consider that the lower limit of fetal viability should represent the threshold for PTB, which further complicates the consistency of data, on the basis that different countries adopt different legal thresholds for this determination. The UK and US, for example, use 24 weeks' gestation as the lower limit, but in less developed settings, a later definition of 28 weeks' gestation is used in accordance with WHO policy.

In Kilifi, standardising these data was further complicated by the management policy amongst paediatric clinicians. Whilst the legal threshold in Kenya was set at 28 weeks' gestation, where newborns were considered to be > 24 weeks' gestation, resuscitation was considered. Thus, where GA estimates were unavailable at birth, decisions with respect to the management of such cases were left to the discretion of individual clinicians. Although considerable effort was made to ensure that clinical staff were able to access GA data from the US service, this could not be guaranteed. Hence, it was determined, that newborns delivered < 24 weeks' gestation by US would be excluded from the analyses presented in this thesis - regardless of their outcomes. Therefore, PTB rates presented in this thesis refer to live births ≥ 24 weeks' gestation, unless otherwise indicated.

Measurements of neonatal and early infant morbidity in low-income settings are complicated by a lack of relevant and consistent case definitions [188, 189]. For this study, it was decided that neonatal morbidity would be determined according to the
need for admission to the neonatal unit at KDH within 7 days of delivery. Reporting of these data was restricted to newborns admitted after delivery at KDH in order to provide a standardised denominator for comparative purposes.

5.3.5 Sample size

Based on retrospective data from 2009, 1003 women presented for their first antenatal clinic appointment at KDH <24 weeks’ gestation and amongst these, the PTB was estimated at 18% by C-GA (unpublished data).

The existing evidence base comparing estimates of GA according to different methods was considered insufficient as a basis for determining a clinically important threshold upon which to base a sample size calculation. Hence, it was decided to consider the level of difference between PTB rates by US-GA and C-GA that would be considered sufficiently important to change local practice. After discussion with clinical and research teams at KDH and KWTRP, it was determined by consensus that a 50% difference in PTB would definitely be sufficient to change routine dating practice and that a difference of 30% could potentially change practice if this were associated improvements in clinical outcomes amongst mothers or newborns.

Table 5.1 illustrates the sample sizes required to achieve 80% power to detect differences in PTB rates between 30 and 50%. All calculations were performed using the ‘sampsi’ command within STATA. For example, ‘sampsi 0.18, 0.22, power(0.8)’ provided the group size required to have 80% power to detect a 50% increase in PTB rates, assuming a 5% type I error rate ($\alpha =0.05$) [190]. In view of the potential that US-GA could produce estimates of PTB that were both higher or lower than estimates by C-GA, the sample sizes were calculated based upon absolute rather than relative percentage differences between groups. Overall, a sample size of 1100 was considered realistic and provided 80% power to detect a 50% increase, or conversely, a 40% decrease in PTB rates by US-GA compared to C-GA.
5.3 Delivery and follow-up

<table>
<thead>
<tr>
<th>Δ PTB rate</th>
<th>↑ / ↓</th>
<th>H₁</th>
<th>H₂</th>
<th>Minimum sample</th>
<th>20% LFU</th>
<th>Sample size</th>
</tr>
</thead>
<tbody>
<tr>
<td>50%</td>
<td>Increase</td>
<td>0.18</td>
<td>0.27</td>
<td>718</td>
<td>144</td>
<td>862</td>
</tr>
<tr>
<td></td>
<td>Decrease</td>
<td>0.18</td>
<td>0.09</td>
<td>494</td>
<td>99</td>
<td>593</td>
</tr>
<tr>
<td>40%</td>
<td>Increase</td>
<td>0.18</td>
<td>0.25</td>
<td>1136</td>
<td>247</td>
<td>1363</td>
</tr>
<tr>
<td></td>
<td>Decrease</td>
<td>0.18</td>
<td>0.11</td>
<td>850</td>
<td>170</td>
<td>1020</td>
</tr>
<tr>
<td>30%</td>
<td>Increase</td>
<td>0.18</td>
<td>0.23</td>
<td>2124</td>
<td>425</td>
<td>2459</td>
</tr>
<tr>
<td></td>
<td>Decrease</td>
<td>0.18</td>
<td>0.15</td>
<td>4938</td>
<td>988</td>
<td>5926</td>
</tr>
</tbody>
</table>

**Table 5.1:** Sample sizes required to detect a range of observed differences in PTB rates by method of GA estimation. A baseline rate of 0.18 was used for all calculations in accordance with historical data from KDH. All calculations were determined using a two-sample difference in proportions methodology with an 80% power at the 5% significance level - \( \alpha = 0.05; \beta = 0.8; H₀ = \) baseline PTB rate by C-GA; \( H₁ = \) PTB rate as determined by US-GA, and LFU = loss to follow-up.

5.3.6 Statistical analysis

The optimal test of PTB status was determined *a priori* to be that which classified the fewest cases as preterm, but retained as ‘test positive’, those pregnancies leading to perinatal mortality or neonatal morbidity. This was assessed by comparing the Hazard Ratios (HR) and absolute proportions of deliveries ending in perinatal death or neonatal morbidity that were classified as PTBs by each dating method.

All analyses were conducted offline using STATA (Version 13, Stata Corp, Texas, USA). Kaplan-Meier product estimates of survivor functions were used to estimate PTB rates. Cox proportional hazard models were used to compare PTB rates for LMP-GA, C-GA and US-GA, with US-GA as the reference group.

Survival analyses were considered appropriate because of their capacity to estimate PTB rates amongst datasets with censored data-points resulting from cases that were ‘lost to follow-up’, as well as their ability to provide estimates of the stability of rate estimates over time [191]. For standardisation purposes, all survival analyses were derived according to the following parameters:

**Failure event:** For the calculation of PTB rates, failure was defined as the date of delivery. For perinatal mortality, failure was defined by the date of delivery for
stillbirths or the date of death for those newborns who died within the first 7 days of life.

**At risk time:** This was arbitrarily assigned as the EDD - 280 for all participants and was used to set both the numerator and denominator for all rate analyses.

**Surveillance time:** For PTBs this began on the date at which GA = 168 when analyses were based on a 24 week lower limit to define PTB status. Analyses were repeated with GA = 196 to compare results according to the WHO definition (28 weeks’ gestation). The surveillance time was adjusted by method when comparing different estimate of GA. Infants with no recorded delivery after EDD + 100 were considered as lost to follow-up and so were automatically removed from both the numerator and denominator of subsequent analyses.

**Conditions for failure:** For PTB rates, failure was restricted to newborns delivered with a detectable heartbeat, or where resuscitation subsequently resulted in a detectable heartbeat at any point. For perinatal mortality indices, both live and stillborn deliveries were included in analyses.

Time variables were entered in their observed states as absolute dates and times rather than according to summary statistics and time intervals between events. Where subgroup analyses were used to compare the performance amongst different methods for estimating GA, each woman was entered into the dataset multiple times, i.e. once for GA by LMP, once for GA by US and so on. This resulted in a dataset with a ‘stack’ of multiple single person entries, which was controlled during analyses by nominating the ‘KIN-Number’ as an indicator of clustering. Hence, significance testing was adjusted to account for the potential influences of multiple single person entries. Specifically, the Huber-White Sandwich Estimator was applied to adjust estimates of variance that were used when determining the CIs for rate calculations [192].

The adjusted Cox regression models were extended using the ‘punafcc’ option within STATA to estimate the population attributable fractions (PAF) of perinatal
5.4 Results

mortality and neonatal morbidity. The models use perinatal death or newborn admissions to the neonatal unit as their clinical outcomes and are compared using three PTB subgroups (by US-GA) as exposure variables: 1) Extremely preterm (24 - < 28 weeks’ gestation), 2) Very preterm (28 - <32 weeks’ gestation), and 3) Late preterm (32 - <37 weeks’ gestation) [193, 194, 195].

5.3.7 Ethics statement and consent

Ethical approval for the studies included in this chapter was provided by the Kenya National Ethic Review Committee - (SSC 1969). See Chapter 4 on 60 for details of ethical approval. Sample consent forms and patient information leaflets are provided in Appendices A.2 and A.3 on pages 200 and 203.

5.4 Results

Between November 2011 and July 2013, 3630 women attended the MCH clinic at KDH for antenatal care. A total of 1827 (50%) had an estimated C-GA ≤ 24 weeks’ gestation and were therefore invited to participate in the study. Of these, 388 (21%) declined to be scanned and a further 268 were deemed ineligible for US after a second round of screening by the US clinician (See Section 5.5.2 on page 91 for a discussion of this). Overall 1171/1827 (65%) of women were scanned. In total, 1107 (61%) women went on to have GA confirmed ≤ 24 weeks’ gestation by US and formed the final study cohort. Full details of the recruitment flow are provided in Figure 5.1.

5.4.1 Demographic characteristics

Baseline demographic and obstetric characteristics indicate that eligible participants (C-GA ≤ 24 weeks’ gestation) were more likely than non-eligible participants (C-GA > 24 weeks’ gestation) to have been educated to secondary school level (16 years of
5.4 Results

C-GA at first presentation:

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>( \leq 24 ) weeks' gestation</th>
<th>&gt;24 weeks' gestation</th>
<th>Test for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( n = 1827 )</td>
<td>( n = 1803 )</td>
<td></td>
</tr>
</tbody>
</table>

Demographics

1. Age (mean - (min, max))
   - 25.77 (17-50) vs. 25.65 (16-46)
   - \( p < 0.001 \)

2. Level of education (percent)
   a. None
      - 14.8 vs. 20.5
      - \( p < 0.001 \)
   b. Primary
      - 57.0 vs. 62.8
   c. Secondary or higher
      - 28.2 vs. 16.7

Obstetric details

3. Parity (median)
   - 1 vs. 2
   - \( p < 0.001 \)

4. Location of last birth (percent)
   a. Home
      - 26.5 vs. 35.8
      - \( p < 0.001 \)
   b. Hospital
      - 34.1 vs. 29.6
   c. Other
      - 39.4 vs. 34.6

5. Stillbirth (percent)
   a. Last delivery
      - 15.0 vs. 9.0
      - \( p < 0.001 \)
   b. Any delivery
      - 18.0 vs. 15.0

Table 5.2: Demographic and obstetric characteristics for all first presentations to the MCH clinic. Please note that stillbirths are self-reported and could not be validated with records of GA. Test for trend = Mann-Whitney U test for continuous data and \( \chi^2 \) test for categorical variables.

Age (28% vs. 16%, \( p < 0.001 \)) and have experienced a previous poor obstetric outcome - last delivery stillborn (15% vs. 9%) and any delivery stillborn (18% vs. 15%, \( p < 0.001 \)) - see Table 5.2.
<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Period 1</th>
<th>Period 2</th>
<th>Period 3</th>
<th>Total</th>
<th>Test for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td></td>
</tr>
<tr>
<td>First ANC visits</td>
<td>1396</td>
<td>1332</td>
<td>902</td>
<td>3630</td>
<td></td>
</tr>
<tr>
<td>C-GA &lt;24 weeks' gestation</td>
<td>785 (56)</td>
<td>617 (46)</td>
<td>425 (47)</td>
<td>1827 (51)</td>
<td></td>
</tr>
<tr>
<td>Inclusion criteria met</td>
<td>265 (34)</td>
<td>493 (80)</td>
<td>349 (82)</td>
<td>1107 (61)</td>
<td></td>
</tr>
<tr>
<td><strong>Demographics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Age (mean)</td>
<td>25.8</td>
<td>24.9</td>
<td>25.5</td>
<td></td>
<td>0.077</td>
</tr>
<tr>
<td>2. Marital status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.005</td>
</tr>
<tr>
<td>a. Single</td>
<td>11 (4)</td>
<td>31 (6)</td>
<td>19 (6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>b. Married</td>
<td>249 (95)</td>
<td>458 (93)</td>
<td>324 (93)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>c. Divorced</td>
<td>3 (1)</td>
<td>1 (&lt;1)</td>
<td>3 (&lt;1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>c. Widowed</td>
<td>0 (-)</td>
<td>3 (&lt;1)</td>
<td>3 (&lt;1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>d. Missing</td>
<td>2 (&lt;1)</td>
<td>- ()</td>
<td>- ()</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Level of education</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.693</td>
</tr>
<tr>
<td>a. None</td>
<td>1(&lt;1)</td>
<td>69 (14)</td>
<td>50 (14)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>b. Primary</td>
<td>135 (52)</td>
<td>281 (58)</td>
<td>211 (60)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>c. Secondary or higher</td>
<td>120 (47)</td>
<td>137 (28)</td>
<td>88 (26)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>d. Missing</td>
<td>9 (3)</td>
<td>6 (&lt;1)</td>
<td>- ()</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Continued overleaf
### Obstetric details

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Period 1</th>
<th>Period 2</th>
<th>Period 3</th>
<th>Total</th>
<th>Test for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>n</em> (%)</td>
<td><em>n</em> (%)</td>
<td><em>n</em> (%)</td>
<td><em>n</em> (%)</td>
<td></td>
</tr>
<tr>
<td><strong>4. Parity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>=0.662</td>
</tr>
<tr>
<td>a. 0</td>
<td>84 (32)</td>
<td>199 (41)</td>
<td>124 (36)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>b. 1</td>
<td>65 (25)</td>
<td>105 (21)</td>
<td>65 (19)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>c. 2</td>
<td>38 (14)</td>
<td>68 (14)</td>
<td>63 (18)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>d. 3</td>
<td>27 (10)</td>
<td>58 (12)</td>
<td>39 (11)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>c. 4</td>
<td>15 (6)</td>
<td>29 (6)</td>
<td>25 (7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>e. ≥ 5</td>
<td>29 (10)</td>
<td>30 (6)</td>
<td>33 (9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>f. Missing</td>
<td>7 (3)</td>
<td>4 (&lt;1)</td>
<td>- ()</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>5. Location of last birth</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>=0.662</td>
</tr>
<tr>
<td>a. Home</td>
<td>91 (57)</td>
<td>175 (61)</td>
<td>121 (55)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>b. Hospital</td>
<td>54 (34)</td>
<td>106 (37)</td>
<td>100 (45)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>c. Other</td>
<td>16 (10)</td>
<td>1 (1&lt;1)</td>
<td>- ()</td>
<td></td>
<td></td>
</tr>
<tr>
<td>d. Missing</td>
<td>20 (10)</td>
<td>12 (2)</td>
<td>4 (&lt;1)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Continued overleaf
<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Period 1</th>
<th>Period 2</th>
<th>Period 3</th>
<th>Total</th>
<th>Test for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td></td>
</tr>
<tr>
<td>6. Stillbirth *</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Last delivery</td>
<td>36 (20)</td>
<td>66 (21)</td>
<td>38 (17)</td>
<td>140 (20)</td>
<td>=0.222</td>
</tr>
<tr>
<td>b. Any delivery</td>
<td>62 (34)</td>
<td>85 (29)</td>
<td>49 (22)</td>
<td>196 (28)</td>
<td>=0.007</td>
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<tr>
<td>Anthropometry</td>
<td></td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>7. MUAC (cm)</td>
<td>25.7</td>
<td>25.6</td>
<td>26.1</td>
<td>-</td>
<td>=0.005</td>
</tr>
<tr>
<td>8. BMI</td>
<td>-</td>
<td>22.4</td>
<td>22.7</td>
<td>-</td>
<td>=0.216</td>
</tr>
<tr>
<td>9. BP (systolic)</td>
<td>-</td>
<td>108</td>
<td>109</td>
<td>-</td>
<td>=0.803</td>
</tr>
<tr>
<td>10. BP (diastolic)</td>
<td>-</td>
<td>65</td>
<td>67</td>
<td>-</td>
<td>=0.087</td>
</tr>
</tbody>
</table>

* Table 5.3: Demographic and obstetric characteristics of the study population over time: Period 1 = 1 November 2011 to 31 May 2012, Period 2 = 1 June 2012 to 31 December 2012, Period 3 = 1 January 2013 to 31 July 2013. P-values represent the probability of association between variables and PTB (US) derived from Cox proportional hazard models. * - The denominator used for reported percentages = Inclusion criteria met period * Parity = 0 period.
5.4 Results

5.4.2 Recruitment flow and outcome data

Outcome data were available for 950 (86%) eligible women. There were 914 (96%) live-births, 25 (3%) stillbirths and 12 (1%) late miscarriages. Amongst live births, 635/914 (69%) women were able to recall a certain menstrual history. C-GA was estimated using SFH in a further 223/914 (24%) participants. Overall, C-GA was unrecordable in 56/914 (6%) women, who were dated using US alone. The recruitment profile of participants over time is shown in Figure 5.2. Details of the demographic, obstetric and anthropometric profiles of all participants are provided in Table 5.3 separated according to three time periods. The timings were specified to coincide with national reporting of data for the Kenyan Government. In period 1 (1 November 2011 to May 31 2012), 34% of women were screened and met the inclusions criteria, compared to 80 and 83% for period 2 (1 June 2012 to 31 December 2012) and period 3 (1 January 2013 to 31 July 2013), respectively.

5.4.3 Gestational age distribution comparisons

The GA at delivery was negatively skewed with a median of 275 days according to all three dating methods, although the interquartile ranges for LMP (IQR=263-285 days) and C-GA (IQR=262-286 days) were higher than US (IQR=267-282 days).

Absolute differences between US-GA and C-GA are shown in Figure 5.3 for PTB and non-PTBs, with median of +1.08 days (IQR=-6 to 10 days) and +0.97 days (IQR=-9 to 14 days), respectively.

5.4.4 Preterm, term and post-term deliveries

PTB rates measured according to US-, LMP- and C-GA were 10.0% (91/914), 16.2% (103/635) and 17.1% (147/858), respectively - see Figure 5.4. Hazard ratios, with adjustment for ‘within-subject’ clustering of results for PTB (using US-GA as the reference group), were 1.69 (1.37- 2.10, \( p < 0.001 \)) and 1.79 (1.48-2.16, \( p < 0.001 \))
for LMP and C-GA indicating that at any point within the study, each subject was
79% more likely to be classed as PTB when rates were estimated according to C-
GA rather than US-GA - see Tables 5.4 and 5.7. US-GA classified more births as
term (37-42 weeks’ gestation) compared to LMP-GA and C-GA - HR 0.65 (0.60-
0.72, \( p < 0.001 \)) and 0.59 (0.55-0.65, \( p < 0.001 \), respectively - and fewer births as
post-term (>42 weeks’ gestation) - HR 4.44 (3.08-6.39, \( p < 0.001 \)) and 5.40 (3.78-
7.71, \( p < 0.001 \)), for LMP-GA and C-GA respectively. Analyses using 28 weeks’
gestation as the threshold for viability (WHO definition) revealed the same trends -
see Table 5.5.
5.4 Results

ANC first visits: 
(n=3630)

Clinical GA >24 weeks: 
(n=1803)

Clinical GA <24 weeks: 
(n=1827)

US performed: 
(n=1171)

Prescan exclusions (n=656):
1. Declined = 388
2. Not pregnant = 68
3. Consent refused = 19
4. Clinical urgency = 8
5. Screening error = 171
6. Other = 2

GA confirmed <24 weeks: 
(n=1107)

Postscan exclusions (n=64):
1. Miscarriage = 43
2. Congenital anomaly = 5
3. Twins = 28

Loss to follow-up: 
(n=157)

Delivery data captured: 
(n=950)
1. Home = 188 (20%)
2. Kilifi District Hospital = 604 (64%)
3. Other government facility = 5 (<1%)
4. Private facility = 55 (6%)
5. Other = 43 (4%)
6. Location unavailable = 53 (6%)
7. Outmigration (<day 7) = 2 (<1%)

Birth outcomes: 
(n=950)
1. Live births = 914 (96 %)
2. Stillbirths = 25 (3 %)
3. Miscarriage (<24 weeks) = 11 (1%)

Figure 5.1: Flow chart of study recruitment. Delivery locations: Other government facility = Coast Province General Hospital, Mombasa and Malindi District Hospital, Malindi. Outmigration includes all women migrated out of HDSS area before day 7 of newborn life. Screening error amongst prescan exclusions refers to those women identified as eligible for US by MCH staff but who were deemed ineligible after validation of screening by the study team. All data are presented in accordance with STARD requirements [196].
Figure 5.2: Bars - number of first attendances at the MCH clinic. Red line - Number of first attendances with C-GA ≤ 24 weeks’ gestation. Blue line - proportion of women with C-GA ≤ 24 weeks’ gestation scanned. Recruitment: 1 November 2011 to 31 July 2013. A national strike by healthcare staff forced recruitment to cease between December 2012 and March 2013.
Figure 5.3: Absolute differences between US-GA - C-GA for preterm and non-preterm groups.
<table>
<thead>
<tr>
<th>Dating Method</th>
<th>24 - &lt;37 weeks' gestation</th>
<th>37-42 weeks' gestation</th>
<th>&gt;42 weeks' gestation</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Rate (95% CI)</td>
<td>HR (95% CI)</td>
<td>n</td>
</tr>
<tr>
<td>1. US-GA</td>
<td>91</td>
<td>10.0 (8.3-12.2)</td>
<td>1.00</td>
<td>789</td>
</tr>
<tr>
<td>2. LMP-GA</td>
<td>103</td>
<td>16.2 (13.7-19.5)</td>
<td>1.69 (1.37-2.10)</td>
<td>446</td>
</tr>
<tr>
<td>3. C-GA</td>
<td>147</td>
<td>17.1 (14.9-20.0)</td>
<td>1.79 (1.48-2.16)</td>
<td>573</td>
</tr>
</tbody>
</table>

Table 5.4: Birth rates for PTB, term and post-term groups estimated by US-GA, LMP-GA and C-GA for women presenting for antenatal care at Kilifi District Hospital ≤24 weeks' gestation (GA used as the basis for PTB rate estimates in this study). HRs and CIs are estimated using Cox proportional hazard models with adjustment for within subject clustering.
### Table 5.5: Birth rates for PTB, term and post-term groups estimated by US-GA, LMP-GA and C-GA for women presenting for antenatal care at Kilifi District Hospital ≤ 28 weeks’ gestation (GA recommended by WHO for PTB rate estimates). HRs and CIs are estimated using Cox proportional hazard models with adjustment for within subject clustering.

<table>
<thead>
<tr>
<th>Dating Method</th>
<th>28 -&lt; 37 weeks’ gestation</th>
<th>37-42 weeks’ gestation</th>
<th>&gt; 42 weeks’ gestation</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Rate (95% CI)</td>
<td>HR (95% CI)</td>
<td>n</td>
</tr>
<tr>
<td>1. US-GA</td>
<td>83</td>
<td>9.2 (7.5-11.2)</td>
<td>1.00 (-)</td>
<td>789</td>
</tr>
<tr>
<td>2. LMP-GA</td>
<td>96</td>
<td>15.4 (12.8-18.4)</td>
<td>1.69 (1.36-2.10)</td>
<td>446</td>
</tr>
<tr>
<td>3. C-GA</td>
<td>135</td>
<td>16.1 (13.8-18.7)</td>
<td>1.74 (1.43-2.12)</td>
<td>570</td>
</tr>
<tr>
<td>Dating Method</td>
<td>28 - &lt;37 weeks' gestation</td>
<td>37-42 weeks' gestation</td>
<td>&gt; 42 weeks' gestation</td>
<td>Total</td>
</tr>
<tr>
<td>---------------</td>
<td>---------------------------</td>
<td>------------------------</td>
<td>----------------------</td>
<td>-------</td>
</tr>
<tr>
<td></td>
<td>( n )</td>
<td>Rate</td>
<td>95% CI</td>
<td>( n )</td>
</tr>
<tr>
<td>US - GA</td>
<td>91</td>
<td>10.0</td>
<td>8.3 - 12.2</td>
<td>789</td>
</tr>
<tr>
<td>US - CRL</td>
<td>11</td>
<td>8.5</td>
<td>4.8 - 14.8</td>
<td>116</td>
</tr>
<tr>
<td>US - HC</td>
<td>80</td>
<td>10.2</td>
<td>7.4 - 12.9</td>
<td>673</td>
</tr>
</tbody>
</table>

Table 5.6: Birth rates and 95% CIs for PTB, term and post-term groups with GA estimated by US with subgroups comparing: 1) US measured by CRL for women where US performed before 14 weeks' gestation, and 2) US by HC for women where US performed between 14 and 24 weeks' gestation. PTB = 24 weeks.
Figure 5.4: A: The distributions of GA at delivery according to US-GA (red), LMP-GA (blue) and C-GA (green). The vertical line at 259 represents the transition from PTB to non-PTB. B: Pie charts illustrating the proportion of PTBs by each ‘dating’ method. C: Absolute proportions of newborns delivered at KDH requiring admission to the neonatal unit who were classified as PTBs. D: Absolute proportions of perinatal deaths classified as PTBs with HRs for perinatal mortality for LMP-GA and C-GA compared to US-GA.
5.4 Results

<table>
<thead>
<tr>
<th></th>
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</tr>
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<tbody>
<tr>
<td></td>
<td>Hazard ratio</td>
<td>95% CI</td>
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<td>24 - &lt;28 weeks’ gestation</td>
<td>0.73</td>
<td>0.31-1.66</td>
</tr>
<tr>
<td>28 - &lt;32 weeks’ gestation</td>
<td>1.58</td>
<td>1.04-2.40</td>
</tr>
<tr>
<td>32 - &lt;37 weeks’ gestation</td>
<td>1.70</td>
<td>1.30-2.20</td>
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Table 5.7A

<table>
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<th>Perinatal mortality</th>
<th>Neonatal morbidity</th>
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<td>Estimate</td>
<td>95% CI</td>
</tr>
<tr>
<td>24 - &lt;28 weeks’ gestation</td>
<td>0.11</td>
<td>0.02-0.20</td>
</tr>
<tr>
<td>28 - &lt;32 weeks’ gestation</td>
<td>0.29</td>
<td>0.13-0.43</td>
</tr>
<tr>
<td>32 - &lt;37 weeks’ gestation</td>
<td>0.06</td>
<td>0.00-0.24</td>
</tr>
</tbody>
</table>

Table 5.7B

Table 5.7: A : Preterm birth subgroup analysis with HRs comparing LMP-GA and C-GA to US-GA. Subgroups of PTBs: 24 - <28 weeks’ gestation = extremely-preterm; 28 - <32 weeks’ gestation = very-preterm, and 32 - <37 weeks’ gestation = late-preterm B: Population attributable fractions for perinatal mortality estimated by extension of Cox proportional hazard models.

There were 28 perinatal deaths, resulting in a perinatal mortality of 29.9/1000 births. The US PTB group identified 18/28 (64%) pregnancies ending in perinatal death, compared to 13/28 (46%) by LMP and 19/28 (6%) by C-GA. The method specific concordance for preterm, perinatal death case ascertainment using US-GA as the reference standard was 12/18 (67%) for LMP and 16/18 (89%) for C-GA respectively. Compared to US, the HRs for perinatal mortality amongst PTBs determined by LMP and C-GA were 0.68 (0.49-0.95, $p=0.025$) and 0.70 (0.55-0.88, $p=0.003$), respectively. Sixty-five (11%) out of 604 newborns who delivered at KDH required
admission to the neonatal unit within 7 days of delivery. Of these, 15/65 (23%) were PTBs by US-GA, compared to 10/65 (15%) by LMP-GA and 16/65 (25%) by C-GA respectively. Despite classifying the lowest number of births as preterm, US detected a similar fraction of those pregnancies that ended in perinatal death or were admitted to the neonatal unit for specialist care. Overall, PTBs determined by US were associated with the greatest risk of perinatal mortality and neonatal morbidity - see Figure 5.4, rows C and D.

5.5 Discussion

5.5.1 Principal findings

This study has found that clinical estimates of GA by LMP-GA or C-GA significantly overestimate the rate of PTB compared to US-GA for women attending the MCH clinic in KDH before 24 weeks’ gestation. Although the PTB rate was lower using US-GA, the perinatal mortality risk was higher, suggesting that US-GA is more specific and therefore that the PTB rate by US-GA is more accurate than by LMP-GA or C-GA.

It is acknowledged that US-GA represents a composite estimate of GA whose accuracy was dependent upon whether CRL or HC was used as its basis. To determine whether the wider variance in error amongst estimates of GA by HC, compared to CRL, was likely to have had a substantial impact on observed outcomes within the study, a subgroup analysis was performed to compare birth outcomes according to CRL and HC individually - see Table 5.6. When compared to GA by CRL, rates of preterm, term and post-term births observed amongst women dated by HC were similar, which suggests that the performance of US-GA as a test of GA was not impaired by the grouping of women for analytical purposes. Overall, such findings validate the accuracy of US-GA when ascertaining PTB status amongst the study group.
Despite providing a more accurate estimate of the PTB rate, it must be emphasised that US-GA as presented in this study is unable to determine ‘true’ GA since the majority of estimates were determined using GA by HC rather than CRL. As such, data from this study do not provide ‘proof’ that PTB rates by US-GA are correct. Similarly, they do not prove that LMP-GA or C-GA are incorrect and for this reason conventional sensitivity analyses of test performance were avoided. It was considered that reporting false positive rates whose accuracy was uncertain would be inappropriate in this setting on the basis that such data could be used to influence decisions about the continued use of LMP-GA and C-GA where these remain the only source of information available for dating purposes. Formal sensitivity testing has been used elsewhere, but in settings where GA estimation is routinely practised and where all three dating methods are routinely available [21, 197]. Nevertheless, the fact that the detection of both perinatal deaths and morbidity was high with US-GA despite a much lower screen positive rate, suggests that this method is best able to discriminate PTBs.

5.5.2 Strengths and weaknesses

This chapter describes one of the largest US-dated birth cohort studies from sSA (outside South Africa) and is the first from the region to report the association between reduced PTB rates using US-GA compared to LMP-GA or C-GA with adequate statistical power to detect the observed differences. An important strength of the study comes from its linkage with the KHDSS, the largest in sSA. This enabled accurate and prospective follow-up of birth outcomes over an extensive geographical area. This is unusual in low-income settings, where vital registration data are often not available and strengthens the findings of these data.

The study also has a number of limitations. Recruitment was problematic in the first reporting period - See Table 5.3. This is reflected by the low percentage of women recruited between November 2011 and May 2012 with 388/1827 (21%) of women being declared by clinic staff as having declined to participate in the study. Once this
5.5 Discussion

became evident, it was clear that there was considerable confusion amongst staff within the MCH clinic with respect to the interface between research and clinical processes. As a consequence, potentially eligible women were not being offered US and were recorded as having declined an invitation to participate in the study.

Furthermore, despite an extensive dialogue between study staff and the community of Kilifi during the preparatory stages of the study, there was anecdotal evidence from feedback to MCH staff that the community perception of the study was adversely affected by historical perceptions of research within the MCH.

The management structures discussed in Chapter 4 were utilised to devise a strategy to address both factors. In response, MCH clinic staff and study research staff met on a daily basis to review the attendance registers. Weekly feedback meetings with senior nursing staff were held and quarterly review meetings with the hospital management teams scheduled to review recruitment performance. Study staff also used the CAST team to meet with community representatives to discuss the study aim and objectives. As a consequence, recruitment performance improved significantly to 80 and 82%, respectively in the remaining 2 reporting periods. The monthly recruitment performance is provided in Figure 5.2.

Selection bias may have been introduced by recruiting only those women presenting for care ≤24 weeks’ gestation and so excluding the 50% of women presenting for care beyond this threshold. Whilst this reflects the upper limit of accuracy for US estimates of GA, it is possible that a study group was recruited whose risk of PTB was different to that within the population of Kilifi as a whole. Similarly, differences such as the lower observed incidence rate of stillbirths compared to that reported amongst participants’ previous deliveries (3% versus 20%) suggest that pregnancies observed by the study may not have been typical of historical pregnancies amongst the study population. There are a wide range of potential explanations for this difference, which include: 1) A lack of GA data when distinguishing between stillbirth and miscarriage for previous pregnancies; 2) Earlier booking of the study pregnancies compared to previous pregnancies, with better pregnancy outcomes.
as a result of interventions offered as part of the WHO focused antenatal care package [168, 169]; and 3) Inaccurate reporting of pregnancy losses amongst women delivering in the community, which has been reported in a range of settings across sSA [198]. Whilst each of these factors may have introduced bias into the estimates of PTB rates observed within the study group, such considerations do not affect analyses assessing the differences in PTB rates, which was the primary aim of the study. Furthermore, with a range of health improvements already linked to early antenatal care, interventions are currently being implemented throughout sSA targeting the early uptake of care, which if successful will increase the proportion of births to which this study’s findings are relevant.

Although the HRs reported for perinatal death amongst PTBs by the three dating methods show statistically significant differences, the study was not powered to detect small differences at this level. The perinatal mortality rate for the study population is 29.9/1000 births using US-GA (26.8/1000 using 28 weeks’ gestation as the cut-off, and 33.9/1000 using the WHO definition), which is considerably lower than national estimates for Kenya (40/1000-Kenya DHS) or (59/1000 WHO) [199, 200]. Whilst these observed differences may relate to the effect of US-GA providing more specific case ascertainment of perinatal deaths compared to the clinical estimates upon which WHO estimates are based, it is also possible that stillbirths and neonatal deaths at earlier GAs were underreported by the study group. To substantiate this claim, unadjusted odds ratios for perinatal mortality by GA were estimated from the dataset and are presented in Figure 5.5. Not surprisingly, there is trend towards higher odds of perinatal death as GA reduces from term. Whilst group sizes are small and, therefore, CIs wide, there is a clear deviation from this trend at GA < 27 weeks’ suggesting that outcomes in this GA group are under-reported. Lastly, it is possible that the observed incidence rate of perinatal mortality was subject to the Hawthorne Effect, [201] whereby patients and clinical outcomes, themselves, were influenced by the processes employed to obtain data during study. These effects are reported in the literature and discussed in the context of this entire thesis in the
concluding chapter.

Despite these limitations, on the basis that the observed trends between dating methods, PTB rates and neonatal outcomes are the same as those for perinatal mortality overall, the principal findings of the study appear valid. The proportions of term and post-term births classified by US-GA compared to LMP-GA and C-GA are consistent with data from elsewhere and are consistent with the mechanisms believed to underly such trends. Hence, the observation of similar findings within the dataset from Kilifi can be used to support the validity of other findings from the data [95, 106]. The observed percentages of post-term pregnancies were 3.2 and 13.2 and 16.7 according to US-GA and LMP-GA and C-GA respectively - see Table 5.4). It is acknowledged that the incidence rate of post-term deliveries by US-GA compares to the rates reported by intervention studies using US-GA [202], which may suggest that clinical decisions were being altered as a consequence of US data being used by clinical staff from KDH when determining the timing of delivery.

![Figure 5.5](image_url)

**Figure 5.5:** Estimated unadjusted odds ratios (blue dots) with 95% CIs for perinatal mortality according to GA at delivery. Delivery at 40 - 42 weeks' gestation was used as the reference category for determining the unadjusted ORs.
Such potential sources of bias were considered in the design phase of the study, but it was determined by consensus amongst clinical and research staff that it would be inappropriate, in the context of an observational study, to withhold clinical data from staff in a setting where such data could not be easily obtained by other means.

With respect to the study design, a number of alternatives were considered, e.g. including control groups to evaluate the use of US as an intervention during antenatal care. However, local feedback suggested that individual randomisation of women to receive US or not would not be acceptable within this community. Similarly, the lack of preliminary data to inform the design of a cluster randomised trial across multiple settings was a major barrier to adopting such an approach, in the first instance. All three methods of estimating GA were, therefore, offered to all study participants and the potential for confounding of outcomes related to ‘within subject’ clustering was adjusted for during the analysis stage.

**Figure 5.6:** The distribution of GA at booking: Period 1 - blue line. Dates = 1 November 2011 to 31 May 2012. Period 3 - red line. Dates = 1 January 2013 to 31 July 2013.
5.5 Discussion

5.5.3 Implications for US in sSA

As global health priorities shift towards an emphasis on maternal and perinatal health, a series of value judgements will become necessary to determine the requirements for estimating GA in low-income settings. In fact, the need to agree upon international standards for the accurate and standardised reporting of GA is highlighted as a research priority in itself [11, 12]. To ensure that any consensus reached through this process is appropriate to the needs of low-income settings, it is essential that data from relevant populations are available and appraised, to which this study contributes.

To this end, a recently published study from Uganda suggests that US has the potential to increase the number and proportion of early ANC attendances. The authors discuss extensively the potential gains that may result as a consequence of providing US in such communities. They refer collectively to such gains as the ‘Magnet Effect of US’ [203]. In view of the potential for a ‘Magnet Effect’ to have emerged in Kilifi, it had to be considered whether any potential bias was introduced into datasets as a result of a ‘shift’ in the GAs at which women presented for MCH services. If US was encouraging the earlier uptake of care, it was feasible that the accuracy of the tests being evaluated, were themselves altered as a result of the intervention being examined i.e. was earlier uptake of US evident and, therefore, increasing the accuracy of PTB rates by US-GA.

To examine for evidence of this effect, a range of demographic, obstetric and anthropometric data were compared across three consecutive time periods during recruitment, with statistical associations between these characteristics and PTB estimated - see Table 5.3. Although fewer women met the inclusion criteria during Period 1, groups remained comparable overall. The distribution of CGA during Periods 1 and 3 were compared to assess for the presence of any ‘Magnet Effect’ are shown in Figure 5.6 where cumulative curves of CGA at presentation are equivalent.

Whilst participation rates increased throughout the study period (Figure 5.2), these
data alone are not sufficient to draw any conclusions relating to: 1) The acceptability of US as an intervention in Kilifi; and 2) The emergence of a ‘Magnet Effect’ through which US can be associated in a causal fashion with increases the early uptake of antenatal care. Such analyses would need to include qualitative data assessing attitudes and perceptions of US amongst patient and provider groups and take into account many of the methodological considerations outlined in the Medical Research Council (UK) guidance on developing and assessing complex interventions [204]. As smaller, portable and more robust US machines enter the market at lower prices, there will be an inevitable increase in the availability of US technology in low-income settings [128, 203]. On this background, it is accepted that research addressing such factors will become a matter of increasing importance, particularly as US-GA estimation is likely to be a component of any agreed international pregnancy dating policy. However, the historical data available in Kilifi were not considered to be of sufficient quality for such comparisons to be made within the framework of this research.

### 5.5.4 Conclusion and future work

This study demonstrates that LMP-GA and C-GA have the potential to overestimate PTB rates significantly compared to US-GA, which has important clinical consequences. For individual women, these include unnecessary costs and risks associated with treatment for imprecise diagnoses of PTB. For health systems in low-income settings, the effective allocation of resources represents a significant challenge, which may benefit from more precise GA estimates. For example, ‘Kangaroo Mother Care’ (KMC) for preterm newborns has been shown to improve morbidity outcomes. However, delivering KMC at scale is a roadblock to its implementation in many low-income settings [205].

In this study, the attributable fraction of morbidity was greatest in the late preterm group, which, not surprisingly, was also the group with the most PTBs and within which the excess of PTBs was greatest by C-GA compared to US-GA (see Table
It is therefore conceivable that if interventions such as KMC were targeted using US-GA to provide more specific case ascertainment, that similar morbidity benefits could be achieved more effectively by identifying a smaller group of newborns who are more likely to benefit. Clearly, future work must establish whether any potential gains would justify the allocation of resources to establish and maintain an US service in the first instance.
Chapter 6

Preterm birth classification: sub-phenotypes in rural Kenya

6.1 Introduction

Many of the themes that emerged when LBW was evaluated as a classifier of birth phenotypes are beginning to appear in the context of PTB. Specifically, it is becoming clear that PTB itself represents a complex group of pregnancies, comprising a range sub-phenotypes, with multiple aetiological determinants that, themselves, share the common characteristic of occurring amongst newborns delivered preterm. Evidence to support this contention has been reported in basic science literature, where molecular diagnostic and genetic studies are delineating the different pathways to PTB, as well as from intervention studies [108, 109, 206, 207].

The consequences that may result from the limited specificity of PTB when classifying birth outcomes are illustrated by an intervention study from Malawi. The authors performed a randomised controlled trial of antibiotic prophylaxis to prevent PTB amongst high-risk women and reported no effect on any of the study’s primary outcomes [208] - see Chapter 2. Amongst several potential explanations for the study’s finding, is the possibility that PTB related to infection was present, but
only in a minority of the intervention group. Hence, no overall treatment effect was observed because analyses used PTB, as a mixed phenotype, for their primary endpoint. In response to the equivocal findings from this study and a range of other intervention studies, a series of scientific meetings were convened to develop the novel classification system presented on page 21.

This chapter applies the novel classification system to analyse the sub-phenotypes of PTB presenting at KDH. Prior to this, a discussion of the various methods available to determine the optimal grouping of data by classification systems is considered. A particular emphasis is placed on the use of the data structures themselves to inform the design of classification systems. An algorithm is then applied to test whether such data driven approaches to phenotype classification are feasible amongst the PTB data from KDH.

6.2 Data classification - a primer

6.2.1 Rule based ontologies

The application of the classification system that was proposed by Villar and colleagues [209] results in the segregation of a large dataset into smaller subgroups, whose constituent members share attributes defined according to the classifier. The resulting classification systems are referred to ‘ontologies’ [210, 211].

Although expert driven ontologies provide the ability to classify large groups of data into a series of smaller, more meaningful subgroups, such approaches inherently assume that scientific consensus alone is able to segregate groups optimally. This contention is challenged frequently as new scientific knowledge emerges. As discussed in Chapter 1, LBW was considered to represent a useful classifier of birth outcomes, until as a consequence of its use during intervention studies, the limited scope provided by its groupings was identified [4, 6]. The use of disease ontologies, derived according to expert consensus, and with clear scientific bases, may
ultimately be deemed inflexible as new knowledge and understanding of disease pathogenesis emerge.

Ontology based classification systems represent a process whose endpoint comprises a number of subgroups of individuals, where each subgroup reflects a numerical partition of the original dataset. A single group of heterogeneous data is divided into several smaller groups that are more homogenous. As a result, if two individuals from the same subgroup were selected and compared at random, the probability of them being alike, in terms of the attributes used to define their groupings, would be greater than if two individuals were randomly selected from the initial group, and significantly greater than if two individuals from different subgroups were compared.

To illustrate this process, the reader is referred to Figure 6.1 on page 103. A rule based ontology for the classification of pre-eclampsia is presented alongside a scatter plot of data that could represent the outcome of such rules being applied amongst a sample of pregnant women. From the scatter plot, it is conceivable that if a green data-point was selected at random from the data and compared to another green data-point, that the pair of data-points would be more likely to have similar measures of BP and protein than if a pairwise comparison were made between a green and red data-point. It is therefore conceivable, that a numerical comparison of the data-points within a dataset alone, has the potential to drive the segregation of the dataset into subgroups, without any prior knowledge of the clinical importance of such groupings.

6.2.2 Data driven cluster analysis

The principle of applying the numerical characteristics amongst data to drive an ‘unsupervised’ partitioning of the dataset, i.e. non-expert driven, represents the branch of statistics and computational processing referred to as cluster analysis and was first described by Robert Tryon in 1939 [212, 213].
Cluster analysis represents a huge field of research in itself and it is far beyond the scope of this Chapter to appraise the methods in detail. However, based on the example provided in Figure 6.1, it follows logically that cluster analysis could be applied to datasets of PTBs for a range of purposes.

Firstly, clustering could be used to provide a mathematical proof that ontology driven disease classification systems produce logical subgroups of data. Naturally occurring phenotypes are more likely to share numerical properties and so are more likely to aggregate within numerically driven cluster analyses [214]. Also, since cluster analyses are independent of any expert knowledge, they provide the ability to examine datasets for novel phenotypes that may not be immediately apparent within expert based classifiers and so offer capacity to improve the performance of ontologies, or even to replace them. In fact, similar methods have been applied to large clinical datasets in cancer and genetic research, and the results used to drive hypotheses and novel phenotype discovery [215, 216].
Figure 6.1: Left - Ontology decision tree that is used to subdivide the data manually into four distinct groups. Right - Scatter plot showing the data-points with colour outlining the groups determined by the classifier. On the basis of the numerical similarity amongst data-points of the same colour, it is conceivable that an automated, mathematical segregation of the data-points may used to determine important groupings amongst the data-points.
A hierarchical clustering algorithm was applied by Villar and colleagues to the data collected from INTERGROWTH-21st in order to determine the numerical validity of their proposed PTB classification system. The hierarchical approach requires the user to manually specify the number of sub-groups that should be used by the algorithm to partition the dataset. This compares to non-hierarchical models that do not group using such specifications [217, 218]. Whilst this approach is widely acknowledged to provide scientifically robust groupings, the process depends upon manual user input and may therefore be subject to bias [215, 216]. Non-hierarchical clustering algorithms, however, use the data structures themselves to determine the optimal number of groups according to a range of mathematical criteria [219, 220]. Such approaches risk the grouping of data into clusters that have no scientific meaning and so must be used assiduously when attempting to derive meaningful groups.

6.2.3 Selection of a clustering algorithm

“It has been said that there are as many cluster analysis methods as there are people performing cluster analysis. This is a gross understatement! There exist infinitely more ways to perform a cluster analysis than people who perform them” [221].

An algorithm was chosen according to the specific requirements for clustering the PTB data. Specifically, it was determined that the clustering algorithm should have the capacity for: 1) Processing non-parametric data; 2) Managing combinations of continuous and discrete variables; 3) Providing a mechanism to reduce the dimensionality of the data for visualisation purposes, whilst retaining the variation amongst pairwise data-points that was present within the initial multidimensional dataset, and 4) Producing reproducible cluster partitions. The final requirement with respect to reproducibility is particularly relevant.

At their core, cluster algorithms use mathematical criteria to decide upon the optimal
groupings of data. Hence, when dealing with datasets that comprise many variables, it is possible that a single solution is provided by several different groupings of the data. This concept of multiple stochastic solutions for the same dataset means that the cluster solution provided by a single numerical algorithm may produce different groupings of the data each time the algorithm is run [222]. Since scientific validity depends upon reproducibility, it was considered an essential requirement that partitions be reproduced upon multiple runs of the algorithm.

Preliminary clustering models were constructed using the $k$-means algorithm, which was selected based upon its relative simplicity and ability to interface with routine statistical software [222, 223, 224, 225]. A literature review also revealed several studies where $k$-means had been applied to describe complex clinical phenotypes [214, 226, 227]. However, upon experimentation, it soon became apparent that $k$-means modelling of the PTB dataset was compromised by the large number of binary variables amongst the data. Despite several approaches to transform these, including dimension reductions using a variant of Principal Component Analysis (PCA) [228], it was decided to investigate alternative algorithms. Following discussion with the Computational Biology Systems Group within the Computer Science Faculty of Oxford University, it was suggested that a model based upon ‘$t$-Distributed Stochastic Neighbor Embedding’ was selected as this was more likely to deal with the specific requirements of the modelling process.

### 6.2.4 $t$-Distributed Stochastic Neighbor Embedding

$t$-Distributed Stochastic Neighbor Embedding ($t$-SNE) describes a complex non-linear data reduction technique that models high-dimensional characteristics (many variables). The process is governed by a complex probabilistic model whose aim is to bring data-points that share common characteristics across many dimensions into close proximity with each other over just two or three dimensions. By conserving the variance that is present amongst data-points within the higher dimensions of the original dataset in such a way, it is possible to demonstrate clusters of similar
data-points graphically using simple two or three-dimensional scatter plots. Conversely, data-points that are dis-similar in the high dimensional space are dispersed from each other over large distances, which can be seen by separation amongst data clusters within the scatter plots. Whilst the final representation of the data is simple and easy to interpret, the complex relationships between the large number variables that comprise the original dataset are conserved within the geometric space between data-points [229, 230]. T-SNE thus represents a powerful tool for the investigation of trends within highly complex datasets and has been applied to a number of medical problems. In particular, an elegant adaptation of the model was applied to visualise a highly complex dataset of Leukaemic cell lines in order to inform hypothesis generation related to cancer phenotypes [231].

6.3 Methods

The methods used to apply the PTB classification system are described in the following section. This is followed by a description of how t-SNE was applied to the same dataset.

6.3.1 PTB classification system

6.3.1.1 Recruitment and study sample definition

The sample includes women that were recruited at the MCH clinic between 1 November 2011 and 30 July 2013, but is restricted to births observed at KDH. Data were collected in accordance with the methods described previously in Chapter 4. Whilst Villar and colleagues extended the definition of PTB to include all newborns delivered with GA \( \leq 38^{+6} \) weeks’ gestation, the analyses presented here are restricted to deliveries from 24 to 36^{+6} to enable comparisons to be made with data from other settings. Pregnancies delivering between 16 and 23^{+6} weeks’ gestation were not included for feasibility purposes. All women were asked to re-confirm their consent at presentation to KDH for delivery.
6.3 Methods

6.3.1.2 Case definitions

Data were collected at the MCH and then, subsequently, at delivery using the KIP-MAT system that is described in Chapter 4 on page 46. Individual women were identified by the KIN-Numbers and datasets merged with birth outcomes for analyses. The case definitions used for each condition are presented in Table 6.1 and were specified according to the definitions provided by Villar and colleagues in their series of papers, unless otherwise stated [108]. To ensure objective classification of outcomes, the case definitions were incorporated into a series of automated extraction scripts to build the dataset and classify the PTBs within.
<table>
<thead>
<tr>
<th>Condition</th>
<th>Case definition</th>
<th>Extraction variables</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal:</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| Extrauterine infection  | Infection - clear focus                | Temperature $\geq 38^\circ C$  
$\pm$ Cough, fever, dysuria, diarrhoea  
$\pm$ White blood cell count $\geq 14 \times 10^9$ |
| Extrauterine infection  | Infection - no clear focus             | Temperature $\geq 38^\circ C$  
No cough, fever, dysuria, diarrhoea, alternative focus  
$\pm$ White blood cell count $\geq 14 \times 10^9$ |
| Clinical chorioamnionitis| Infection - clear uterine focus        | Offensive liquor $\pm$ vaginal discharge  
$\pm$ Temperature $\geq 38^\circ C$  
$\pm$ Maternal heart rate $>100$ bpm  
$\pm$ Fetal heart rate $>160$ bpm  
$\pm$ Uterine tenderness on palpation  
$\pm$ White blood cell count $\geq 14 \times 10^9$ |
| Maternal trauma         | Abdominal trauma on admission          | Free text history                      |
| Uterine rupture         | Uterine rupture: surgically confirmed  | Uterine rupture                       |
| Pre-eclampsia/eclampsia | Pre-eclampsia                          | Known pre-eclampsia this pregnancy  
BP $\geq 140/90 \times 2$, BP $\geq 160/100 \times 1$  
Urine dipstick: protein $\geq 2+$  
Eclampsia                |  
Tonic-clonic seizure at any time during pregnancy  
Magnesium sulphate required |
<table>
<thead>
<tr>
<th>Condition</th>
<th>Case definition</th>
<th>Extraction variables</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fetal:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intrauterine fetal death</td>
<td>No fetal heart at birth</td>
<td>Admitted IUFD confirmed</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Intrapartum - FH not recorded</td>
</tr>
<tr>
<td>Intrauterine fetal growth restriction</td>
<td>Birthweight &lt;10(^{th}) centile for GA</td>
<td>Birthweight</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gender</td>
</tr>
<tr>
<td>Abnormal fetal heart rate</td>
<td>Fetal bradycardia</td>
<td>FHR - pinnard &lt; 110 bpm, no maternal cause</td>
</tr>
<tr>
<td></td>
<td>Fetal tachycardia</td>
<td>FHR - pinnard &gt; 160 bpm, no maternal cause</td>
</tr>
<tr>
<td>Fetal anomaly</td>
<td>Structural anomaly</td>
<td>Neonatal check anomaly</td>
</tr>
<tr>
<td>Multiple fetuses</td>
<td>Excluded from study</td>
<td>-</td>
</tr>
<tr>
<td><strong>Placental pathologies:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placental abruption</td>
<td>Indication to deliver- antepartum haemorrhage (APH)</td>
<td>Induction of labour (APH)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Caesarean section (APH)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Delivery complication (APH)</td>
</tr>
<tr>
<td>Placental praevia</td>
<td>Confirmed &lt;2cm from internal cervical Os</td>
<td>Confirmed after 36 weeks by US</td>
</tr>
<tr>
<td>Other placental abnormality</td>
<td>Placenta weight &lt;10(^{th}) centile for GA</td>
<td>Membrane free placental weight as described [232]</td>
</tr>
</tbody>
</table>

**Table 6.1:** Case definitions used to extract data for novel PTB classification system. All criteria defined in accordance with Villar and colleagues unless otherwise stated [108]. Fetal anaemia, fetal polyhydramnios, and histological chorioamnionitis were included in the original phenotype, but could not be classified amongst births at KDH. Birthweight for GA centiles adapted according to Table A.1 in Appendix A.9 on page 227. Full blood counts were obtained using a closed system EDTA collection tube and analysed using a Coulter AcT SDiff analyser. Blood pressures measured using the automated Microlife BP 3BTO-A(2) device, validated for use in pregnancy [233]. Abbreviations: BP - blood pressure; IUFD - Intrauterine fetal death; FH - fetal heart; FHR - Fetal Heartrate, and APH - Antepartum haemorrhage.
6.3 Methods

6.3.1.3 Measurements

All maternal venepuncture was performed in labour and fetal anthropometric measurements obtained within 24 hours of birth. Birthweight was measured using an electronic scale (SECA, Germany). All measurements were performed in accordance with WHO recommendations [234] and protocols based upon the standards applied during the Multi-centre Growth Reference Study [32]. All nursing staff performing anthropometry underwent frequent standardisation exercises with visiting teams from the INTERBIO-21st coordinating centre [235].

6.3.1.4 Follow up

Newborns were followed up using hospital and KHDSS surveillance systems. Neonatal morbidity was defined according to the need for newborn admission to the paediatric unit within 7 days of birth. Perinatal mortality was defined as a stillbirth ≥24 weeks’ gestation or an early neonatal death at ≤7 days after birth.

6.3.2 PTB cluster analysis using t-SNE

The source code to run the t-SNE model is freely available from the authors¹ and was used within the MATLAB mathematical programming environment [236]. The algorithm was applied to the dataset of PTBs described above.

6.3.2.1 Data pre-processing

Data were pre-processed to standardise their numerical weight within the datastructure. This was to ensure that each variable had equivalent relative weighting within the t-SNE selection process. For example, in an untransformed state, fetal weight would have a variance that was, in absolute terms, much greater than a binary variable such as placenta praevia that existed in a ‘1’ or ‘0’ state. Since

¹http://homepage.tudelft.nl/19j49/t-SNE.html.
variance across many dimensions determined feature selection by $t$-SNE, the effect of units of observation for single variables represented a source of selection bias within the model. To overcome this, all variables were standardised to have a mean of 0 and a range of 1. With no further processing required, $t$-SNE was performed on the dataset comprising the extraction variables used by the phenotype classifier discussed previously - see Table 6.1, column 3 for a list of variables and associated conditions used to construct the cluster groupings presented in Section 6.4.2.

6.4 Results

Outcome data were available for 604 births at KDH with GAs between $24^{+1}$ and $44^{+1}$ weeks’ gestation. Of these, 99/604 (16%) delivered at <37 weeks’ gestation. This group constituted the study population for this analysis ($n = 99$). There were 20/99 (20%) stillbirths, of which 11/20 (55%) were diagnosed before labour (pre-labour IUFDs) and 9/20 (45%) occurred intra-partum. The mean GA at birth was 32.8 weeks and mean birthweight was 2303g. Amongst the study population, 57/99 (58%) of newborns had a birthweight <2500g - See Table 6.2.

6.4.1 PTB classification system

The results of the classification system, as determined by the case definitions in Table 6.1, is provided in Figure 6.2 and Table 6.3. Sufficient data were collected to classify 88/99 (89%) of PTBs. A single maternal, fetal or placental condition was identified in 38/99 (38%) of cases and 50/99 cases (51%) of PTBs were the result of a combination of two or three coexisting conditions - see panel A in Figure 6.2. The individual itemisation of respective maternal, fetal and placental conditions are provided in the three pie charts of Figure 6.2. 44/99 (44%), 23/99 (23%) and 77/99 (78%) cases of PTB were associated with maternal, placental and fetal conditions, respectively. Amongst maternal conditions, infection was associated with 24/44 (55%) of PTBs, although this only met the clinical definition of chorioamnioni-
tis in 3 cases. With respect to placental and fetal conditions, 15/23 (65\%) and 61/78 (78\%) of PTB cases, respectively, were associated with placental weight <10\textsuperscript{th} centile for GA and SGA, respectively.

There were 24 perinatal deaths in total. 16/24 (67\%) were in infants with SGA. Maternal factors alone were associated with 8/24 (33\%) of these. 1/24 (4\%) was associated with maternal infection as its single determinant, although the focus could not be classified by clinical definitions alone.

<table>
<thead>
<tr>
<th>Gestational Age</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. &lt;28 weeks</td>
<td>11 (11)</td>
</tr>
<tr>
<td>2. 28 - 31\textsuperscript{+6} weeks</td>
<td>28 (28)</td>
</tr>
<tr>
<td>3. 32 - 33\textsuperscript{+6} weeks</td>
<td>14 (14)</td>
</tr>
<tr>
<td>4. 34 - 36\textsuperscript{+6} weeks</td>
<td>46 (47)</td>
</tr>
<tr>
<td>Total</td>
<td>99 (100)</td>
</tr>
</tbody>
</table>

\textit{(Mean = 32.8 weeks (SD = 3.3 weeks), median = 33.7).}

<table>
<thead>
<tr>
<th>Birthweight</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. &lt;1500g</td>
<td>17 (17)</td>
</tr>
<tr>
<td>2. 1500 - 2499g</td>
<td>40 (41)</td>
</tr>
<tr>
<td>3. 2500 - 2999g</td>
<td>25 (25)</td>
</tr>
<tr>
<td>4. &gt;3000g</td>
<td>17 (17)</td>
</tr>
<tr>
<td>Total</td>
<td>99 (100)</td>
</tr>
</tbody>
</table>

\textit{(Mean = 2303g (SD = 788g), range = 780-4285g).}

\textbf{Table 6.2: Demographic and obstetric characteristics for all first presentations to the MCH clinic.}
Figure 6.2: Prevalence of preterm subphenotypes of delivers at KDH. GA = 168 - 258 days (24 to 36\textsuperscript{th} weeks).
### 6.4 Results

<table>
<thead>
<tr>
<th>Preterm group</th>
<th>Number (%)</th>
<th>NICU (%)</th>
<th>PND (%)</th>
<th>PMR / 1000</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Maternal only</td>
<td>10 (10)</td>
<td>1 (7)</td>
<td>1 (4)</td>
<td>10.2</td>
</tr>
<tr>
<td>2. Fetal only</td>
<td>28 (29)</td>
<td>4 (27)</td>
<td>6 (25)</td>
<td>60.6</td>
</tr>
<tr>
<td>3. Placental only</td>
<td>0 (-)</td>
<td>0 (-)</td>
<td>0 (-)</td>
<td>0</td>
</tr>
<tr>
<td>4. Maternal + fetal</td>
<td>27 (27)</td>
<td>7 (47)</td>
<td>5 (21)</td>
<td>50.5</td>
</tr>
<tr>
<td>5. Maternal + placental</td>
<td>1 (&lt;1)</td>
<td>0 (-)</td>
<td>0 (-)</td>
<td>0</td>
</tr>
<tr>
<td>6. Placental + fetal</td>
<td>16 (16)</td>
<td>3 (13.8)</td>
<td>8 (33)</td>
<td>80.8</td>
</tr>
<tr>
<td>7. Maternal + fetal + placental</td>
<td>6 (6)</td>
<td>0 (-)</td>
<td>2 (8)</td>
<td>20.2</td>
</tr>
<tr>
<td>8. Unclassified</td>
<td>11 (11)</td>
<td>0 (-)</td>
<td>2 (8)</td>
<td>20.2</td>
</tr>
<tr>
<td>Total</td>
<td>99 (100)</td>
<td>15 (100)</td>
<td>24</td>
<td>242</td>
</tr>
</tbody>
</table>

**Table 6.3**: Morbidity and mortality indicators for PTB subgroups. Abbreviations: NICU = admissions to the neonatal care unit at KDH; PND = perinatal deaths, and PMR/1000 = Estimated perinatal mortality rate/1000 births.

### 6.4.2 PTB cluster analysis using t-SNE

Figure 6.3 illustrates the outcome of t-SNE clustering of the dataset and compares the results to a PCA reduction of the same dataset from 47 to 3 dimensions. The purpose of this comparison is to demonstrate the extent to which t-SNE groups data compared to a more straightforward variable reduction process such as PCA.

#### 6.4.2.1 t-SNE: preliminary validation

After partitioning the PTB dataset using t-SNE, five distinct clusters of data-points were identified. The cluster sizes comprised 24, 9, 21, 18 and 27 PTBs, respectively. As a first-line test of the potential clinical validity of the clusters, preterm IUFDs were colour coded in green and their presence determined within output clusters. As a naturally occurring phenotype that was easily detected using the ontology, the ability of the t-SNE to group such cases was considered as an appropriate first line test of cluster performance. The 10 cases of pre-labour IUFD (see Figure 6.4) were all grouped within the same cluster (cluster size = 18) with clear separation from the
two larger and two smaller clusters. With evidence of distinct groupings within the data, that on first line testing have some clinical meaning, the model was subsequently tested for stability by running the same procedure 1000 times. Plots were constructed for after 250, 500, 750 and 1000 cycles and are shown in Figure 6.4. It should be noted that the axes on all $t$-SNE plots represent a ‘conceptual’ cluster space and have no geometric meaning in terms of classifying individual data-points by anything beyond their cluster groupings. Whilst the relative position of clusters moves within the axes, there are clearly five groups of the same sizes present (cluster sizes = 24, 9, 21, 18 and 27) that are in similar relative positions within the cluster axes amongst the different model plots. Furthermore, the IUFD cases are consistently grouped into the same cluster by each model.
Figure 6.3: Left - post PCA 47 to 3 dimension reduction. Right - post t-SNE 47 to 3 dimension reduction with clustering. Blue data-points = PTBs. Green data-points = PTB with confirmed prenatal IUFD.
Figure 6.4: Post clustering plots after 1000 runs of the t-SNE model. Axes represent a conceptual cluster space and are variable amongst models. t-SNE conserves the relative pair-wise location of data-points within the dataset and therefore can be used to group data by assigning individuals to a specific cluster group. The relative position of data-points within the axis space has no geometric origin and cannot be used to measure variable variable phenotypes. Blue data-points = PTBs. Green data-points = PTB with confirmed prenatal IUFD. Scatter plots in this figure are presented in 2D for ease of counting group sizes.
### 6.4.2.2 t-SNE: results

After preliminary validation, the clusters were extracted and the characteristics of each group determined by reference to the original ontology case definitions. The cluster scatter plots with respective labels are illustrated in Figure 6.5. The data for the marked t-SNE cluster groups are presented in Table 6.4 for comparison with the grouping produced by the PTB ontology.

![Figure 6.5: Scatter plot illustrating the groupings identified by t-SNE. Groups 1 to 5 were extracted from the dataset and are summarised in Table 6.4.](image-url)
<table>
<thead>
<tr>
<th>t-SNE group</th>
<th>Number</th>
<th>NICU</th>
<th>PND</th>
<th>Maternal</th>
<th>Fetal</th>
<th>Placental</th>
<th>Maternal + fetal</th>
<th>Maternal + placental</th>
<th>Placental + fetal</th>
<th>All three</th>
<th>Not classified</th>
</tr>
</thead>
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**Table 6.4**: Results of the t-SNE data clustering. Perinatal mortality and neonatal morbidity data are provided along with the frequencies according to grouping by the PTB ontology in Table 6.3.
Figures 6.3, 6.4, 6.5 and Table 6.4 suggest that the application of $t$-SNE to the PTB dataset produced cluster groupings that were both stable, reproducible and consistent. $T$-SNE groupings were compared to the case definitions used to construct ontology groupings. Figure 6.5 was used as a basis to consider the clinical relevance of the $t$-SNE groupings. Maternal, fetal and placental $t$-SNE sub-groups were extracted and are presented with their associated individual sub-phenotypes, which are colour coded according to the case definitions provided by the original PTB ontology presented by Villar et-al. The clinical relevance of the groups was considered and summarised as follows:

**Group 1** All cases of maternal pre-eclampsia are in this group. For some PTBs, there are co-existing phenotypes, including pre-labour IUFD, an abnormal fetal heart rate or antepartum haemorrhage that is distinct from placenta praevia. This group of PTBs can be explained on the basis that each case shares one of the pathological variants associated with pre-eclampsia i.e. maternal disease associated with placental impairment resulting in SGA, IUFD and/ or antepartum haemorrhage (placental abruption).

**Group 2** A cluster of PTBs with mainly placental phenotypes comprising APHs. Some cases demonstrate coexisting fetal sub-phenotypes. For example, APH that is associated with SGA or IUFD but that are not associated with maternal pre-eclampsia.

**Group 3** A mixed group comprising the majority of ‘unclassified’ cases along with cases of SGA that are not associated with maternal pre-eclampsia or other fetal sub-phenotypes as in Group 2.

**Group 4** All cases of maternal, extrauterine infection are present within this group. Some infected PTBs have coexistent SGA, although none of the infections were localised to a uterine focus.

**Group 5** A group mostly comprising fetal PTBs related to SGA. Whilst there is some cross-over with placental weight <10th centile, the majority of cases in this
group comprise isolated fetal-SGA and are not associated with pre-eclampsia. Whilst forming distinct clusters after t-SNE, Groups 1 and 3 may be considered to represent composite groups with two further sub-groupings embedded within them. For Group 1, these subgroups comprise PTBs resulting from maternal pre-eclampsia and pre-labour IUDs and in Group 3, PTBs are the result of fetal-SGA and unclassified cases. Such clustering of unclassified cases suggests that there may be phenotypic similarities between these PTBs that are not classified by the ontology classifier, or that the case definitions applied to the data were inadequate.
Figure 6.6: t-SNE cluster analyses with individual sub-phenotypes determined by the novel PTB classification system displayed. A - PTBs associated with maternal sub-phenotypes, B - PTBs associated with fetal sub-phenotypes, C - PTBs associated with placental sub-phenotypes and D - PTBs that were unclassified by the ontology. Groups 1 to 5 are indicated by numbers adjacent to groups.
6.5 Discussion

6.5.1 Principal findings

Work from this chapter presents two novel contributions to the evidence examining PTB in sSA. In the first section, data presented in Figure 6.2 are the first attempt to examine the sub-phenotypes responsible for PTBs in sSA. The application of the t-SNE clustering algorithm has demonstrated that an entirely data driven approach to analysing the PTB dataset is able to ‘rediscover’ clinically relevant groupings within the data using a purely data driven methodology that is independent of ‘expert knowledge’.

Overall, SGA was associated alone with 61/99 (62%) cases of PTB and was associated with 13/24 (52%) of the perinatal deaths amongst PTBs. Infective causes are widely reported in association with PTB in sSA. From this study, there was clinical evidence of infection amongst 55% of cases, although the focus of infection was determined for only 3 cases. It is likely that the clinical case definitions applied were not sufficiently specific and that more precise diagnoses would be achieved after processing of biological samples. To this end, maternal and umbilical cord blood and placental tissues and membranes have been collected from all women included in this dataset.

Data examining the causes PTBs in sSA are poorly defined. However the results from both the classification system and t-SNE analysis show clustering of phenotypes with respect to infection as well as SGA related phenotypes and maternal pre-eclampsia. The exploration of data by maternal, fetal and placental sub-phenotypes was considered important both to enable comparisons to be made with the original ontology, but also to highlight groups demonstrating obvious linkage between maternal, fetal and placental sub-phenotypes. In the first instance, this enabled current understanding of how pathological processes may have resulted in maternal and fetal phenotypes linking in to one PTB subgroup, such as the grouping of
pre-eclampsia in Group 1. Furthermore, the distinct separation of cases of maternal infection that is not clearly of uterine origin as well as cases of SGA (Groups 4 and 5, respectively) provides a basis for developing and testing new hypotheses that explore the linkage between these observed phenotypes. It will be important to examine microbiological hypotheses, as well as those which question the links between maternal and fetal nutritional status, to delineate these links using placental tissue, maternal serum and cord blood as these data become available from the PTBs in Kilifi.

It is, therefore, feasible that with more precise phenotype data that intervention studies, whose results were previously inconclusive, should be revisited [? ]. It is plausible that studies where positive effects on birthweight or GA were demonstrated, but where no overall change in rates of PTB or SGA were observed, would have different outcomes if interventions were targeted by more specific case definitions [? ].

6.5.2 Appraisal of methodology

The maternal and newborn anthropometry included in this chapter were collected using rigorously standardised protocols in line with WHO policy employed as part of the Newborn Growth Reference Study [32]. All staff and equipment were standardised at three monthly intervals throughout the study to ensure that measurements were obtained to a standard that was suitable for comparisons with other INTERBIO-21st centres. Despite these measures, the absence of relevant international fetal growth standards at the time of publishing these data means that estimates of birthweight for GA centiles were determined according to International birthweight charts from the UK.
It is conceivable that estimates of the prevalence of SGA as a cause of PTB may alter if alternative fetal birthweight for GA reference standards were used for the analysis. Landis and colleagues reported that fetal growth amongst populations in sSA should be assessed according to customised centiles. They suggest that the arbitrary use of international fetal growth and newborn weight standards results in artificially high rates of SGA in sSA. They further suggest that the high prevalence of adverse maternal phenotypes in sSA is associated with a lower ‘normal’ birthweight and hence, that international birthweight standards overdiagnose SGA. This contention is, however, based upon a statistical definition derived using the 10\textsuperscript{th} centile from a sample of data collected during their study [?]. Whilst the group’s analyses were methodologically rigorous and sensitive to the challenges of data collection in sSA, they do not provide any independent clinical data with which to validate the performance of their centiles estimates. Without reference to outcomes such as perinatal or neonatal morbidity or mortality, it is impossible to determine the extent to which their use of statistical definitions of SGA accurately capture high risk clinical phenotypes.

In contrast, results from INTERGROWTH-21\textsuperscript{st}, which include data from Kenya, strongly refute the philosophy of customised growth and demonstrate clearly that fetuses from sSA can achieve the same growth potential as newborns from high-income nations. It will, therefore, be important to re-analyse this dataset according to the newborn standards that are due to be published from INTERGROWTH-21\textsuperscript{st}. Whilst it may be the case that such standards result in more than 10\% of infants being diagnosed as SGA, this will represent the prevalence of SGA within the Kilifi population, i.e. the number of infants that failed to meet their growth potential rather than a number defined arbitrarily by customised centiles that themselves were selected to over-represent high-risk phenotypes. With ‘predictable’ fractions of SGA diagnoses, it may be attractive for healthcare providers to apply customised centiles.
derived from their local populations as screening tools in clinical practice. However, what may be useful as a screening test is conceptually very different to what represents optimal phenotype classification. As such, the use of customised centiles introduces as a possibility that newborns may be labelled as ‘normal’ when, in fact, they have not achieved their growth potential.

In the latter half of the chapter, a novel analytical approach for examining PTB phenotypes has been implemented and tested as a first-line method for exploring complex clinical datasets that consist of many variables. The use of t-SNE not only confirms that there is a mathematical basis for groupings proposed by Villar and colleagues, but also provides a methodology with the capacity to examine datasets for novel phenotype variants. In their present form, the groupings are based upon potentially subjective clinical diagnoses. The addition of quantitative data from laboratory analyses and US data had the potential to improve cluster performance, which is also likely to benefit from a larger sample size. As the scope for molecular diagnostics advances, it is almost certain that new data will emerge at a rate that is faster than the capacity offered by conventional analytical methods to examine such data. Hence, new methods such as t-SNE must be explored as a means of utilising such data to their full potential. Whilst the methodology itself does not provide a classification system with which to classify the data de novo, it provides a very simple representation of extremely complex datasets for the exploration of trends and formulation of hypotheses.

6.5.4 Conclusion and future work

The methods presented in this chapter offer an insight into the feasibility of data collection within a low-income setting. The results from this chapter provide proof of concept that data of sufficient quality can be collected to support the implementation of a novel PTB classification system. Furthermore, by linking the dataset to analytical methods, such as data clustering and the t-SNE algorithm, is a further demonstration of how such data can be used to drive novel hypotheses in the future.
Chapter 7

Third trimester dating: novel equations from US biometry

7.1 Introduction

Current US derived estimates of GA offer little clinical value beyond 24 weeks’ gestation. Whilst there is no definitive consensus that has determined this threshold, the predictive error beyond such GAs renders estimates of GA by US to be unsuitable for dating purposes.

As changes during development of the fetal cerebral cortex can be visualised using US, and follow a time sequence that closely reflects GA, this thesis presented the hypothesis that such changes could be used as a biomarker for estimating GA.

In particular, progressive changes in the appearance of the brain as sulci and gyri develop upon the cortical surface were considered as candidate markers upon which to build a model to estimate GA between 18 and 34 weeks’ gestation. Such a model would have important implications for clinical services in Kilifi and other low-income settings. As discussed in Chapter 5, 50% of women presented for their first ANC visit after 24 weeks’ gestation and were therefore unable to benefit from the improved specificity of US dating with respect to PTB - See Chapter 5. As well
as the potential gains for individual women afforded by such improvements, access to more accurate dating beyond 24 weeks' gestation would enhance our understanding of the epidemiology of PTB in a large group of women who remain poorly phenotyped as a result of late presentation for care.

The work presented in this Chapter was developed to provide a reference standard to support the development of a novel, automated method for estimating GA, which is presented in Chapter 8. Some of the concepts underlying the automated model are common to both this Chapter and that which follows. Hence, the content of the remainder of this thesis shares the common objective of extending the limits within which US can be used to estimate GA.

The strategic vision was that expertise in global health, fetal medicine and ultrasound image processing could be applied to deliver an automated model. By automating, or semi-automating the process, the intention was to provide a model that was minimally dependent upon specialist sonographers for image acquisition. Similarly, the approach of using the binary data from ‘raw image’ file formats enabled the exploration of brain data in previously unexplored dimensions.

### 7.2 Model requirements

In order to build a dating model that was suitable for clinical practice in low-income settings, the following model specification was agreed *a priori*:

1. GA should be assessed using data acquired from a single 2D image and/or 3D volume acquired of the fetal brain;

2. The model should predict GA between 18 and 34+ weeks' gestation. This threshold was determined by consensus amongst clinicians and academic teams within the UK and Kenya. It is based upon the evidence suggesting that knowledge of GA before 34 weeks' gestation can be used to guide the initiation of treatments such as corticosteroid administration to reduce the in-
cidence of respiratory complications associated with PTB;

3. For feasibility purposes, the data should be acquired during a single US scan at a single time point in pregnancy.

7.3 Model validation - reference standard

As a pre-requisite for developing the GA model, a reference standard was required against which the model design could be evaluated. The standard would take the form of an accepted threshold of error for GA predictions delivered by any novel methods.

The candidates available to define such a standard were not considered to be fit for purpose. One option was to define an error threshold according to expert clinical judgement. Whilst certainly feasible, this did not represent a sound, evidence based approach. Similarly, comparing the error of a predictive model in the third trimester with that of CRL before 14 weeks’ gestation was considered to be inappropriate.

As discussed in Chapter 2, the best clinical alternatives using US, such as the models used to derive Hadlock’s multivariate equations [55], were associated with sub-optimal model fitting and unacceptably wide 95% CIs and so did not represent a reasonable target standard with which to assess the performance of new models. Hadlock’s equation used a stepwise linear regression model to predict GA using HC, BPD, AC and FL [55] - see Chapter 2, page 11. On the basis that such variables are related to GA in a curvilinear rather than linear fashion [52, 140, 237, 238], it is not surprising that attempts to regress such data using linear methods provides a sub-optimal fit of the data ($r^2 = 86.9$) and thus results in 95% CIs that render estimates unsuitable for dating purposes.

In contrast, the analytical approach adopted to model univariate predictors of GA employs fractional polynomial regression analyses instead of the linear approach and achieves adequate fits of data. Chitty’s equation using HC for dating was based
on such methods [52]. Hence, it was decided to explore, as an additional hypothe-
sis, whether a multivariate polynomial regression model could be applied to derive
novel candidates for a dating equation for use in the late second and early third
trimesters of pregnancy. If feasible, a new equation and the associated estimates of
the centiles (3rd and 97th) would provide a reference standard to guide development
of the automated model. Any new equation could also be tested as a stand alone
predictor of GA between 18 and 34+ weeks’ gestation.

7.4 Novel dating equations

An automated and supervised machine learning algorithm was employed to build
the novel dating equations. The process was designed and automated specifically
to generate a series of candidate polynomial equations; assess their performance
using pre-specified mathematical criteria, and subsequently, to adapt the structure
of the equations using a system to identify those that provide the best descriptions
of the data. By automating the process in such a way, the algorithm was able to
search for the optimal solution in a computationally efficient manner and specifically
avoided some of the pitfalls associated with conventional stepwise models. With
access to the ‘ground truth’ state of the associated variables (GA by LMP/CRL),
such algorithms are considered to represent a form of ‘supervised’ machine learn-
ing. These concepts are discussed in more detail in the next section before the
specific methods are described.

All models were constructed according to the recommendations of Royston, Alt-
man and colleagues, in their extensive accounts of applying such methods to derive
univariate equations to predict GA [238, 239, 240, 241, 242]. Specifically, it is rec-
ommended that equations are restricted to using powers between 0 and 3 in order
to avoid models that overfit the data and also to avoid developing equations that are
overly divergent at the extreme ranges of the predictor variables, or that demonstrate
‘wave-like’ forms between training data-points.
7.5 Machine learning - a primer

A number of important concepts are discussed in the following text. These are not designed to provide an exhaustive review of the techniques, but to provide sufficient background information to facilitate an understanding of the methodologies that were applied to determine the final clinical dating equations.

7.5.1 Model ‘search-space’

The objective of applying the machine learning algorithm to the dating problem was simple: to determine the equation that best describes the relationship between a series of candidate biometric parameters and GA. In order to achieve this, an exhaustive search of the prediction error of all potential combinations of candidate variables combined into a sequence that was representative of all potential forms of individual polynomial equations was necessary. The best equation, in numerical terms, was determined as that which provided the lowest prediction error. Thus, the process that was embedded within the machine learning algorithm was designed to resolve a series of candidate models that each met the ‘lowest prediction error’ criterion.

As an abstract principle, it is possible to conceptualise a data structure (a table, a matrix or series of matrices) that consists of all of the potential model errors that would result from equations that encompassed all possible combinations of the predictor variable sequence. When such a data structure is organised using a topology that represents the structure of the underlying equations, the loci within which the associated errors are stored correlate to the degree of similarity between the equations from which the error scores were derived. Data structures of this nature are referred to as search-spaces [243]. Search-spaces that are organised according to such principles provide a hierarchical system, whose structure can be used as the basis of a search that identifies the optimal model. ‘Search-spaces’ and their
associated search methodologies have been studied extensively and are described in detail elsewhere [243, 244].

### 7.5.2 Fitness functions

Once a hierarchical structure such as the 'search-space' has been defined, a series of numerical operators can be applied to execute an iterative search within the space that identifies the optimal model. Since the ‘ideal’ model has a predictive error equal to 0, the optimal model, in numerical terms, can be identified by a search that resolves the model whose error is closest to 0.

Comparisons of error for single parameter dating models use the SD and 95% CI of data-points from the mean. For multivariate models, comparisons of error conventionally use the square root of the mean error (RMSE). Once the RMSE has been determined, it is straightforward to use simple conditional operators to compare the ‘fitness’ of each model with others in the search-space and, therefore, to identify those models with the lowest RMSE values automatically. From this point onwards, the RMSE will be referred to in generic terms as the ‘fitness score’.

In order to initiate the search, it is necessary in the first instance, to nominate arbitrarily the ‘fitness score’ of a single model as the index case. This can be selected at random. From the index case, comparisons are then made with the terms of adjacent loci within the search-space using conditional operators that determine, which, if any has the better ‘fitness score’. If this ‘fitness criterion’ is met, the model transitions to a new index case and the process is repeated. Ultimately, over many iterations, the search resolves the model whose prediction error is the lowest, and, therefore, has the best ‘fitness score’ within the search-space. Such an approach represents an example of a ‘Trust-region’ method of searching and can be illustrated by the ‘Hill climbing’ paradigm [245].
7.5.3 Simple and complex models

‘Hill-climbing’ is an illustrative concept and is based on the observation that the ‘fittest’ model(s) within a group of models will reside at the peak of an axis (1 - error) within a search-space. Thus, when the fitness function is used as the basis for navigating within the search-space, the relative change in gradient between two adjacent models being considered at any single locus can be used to determine the direction and orientation of the search process, i.e. a positive gradient would be defined when the transition is towards the optimal solution and, conversely, a negative gradient would indicate a transition away from the optimal solution. As illustrated by Figure 7.1, if a hypothetical search were to commence at point A within the search-space presented, the gradient alone would provide sufficient information to determine when point B has been reached; the algorithm would traverse in a stepwise fashion along a positive gradient that tends towards no gradient once the top of the hill, and hence the optimal solution, has been determined.
Figure 7.1: Left - the search-space error contour of variables, ‘var a’ and ‘var b’. Right - The trajectory profiles of all potential variants of a search pathway between A and B. It is clear that a simple fitness stop criterion based upon comparisons of the gradient between models would terminate at B.
Searching according to ‘Hill climbing’ principles can be very successful [245, 246, 247]. However, the approach described thus far assumes that the ‘landscape’ of the search-space exists in a simple form such as that represented by the contour of Figure 7.1. In the context of GA modelling by the use of US biometry data, it is impossible to define in advance the exact nature of the search-space contour. Hence, reliance on such methods has the potential to oversimplify the search, resulting in an equation being incorrectly declared as the ‘fittest’. Using a simplistic analogy, it cannot be assumed that the GA search-space comprises a single hill with a simple contour.

When the search topology is complex, or unknown, alternative methods of defining the model with the optimal fitness have to be considered. Figure 7.2 illustrates a hypothetical complex search-space and demonstrates how a search algorithm in such a dataset has the potential to resolve a suboptimal solution. From Figure 7.2, it is clear that point C represents the global maximum ‘fittest’ solution within the search-space and, consequently, the target for any search algorithm to identify. However, point B and other similar points, that themselves, represent ‘local’ optima throughout the search-space would potentially be selected by a stepwise model.
Figure 7.2: Left - the search-space contour of a complex search-space of variables, ‘var a’ and ‘var b’. A represents the starting point for any search algorithm. B represents the ‘fittest’ function and is termed the ‘global optimum’. However, the complex nature of the search-space is such that a single, stepwise approach to finding the ‘global maximum’ may resolve a model whose error reflects a ‘local maximum’ - represented at point C in the search-space. Right - The trajectory profiles of all variants of the search pathways between A and B. It is clear that a simple fitness stop criterion based on a gradient change between search-space loci has the potential to terminate at multiple ‘local optima’ in as well as the ‘global optimum’ at point B.
Search-space complexity has important implications when determining how to approach the identification of an optimal solution amongst all potential alternatives. Conventionally, medical analyses apply the principles of stepwise modelling whereby a model is incrementally altered by a single term until the ‘optimal solution’ is determined. Having demonstrated the complexities that can be associated with search-space topology, it is conceivable that such stepwise approaches risk resolving sub-optimal solutions that, themselves, represent a local, rather than global maximum. For this reason, a stepwise regression analysis was not undertaken to generate the prediction equations presented below.

### 7.5.4 Genetic Algorithms

A genetic algorithm is a computational search methodology with the capacity to select a subset of variables from a candidate dataset that provides the optimal solution to a user specified problem [248]. With respect to predicting GA, the genetic algorithm provides one method of searching within a complex ‘search-space’ to define a subset of variables (biometric parameters) that best predict GA defined using polynomial regression analyses.

Using an iterative methodology, the genetic algorithm is based upon a modelling approach that adapts itself across multiple ‘generations’ until an optimal solution is found. The algorithm initiates itself by generating many copies of a dataset that, themselves, are each variants of the original datasets in terms of the number and order of constituent variables. With respect to predicting GA using US biometric parameters, the genetic algorithm would randomly initiate itself with 1000 randomly assembled polynomial equations comprising a specified number of terms, that themselves are random combinations of variables from the candidate dataset.

Each equation within a generation is conceptually analogous to a sequence of DNA, and represents a string of constituent variables i.e. a group of variables that themselves behave like genes and are in ‘active’ or ‘inactive’ states. Combinations of the
variables, or genes, are brought together to form terms that are used to build each variant polynomial equation. The equations vary such that within any generation there are individual sequences of variables that differ according to the 1) The number of variables that are switched ‘on’ (and therefore available for use); and 2) the order or sequence with which those variables that are switched on are seen by any subsequent function. The latter stage is important as it allows the extent to which different sequences of the same variables are able to affect the performance of functions that use the dataset to be examined; a process that mirrors the assessment of covariance [249].

Each term within the equation is randomly assigned a coefficient and a power specified in the range of 0 - 3, as discussed above. The individual predictor variables are combined as products of each other to build the terms of each equation - therefore a term that is limited to the power 2, can have a single variable, \( a \), represented as \( a^2 \) or can be combined with a second variable, \( b \), to produce a term, \( a \times b \), and so on. Terms are combined into equations and, finally, each equation is used to predict GA. The predicted GAs for the whole dataset are evaluated against GA\(_{truth}\) to determine the model error, or the RMSE.

The results of all equations from a single generation of the genetic algorithm are then assembled and their respective RMSEs compared. According to a user defined fraction of ‘best performing’ models, with the lowest RMSE values, a subset of ‘fittest’ models from the complete generation are selected. Single variables within each of these ‘fittest’ equations are subsequently switched between the on and off state at random. Whole terms are also ‘exchanged’ between adjacent equations. Overall, these processes are implemented to address the hypothesis that random changes within the sub-structure of an equation, with respect to whether or not variables are in the ‘on’ or ‘off’ state, have the potential to confer improvements in the resulting equation’s predictive performance; or a ‘survival advantage’ [250, 251]. If such survival advantages are demonstrated, by a better fitness score from a subsequent round of regression, the change is conserved into later generations. If such
changes ‘fail’, then the equation is discarded and the negative effect thus rejected. Finally, the remaining equation variants are cloned to produce an ‘offspring’ of identical equations and the whole process is repeated.

Over many iterations, the genetic algorithm converges upon an equation that optimally predicts the desired outcome. For the prediction of GA, this will be list of variables that optimally predict GA with their respective polynomial equation. Whilst the description of the genetic algorithm thus far has focused upon adapting the structure of the original dataset of variables, the same principles of adaptation are applied to select the coefficients of each term and their polynomial forms (powers).

With respect to traversing a search-space, the genetic algorithm offers a number of advantages over conventional approaches. By selecting only the optimally performing predictive variants to propagate into each subsequent generation, the algorithm terminates pathways through the search-space that are uneconomical i.e. those searches that are clearly directed away from the solution. Similarly, by traversing the search-space using a population of individual variants of the dataset, that each begin their search for the optimum model from a different point that is randomly assigned, the genetic algorithm has been likened, by a leading scientist in the field, to “dropping 1000 [i.e. population size] parachutists, rather than 1, on (to search) the landscape” [252].

As such, it is conceivable that through its application of multiple searches in parallel that each start from initially random points within the search-space, that a genetic algorithm is more likely to discover the global optimum solution than if random or stepwise enumerative searches following a single pathway were employed [252, 253, 254]. These hypotheses have been been tested and validated repeatedly by extensive research within computer science disciplines. Genetic algorithms are used widely to select important predictive features within biological datasets [255, 256, 257, 258]. They have also been tested against other state-of-the-art feature selection techniques and have demonstrated equivalent levels of performance [259, 260].
On the basis that the genetic algorithm independently assesses the value of terms presented to it using the ‘fitness function’ and adapts the model based upon these findings, the algorithm represents a form of supervised machine learning.

7.6 Methods

7.6.1 Recruitment and study sample definition

The model was designed using a test dataset acquired by the INTERGROWTH-21\textsuperscript{st} study - See Section 7.6.2. A sample of US images \((n=2000)\) was selected randomly from a total of 4315 subjects using a standard function provided by STATA. HC, BPD, OFD, FL and AC biometric measurements were obtained in triplicate with their corresponding 2D image and 3D volume data. Whilst each woman was scanned longitudinally during INTERGROWTH-21\textsuperscript{st}, a cross-sectional sample of the larger group was obtained for this study, where each fetus was included only once and where each day of gestation between 18 and 35 weeks’ gestation was represented in the sample equally. Whilst repeated measure studies, based upon longitudinal datasets with multiple entries for a single fetus, are necessary for studies describing fetal growth, the use of such sampling methods for describing GA can introduce bias into the variance observed by analyses. It is important to emphasise that size, measured at a single episode, is the predictor variable for GA. Whilst size is clearly related to growth and growth velocity, the two entities represent distinctly separate concepts with respect to evaluating fetal development. Hence, such distinctions at the level of sampling within datasets were essential to maximise the extent to which candidate models are generalisable across different samples and populations [52].

7.6.2 Model design - INTERGROWTH-21\textsuperscript{st} dataset

INTERGROWTH-21\textsuperscript{st} is a multi-centre, multinational study whose aim was to define fetal growth standards within an optimally healthy, geographically diverse population
Recruitment Criteria

1. Aged $\geq 18$ and $< 35$ years
2. BMI $\geq 18.5$ and $< 30$ kg/m$^2$
3. Height $\geq 153$ cm
4. Singleton pregnancy
5. Known LMP with regular cycles (defined as 28 days $\pm 4$ days)
   No hormonal contraceptive or breastfeeding in the 2 months before pregnancy
6. Natural conception
7. No significant past medical history and no need for long-term medication
   (excluding routine iron, folate, calcium, iodine or multivitamin supplements)
8. No evidence of socio-economic deprivation
9. No use of tobacco or recreational drugs such as cannabis
10. Limited alcohol use (defined as $< 5$ units (50ml pure alcohol) per week) since
11. $\leq 1$ miscarriage in the 2 previous consecutive pregnancies
12. No previous baby delivered pre-term ($< 37$ weeks) or with a birth weight $< 2500$g or $> 4500$g
13. No previous neonatal or fetal death or previous baby with any congenital malformations
14. No previous pregnancy affected by pre-eclampsia/eclampsia, HELLP syndrome
15. No clinically significant atypical red cell alloantibodies
16. Negative urinalysis
17. Systolic blood pressure $< 140$ mmHg and diastolic blood pressure $< 90$ mmHg
18. No diagnosis or treatment for anaemia during current pregnancy
19. No clinical evidence of sexually transmitted diseases
20. Not in an occupation with exposure to chemicals or toxic substances or very physically demanding activity

Table 7.1: Selection criteria applied for recruitment of participants of the INTERGROWTH-21 study.

of women recruited across eight centres$^1$. The study employed rigorous selection criteria, which are summarised in Table 7.1. Importantly, only those women who were able to recall the specific date of their LMP were recruited. Eligibility was further restricted to women with regular cycles, of a fixed length, and in whom there was no reported use of hormonal contraception or breast feeding in the 2 months prior to entry. The GA by LMP was validated by US measured CRL, which was performed according to strict US measurement protocols. Specifically, the mean of 3 CRL estimates was calculated after measurements had been obtained in a blinded fashion, using the same process outlined in Chapter 5. Only women in whom LMP and CRL estimates of GA agreed to within 7 days were subsequently selected. Hence, the GA reference standard, referred to as $GA_{truth}$ from this point, was rigorously standardised to provide a highly accurate dataset.

$^1$www.intergrowth21.org.uk
7.6 Methods

7.6.3 Model validation - INTERBIO-21\textsuperscript{st} dataset

A second validation dataset was acquired prospectively whilst the model was being developed (n=200). These data were collected from women being scanned as part of the INTERBIO-21\textsuperscript{st} study in Oxford, UK. The same rigorous process was used to determine GA, but women were not selected to represent an optimally healthy population. As such, the validation sample was considered to represent a clinically representative population within which to perform first line testing of the model.

7.6.4 Data cleaning

Data were preprocessed before modelling was undertaken. Scatter plots of each parameter were plotted against GA\textsubscript{truth}. Obvious outliers were identified and referred back to the original datasource for correction. Persistent outlying values remained and so further scrutiny was necessary. To manage these data-points, a fractional polynomial regression analysis of each parameter was undertaken using GA\textsubscript{truth} as the predictor variable; the approach taken for growth studies. Plots of the mean and centiles (3\textsuperscript{rd} and 97\textsuperscript{th}) were generated. Data points with a z-score of $\geq \pm 2.5$ were then excluded from the dataset. Whilst this was an arbitrary threshold, a z-score of $\pm 2$ was considered to exclude too many data-points; particularly since the sample was so highly selected to exclude women at risk of abnormal fetal growth. All of the pre-processing analysis was undertaken using the ‘Xrigls’ function in STATA [261].

Figures 7.3 - 7.7 illustrate the pre- and post-cleaning distributions for each of the datasets. The clean dataset was used for all subsequent analyses, with no further exclusions made. The sample size for model development was $n = 1945/2000$ (97\%).

\textsuperscript{2}www.interbio21.org.uk
Figure 7.3: A - log HC scatter plot with obvious outliers. B - same data plotted after cleaning. Solid blue line = log HC, bottomed blue line = 3rd and 97th centiles.
Figure 7.4: A - BPD scatter plot with obvious outliers. B - same data plotted after cleaning. Solid blue line = mean BPD, dotted blue line = 3rd and 97th centiles.
Figure 7.5: A - OFD scatter plot with obvious outliers. B - same data plotted after cleaning. Solid blue line = mean OFD, dotted blue line = 3rd and 97th centiles.
Figure 7.6: A - AC scatter plot with obvious outliers. B - same data plotted after cleaning. Solid blue line = mean AC, dotted blue line = 3rd and 97th centiles.
Figure 7.7: A - FL scatter plot with obvious outliers. B - same data plotted after cleaning.
Solid blue line = mean FL, dotted blue line = 3rd and 97th centiles.
7.6.5 Polynomial regression analysis via the Genetic Algorithm

A customised algorithm was developed to produce GA equations using MATLAB (The Mathwork's, MA, USA) [236]. A toolbox - ‘GAPolyfitn’ - was obtained under the MATLAB licence using source code from the Engineering Department of the University of Sheffield, UK [262]. The methods were reviewed and approved by an academic computer scientist within the Nuffield Department of Obstetrics & Gynaecology (NDOG). A number of conditions for the genetic algorithm can be defined by the user, such as the size of the initial population of chromosomes, the crossover fraction and mutation frequency. These parameters were specified according to pre-defined guidelines [262]. MATLAB was chosen on the basis of its inbuilt functionality in an ‘off-the-shelf’ package and, also, that its functionality is provided with access to the software code. This provides the versatility to adapt certain features according to specific requirements such as pre-specifying the limits for polynomial forms that are presented.

The output of ‘GAPolyfitn’ consisted of a polynomial equation, a matrix of the predicted GA, GA_{model}, alongside performance indicators including the RMSE, regression coefficient (r^2) and the standard deviation of each model term.

7.6.6 Fitness function

The RMSE for each model was used to determine model fitness. Conditional operators were coded to positively select equations for which the RMSE of GA_{model} tended to 0.
7.6 Methods

7.6.7 Data extraction and model preparation

The following data structures were assembled from the INTERGROWTH-21 dataset for modelling with ‘GAPolyfitn’.

1. HC
2. HC + log HC
3. HC + log HC + BPD
4. HC + log HC + BPD + log BPD
5. HC + log HC + BPD + log BPD + OFD
6. HC + log HC + BPD + log BPD + OFD + log OFD
7. HC + log HC + BPD + log BPD + OFD + log OFD + AC
8. HC + log HC + BPD + log BPD + OFD + log OFD + AC + log AC
9. HC + log HC + BPD + log BPD + OFD + log OFD + AC + log AC + FL
10. HC + log HC + BPD + log BPD + OFD + log OFD + AC + log AC + FL = log FL

Variables were entered in their observed state and their log transformed state for two reasons:

1. Based on first principles, the regression equations derived are likely to provide a better fit of the data if their constituent predictor variables are normally distributed. Since the extent to which data are 'skewed' represents a continuous rather than a binary classification, the data were presented to the GA using the log transformation as a means of providing greater symmetry to individually 'skewed' variables. The algorithm was left to determine which variant was selected based upon an objective assessment of performance;

2. To transform outlying values that may well be associated with abnormal clinical phenotypes in order to reduce the potential for misclassifying GA. For example, short femur length is associated with fetal growth restriction and so a dating equation based on size has the potential to determine GA for such cases incorrectly. This represents a significant risk associated with the method.
of dating by size. The performance of subsequent equations from this chapter of work and also the final automated model must be tested rigorously on data with adequate fetal growth restriction representation.

As well as restricting the polynomial form of equations, ‘GA-polyfitn’ requires the user to specify the number of terms to be returned within the equations generated i.e., a generic equation is produced in the form \( \text{GA} = aX_1^i + bX_{b+1}^j + cX_{b+2}^k \ldots \), which requires the user to specify the limits of \( X_b \). Forms were restricted to equations of 2, 3 or 4 terms.

Hence, the structure of the polynomial equations derived were:

\[
\begin{align*}
\text{GA} &= aX_1^{0-3} + bX_2^{0-3} + \text{constant} \\
\text{GA} &= aX_1^{0-3} + bX_2^{0-3} + cX_3^{0-3} + \text{constant} \\
\text{GA} &= aX_1^{0-3} + bX_2^{0-3} + cX_3^{0-3} + dX_4^{0-3} + \text{constant}
\end{align*}
\]

where:

- \( a, b, c, d \) : Coefficients determined by ‘GA-polyfitn’.
- \( X_1, \ldots, X_4 \) : Combinations of variables determined by genomic selection from the genetic algorithm.

Henceforth, the combined methodology for generating polynomial equations to predict GA will be referred to as ‘GA-polyfitn’.

### 7.6.8 Analytical approach - model comparison

Visual inspection of scatter plots enabled the overall fit of models to be determined and so provided a rapid, subjective assessment of model performance. Such plots are easily constructed when data are modelled in two dimensions. Once models were extended into multivariate analyses, constructing scatter plots of several variables in their untransformed states was not possible. To overcome this limitation scatter plots of \( \text{GA}_{\text{model}} \) versus \( \text{GA}_{\text{truth}} \) were constructed with a line of equality.
7.6 Methods

7.6.9 Analytical approach - heterostochastic standard deviations

Assessment of model performance over the range of $\text{GA}_{\text{truth}}$ required specific adaptations to the data analysis. Whilst error within the ‘fitness function’ of the genetic algorithm was based on a single, uniform estimate of the RMSE, this was likely to be insufficient when assessing the clinical implications of the error associated with final models. It is widely reported that the SDs of fetal biometric parameters when expressed as a function of GA are heterostochastic [238]. This means that the SDs, themselves, vary with respect to GA. However, conventional modelling software provides equations for SD based on a single candidate predictor variable. A method was therefore needed to estimate the SD of $\text{GA}_{\text{model}}$ as a function of $\text{GA}_{\text{truth}}$.

The equation for SD is:

$$s = \sqrt{\frac{\sum (x_i - \bar{x})^2}{n - 1}}$$

Conceptually, the SD represents a simple transformation of the residuals between an observed value and its mean within a sample of data-points. With respect to the GA prediction equations, the residual to be transformed can be estimated by calculating the difference between $\text{GA}_{\text{model}}$ and $\text{GA}_{\text{truth}}$, for each value of $\text{GA}_{\text{model}}$. Hence, calculation of the residuals results in a series of positive and negative residual values that fall above and below a line of equality between $\text{GA}_{\text{model}}$ and $\text{GA}_{\text{truth}}$.

To estimate how such residual values vary as a function of time, it is then possible to regress the observed residuals against $\text{GA}_{\text{truth}}$ to provide two equations: 1) for the positive SD and 2) for the negative SD. Both equations can then be applied to derive centiles for $\text{GA}_{\text{model}}$ accordingly by multiplication of respective $z$ scores - ± 1.96 for the $3^{rd}$ and $97^{th}$ centiles, respectively.

The technique was validated using a single variable dataset where the SD function was also determined using standard statistical packages. The method was reviewed and approved by a statistical advisor in NDOG.
7.6.10 Model testing

‘GA-polyfitn’ was tested extensively before running analyses to validate the algorithm’s performance. Specifically, 40 test datasets were assembled within which the constituent variables were replaced by random data generated within the same range as the observed data using the ‘runiform’ random number generator in STATA. As an indicator of the robustness of ‘GA-polyfitn’, the test datasets were used to determine if any of the random variables would be selected as predictors of GA by the model. This was not the case.

7.7 Results

Each equation was developed by comparing upto 64,000 variants over 500 generations of the genetic algorithm. The results from the output from ‘GA-polyfitn’ are presented in Figure 7.10. The plots display the RMSE for each function presented by ‘GA-polyfitn’ and are organised into three columns according to the equation variant from which they are derived (i.e. equations 7.1, 7.2 and 7.3). The upper panel of each column presents the output when each equation was limited to a power of 2 (plots A, B and C) and the lower panel when limited to a power of 3 (plots D, E and F).

7.7.1 Preliminary results

The ‘fittest models’ from each panel of Figure 7.10 were selected and used to predict $GA_{model}$. Results were assembled into scatter plots comparing $GA_{model}$ and $GA_{truth}$. The line of equality was plotted with the centiles ($3^{rd}$ and $97^{th}$) using the previously described methodology. Each plot was then visually inspected as a first line evaluation of model fit. Specifically, the symmetry of scattered data-points around the line of equality was examined as well as the symmetry to which the plotted centiles captured variance across the range of GA. It was immediately apparent from predicted
data that the models were demonstrating suboptimal fitting. The rationale for this assessment is demonstrated in Figure 7.9. Subjectively, the data in top panel are asymmetrically scattered around the line of equality, which itself does not traverse the centre of the distribution. This means that the predicted GAs were not being determined uniformly across the range of $\text{GA}_{\text{truth}}$ and, therefore, that the models were not stable.

Although the degree of instability was variable, there was evidence of poor fitting throughout. It is clear from the top panel of Figure 7.9 that beyond approximately 250 days on the $\text{GA}_{\text{truth}}$ axis, the variance amongst predicted values increases significantly. Such a trend was not surprising given the extent to which individual growth parameters vary at such GAs. Hence, it was considered that such variance was compromising the performance of predictive models at earlier GAs by attempting to predict GA later in pregnancy using a single equation.

Whilst limiting the range of the training dataset is controversial [52], a decision to restrict the upper limit of GA of the training dataset used to develop equations to 245 (35 weeks) days was made. The clinical consequences of this decision were considered to favour such an approach and formed a simple trade-off: The critical period for estimating GA was $\leq 34$ week threshold where such data were important to guide the use of the therapeutic interventions discussed previously. A precise GA at 36 weeks' gestation was less critical. Therefore, a cut off with the training set at 245 days (35 weeks) would enable a GA $>34$ weeks' gestation to be provided by the model, whilst not exposing the regression equation to the increased variation at GAs beyond this when resolving its equations. By adopting this approach, all of subsequently derived models provided much better fits of the data using the same subjective assessments - as shown in the bottom panel of Figure 7.9. Interestingly, whilst this approach provided a much better capture of variance, the RMSE values of the restricted GA ranges were higher than the un-restricted, which can be seen by comparing Figures 7.10 and 7.8. This observation probably represents the better capture of true variance within the restricted models, rather than the compromise introduced by suboptimal fitting of the centiles across the wide range of GA.
Figure 7.8: Model output 1: Best performing GA prediction models selected by the genetic algorithm $GA_{truth} = 120-260$.
7.7 Results

7.7.2 Final candidate GA equations

‘GA-polyfitn’ was subsequently re-configured with the restricted datasets and the models re-constructed using the same technique. A second model selection plot from the new data is provided in Figure 7.8. Using the RMSE as the fitness score, the single best performing equation was based on 4 term, 2 power polynomial equation: RMSE = 4.7 days, $r^2 = 0.99$.

The equation for GA is:

$$GA = 18.0461 \times HC^2 + 5.7299 \times \log AC^2 \ldots$$

$$\ldots + 10.9393 \times \log FL \times FL - 26.8838 \times \log HC \times \log FL$$ (7.4)

In comparison, the worst performing model was based on a cubic form of equation 7.2 with an RMSE of 6.8 days, $r^2 = 0.99$. Whilst feasible to obtain, the optimum equation (7.4) was complex. Since the range of error for RMSE between the best and worst performing models was 2.1 days and therefore of questionable clinical significance, alternative equations were examined for a less complex alternative.

The three best performing equations for each of plots A to F from Figure 7.8 were extracted. Scatter plots with the line of equality and centiles were generated with a QQ plot to assess model quality in terms of the shape of data predicted [52].

A QQ plot uses a probability based methodology to provide a graphical representation of the quantiles of two separate distributions of data against one other. Where the plot results in a line of equality, two samples can be considered to have been selected from the same population. As such, if a line of equality is assumed by the QQ plot, the spread of residuals between data from one sample can be considered to be normally distributed for each value of the second sample [263]. Based on these properties, QQ plots were used to visually inspect the distributions of $GA_{model}$ for each value of $GA_{truth}$ for each equation generated by ‘GA-polyfitn’. Best performing models, with the most reliable estimates of $GA_{model}$ were demonstrated by those
models whose QQ plots approximated a line of equality, which confirmed that the
distribution of $GA_{model}$ was normally distributed around $GA_{truth}$. The demonstration
of such proof enabled the assumption to be accepted that the mean of $GA_{model}$, pro-
vided by the regression formula, was likely to be an accurate reflection of $GA_{truth}$. In
the absence of such proof, or where there was significant deviation from the line of
equality on QQ plots and, therefore, a skewed distribution of residuals, GA models
that were based upon regression to mean would not provide an adequate estimate
of the typical value of $GA_{truth}$.

The equations are listed in Table 7.2. On the basis that the model is searching for
optimal performance in the third trimester, this extraction includes estimates of the
95\% CIs for each model at 26, 30 and 34 weeks’ gestation. The percentage of
observed values outside the limits of the predicted 95\% CIs was also examined as
an indicator the accuracy of centile estimates. These additional indicators required
considerable manipulation of the dataset and so were not feasible as outputs of the
initial model selection using ‘$GA$-polyfitn’.
Figure 7.9: Top - Scatter plot of $GA_{\text{model}}$ versus $GA_{\text{truth}}$ for GA range of 120 to 260 days. There is evidence of suboptimal model fitting with asymmetry of data-points around the line of equality and poor capture of variance between centiles. Bottom - Scatter plot of $GA_{\text{model}}$ versus $GA_{\text{truth}}$ using the same training model parameters although with the training data sample restricted to include a GA range of 126 to 245 days. Data points are more evenly distributed around the line of equality and estimates of centiles capture data more precisely.
7.7 Results

Figure 7.10: Model output 1: Best performing GA prediction models selected by the genetic algorithm $GA_{truth} = 126-245$.)
Figure 7.11: Models - 2 terms, 2 powers: Nomograms with centiles (3rd, 50th (= mean) and 97th) and QQ-plots. $GA_{\text{truth}} = 126-245$. Equations references from Table 7.2.
Equation 3.1

Equation 3.2

Equation 3.3

Figure 7.12: Models - 2 terms, 3 powers: Nomograms with centiles ($3^{rd}$, $50^{th}$ (= mean) and $97^{th}$) and QQ-plots. $GA_{truth}=126-245$. Equations references from Table 7.2.
Equation 2.3  
Equation 2.5  
Equation 2.8

Figure 7.13: Models - 3 terms, 2 powers: Nomograms with centiles ($3^{rd}$, $50^{th}$ (= mean) and $97^{th}$) and QQ-plots. $GA_{\text{truth}} = 126-245$. Equations references from Table 7.2.
Figure 7.14: Models - 3 terms, 3 powers: Nomograms with centiles (3\textsuperscript{rd}, 50\textsuperscript{th} (= mean) and 97\textsuperscript{th}) and QQ-plots. $GA_{\text{truth}}=126-245$.
Equations references from Table 7.2.
Figure 7.15: Models - 4 terms, 2 powers: Nomograms with centiles (3\textsuperscript{rd}, 50\textsuperscript{th} (= mean) and 97\textsuperscript{th}) and QQ-plots. \(GA_{truth}=126-245\). Equations references from Table 7.2.
Figure 7.16: Models - 4 terms, 3 powers: Nomograms with centiles (3\textsuperscript{rd}, 50\textsuperscript{th} (= mean) and 97\textsuperscript{th}) and QQ-plots. GA\textsubscript{truth} = 126-245. Equations references from Table 7.2.
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Table 7.2: Candidate dating equations with error indices - RMSE, $r^2$, and 95% CIs at 26, 30 and 34 weeks’ gestation (CI_{26}, CI_{30} and CI_{34} and absolute percentage of outliers observed outside the limits of the calculated 95% CIs.)
7.7 Results

7.7.3 Final model selection

The difference between maximum and minimum RMSE for each of the 18 equations derived was 1.3 days, which is unlikely to be clinically important. The shape of QQ plots was therefore considered along with the absolute percentage of observed data-points classified as outliers by the estimated centiles. Evidence of a uniform fit across the range of $GA_{truth}$ on the QQ plot in conjunction with an observed percentage of outliers close to 5% were considered as indicators of good model fitting of predicted GA. Using these criteria, equations 2.5, 2.6, 2.8, 2.9 and 3.7 were selected as a final subgroup. The centiles of these equations were superimposed into a single graph to compare their performance visually. The rationale for representing the data using this technique was that the optimal model, if there was a single best model, would have centiles closest to the line of equality - see Figure 7.17.

From Figure 7.17, there was no distinct separation of models and so the structure of the candidates was considered. Hence, with relative uniformity with respect to the error associated with each equation across the range of $GA_{truth}$, the final candidate could be selected according to a ‘trade off’ between the complexity of its form and the required application of the equation.

To this end, as a standard for developing an automated dating model, the greater accuracy of equation 2.9, albeit very minor, fulfils to numerical criteria for the optimal equation. However, equation 2.5 based upon two US parameters (HC and FL) has a 95% CI of 13.6 days at 34 weeks’ gestation. The equation provides a similarly good model fit and the performance from the test dataset is comparable to that of BPD or HC at 22-23 weeks’ gestation as reported by Altman and Chitty. As such, both of these equations will be presented for comparison with the automated dating model as stand alone novel dating methods for the third trimester. A final plot comparing the centiles of equations 2.5 and 2.9 is shown in the lower panel of Figure 7.17 and clearly demonstrates almost equivalent performance based upon prediction error margins. Dating tables for equations 2.5 and 2.9 listing the 3rd and 97th centiles
and uncertainty estimates are provided in Appendix A.10 and A.11 on pages 229 to 232.

7.7.4 Model validation and final equations

Equations 2.5 and 2.9 were used to generate $GA_{model}$ using the clinical validation dataset described previously (7.6.1). The plots compare the absolute difference between $GA_{model}$ and $GA_{truth}$. For equation 2.5, a systematic difference of 0.05 days was observed with 95\% limits of agreement (LOA) of -8.41 to 8.50 days respectively. From equation 2.9, the systematic difference and 95\% LOA were -0.30 days and -8.66 to 8.07 days, respectively.

With minimal objective difference in performance between equations 2.5 and 2.9, the decision as to which should form the final solution was determined according to model complexity. On the basis that a model with fewer parameters was likely to provide a more feasible clinical solution, equation 2.5 was presented as the final equation. In comparison to equation 2.9, the difference in predictive error at 34 weeks (1.2 days), systematic difference and LOA are unlikely to be clinically important and so the less complex form of equation 2.5 was considered to offer a superior clinical solution.

7.8 Discussion

The objective of this chapter was to present a candidate dating equation to use as a reference standard for developing an automated tool to estimate GA in the third trimester.

7.8.1 Principal findings

This study has demonstrated that a novel methodology applied to direct an automated search algorithm was able to generate dating equations with the capacity to
Figure 7.17: Top - Final equation selection: Overlaid centiles (3rd and 97th) for 5 best equations. Bottom - Final equation selection: Overlaid centiles (3rd, 50th (= mean) and 97th) for 2 final equations selected as standalone dating equations between 126 - 245 days.
Figure 7.18: Top - Bland Altman plot of the absolute residuals of \( \text{GA}_{\text{model}} \) from equation 2.5 for GA prediction. Bottom - Bland Altman plot of the absolute residuals of \( \text{GA}_{\text{model}} \) from equation 2.5 for GA prediction. Ground truth = LMP/ CRL based GA estimates as per INTERGROWTH-21\textsuperscript{st} dating protocols [175].

estimate GA with an error (95% CI) of 13.6 days at 34 weeks’ gestation (equation 2.5). The final equation has uniformly distributed performance across the entire GA
range (i.e. are not skewed at any point) and produces an estimate of the centiles that accurately exclude 5\% of data-points; i.e. accurately describe 95\% CIs of the proposed equations.

### 7.8.2 Strengths and weaknesses

When the equations were applied to a novel dataset, whose data-points had not been used to derive the equations, performance indicators consistent with the test dataset were obtained: absolute differences compared using Bland Altman plots showed minimal systematic differences and 95\% LOA that were consistent with error estimates from the centiles derived with the original equations. This suggests that the novel equations are stable and are thus unlikely to be overfitting the training dataset from which they were developed.

The rationale for the methodologies used to generate these equations has been discussed extensively throughout the chapter. The results, overall, are comparable to dating equations currently applied in routine clinical practice in many settings: the 95\% CI, systematic error and LOA are all consistent with the performance of single variable equations based upon HC or BPD at 22 weeks’ gestation [52].

The study has a number of limitations in its current form. Firstly, whilst the sampling technique employed by INTERGROWTH-21st provided an optimally healthy dataset upon which to develop the equations, and thus, was unlikely to introduce bias as a consequence of the presence of biologically extreme phenotypes, the extent to which findings from such datasets can be translated into routine clinical practice in high risk settings, such as Kilifi, must be questioned.

On the basis of the selective sampling used to define the datasets, it will be important to examine the performance of the equations in specific circumstances: 1) Using data acquired by clinical sonographers. The extent to which inter- and intra-operator variability with respect to the acquisition of measurements obtained by a group of ‘expert’ research sonographers cannot be assumed to be representative
of the quality of measurements acquired in the field; and 2) Validation studies in fetuses displaying evidence of growth restriction in order to determine the extent to which estimates of GA can be considered valid across the entire third trimester.

With respect to point 1) above, published data have previously described the tendency for variation in operator reproducibility to increase as a function of GA [264, 265]. However, and somewhat surprisingly, very few studies have examined these effects using methodologies that are sufficiently robust to enable comparisons with other datasets to be made. Where data are available, the studies have been subject to a range of criticisms, including the use of small sample sizes, obtained over narrow windows of GA that use sub-optimal statistical assessments to compare operator performance [266, 267, 268]. Sarris and colleagues addressed many of these limitations and undertook a systematic assessment to estimate the extent to which operator variation influenced estimates of fetal biometry throughout pregnancy. Their study demonstrated that measurements varied between ± 3.0 and 11.1% depending upon the structure measured and the performance indicator assessed (inter- or intra-operator variation). Rijken and colleagues went on to examine the performance of non-specialist sonographers in a low-income setting on the Thai-Burmese border and demonstrated that locally trained health-workers were able to obtain biometry measurements with acceptable limits of accuracy and precision. This study reported LOA of ± 1 week between locally trained health-workers and a specialist obstetrician [128]. They showed a trend towards less reproducible measurements later in pregnancy with an error in the third trimester of ± 2 weeks, which is consistent with data elsewhere [61, 63].

Overall, these data suggest that high quality estimates of the biometry of fetal structures can be obtained in low-income settings, but that the natural tendency for inter- and intra-observer variation to increase as a function of GA has the potential to introduce important differences in performance when such equations are presented for use as standalone tests of GA. Thus, the final equations must be validated as standalone screening interventions that consider the consequences of such varia-
7.8 Discussion

Validating the equations amongst samples with abnormal phenotypes will be an additional essential component of model validation. Whilst INTERGROWTH-21st provides persuasive evidence that the geographical dispersion of women has no impact upon the ‘potential’ growth of a fetus, the extent to which stressors within the maternal environment predispose to variants in the fetal and newborn phenotype are less well described. Therefore, validating the equation in groups at high risk of growth restriction will be important.

7.8.3 Conclusion and future work

In summary, this chapter presents two novel equations that use routinely collected US biometric data to extend the prediction of GA by US to 34 weeks’ gestation. This was done using multivariate polynomial equations to achieve a magnitude of error that is comparable to single parameter estimates, using HC or BPD, at GAs of \( \leq 22 \) weeks’ gestation. Hence, the new methods offer the potential to extend the range with which US can be used to provide clinically useful estimates of GA (up to at least 34 weeks’ gestation) and should be considered for further clinical evaluation.

Although the novel approach used the derive the equations warrants a cautious approach with respect to determining their clinical value, all of the presented validation for each equation has been undertaken according to the systematic methodologies that were used to validate the single parameter equations [238]. Furthermore, preliminary validation of the equations using an independently acquired dataset suggests that their predictive performance is stable, and thus, that the equations are unlikely to represent an overfitting of the dataset from which they were derived.

Whilst the evidence relating to operator variation provides an important framework to consider how differences in the quality of data collected for research purposes may differ from the standard of data available through routine clinical practice, such data should be used to infer the likely performance of the new equations. Perfor-
Performance testing requires prospective clinical validation. Many of the reported thresholds of ‘acceptable error’ are based upon clinical consensus and were not determined according to a scientific evidence based. Hence, whilst understanding how sonographer performance in the ‘field’ affects the performance of equations will be essential, data on variation alone can not be used to determine if such ‘differences’ are clinically important. This suggests, overall, that the only objective mechanism to test the utility of models formally would be through an interventional study and, ideally, a (cluster) randomised trial.

Throughout this thesis, it has been repeatedly emphasised that the evaluation of dating interventions for low-income settings must be based upon hypotheses that consider the very different nature of the clinical questions that are relevant to assessing GA in such settings. To this end, the reader is asked to consider the results from Chapter 5. Here, it was demonstrated clearly that the use of an US dating policy up to 24 weeks’ gestation was able to improve the specificity of diagnoses of PTB with potentially important clinical gains. The scope for these gains would be significantly enhanced if US were extended to 34+ weeks’ gestation.
Chapter 8

Third trimester dating: an automated method to estimate GA

8.1 Introduction

This chapter describes the development and evaluation of an Automated Dating Model (ADM) designed to estimate GA using a single 3D US volume file acquired from the fetal brain. ADM was designed using a random subsample of 158 image volume files selected from the INTERGROWTH-21st dataset, with GAs ranging between 126 and 238 days. A description of the background and rationale leading up to this work was provided in Chapter 7. The software was developed by imaging processing experts at the IBME. Prospective evaluation of model performance was then performed by the author of this thesis. A summary of the model development process is provided in the methods section before the prospective clinical validation and results are presented.
8.2 Methods

8.2.1 Model development: data standardisation

In the first instance, data captured during brain volume acquisition were preprocessed to standardise their temperospatial orientation. Since the fetus floats freely \textit{in utero}, the position at which individual structures within the brain were captured, with respect to their position on screen, was variable. As such, data relating to a single structure were present at different loci throughout the image file. Since data acquisition in a specific and reproducible temperospatial order was, and would remain, impractical, processes were developed to orientate and standardise volume files, such that data from specific regions of the brain could be localised to specific loci within datasets in a consistent and reproducible manner. To achieve this, software was developed for non-specialist users to align fixed points upon the bony skull using an onscreen virtual cap.

8.2.2 Model development: Random Forest Classifiers

At its core, ADM was designed using a branch of machine learning called Regression Forest Classifiers (RFC) [269, 270, 271]. Over many iterations, RFCs were used to assemble a classification system with the capacity to link the binary representation of the appearance the surface of the fetal cerebral cortex with GA, such that a volume of unknown GA could then be passed through the ADM to obtain an estimate of GA automatically.

In the first instance, the cortical surfaces of the brains were ‘parameterised’ to define a collection of ‘features’, or segments of data, which were used to build the ADM. Each ‘feature’ was defined by a cuboidal ‘volume of interest’ and was sampled at random to yield a collection of numerical descriptors to represent the shape, size and other ‘data-driven’, contextual characteristics of the cerebral cortex. A user specified number of ‘features’ were passed subsequently to the RFC classifier and
used to construct a decision tree to estimate GA. The structure of the tree was determined by a mathematical function that linked each feature to $GA_{\text{truth}}$ as a continuous variable using probability estimates. The probability that each feature’s association with GA was estimated in a stepwise fashion to generate a decision tree linking ‘features’ with GA. Thereafter, many RFC trees were developed using the same methods, which are collectively referred to as a ‘fores’. Finally, the best performing nodes from the forest were identified and combined to define a single tree whose structure represented those features best able to discriminate GA from the brain volume datasets. This final process was performed over multiple iterations using a fitness function to minimise the error between $GA_{\text{model}}$ and $GA_{\text{truth}}$.

For the ADM, a training cycle of 10 trees with a maximum depth of 15 feature levels was pre-specified and 500 candidate ‘features’ selected and evaluated. A full description of the technical aspects of this development are provided elsewhere [272]. Initial validation of model performance was performed using a bootstrap ‘leave $n$ out’ approach, whose results were used to iterate and refine ADM. The RMSE of prediction error for the automated model was ±4.12 days for the INTERGROWTH-21st dataset. This compares to the best RMSE for the final polynomial equation of ±4.7 days. All technical aspects of model development described in this subsection of the methods were implemented by Ana Namburete, DPhil candidate from the IBME.

8.2.3 Model validation: INTERBIO dataset

The INTERBIO1st validation dataset described in Section 7.6.3 on page 142 was used to prospectively validate performance of the automated model - see Section 7.6.2 on page 140. Data files were extracted and provided to Ana Namburete (DPhil Candidate) from the IBME. The structural regions within the cortex that were best able to discriminate GA amongst the validation dataset were identified and visualised using ‘hotspots’ that were superimposed upon US images. For each dataset, a predicted $GA_{\text{model}}$ was provided, and these were compared using a scatter and QQ plot. Estimates of the systematic difference and 95% limits of agreement were
also estimated and are illustrated using Bland Altman plots. All validation described in this subsection of the methods was performed by the author of this thesis.

8.3 Results

158/200 data volume files were deemed to be of sufficient quality to validate the model. At the time of reporting, this decision was based upon a subjective assessment of image quality. Figure 8.1 illustrates a sequence of 2D images extracted from the 3D volume structures from the training dataset. The ‘hotspot’ areas where change in structure, as determined by numerical features, are represented by red pixels. The thalamus and Sylvian fissure were consistently selected and are highlighted in red by the model. Whilst the data are presented in separate two week windows, the regression model provided by the software was able to provide GA as a continuous variable in the same way that the previous novel clinical models were able to.
**Figure 8.1:** Automated dating model - structural hotspots associated with GA that were identified using regression random forests. Th - thalamus, SF - Sylvian fissure.
Figure 8.2 shows the nomogram, centile estimates and QQ plots for the output from the model. From the QQ plot, it is clear that there are insufficient data-points to comment on the stability of the model. The 95% CI at 26, 30 and 34 weeks’ gestation were estimated at 8.8, 11.2 and 14.2 days, respectively. The systematic difference for the ADM compared to $\text{GA}_\text{truth}$ was estimated at 0.8 and the 95% LOA were -10.92 and 10.52 days, respectively.

8.4 Discussion

8.4.1 Principal findings

This chapter demonstrates that an automated model to estimate GA using data from a single 3D volume of the fetal head acquired during a single episode is practically feasible. Moreover, the model uses numerical ‘features’ that are associated with distinct structural regions of interest rather than providing estimates of GA that are determined by size alone, suggesting that the original hypothesis presented by this thesis may be valid.

8.4.2 Strengths and weaknesses

The model's selection of brain regions within the territory of the Sylvian fissure as important predictors of GA is biologically consistent with the observations from previous studies. From 14 weeks’ gestation onwards, the Sylvian fissure develops according to a well described temporal sequence [156, 157, 159, 273, 274]. In fact, various groups have attempted to quantify this process, referred to as the ‘operculization of the Sylvian fissure’, and to correlate these findings with GA. Various scoring systems have been presented that associate the findings of such classifications to GA. Using 2D images slices, groups have measured the distance between the midpoint of the Sylvian fissure and the parietal bone [275, 276], and have used the angle between the insular and temporal lobes to assign a score that reflects the
8.4 Discussion

level of maturation of the regions [157, 277]. Such systems depend upon subjectively determined operator assigned scores, and are thus subject to significant variation and overlap when appearances are correlated with GA [277]. Despite using the same reference standard for GA (CRL before 14 weeks’ gestation), and despite using similar morphological grading systems, the system presented by Quarello and colleagues differs markedly from that of Pistorius in terms of overall correlation with GA. As such, it seemed unrealistic to consider developing a parallel approach to the automated model that was based upon user determined structural assessments of the region.

The need to validate the automated model using a reference standard was the primary motive underlying the development of the equations presented in the previous chapter: both to provide a reference standard against which the model could be evaluated, but also to provide a ‘back-up’ supporting method for estimating GA where head volume acquisition was not feasible, or where there was suspicion of model error in clinical situations where no alternative dating methods were available.

8.4.3 Conclusion and future work

This study was initially conceived on the basis of a consensus of opinion amongst neuropathologists that, for a given GA, it is feasible to determine whether a brain is ‘normal’ or ‘abnormal’ based upon the morphology of the cerebral cortex. The findings of this work have demonstrated that it is feasible to reverse this hypothesis and use the appearance of the cerebral cortex within a normally developing brain to estimate GA. Future work is in progress to extend the predictive range of the model and to validate the data on a larger clinical dataset.

Many of the discussion points from the previous chapter also apply to this model with respect to clinical validation in low-income settings. Compared to the ‘novel clinical model’, the automated model offers a number of potential advantages. Specifically, the ability to provide automated GA with minimal training with respect to image ac-
quisition is particularly relevant to low-income settings. As discussed in Chapter 2 on page 22, US remains unregulated in many low-income settings and the availability of trained sonographers is limited. Thus, an automated approach has many potential benefits with respect to delivering a dating service.
8.4 Discussion

Figure 8.2: Models - automated brain model: Nomograms with centiles (3rd, 50th and 97th) and QQ-plots. $G_{\text{truth}} = 126-238$. 
Figure 8.3: Bland Altman plot of the predictive performance of the automated 3D volume prediction model for GA. Ground truth = LMP/ CRL based GA estimates as per INTERGROWTH-21st.
Chapter 9

Conclusion

The objective of this thesis was to determine if GA estimation could be improved in a low-income setting. The work undertaken to investigate this has established a perinatal surveillance platform at KWTRP. The extensive background work undertaken to inform the design and implementation of this system has enabled data collection for research purposes to be integrated into routine clinical services within the MCH clinic and maternity department.

A discussion of the strengths and weaknesses related to each chapter of results was provided with the conclusions of the relevant chapter. This closing chapter of the thesis presents the principal findings of the research and outlines possibilities for future research.

9.1 Principal findings

The principal findings from this thesis are summarised as follows:

1. Clinical estimates of GA by LMP-GA or C-GA significantly overestimate the rate of PTB compared to US-GA for women attending the MCH clinic in KDH before 24 weeks of gestation. Although the PTB rate was lower using US-GA, the perinatal mortality risk was higher, suggesting that US-GA is more specific
and therefore that the PTB rate by US-GA is more accurate than by LMP-GA or C-GA.

2. The implementation of a classification system to characterise sub-phenotypes of PTBs is feasible. Analyses suggest that SGA phenotypes explain a greater proportion of PTBs than infection. Biological samples are being collected for processing through a biobank and are likely to provide more specific case ascertainment.

3. Data driven cluster analyses using t-SNE provides a feasible methodology for investigating PTB phenotypes. t-SNE provides the potential to modify expert driven ontologies and to inform hypothesis generation towards describing new PTB sub-phenotypes.

4. Estimating GA during the third trimester of pregnancy:

(a) New polynomial equations can estimate GA using standard US biometric data up to 34 weeks’ gestation. The prediction error at 34 weeks’ gestation (12.4 days) is equivalent to estimates by HC at ≤22 weeks [52]. These findings were confirmed by first-line tests using an independent validation dataset.

(b) A fully automated machine learning algorithm can predict GA between 18 and 34 weeks’ gestation with an accuracy of 14.2 days at 34 weeks’ gestation. First line clinical validation using the same independent dataset suggests that such findings are reproducible. However, preliminary plots suggest that more data are required to achieve stable predictions from the model.

Collaborations from the work include an ongoing partnership with the Institute of Biomedical Engineering at the University of Oxford to develop a standalone software package that is able to provide a fully automated estimate of GA based upon a single US 3D volume file of the fetal brain. The qualitative work in Kilifi has also been developed to examine the factors the influence the uptake of ANC amongst women in Kilifi.
9.2 Summary and discussion

A more precise understanding of the relationship between pregnancy dating methods, preterm birth rates and associated newborn clinical outcomes in high risk, low-income settings is a pre-requisite for future research to address the excess of perinatal mortality in such settings. As a consequence of huge data gaps with respect to data assessing GA amongst hard-to-reach populations, there have been numerous calls to establish an evidence base to guide the debate towards an international dating policy. This work presents one of the first studies from sub-Saharan Africa (outside South Africa) to directly assess the relationship between methods used to estimate GA and clinical outcomes.

The study adopted a prospective, observational design and invited all eligible women attending the MCH clinic at KDH to participate. Sample sizes were determined *a priori* to provide adequate statistical power to detect the observed outcomes. A particular strength of the work comes from the linked population surveillance. KHDSS provided the capacity for active follow up of women in a logistically challenging environment and so enabled birth outcomes to be reported across the population. This is unusual in sSA where the absence of vital registration systems can be a significant source of bias amongst studies by limiting investigators to reporting directly observed findings from institutional settings. Despite all attempts to recruit participants using an ‘inclusive’ selection policy that offered US to all women \( \leq 24 \) weeks, the nature of the operating environment was such that 1107/1827 (61%) eligible women were scanned and recruited.

A large component of the research was the establishment of a perinatal surveillance network. The study was committed to implementing a research policy with community integration and sustainability at its core. The recruitment and training of local Government healthcare employees at all stages of the research cycle represented a strategic shift from previous work at KWTRP, but was considered essential to build the foundations of a long-term, and sustainable, perinatal health research
programme. As a result, several Kenyan clinical and research staff have been able to secure higher academic and clinical training as a direct consequence of their involvement with this research.

Overall, the work represents one of the largest birth cohort studies from SSA. A number of factors must be considered in terms of the extent to which the findings can be generalised to other settings in Kenya and elsewhere. To this end, it must be acknowledged, that whilst the operating environment at KWTRP provides a platform that is essential for conducting high quality research amongst hard-to-reach populations, the longstanding interaction between KWTRP and the community cannot be considered to be typical of the relationship that exists between healthcare providers and the wider community elsewhere in Kenya.

KWTRP was established to facilitate the conduct of high quality epidemiological research and clinical trials. Research staff and the wider community are, therefore, very familiar with the ‘research process’ and with the consequences that being involved in research may or may not have for individuals, households and the clinical care provided at KDH. The unique nature of this relationship means that the behaviour amongst women participating in this study was potentially altered as a consequence of their involvement in the research. This introduces as a possibility that the observed outcomes were influenced by the ‘Trial effect’ [278]. The trial effect is related to the Hawthorne Effect [201] and is not unique to Kilifi. However, the magnitude of the difference in the standard of care that is provided as a consequence of the research programme at KDH compared to the standard available elsewhere in Kenya is considerably greater than in high-income settings.

Nevertheless, the infrastructure provided by KWTRP was an essential requirement for the first-line hypothesis testing undertaken during this research. KWTRP enabled the research to recruit women from a routine clinical population whilst at the same time providing essential safety and risk management processes to manage the introduction of a new clinical service introduced primarily for research purposes.

With respect to the study’s findings, although a Trial effect may have introduced bias
in to the rates of PTB observed amongst the study group, this was unlikely to have altered the ability of the study to detect differences in PTB rates, which was the study’s primary aim.

The tendency amongst women in sSA to present for ANC >24 weeks’ gestation represents a significant limitation with respect to estimating GA using US. In this study, 50% of women attended >24 week threshold for US and thus were excluded from the study. The final two chapters of the thesis examined hypotheses related to the extension of US based GA estimation into the third trimester. The consensus of opinion amongst clinicians in Kilifi and the UK was that the capacity to estimate GA ≤34 weeks was likely to enhance care significantly by guiding the appropriate administration of corticosteroids to manage the risks associated with PTB.

As discussed in Chapters 2 and 7, the literature examining third trimester dating models focused upon linear regression analyses of routinely available biometric parameters. With evidence that biometry varies according to a curvilinear relationship with GA, the hypothesis was presented that multivariate polynomial equations offered the potential to provide a more precise fit of the data.

A cross sectional data sample was obtained from the INTERGROWTH-21st dataset. A novel approach using a machine learning algorithm was applied to build a series of candidate equations, which were subsequently evaluated according to the methodology recommended for developing new GA standards. The main advantage offered by the Genetic Algorithm was the capacity to adapt the structure of models autonomously such that an entire search-space was explored with minimal dependence upon user input. For each equation presented in Table 7.2, the Genetic Algorithm compared up to 64,000 variants over 500 generations.

The algorithm presented a series of candidate equations and selected two as the final variants for testing. The results of a clinical validation using an independent dataset suggest that the performance of models is stable and hence that the derived equations are unlikely to represent an overfitting of the training dataset. Whilst there
was minimal selection of participants comprising the initial validation dataset, there were only four PTBs and one case of fetal SGA.

Finally, the concluding body of work has developed a preliminary model with the capacity to estimate GA using structural biomarkers from the fetal brain. The primary objective for this section was to provide a solution to estimate GA with minimal sonographer training. Whist the equations provided a basis for testing in future work, their dependence upon the availability of skilled sonographers was considered a potential barrier to their implementation. Since formal training in US and the regulation of its service provision are not common-place in low-income settings, equations that are dependent upon expert sonographers to acquire data may not be feasible in all settings.

As part of a collaboration with an Image Processing DPhil candidate, an automated model was developed with the capacity to predict GA using a single 3D US volume of the fetal head. In its current form, the software comprises a multi-program pipeline that requires significant user input. Based upon results from preliminary testing using the validation dataset acquired by INTERBIO-21[^1], the model offers GA at 30 weeks with a prediction error of 11.2 days. However, the distribution of model outcomes from a QQ plot suggests that further training data are required. Since equation 2.9 (Table 7.2) suggests that a stable predictive model can predict GA at 34 weeks with an error of 12.4 days, it is reasonable that such a similar error threshold should be the target for the automated model.

### 9.3 Future work

The findings presented by Chapters 7 and 8 have validated hypotheses related to the estimation of GA in the third trimester. Both methods require extensive clinical validation to determine their performance in the field and amongst groups comprising ‘high-risk’ clinical phenotypes. With respect to the novel equations, the discussion in Chapter 7 on Page 171 outlined a number of important differences between
the INTERGROWTH-21$^{st}$ sample used to develop the model and the clinical field in low-income settings for which such equations presented. To this end a study is currently being designed to examine the performance of the novel dating equations and the automated model amongst a group of SGA newborns recruited by INTERGROWTH-21$^{st}$. It is envisaged that the study will adopt a case control design to ensure adequate representation of high risk groups within the dataset and use analytical methods (Bland Altman plots, systematic differences and LOA) adopted when examining test performance during the first line validation that has been presented.

In the first instance, testing should be performed in a setting such as that provided by KWTRP, where adequate clinical support is available and where the level of risk can be managed in an appropriately resourced environment. If first-line testing demonstrates that similar findings can be reproduced at the ‘front-line’ of clinical services, the next stage of testing would be in the form of a randomised controlled trial. In a setting such as Kilifi, a cluster design would be meet many of the requirements in terms of experimental design and can be used to mitigate potential sources of bias, such as those associated with trial effects.

9.4 Conclusion

It is my belief that this thesis provides a new perspective towards assessing GA and the epidemiology of PTB in low-income settings. The findings from this thesis are important because they should inform not only policies relating to future pregnancy dating and resource utilisation but also how public health, epidemiological and interventional research should be conducted in the future. If the diagnosis of PTB is inaccurate and case definitions non-specific, efforts to understand better the causes underlying PTB and to develop and test interventions to reduce PTB rates in high burden settings will prove to be an even greater challenge than they already are.
Appendices
Appendix A

A.1 Systematic review: data extraction form and extracted data
A.1 Systematic review: data extraction form and extracted data

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**Setting:** University Hospital, Cairo, Egypt  
**Design:** Prospective, cohort study  
**Objective:** Detection of IUGR using antenatal ultrasound | Infants delivered with confirmed growth restriction                                       | US evaluation able to predict 89.7% IUGR cases v 34.7% fundal height palpation.       | No assessment of the value of the diagnosis to the managements of its antenatal population. |
| Nzeh et al; 1992 | Objective: Compare the measured diameter of the internal cervical os in women with and without cervical incompetence | Mean diameter (+95% confidence intervals) of internal os in cases and controls         | Statistically significant difference between case and control group internal os diameter |                                                                     |
| Theron et al;1992 | Evaluation of Umbilical Doppler waveform and resistance indices in the prediction of IUGR amongst small for gestational height pregnancies  
**Setting:** MRC Perinatal Research Unit, University of Stellenbosch, South Africa.  
**Design:** Prospective cohort study | Antenatal complications (mild, moderate or severe)  
Mode of delivery  
APGAR at 5 mins  
Perinatal death | Association between absent end-diastolic flow and –  
IUGR Kappa Index -0.02  
Perinatal mortality Kappa Index 0.50 | Umbilical arterial doppler waveforms are not a good screening test for IUGR or SGA.       |
| Dissanayake et al; 1993 | Objective: Comparison of sono graphic diagnosis with discharge diagnosis in cases of suspected ectopic pregnancy  
**Setting:** University of Nairobi, Kenya  
**Design:** Retrospective cohort study | Clinical and sonographic findings in women in whom there was a clinical suspicion of ectopic pregnancy | Clinical suspicion of ectopic pregnancy in 127 patients. Ultrasound excluded this in 96 cases. 15/127 cases were diagnosed with ectopic pregnancy at surgery. | Sonography useful in the evaluation of women with potential ectopic pregnancy |
| Geerts et al, 1996 | Comparison of routine v selected ultrasound at 18 - 24 weeks of gestation  
**Setting:** Urban, Tertiary Centre, South Africa  
**Design:** Prospective, randomised controlled trial  
**Objective:**  
1. Perinatal mortality  
2. Admissions to Neonatal Intensive Care  
3. Admission to neonatal wards > 2days  
Scanner: Fixed machine  
Operator: Local doctor  
N = 988 women randomized | Increase in low birth weight babies in intervention group  
(OR 1.73; 95% CI 1.15-2.63; p=0.01)  
Perinatal mortality: No significant difference (OR 0.70; 95% CI 0.19-2.47) | No difference for selective v routine US:  
- perinatal mortality  
- adverse outcome  
Cost savings for selective scans. |
<table>
<thead>
<tr>
<th>Study</th>
<th>Setting</th>
<th>Design</th>
<th>Objective</th>
<th>Data extraction form</th>
<th>Extracted data</th>
<th>Case definitions used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seffah et al; 1999</td>
<td>Teaching Hospital, Accra, Ghana</td>
<td>Retrospective comparative study</td>
<td>Determine whether a single measurement of AFI could be used to screen for adverse birth outcomes late in pregnancy</td>
<td>Scanner: Fixed machine Operator: Not stated</td>
<td>Amniotic fluid index and rate of induction, caesarean section and fetal distress</td>
<td>AFI &lt;5 associated with worse perinatal outcome. No case definitions used.</td>
</tr>
<tr>
<td>Steinmetz et al, 1999</td>
<td>Rural Private Hospital, Cameroon</td>
<td>Prospective cohort, with intervention</td>
<td>Comparison of clinical diagnosis vs ultrasound diagnosis in selected cases.</td>
<td>Scanner: Fixed Operator: Trained research doctor, non – local N = 323 scans performed</td>
<td>Agreement between clinical and US diagnoses Number of diagnoses aided by US.</td>
<td>Ultrasound ‘useful’ in 68% of cases No evaluation of patient oriented outcomes.</td>
</tr>
<tr>
<td>Geerts et al, 2004.</td>
<td>Two community midwifery units, South Africa</td>
<td>Prospective trial.</td>
<td>Investigate the impact of ultrasound dating on current obstetric services. Routine ultrasound (for dating, placental position, identification of multiple gestation)</td>
<td>Scanner: Fixed machine Operator: Local doctor N = 3009 (recruited). Data available for 311 in routine US group and 349 in standard care group.</td>
<td>Clinic workload (number of visits and referrals made): Number of pre/ post-term deliveries, Number of elective deliveries Number of small for gestational age babies.</td>
<td>Discrepancy in gestation age of ≥3 weeks for 28.4% of cases dated from menses and 29.8% of cases dated clinically. Reduced referrals to regional referral clinics 15.6% v 29.6% (p&lt;.0001). Increase in term deliveries 79.9 v 72.5 % (p&lt;0.001) No difference in maternal or perinatal outcomes. Community based obstetric sonography reduces the need to refer on to regional centres.</td>
</tr>
<tr>
<td>Study</td>
<td>Setting</td>
<td>Design</td>
<td>Objective</td>
<td>Methods</td>
<td>Findings</td>
<td></td>
</tr>
<tr>
<td>------------------------------</td>
<td>--------------------------------------------</td>
<td>---------------------------------</td>
<td>---------------------------------------------------------------------------</td>
<td>-------------------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Lamina et al, 2004</td>
<td>Awolowo College of Health Science, University of Sagamu</td>
<td>Retrospective study</td>
<td>Assess the role of routine ultrasound scanning for all pregnant women</td>
<td>Scanner: Fixed machine Operator: Not stated</td>
<td>Positive findings in 34 (5.6%) of unselected group vs 294 (75%) of selected scans. Ultrasound should be performed for clear obstetric reasons.</td>
<td></td>
</tr>
<tr>
<td>Shittu et al, 2007</td>
<td>Obafemi Awolowo University Teaching Hospital, Osun State, Nigeria</td>
<td>Prospective cohort study</td>
<td>Evaluation of clinical vs ultrasound estimation of fetal weight at full term delivery</td>
<td>Scanner: Static machine Operator: Specialist physician N = 100</td>
<td>Clinical and Sonographic estimation of fetal weight within 24 hours of delivery compared to actual fetal weight. Mean absolute percentage error for &lt;2500g = 12.6 ± 11.7 percent for US v 16.1 ± 14.6 for clinical methods. Mean absolute percentage error for &gt;4000g = -4.3 ± 6.9 for US v 6.5 ± 7.2 for clinical methods. Both clinical and ultrasonographic methods overestimate birthweight for infants delivered &lt;2500g.</td>
<td></td>
</tr>
<tr>
<td>Study</td>
<td>Setting</td>
<td>Design</td>
<td>Intervention</td>
<td>Data extraction form and extracted data</td>
<td></td>
<td></td>
</tr>
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<td>-------------------------</td>
<td>-------------------------------------------------------------------------</td>
<td>----------------------------------------------------</td>
<td>--------------------------------------------------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------------------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spencer, 2008</td>
<td>2 primary care centres and 2 hospitals in Ghana</td>
<td>Prospective cohort study, with intervention</td>
<td>Ultrasound performed according to need for diagnostic imaging (selection by single Radiologist).</td>
<td>Descriptive account of use of ultrasound. No assessment of intervention.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Van Dyk et al; 2008</td>
<td>Distric and regional referral hospital in Western Gauteng, South Africa</td>
<td>Open cluster, randomised controlled trial</td>
<td>Comparison of a routine single second trimester ultrasound scan to standard antenatal care offering selected scanning.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neufeld, 2009</td>
<td>Health centres in Rural Bangladesh</td>
<td>Prospective cohort study, with intervention</td>
<td>Delivery of US training in district health centres. 6 week US training course.</td>
<td>Agreement in biometric measurements, operator unblinded to measurements. Intraobserver error 1.8%.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rijken et al; 2009</td>
<td>Refugee camp on Thai-Burmese border</td>
<td>Prospective cohort study, with intervention</td>
<td>Objective: 3 month practical and theoretical training course for local workers (educated to 16 years).</td>
<td>Agreement in biometric measurements between local healthcare workers and a European trained obstetrician.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| Kimberley et al; 2010 | **Objective:** Teach obstetric ultrasound to a cohort of midwives.  
Scanner: Portable machine.  
Operator: Local midwives  
N = 21 | OSCE scores at 2 and 6 months after training.  
Agreement between midwife and physician for:  
Fetal heart rate measurement  
Placental localization  
Fetal biometry (AC and FL) | Midwives were able to acquire skills required to perform US.  
At one-year follow up, each midwife was performing 10 scans per week. |
A.2 Methods: patient information leaflet
How safe is the procedure?

- Unlike x-rays, ultrasound does not use radiation and has been used safely for over 40 years to provide images during pregnancy.

What are the benefits of the scan?

- Knowing how many weeks pregnant you are will help you plan the pregnancy and provides doctors and nurses with important information if further care is needed later on in pregnancy.

- An ultrasound scan can also show if you are expecting twins and if the afterbirth (placenta) is likely to cause any problems.

- The results will be interpreted by medical staff who will advise you if any extra care is needed in your pregnancy.

- You will have the opportunity to see your baby on the screen if you want to.

IMPORTANT HEALTH MESSAGES

- Please remember to come for all of your subsequent antenatal clinic appointments.

- It is also important to go to hospital for delivery irrespective of your scan results.

- It is advisable that you visit a qualified clinician for any check up or procedure relating to your pregnancy.

What is I have any questions?

- If you have any questions, please ask the KEMRI staff or hospital staff in the MCH department

OR

- Contact a member of the study team on 0705 206 152
What is KEMRI
• KEMRI is a national research organisation whose work is to find better ways of preventing and treating illness for the benefit of everyone.
• Kilifi District Hospital and KEMRI work together.

What is this study about?
• KEMRI is conducting research here in Kilifi District Hospital antenatal clinic to learn more about how babies grow during typical pregnancies.
• This is being done in order to improve maternal health services here and in other parts of Kenya and the rest of the world.

Who is invited to participate in the study?
• All women attending for antenatal care at Kilifi District Hospital at less than 24 weeks of pregnancy (about 6 months) will be invited.
• Participation in research is voluntary.

What happens next if you are eligible?
• All women will be offered routine care whether or not they participate in this study

What is an ultrasound scan?
• An ultrasound scan is a harmless way of producing pictures of a baby and the afterbirth (placenta) inside the womb.

What is an ultrasound scan?
• As part of the preparation, the doctor will apply a liquid called the ultrasound gel on your stomach.
• This gel has no medicinal value and is harmless.
• The gel helps the machine and reduces friction against the body during scanning.
A.3 Consent form: ultrasound and KIPMAT
Ultrasound Patient Information Leaflet Content and Consent Form

Joint KEMRI / MoH Research into Risk Factors Associated With Fetal Growth in Kilifi District Hospital

A.1 What is an ultrasound scan?

Ultrasound is a harmless way of producing pictures of a baby and the afterbirth (placenta) inside the womb. Unlike x-rays, ultrasound does not use radiation and has been used safely for over 40 years to provide images during pregnancy.

A.2 What is this study about?

This research is part of an international programme that has been running for 18 months in parts of Kenya and the rest of the world.

A.3 Consent form: ultrasound and KIPMAT 204

Your antenatal care is being provided at Kilifi District Hospital (KDH). As part of routine care, nursing staff will measure your blood pressure, test your urine and recommend that you have blood tests. In addition to these routine checks, we are asking your permission to be part of a study being conducted by KEMRI that wants to learn more about babies' growth during pregnancy.

A.4 What is KEMRI?

Kilifi District Hospital and KEMRI work together. KEMRI is a national research organization whose work is to find better ways of preventing and treating illness for the benefit of everyone.

A.5 What is this study about?

KEMRI is conducting research in KDH antenatal clinic to learn more about the growth of babies during typical pregnancies in Kilifi in order to improve maternal health services here and in other parts of Kenya and the rest of the world.

A.6 What is an ultrasound scan?

An ultrasound scan is a harmless way of producing pictures of a baby and the afterbirth (placenta) inside the womb. Unlike x-rays, ultrasound does not use radiation and has been used safely for over 40 years to provide images during pregnancy.

Before 24 weeks, an ultrasound scan allows us to be more certain about how many weeks pregnant you are. Later ultrasound scans can look to see how well the baby is growing. Where there is a problem seen with a baby, the ultrasound scan may alert doctors to this before it would otherwise be known about. An ultrasound scan does not affect a baby in the womb and does not cause problems with a baby's development and growth.

A.7 It is important to remember that:

- Ultrasound scans can identify some but not all problems with babies in the womb, so it is still possible that babies can have problems after birth even if their ultrasound picture looked normal before birth.
- Ultrasound scans are a form of 'test' and not a treatment.

A.8 Will it involve for my baby and me if I agree to participate?

1. First, you will have an ultrasound picture of your stomach during this visit to check how many weeks pregnant you are. If you are more than 24 weeks pregnant (about 6 months), an ultrasound picture cannot tell you how many weeks pregnant you are. If this is the case, your due date will depend on the dates since your last period. The rest of your pregnancy will be looked after by the team at KDH, which is supported by its partnership with KEMRI.

A.9 Are there any risks or disadvantages to me taking part?

- Coming back to KEMRI for ultrasound scans during your pregnancy will take your time, but we will reimburse all costs of transport for these visits.

A.10 Are there any benefits to me by taking part?

- We will gather information about how your baby is growing, so that we can understand what might be happening in more detail, and how best to help you.
- We will do a second ultrasound scan at the end of your pregnancy to check that your baby has grown correctly.
- We may do this ultrasound before you or your doctors are aware of it. It is important to know before birth if there are any problems with the baby, so that medical advice and treatment can be given at the same time.

A.11 Are there any benefits to me by taking part?

- We will gather information about how your baby is growing, so that we can understand what might be happening in more detail, and how best to help you.
- We will do a second ultrasound scan at the end of your pregnancy to check that your baby has grown correctly.
- We may do this ultrasound before you or your doctors are aware of it. It is important to know before birth if there are any problems with the baby, so that medical advice and treatment can be given at the same time.

A.12 Are there any benefits to me by taking part?

- We will gather information about how your baby is growing, so that we can understand what might be happening in more detail, and how best to help you.
- We will do a second ultrasound scan at the end of your pregnancy to check that your baby has grown correctly.
- We may do this ultrasound before you or your doctors are aware of it. It is important to know before birth if there are any problems with the baby, so that medical advice and treatment can be given at the same time.
Participation in the research is voluntary. Deciding not to participate will not affect your health care now or in the future. If you do agree now, you can change your mind at any time in the future. Contact details are provided on your copy of this form to enable you to withdraw from the study in the future.

Who will have access to information about me/my child in this research?
All our research records are stored securely in locked cabinets and password protected computers. Only a few people who are closely concerned with the research will be able to view information from mothers.

Who has allowed this research to take place?
An independent national committee and a committee in Kilifi have looked carefully at this work and agreed that the research is important, that it will be conducted properly, and that mothers’ safety and rights have been respected.

Will I have to pay for any of the tests?
All ultrasound scans provided as part of this research are free of charge. You will also be reimbursed for the cost of travel when returning to the hospital for these scans. If the doctors at KDH later advise that you have a scan for reasons during your pregnancy that are not part of this research, charges may be made according to hospital policy. It is important to understand that the cost of having your baby in hospital cannot be reimbursed by KEMRI.

Will I have to fast before the ultrasound scan?
You will not have to fast before the ultrasound scan.

What if I have any questions?
You may ask any of our staff questions at any time. You will be given a copy of the information contained on this form to take away with you. You can also contact those who are responsible for the care of you and your child and this research:

PI’s name(s) and contacts
Dr. Bryn Kemp KEMRI - Wellcome Trust
P.O.Box. 230, Kenya. Telephone: 0715 052318

If you want to ask someone independent anything about this research please contact Community Liaison Manager, KEMRI – Wellcome Trust
P.O.Box 230, Kilifi. Telephone: 072342780 or 041 7522083

Or
The Secretary, - KEMRI/National Ethics Review Committee
P. O. BOX 54840
Nairobi, Tel number: 020 272 2541 Mobile: 0722209901 or 0733400003

A witness is a person who is independent from the trial or a member of staff who was not involved in gaining the consent.

Subject’s signature: ___________________________ Date _____________
Subject’s name: _____________________________ Time ___________

I certify that I have followed the study SOP to obtain consent from the participant. She apparently understood the nature and the purpose of the study and consents to participation in the study. She has been given opportunity to ask questions which have been answered satisfactorily.

Designee/investigator’s signature: _______________________ Date ____________
Designee/investigator’s name: ________________________ Time ______

I attest that the information concerning this research was accurately explained to and apparently understood by the subject and that informed consent was freely given by the participant.

Witness’ signature: ______________________ Date ____________
Witness’ name: ___________________________ Time _____________

*Thumbprint of the subject as named above if they cannot write:    

THE PARTICIPANT SHOULD NOW BE GIVEN A SIGNED COPY TO KEEP.
Admission Record – Kilifi District Hospital Maternity Unit

1. Mother’s admission details (midwife)

- Mother’s Name _______________________
- Mother’s Date of Birth ___/___/______
- Date of birth _______________________
- Place of delivery: Hospital ( ), Clinic ( ), Home/dwelling ( ), Other ( )
- Type of birth: Vaginal ( ), Cesarean section (C/S), Other ( )
- Date of delivery ___/___/______
- Time of delivery AM / PM
- Does the patient meet any of the following criteria? Y N
  - Abnormality not patent
  - Haemorrhage > 300 ml
  - Syphilis RPR > 1:8
  - Discharge > 300 ml
  - Anaemia ≥ 100 g/l
  - Unconscious or alert only to pain?
  - Other: _______________________
- Does the patient require emergency intervention? Y N
-/admission details

2. Household / social history (field-worker)

- Number of people live in the house: 1 2 3 4 5
- Type of house mother lives in: Own house ( ), Rental house ( ), Other ( )
- What is the main water source? Tap in house or compound ( ), Tap in community ( ), Borehole in compound ( ), Borehole in community ( ), Water vendor ( ), Other ( )
- Type of latrine household uses: flush toilet ( ), Pit latrine ( ), Other ( )
- Main source of fuel household uses for cooking: Wood ( ), charcoal ( ), Gas ( ), Other ( )
- How many cattle does the household look after? 1 2 3 4 5
- What is the average household monthly cash income? KES 1,000 - 2,999, KES 3,000 - 4,999, KES 5,000 - 6,999, KES 7,000 - 8,999, KES 10,000 - 14,999, Other ( )
- Description of the mother: _______________________
- Occupation of the father: _______________________
- Has the mother during pregnancy looked after cattle? Y N
- Does the father routinely look after cattle? Y N
- Marital status: Married ( ), Single ( ), Other ( )
- Educational level: None ( ), Prim ( ), Sec ( ), Higher ( )
- Occupation of the father: _______________________
- Occupation of the mother: _______________________
- Main source of fuel household uses for cooking: _______________________
- Does the father routinely look after cattle? Y N

3. Consent for treatment (midwife)

If the case merit urgent treatment and for any medical or surgical treatment which the doctor may consider necessary to be performed upon my/ my wife/my child... Nakubali dawa ya kupoteza shaharani kuu na pia daktari wa kufanya utibabu wao kama umeme wako.

- Date / _______________________
- Signature / Seini _______________________
- Witness / Guardian: _______________________
- Student Midwife: _______________________

4. Admission by (midwife/clinician)

- UAP: Known ( ), Not Known ( ), Month only known ( ), UAP Date ___/___/______
- EOB by date ( ): ___/___/______
- EOB by ultrasound (If dating scan was done at <24 weeks): ___/___/______

5. Antenatal History at admission (midwife)

- Total number of pregnancies (last current): 1 2 3 4 5
- No. of pregn. >28 weeks: 1 2 3 4 5
- No. of pregns >35 weeks: 1 2 3 4 5
- Miscarriage: 1 2 3 4 5
- Early pregnancy termination: 1 2 3 4 5
- Mode of delivery: Hospital ( ), Home/bathing ( ), Other ( )
- Mode of delivery: Vaginal ( ), Cesarean section (C/S), Other ( )
- Complications: None ( ), Pre-eclampsia ( ), Pre-eclampsia w/haemorrhage ( ), Pre-eclampsia w/o haemorrhage ( ), High blood pressure ( ), Polyhydramnios ( ), Other ( )
- Delivery date (MM/YY/TY): ___/___/______
- Place of delivery: Hospital ( ), Clinic ( ), Home/dwelling ( ), Other ( )
- Mode of delivery: Vaginal ( ), Cesarean section (C/S), Other ( )
- Baby alive: Y N
- If NO: complete table below.
Mother’s Name _______________________ Mother’s Date of Birth ___/___/______  No. ANC attendances this pregnancy? ____________

PMTCT

Observations

Viral load (VL)

Date of result

Blood pressure (BP)

Date of result

Venous blood samples

Date of result

Full blood count (FBC)

Date of result

Medical problems

Before pregnancy

During this pregnancy

Previous delivery

Examination:

Admitted on day of admission: _______ days _______ hours

Examination:

Admitted by (initials) ____________

7. Routine investigations on admission (clinician/nurse)

Mother’s Name _______________________ Mother’s Date of Birth ___/___/______  No. ANC attendances this pregnancy? ____________

PMTCT

Observations

Viral load (VL)

Date of result

Blood pressure (BP)

Date of result

Venous blood samples

Date of result

Full blood count (FBC)

Date of result

Medical problems

Before pregnancy

During this pregnancy

Previous delivery

Examination:

Admitted on day of admission: _______ days _______ hours

Examination:

Admitted by (initials) ____________

7. Routine investigations on admission (clinician/nurse)

Mother’s Name _______________________ Mother’s Date of Birth ___/___/______  No. ANC attendances this pregnancy? ____________

PMTCT

Observations

Viral load (VL)

Date of result

Blood pressure (BP)

Date of result

Venous blood samples

Date of result

Full blood count (FBC)

Date of result

Medical problems

Before pregnancy

During this pregnancy

Previous delivery

Examination:

Admitted on day of admission: _______ days _______ hours

Examination:

Admitted by (initials) ____________

7. Routine investigations on admission (clinician/nurse)
10. Partogram (midwife)

11. Delivery details (midwife/clinician)

<table>
<thead>
<tr>
<th>Delivery</th>
<th>Y</th>
<th>N</th>
<th>BBA</th>
<th>If No, go to section 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Client of labour?</td>
<td>No Labour</td>
<td>Spontaneous</td>
<td>Induced</td>
<td>Induced Caesarean</td>
</tr>
<tr>
<td>PROM (&gt;=18hrs)?</td>
<td>Y</td>
<td>N</td>
<td>If Y, go to section 14</td>
<td></td>
</tr>
<tr>
<td>Antibiotic before delivery?</td>
<td>IV</td>
<td>Oral</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>Fetal bradycardia (&lt;110) for 3 mins</td>
<td>Y</td>
<td>N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fetal tachycardia (&gt;180) for 3 mins</td>
<td>Y</td>
<td>N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thick meconium?</td>
<td>Y</td>
<td>N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Offensive amniotic fluid?</td>
<td>Y</td>
<td>N</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| Mode of delivery | Vaginal 
  cephalic | Vaginal 
  breech | Ventouse |
| Induction of Labour: | Caesarean Section |
| Post term | IUGR | Poor progress | Prev CS |
| PROM | APH | Multiple preg | Fet. distress |
| PET | IUPO | Cord prolapse | Plac. Previa |
| Other fetal | Malpresent | Breach |
| Other mat | PET | Failed induction |
| Indication if induction of labour? | CS |
| Duration 2nd stage | Min |
| Placenta Complete | Y | N |
| Episiotomy | Y | N |
| 3rd Stage | Min |
| Delivery attended by (tick all that apply): | Stud nurse | Nurse | COI | COC | MOI | MOC | Obstet |

Completed by (initials) | Date |
Mother’s Name _______________________  Mother’s Date of Birth ___/___/______  Completed)by)(initials)  Date) /)))))))))/) ) )

### 15. Mother Outcome (fieldworker)

<table>
<thead>
<tr>
<th>Date of Actual Discharge</th>
<th>/</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delivery</td>
<td></td>
</tr>
<tr>
<td>No □</td>
<td>Yes – KDH □</td>
</tr>
<tr>
<td>Discharged to Maternal Shelter □</td>
<td>Abducted □</td>
</tr>
<tr>
<td>Transferred other hospital □</td>
<td>Transferred other ward KDH □</td>
</tr>
<tr>
<td>Maternal weight at discharge</td>
<td>kg</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### Mother final outcome

- Discharged  ☐  Home  ☐
- Transferred other hospital  ☐
- Transferred other ward KDH  ☐
- Died  ☐

Completed by (initials)  Date  / /

### 16. Baby outcome (fieldworker)

<table>
<thead>
<tr>
<th>Baby name (if known)</th>
<th>/</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baby name (if known)</td>
<td></td>
</tr>
</tbody>
</table>

#### Baby outcome

- S = stillborn, BD = born alive who died in maternity, T = transferred, D = discharged alive, A = absconded

<table>
<thead>
<tr>
<th>S</th>
<th>BD</th>
<th>T</th>
<th>D</th>
<th>A</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>BD</td>
<td>T</td>
<td>D</td>
<td>A</td>
</tr>
<tr>
<td>S</td>
<td>BD</td>
<td>T</td>
<td>D</td>
<td>A</td>
</tr>
</tbody>
</table>

Completed by (initials)  Date  / /

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A.5 Clinical record form: MCH clinic
A.6 Kilifi Ultrasound System and Clinical report template
## Interbio 21st Kilifi: Ultrasound System

### Data Collection
- Record Status Dashboard
- Add / Edit Records
- KIN Number 2
- Demographics
  - MCH Data Current Pregnancy
  - MCH Data Previous Pregnancies
  - PIP Allocation and Consent
  - Scan 1 Ultrasound Report
  - Scan 1 Ultrasound Report Us Biomathemical Data
  - Scan 2 Ultrasound Report
  - Scan 2 Ultrasound Report Us Biomathemical Data
- Lock all forms
- Unlock all forms

### Applications
- Calendar
- Data Export Tool
- Data Comparison Tool
- Field Comment Log
- File Repository
- Record Locking Customization
- E-signature and Locking Mgmt
- Graphical Data View & Stats
- Report Builder

### Reports
- Monthly Report
- Participation

### Help & Information
- Help & FAQ
- Video Tutorials
- Suggest a New Feature

---

### Locked by hmwangudzah (hope mwangudzah) on 09/04/2013 9:14am

A user has locked record "2" for the form "Demographics". If you have looking/unlocking privileges, you may unlock this record at the bottom of the page.

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### Editing existing KIN Number 2

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### Form Status
- Complete? Unverified

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Locked by hmwangudzah (hope mwangudzah) on 09/04/2013 9:14am
Scan 1 Ultrasound Report

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This patient had an ultrasound scan as part of the INTERBIO - 21st study at Kilifi District Hospital. All data are obtained by trained obstetric sonographers. Data do not replace clinical judgement and are used at the discretion of the attending clinician.

Number of fetuses: [ ] 1  [ ] 2
Number of fetal heartbeats: [ ] 1  [ ] 2
Placental position
[ ] Fundal
[ ] High anterior
[ ] High posterior
[ ] Low anterior
[ ] Low posterior
[ ] High left lateral
[ ] Low left lateral
[ ] High right lateral
[ ] Low right lateral
Amniotic fluid comment
[ ] Normal
[ ] Increased
[ ] Reduced
Gestation (US) on scan

EDD (US)

Further research scan required? [ ] No
[ ] Yes - Placenta
[ ] Yes - Placenta and biometry
Follow up appointment date

Pregnancy redated [ ] Yes
[ ] No
Agreed EDD

Agreed EDD according to
[ ] LMP
[ ] US
Sonographer name
[ ] Hope Mwangudzah
[ ] Angela Koech
[ ] Bryn Kemp
[ ] Stella Mwakio
[ ] Other
A.7 Standard operating procedures
ULTRASOUND KILIFI

SOP Title: Gestational Age Estimation

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<td>Bryn Kemp</td>
<td>30/9/11</td>
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<tr>
<td>REVIEWING AUTHORITY</td>
<td>Aris Papageorgiou</td>
<td>30/9/11</td>
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<td>QA UNIT AUTHORITY</td>
<td>James Berkley</td>
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1.0 PURPOSE / INTRODUCTION:

The gestational age of a pregnancy can be accurately measured using ultrasound between 6 and 24 weeks' gestation. The crown-rump length (CRL) should be used between 6 and 13 weeks' followed by the head circumference (HC) for later gestations. This SOP outlines the process of assigning a gestational age using ultrasound and includes guidance on assigning an estimated date of delivery where there is a disagreement between ultrasound measurements and calculated dates in women who are certain of their menstrual history.

Gestational age assessment using ultrasound should only be offered to women who are less than 24 weeks' gestation according to best clinical estimate. Calculated dates according to the first day of the last menstrual period (LMP) should be used when a woman is sure of dates. Where there is uncertainty with recalling this, the symphysis fundal height should be measured and women offered a scan if this measures less than 24 cms. Where there is disagreement between a certain menstrual history and symphysis fundal height, the menstrual history should be used to estimate gestational age and determine eligibility for a dating ultrasound scan.

2.0 SCOPE / RESPONSIBILITY:

All women scanned in Kilifi District Hospital should have their estimated date of delivery determined using this protocol. All sonographers are responsible for ensuring that they are familiar with this policy.

3.0 EQUIPMENT / MATERIALS/ REAGENTS:

- Ultrasound machine
- Tape measure
- Obstetric wheel
**SOP Title: Gestational Age Measurement**  
**Version: 1**  
**SOP No. 3**

### 4.0 METHODOLOGY:

**Determine eligibility for Dating Ultrasound**

Women attending for antenatal care at the maternal and child health clinic are screened by trained nursing staff to determine eligibility for a dating ultrasound.

Before scanning, ensure that inclusion criteria are met:
- A certain LMP giving an EDD and gestational age of <24 weeks.
- OR
- Where LMP is not certain, a SFH >24 cm.

Where LMP and SFH disagree, confirm that the LMP is certain and use this to calculate an EDD for determining eligibility for scan.

Women who are eligible according to LMP, but have no abdominal mass should have a urine pregnancy test performed prior to ultrasound.

**Ultrasound examination for assigning gestational age**

All ultrasound examinations should document the number of fetuses and their viability (presence of a fetal heart beat) as well as the position of placental insertion in the uterus. Where the fetal heart is absent, the patient should be managed according to SOP 4 (Management of a non-viable pregnancy).

Where a low lying placenta is identified, women should be counselled about the need to seek medical care after any vaginal bleeding and should be booked for a rescan at 36 weeks to document placental position.

**Assigning gestational age up to 13+6 weeks’ gestation**

The CRL is the distance between the top of the fetal head (crown) and the base of the trunk (rump) and should be used for all dating scans <14 weeks’ gestation. The CRL should be measured from three different images and measurements should agree to within 5mm of each other. Where fetal flexion prevents a measurement being taken, further attempts should be made after a brief pause. If it is not possible to obtain the correct image for measurement, please measure the patient about fetal viability and make an appointment for a scan with the senior sonographer.

**Assigning gestational age from 14 to 24 weeks’ gestation**

Head circumference should be used from 14 to 24 weeks’ gestation. It is obtained from an image taken in the transverse plane of the fetal head at the level of the thalami. The correct plane is identified by locating the falx cerebri in the midline and should include the septum pellucidum anteriorly. The plane lies above the posterior fossa and so no cerebellum should be visible. Calipers are placed around the outer border of the cranium to obtain the circumference. Three measurements should be obtained.

**Discrepancy between US measured estimated delivery date and certain menstrual history**

The following criteria should be used for redating pregnancies for clinical reporting where there is a discrepancy between US measured EDD and that calculated from a certain LMP.

<table>
<thead>
<tr>
<th>Gestation</th>
<th>US Measurement</th>
<th>Difference before EDD adjusted</th>
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<tr>
<td>5 – 12th weeks</td>
<td>CRL</td>
<td>&gt; 5 days</td>
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<tr>
<td>12th – 20 weeks</td>
<td>CRL &lt;13th weeks HC 14 – 24 weeks</td>
<td>&gt; 7 days</td>
</tr>
<tr>
<td>20th – 24 weeks</td>
<td>HC</td>
<td>&gt; 14 days</td>
</tr>
</tbody>
</table>

Prior to redating the pregnancy, the femur length should be checked. The estimated gestational age from the femur length should equate to that of the head circumference to within 5, 7 or 14 days according to the gestations listed above.
Ultrasound Report

An antenatal ultrasound report should be completed for all women scanned within the INTERGROWTH scan facility. All fields should be completed for all women. A copy of this report should then be fixed into the maternal handheld antenatal care record.

Follow-up appointments

Appointments for later ultrasound scans should be made immediately and entered on to the INTERGROWTH US follow-up calendar. Women should have the appointment logged in their maternal handheld record. The cost of return visits for clinically indicated scans will not be reimbursed. If a patient is being specifically requested to return for an ultrasound scan purely for research purposes, then a reimbursement will be made according to standard KEMRI procedures.

5.0 APPENDICES

Antenatal Ultrasound Report

6.0 DOCUMENT CHANGE HISTORY

This section is to be completed by the Quality Management or designee

Version Table:

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NOTE: This is a CONTROLLED document. Any documents that are not stamped in red "APPROVED" are not controlled. Anyone using an uncontrolled copy is responsible for checking that they have the latest revision of the document prior to use.
1.0 PURPOSE / INTRODUCTION:

Ultrasound may identify a non-viable fetus before the mother identifies a problem with the pregnancy. All women are counselled that this is a possibility during the consent procedure for study participation. This SOP and supporting documentation should be used when ultrasound identifies a fetus with no heart beat activity.

2.0 SCOPE / RESPONSIBILITY:

All sonographers are responsible for ensuring that they are familiar with this policy.

3.0 EQUIPMENT / MATERIALS/ REAGENTS:

Ultrasound machine
Adverse fetal outcome forms

4.0 METHODOLOGY

When a scan reveals that there is no fetal heart beat present, a failed pregnancy or miscarriage is diagnosed if the gestation is <28 weeks. Beyond this gestation, these findings are termed an intrauterine death.

Sonographers who identify an absent fetal heart beat should immediately call for a second opinion from an authorised study sonographer. A list of current sonographers available to provide second opinions is always available in the INTERGROWTH scanning room. Both sonographers should be present and confirm the absence of a fetal heart beat after a period of 30 seconds of observation of the fetal heart structures.

Condolences should be offered to the mother in her mother language. She should be referred to the Gynaecology ward after a verbal referral to the oncall medical officer intern or clinical officer intern.
All women should be offered an appointment with a trained INTERGROWTH counsellor. If this is accepted, contact details should be taken and passed to one of the designated INTERGROWTH counsellors (see list in Study Room) by the clinician/sonographer scanning for that session. The counsellor or clinician should then contact the patient and arrange to meet whilst in hospital or provide a follow up appointment at a convenient time for the woman. Those women declining an appointment should be provided with contact details of the INTERGROWTH team in order that they can contact a study member if their wishes change.

Any follow up appointments or investigations scheduled for the pregnancy should be cancelled by the duty Fieldworker for INTERGROWTH.

5.0 APPENDICES

Adverse Pregnancy Outcome form

6.0 DOCUMENT CHANGE HISTORY

This section is to be completed by the Quality Management or designee

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NOTE: This is a CONTROLLED document. Any documents that are not stamped in red "APPROVED" are not controlled. Anyone using an uncontrolled copy is responsible for checking that they have the latest revision of the document prior to use.
1.0 PURPOSE / INTRODUCTION:

All women referred for ultrasound who have no palpable abdominal mass should have a pregnancy test conducted before consent is taken for ultrasound scanning. Only women with positive pregnancy test or obvious gravid uterus are eligible for ultrasound scanning.

2.0 SCOPE / RESPONSIBILITY:

This protocol applies to all women attending for antenatal care at Kilifi District Hospital and who are referred for ultrasound scanning as part of the INTERGROWTH study.

3.0 EQUIPMENT / MATERIALS/ REAGENTS:

Urine pregnancy test kits
Urine bottles
Latex examination gloves
Clinical waste disposal unit
Watch or stopwatch

4.0 METHODOLOGY

The duty fieldworker should check the palpation section of page 6 of the antenatal clinic hand held record. Where no fundal height has been recorded or "no mass" is documented, the duty fieldworker should inform the study clinician, who will review the notes at the next available opportunity and authorise the fieldworker to request a urine specimen from the woman.

The woman should be provided with a urine specimen bottle and directed to the nearest toilet facility.

Testing of urine should be performed by trained clinical personnel only. It is not acceptable to delegate the task to a fieldworker.
A single pregnancy test strip should be opened and removed from the foil packaging. In accordance with manufacturers’ instructions, the strip should be immersed in the urine for at least 10 to 15 seconds and then removed. Care should be taken not to immerse the strip beyond the max line.

After three minutes, a reading should be taken.

Results

- **Positive**
- **Negative**
- **Invalid**

Only women with a valid and positive result should have consent taken for an ultrasound scan.

### 5.0 APPENDICES

All staff are responsible for reading the information leaflet provided by ACON – “One Step Pregnancy Test Strip (Urine)” Reference FHC-101 and FHC-111 attached to this SOP.
A.8 Study authorisation: ethics and buildingwork approval
KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840-00200, NAIROBI, Kenya
Tel (254) (020) 2722541, 2713349, 0722-209801, 0733-400000; Fax: (254) (020) 2720030
E-mail: director@kemri.org  info@kemri.org  Website:www.kemri.org

KEMRI/RES/7/3/1

TO:  DR. BRYN KEMP (PRINCIPAL INVESTIGATOR)

THROUGH:  DR. SABAH OMAR,
THE DIRECTOR, CGMR-C,
KILI

June 5, 2013

Dear Sir,

RE:  SSC PROTOCOL No.1969 (REQUEST FOR ANNUAL RENEWAL); INTERGROWTH 21ST.

Reference is made to your letter dated 30th May 2013. The ERC Secretariat acknowledges receipt of your response to the ERC letter dated May 21, 2013 on 3rd June 2013.

The Committee determines that the issues raised at the 215th meeting of 21st May 2013 are adequately addressed. Consequently, the study is granted approval for continuation effective this 5th day of June 2013. Please note that authorization to conduct this study will automatically expire on June 4, 2014.

If you plan to continue with data collection or analysis beyond this date, please submit an application for continuing approval to the ERC Secretariat by April 24, 2014. You are required to submit any proposed changes to this study to the SSC and ERC for review and the changes should not be initiated until written approval from the ERC is received.

Please note that any unanticipated problems resulting from the conduct of this study should be brought to the attention of the ERC and you should advise the ERC when the study is completed or discontinued.

You may continue with the study.

Yours faithfully,

DR. ELIZABETH BUKUSI,
ACTING SECRETARY,
KEMRI ETHICS REVIEW COMMITTEE

In Search of Better Health
The Director
KEMRI-Kilifi

Dear Dr Peshu

Re: Allocation of room to conduct Inter-Growth Study

This is to confirm to you that your request for a room within the hospital to carry out the above mentioned study has been approved. The hospital has thus allocated the old incinerator room, behind the hospital radiology department; to the study team to carry out this work.

The room will however require some minor renovations and partitioning for it to be habitable by the study team.

Thanks

[Signature]

Dr. Maureen Owiti
Medical Superintendent
Kilifi District Hospital
A.9 Fetal birthweight for GA reference data
### Table A.1: Birthweight for GA centiles adapted from Oxford University Hospitals NHS Trust, UK.

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<th>10\textsuperscript{th} centile</th>
<th>50\textsuperscript{th} centile</th>
<th>90\textsuperscript{th} centile</th>
<th>97\textsuperscript{th} centile</th>
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A.10 Dating chart: equation 2.5

\[ GA = 20.5624 \times \log HC^2 + 3.6027 \times FL^2 - 6.4919 \times \log HC \times FL \]
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<thead>
<tr>
<th>Estimated GA (ww+d)</th>
<th>3rd centile (ww+d)</th>
<th>97th centile (ww+d)</th>
<th>Uncertainty ± days</th>
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A.11 Dating chart: equation 2.9

\[ GA = 18.0461 \times HC^2 + 5.7299 \times \log AC^2 + 10.9393 \times \log FL \times FL - 26.8838 \times \log HC \times \log FL \]
### A.11 Dating chart: equation 2.9

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A.12 Thesis contributions

The following contributions are acknowledged for each chapter of this thesis:

Chapter 1 Author only.

Chapter 2 Dr Angela Koech contributed to data extraction for the Systematic Review of Methodologies (Section 2.7 on page 22). All remaining sections are the author's own work.

Chapter 3 All clinical data collection within the maternity inpatient department was undertaken in collaboration with Dr Anna Seale and supervised by Dr Jay Berkley from the KEMRI-Wellcome Trust Research Programme, Kilifi, Kenya. All remaining sections describe the author's own work.

Chapter 4 All clinical and US data were obtained by Dr Angela Koech, Hellen Cherono, Hope Mwangudzah and Stella Mwakio along with nursing staff from KDH operating on research contracts. All team members were directly supervised by the author of this thesis. Statistical advice was obtained from Eric Ohuma, Medical Statistician within NDOG. All analyses and results were undertaken by the author.

Chapter 5 All clinical and US data were obtained by Dr Angela Koech, Hellen Cherono, Hope Mwangudzah and Stella Mwakio along with nursing staff from KDH operating on research contracts. All team members were directly supervised by the author of this thesis. The t-SNE methodology was developed with advice from Dr Alfonso Bueno from the Computational Biology Systems Groups at the University of Oxford. MATLAB source code was obtained and used under ‘open-source’ licence from Tilburg University [229, 230]. All presented models and data are the author’s own work.

Chapter 6 Clinical data were obtained by the INTERGROWTH-21\textsuperscript{st} team, based within NDOG at the University of Oxford. The Genetic Algorithm was used under an ‘open-source’ MATLAB licence from the University of Sheffield [262].
Methods were discussed with Dr Antoniya Georgieva, Computer Scientist within NDOG. All models and results are the author's own work.

**Chapter 7** The automated dating model was developed by Ana Namburete, DPhil candidate and the IBME. Clinical guidance was provided by the author along with Dr Aris Papageorghiou. Prospective validation was performed by the author. Specific contributions to each section of this chapter are provided within the body text.
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