




BMJ Open Can the date of last menstrual period be trusted in the first trimester? Comparisons of gestational age measures from a prospective cohort study in six low-income to middle-income countries

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ABSTRACT

Objectives We examined gestational age (GA) estimates for live and still births, and prematurity rates based on last menstrual period (LMP) compared with ultrasonography (USG) among pregnant women at seven sites in six low-resource countries.

Design Prospective cohort study

Setting and participants This study included data from the Global Network's population-based Maternal and Newborn Health Registry which follows pregnant women in six low-income and middle-income countries (Democratic Republic of the Congo, Guatemala, India, Kenya, Pakistan and Zambia). Participants in this analysis were 42 803 women, including their 43 230 babies, who registered for the study in their first trimester based on GA estimated either by LMP or USG and had a live or stillbirth with an estimated GA of 20–42 weeks.

Outcome measures GA was estimated in weeks and days based on LMP and/or USG. Prematurity was defined as GA of 20 weeks+0 days through 36 weeks+6 days, calculated by both USG and LMP.

Results Overall, average GA varied ≤ 1 week between LMP and USG. Mean GA for live births by LMP was lower than by USG (adjusted mean difference (95% CI) = -0.23 (-0.20 to -0.17) weeks). Among stillbirths, a higher GA was estimated by LMP than USG (adjusted mean difference (95% CI) = 0.42 (0.11 to 0.72) weeks). Preterm birth rates for live births were significantly higher when dated by LMP (adjusted rate difference (95% CI) = 4.20 (3.56 to 4.85)). There was no significant difference in preterm birth rates for stillbirths.

Conclusion The small differences in GA for LMP versus USG in the Guatemalan and Indian sites suggest that LMP may be a useful alternative to USG for GA dating during the first trimester until availability of USG improves in those areas. Further research is needed to assess LMP for first-trimester GA dating in other regions with limited access to USG.

Trial registration number NCT01073475.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- ⇒ This study examined data from the Global Network for Women's and Children's Health Research Maternal and Newborn Health Registry, a large community-based registry of pregnant women in six low-middle income countries in Asia, Africa and South America.
- ⇒ The study implemented a prospective cohort design, following participants from the first trimester of pregnancy through delivery, and utilised a standardised protocol across sites to enhance quality and timeliness of data collection.
- ⇒ The study examined gestational age estimation for both live births and stillbirths.
- ⇒ Access to ultrasonography varied across sites, limiting the number of direct comparisons between the two methods of gestational age estimation.
- ⇒ Due to the large sample size for live births, analyses were conducted both for the overall sample and for individual sites; however, the smaller sample size for stillbirths did not allow for site-level analyses.

INTRODUCTION

Epidemiological research and public health interventions directed at improving women's and neonates' health during pregnancy, intrapartum and postpartum, particularly in low-income and middle-income countries (LMICs) focus on key health indicators such as preterm birth or small for gestational age (SGA) infants as they contribute to the majority of neonatal morbidities and mortality.¹ Estimation of burden of preterm births or SGA infants relies on accurate estimation of gestational age (GA). GA also needs to be evaluated in an accurate, reliable

and consistent way for caregiving. In the absence of reliable GA dating, estimates of preterm birth and SGA rely on complex modelling approaches from limited data.^{2 3}

Fetal biometry in the first trimester using ultrasonography (USG) is considered as the gold standard method of estimating GA.^{2 4 5} Accuracy diminishes in later trimesters if intrauterine growth of the fetus is not commensurate with that of GA. First trimester USG is often not available in low resources settings, because pregnant women rarely register for antenatal care during their first trimester and many rural clinics do not have USG machines or staff trained to estimate GA. As a result, globally, GA is mostly estimated from the first day of the last menstrual period (LMP) to the day the woman registers for antenatal care.⁶ However, this date may be inaccurate if the woman has irregular menstrual cycles, is calendar illiterate, does not track the date of the LMP or has poor recall due to registration for antenatal care in later trimesters.^{7 8}

In recent years, access to easy-to-use urine pregnancy testing kits and training of community health workers to facilitate early registration of pregnant women at antenatal clinics has improved estimation of GA and estimated dates of delivery using LMP in low-resource populations.^{9 10} However, few population-based studies, with prospective data collection, have compared the impact of GA estimated by LMP and USG, on estimates of rates of preterm birth in women registering for antenatal care in the first trimester of pregnancy.^{2 3} The *Eunice Kennedy Shriver* National Institute of Child Health and Human Development's Global Network Maternal and Newborn Health Registry (MNHR) has one of the largest prospectively collected population-based pregnancy registries, with increasing registration in the first trimester. Data from the MNHR are available through National Institute of Child Health and Human Development (NICHD) Data and Specimen Hub.¹¹ We used MNHR data from seven Global Network (GN) sites of six LMICs during 2017–2018 to assess the reliability of GA estimated by LMP among live births and stillbirths of women registered in the first trimester for antenatal care. Our aims were to: (1) compare distributions of GA assessed by LMP to those assessed by USG; (2) estimate and compare population rates of preterm births, overall and by site, based on GA estimates by LMP and USG and (3) determine the proportion of women whose GA estimated by first trimester USG and LMP were within 1 week of each other, when both estimates were available, stratified by term and preterm status.

METHODS

The GN's MNHR follows pregnant women in the catchment area of seven locations in six LMICs in rural sites in Guatemala and India (two sites: Nagpur and Belagavi), Pakistan, Kenya, Zambia and the Democratic Republic of the Congo (DRC) from the time of antenatal care registration through labour and delivery and up to 6 weeks postpartum using standardised data collection forms

completed by trained data collectors. Data quality has been monitored consistently since 2009,^{12–16} and since 2014, GA dating has improved with increased access to USG. USG was either part of routine antenatal care or occurred in GN studies using standardised protocols.^{17–20}

Patient and public involvement in research

The MNH Registry was initiated in 2009 to monitor outcomes in pregnant women and their babies in low resource settings globally. Community meetings were held in all study sites to discuss important mother and baby problems in the community using core-group meetings that helped to frame the research questions for the registry. This study is secondary data analysis of the data collected in the registry. The study has been monitored annually by US and site-specific Institutional Review Boards as well as by an international data and safety monitoring board assembled by the National Institutes of Health. Results of the longstanding observational studies are discussed with the communities at least annually as not all participants can reach dedicated websites.

Study population and eligibility criteria

For this analysis, we included women in the MNHR who registered for the study in their first trimester based on GA estimated either by LMP or USG and had a live or stillbirth with an estimated GA of 20–42 weeks (biologically plausible range). The LMP was used to define first trimester (GA between 0–13 weeks and 6 days) when there was conflict between GA determined by USG or LMP. We excluded women who died prior to delivery and those who had miscarriages, medical terminations of pregnancy and pregnancies for which no birth outcome was obtained (figure 1).

Measures

Estimated gestational age

GA was estimated in weeks and days based on one or both of these methods: LMP and USG. GA by LMP was calculated as the date of enrolment minus LMP divided by seven and rounded to the nearest week. GA was estimated on the date the USG was performed. Prematurity was defined as GA of 20 weeks+0 days through 36 weeks+6 days, calculated by both USG and LMP.

Statistical analysis

For aim 1, we compared the distributions of GA assessed by LMP to those assessed by USG, overall and for all sites. All available observations with first trimester USG were included in the distribution of GA by USG. The distribution of GA by first trimester LMP was assessed using all observations with first trimester LMP. We calculated descriptive statistics (mean and SD) of the continuous GA measurements from each source. Overlapping density plots were used to visually assess the level of divergence between the distribution for the first trimester USG and LMP for first trimester enrollees. We obtained model-adjusted mean GA and associated 95% CI and estimated

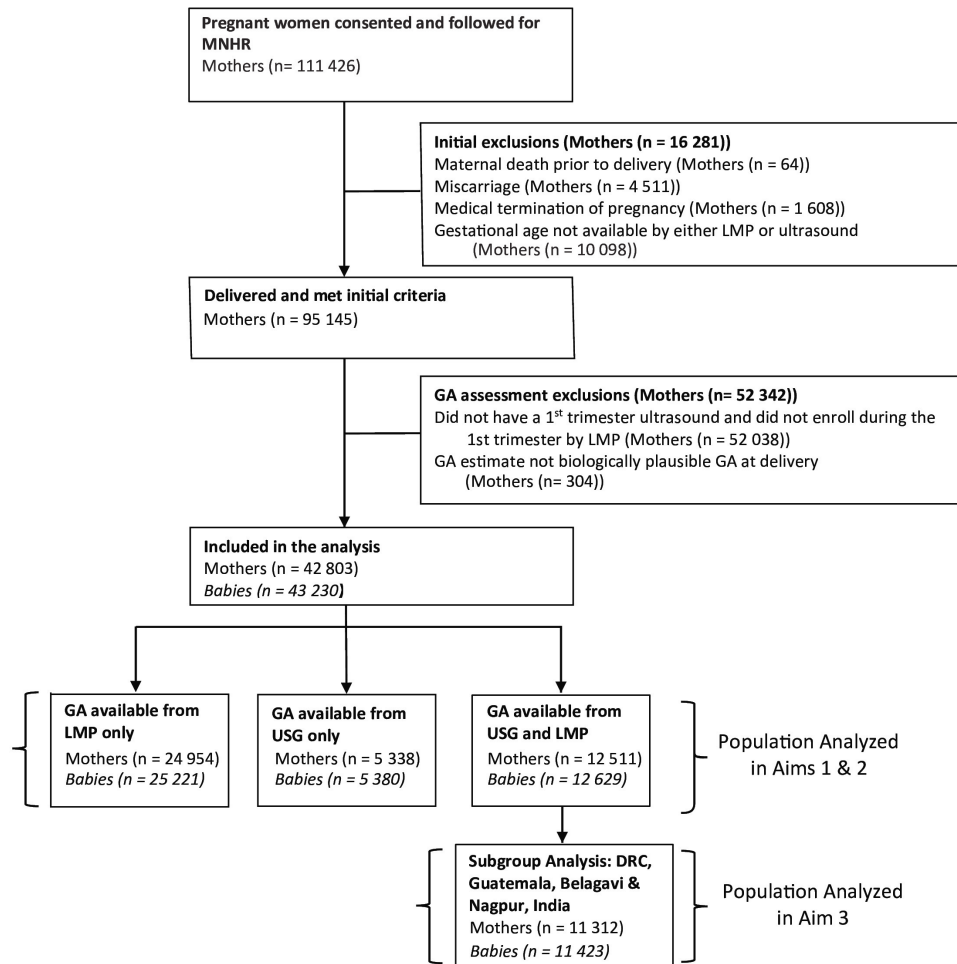


Figure 1 Consort diagram, 2017–2018. DRC, Democratic Republic of the Congo; GA, gestational age; LMP, last menstrual period; MNHR, Maternal and Newborn Health Registry; USG, ultrasonography.

mean differences and corresponding 95% CI comparing LMP and USG. We computed multivariable generalised linear mixed-effect models of GA by method (LMP or USG) to account for repeated measures for participants with GA calculations by more than one method. We used an unstructured covariance matrix to account for the correlation within pregnancy across methods of the GA calculation. If the model did not converge with the unstructured covariance matrix, we used a variance components covariance matrix. The models included site, method and site-by-method interaction to compute site-level estimates and controlled for maternal age, maternal education and parity. Babies were analysed as independent observations. Stillbirths and live births were analysed separately because the GA distributions for these outcomes are different.

For aim 2, we applied a similar approach to examine the differences in rates of preterm births, when GA is assessed by LMP and when the rates are assessed by USG. We fit binary mixed-effect models with preterm birth as the outcome to account for repeated measures for participants and used an identity link function to obtain adjusted preterm birth rates and 95% CI and differences in preterm birth rates with 95% CI comparing the

two methods (LMP or USG). Similar to the models for GA, site, method and site-by-method interaction were included in each model of preterm birth with maternal age, maternal education and parity as control variables.

For aim 3, we compared GA estimates among the subset of women who had GA measured by both LMP and USG. We computed the difference in the estimated GA in days by USG and LMP using three categories: ± 7 , ± 8 to ± 14 , or $\geq \pm 14$ days. This analysis was stratified by term and preterm using only GA estimated by USG. The analysis excluded Zambia, Kenya and Pakistan because of insufficient women who had both a first trimester USG and date of LMP.

All analyses were conducted using SAS V.9.4. Statistical analyses were conducted with babies as the unit of analysis with the exception of the descriptions of maternal characteristics, such as [table 1](#), which included mothers as the unit of analysis.

RESULTS

Participant characteristics

Of the 111 426 pregnant women enrolled in the MNHR between January 2017 and December 2018, 95 145

Table 1 Distribution of maternal characteristics and birth outcomes among women with GA measured by either first trimester enrolment as per LMP or first trimester ultrasound, overall and by site 2017–2018

Characteristics	Site							
	Overall	DRC	Zambia	Kenya	Guatemala	Belagavi	Nagpur	Pakistan
Women, N	42 803	3528	2571	2476	5730	11 471	10 196	6831
Maternal age	42 802	3528	2571	2475	5730	11 471	10 196	6831
<20	6745 (15.8)	1302 (36.9)	894 (34.8)	815 (32.9)	1218 (21.3)	1807 (15.8)	330 (3.2)	379 (5.5)
20–35	34 902 (81.5)	2040 (57.8)	1516 (59.0)	1616 (65.3)	4195 (73.2)	9620 (83.9)	9813 (96.2)	6102 (89.3)
>35	1155 (2.7)	186 (5.3)	161 (6.3)	44 (1.8)	317 (5.5)	44 (0.4)	53 (0.5)	350 (5.1)
Maternal education	42 800	3528	2571	2476	5730	11 470	10 194	6831
No formal education	8537 (19.9)	1160 (32.9)	154 (6.0)	22 (0.9)	354 (6.2)	1002 (8.7)	142 (1.4)	5703 (83.5)
Primary/secondary	30 062 (70.2)	2355 (66.8)	2374 (92.3)	2199 (88.8)	4716 (82.3)	9069 (79.1)	8291 (81.3)	1058 (15.5)
University+	4201 (9.8)	13 (0.4)	43 (1.7)	255 (10.3)	660 (11.5)	1399 (12.2)	1761 (17.3)	70 (1.0)
Parity	42 798	3528	2571	2476	5730	11 471	10 195	6827
0	18 704 (43.7)	1312 (37.2)	1255 (48.8)	1487 (60.1)	2671 (46.6)	4442 (38.7)	5632 (55.2)	1905 (27.9)
1–2	17 489 (40.9)	930 (26.4)	839 (32.6)	613 (24.8)	2122 (37.0)	6286 (54.8)	4437 (43.5)	2262 (33.1)
>2	6605 (15.4)	1286 (36.5)	477 (18.6)	376 (15.2)	937 (16.4)	743 (6.5)	126 (1.2)	2660 (39.0)
ANC visits	42 795	3528	2571	2470	5730	11 470	10 196	6830
0	332 (0.8)	101 (2.9)	1 (0.0)	39 (1.6)	51 (0.9)	1 (0.0)	1 (0.0)	138 (2.0)
1–3	9430 (22.0)	1475 (41.8)	558 (21.7)	614 (24.9)	1150 (20.1)	1731 (15.1)	463 (4.5)	3439 (50.4)
4+	33 033 (77.2)	1952 (55.3)	2012 (78.3)	1817 (73.6)	4529 (79.0)	9738 (84.9)	9732 (95.4)	3253 (47.6)
Babies, N	43 230	3597	2601	2502	5778	11 576	10 272	6904
Birth outcome	43 230	3597	2601	2502	5778	11 576	10 272	6904
Stillbirths	1189 (2.8)	154 (4.3)	65 (2.5)	79 (3.2)	93 (1.6)	270 (2.3)	207 (2.0)	321 (4.6)
Live births	42 041 (97.2)	3443 (95.7)	2536 (97.5)	2423 (96.8)	5685 (98.4)	11 306 (97.7)	10 065 (98.0)	6583 (95.4)

ANC, antenatal care; DRC, Democratic Republic of the Congo; GA, gestational age; N, number.

(85.4%) met initial eligibility criteria, and of these, 42 803 (45.0%) were in their first trimester based on date of the LMP or USG (figure 1; online supplemental table 1). GA calculated to be in the first trimester was available from both LMP and USG for 29.2%, from only LMP for 58.3% and from only USG for 12.4%. The percentages of women with both LMP and USG varied by site from 5.8% in Kenya to 72.7% in Belagavi, India (online supplemental table 1). The analytic sample included the 43 230 babies of the 42 803 mothers who fit the study inclusion criteria (figure 1). The number of babies exceeds the number of mothers due to some mothers having more than one pregnancy during the study period and/or giving birth to multiple babies from a single pregnancy (eg, twins).

Distribution of maternal characteristics, overall and by site, are shown in table 1. Overall, 81.5% were 20–35 years of age, 70.2% had completed some primary or secondary education, 43.7% were nulliparous and 77.2% had four or more antenatal care visits. Women in Guatemala and the two Indian sites tended to be older, were more likely to have education beyond secondary school and were more likely to have had four or more antenatal care visits than women in the three African sites and Pakistan.

Online supplemental table 2 shows maternal characteristics by method of GA dating and are similar in both groups. Distribution of the characteristics of first trimester enrollees with GA dating by LMP was similar to those with USG in the first trimester except for a lower proportion of younger women (15.7% vs 23.6%) and nulliparous (42.0% vs 60.8%) registering in the first trimester based on the estimated GA date using LMP versus USG.

Aim 1: gestational age estimates

Gestational age estimates by dating method for stillbirths and live births are shown in figure 2. For stillbirths, the mean GA at delivery ranged from 31.7 to 31.9 weeks across the methods and about 38.5 weeks among live births. The average GA varied ≤ 1 week across sites and across methods within sites. Among live births, the distributions for LMP and USG overlap almost entirely for Guatemala (online supplemental figure 1). In addition, the distributions

for the two sites in India (Belagavi and Nagpur) are very similar with LMP shifted slightly to the right (ie, higher values). In DRC, Zambia, Kenya and Pakistan, the GA distribution by USG is narrower, around a higher mean than GA by LMP. Overall, the distributions for LMP and USG exhibited similar patterns among stillbirths in the overall sample (online supplemental figure 2).

Figure 2 displays the model-adjusted mean differences in weeks and associated 95% CIs between GA estimated by USG and LMP, after controlling for age, education and parity. Overall, for live births, the estimation of mean GA by LMP was lower than the estimation of mean GA by USG (adjusted mean difference (95% CI): -0.23 (-0.29 to -0.17) weeks). In the DRC, Zambia, Kenya and Pakistan, LMP estimated the mean GA to be lower than that estimated by USG by 0.35 weeks (Pakistan) to 0.84 weeks (Zambia), while in India and Guatemala, LMP estimated the GA higher by 0.06 weeks (Guatemala) to 0.37 weeks (Belagavi, India). As shown in figure 2, overall, LMP estimated a higher GA (adjusted mean difference 0.42 weeks (95% CI) (-0.72 to 0.11)) for stillbirths.

Aim 2: preterm birth rates

Overall, preterm birth rates for stillbirths (70%) were similar for USG and LMP dating methods (figure 3). Among the live births, preterm rates were 4% higher when dated by LMP (20.0%) than when dated by USG (15.7%). Site-specific analysis showed that only at the two Indian sites, the preterm rates by LMP dating method were lower as compared with the rates by USG (12.2% vs 13.7% at Belagavi and 14.3% vs 14.8% at Nagpur). GA by LMP overestimated the rates as compared with USG by 0.3%, 8.2%, 9.5%, 7.5% and 8.8%, respectively, at Guatemala, DRC, Zambia, Kenya, and Pakistan (figure 3).

Aim 3: direct comparison of LMP and USG dating

Results from the analysis for individual participants who had GA dating by both USG and LMP are shown in figure 4 for four sites: DRC, Guatemala, and Belagavi and Nagpur in India as there was insufficient data to directly compare GA by LMP and USG dating for other sites. The

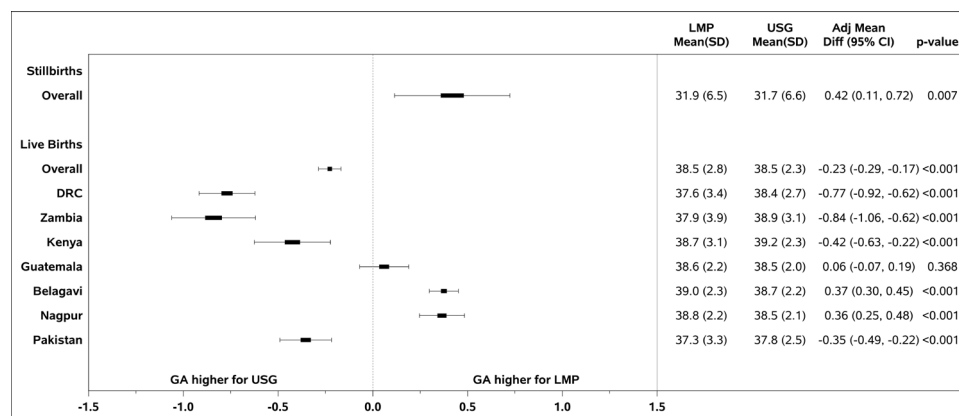


Figure 2 Model-adjusted mean differences (95% CIs) for gestational age in weeks by method of calculating gestational age within site among stillbirths and live births. DRC, Democratic Republic of the Congo; GA, gestational age; LMP, last menstrual period; USG, ultrasonography.

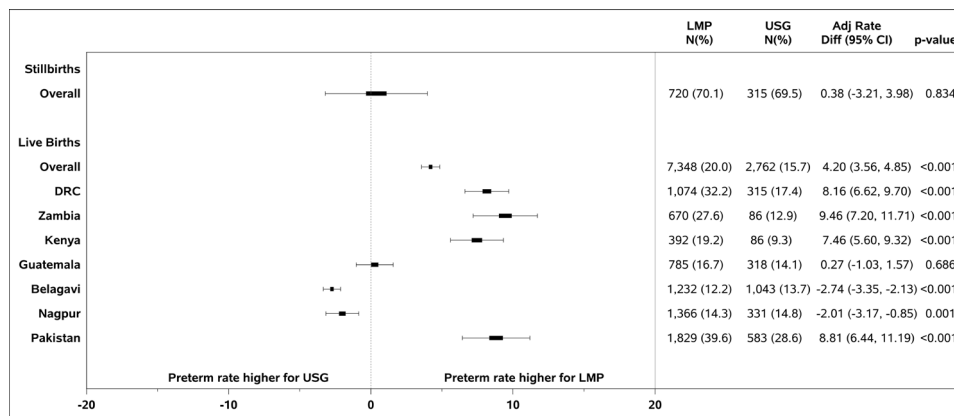


Figure 3 Model-adjusted differences (95% CIs) in preterm birth rates by method of calculating gestational age within site among stillbirths and live births. DRC, Democratic Republic of the Congo; LMP, last menstrual period; USG, ultrasonography.

results are stratified by preterm versus term birth status according to USG. GA dating by these methods was within 7 days of each other for 76.2% of preterm stillbirths and 63.7% of preterm live births, 69.7% of all term stillbirths and 78.9% of all term live births. All four sites had agreement within 7 days for at least 78.4% term live births but lower levels of agreement of the dates in preterm births and term stillbirths.

DISCUSSION

Using data from a large prospectively collected population-based MNHR with a standardised protocol, training and monitoring of data quality in seven rural locations in six countries, we found that GA estimates for LMP and USG varied by less than 1 week on average for women who registered for antenatal care in their first trimester, when comparing mean values. In addition, distributions of GA values were similar for Guatemala and the two sites in India. However, it should be noted that similarity between means and distributions is a necessary but not sufficient condition to establish equality between the two GA methods at the individual level. Therefore, we also conducted direct comparisons of USG and LMP for the sites with large enough samples of participants having both measurements. Based on these analyses, while agreement (within 1 week) between estimated GA by USG and LMP when both were available was almost 80% for term live births, agreement rates for stillbirths and preterm births were lower. Three of the sites (Kenya, Pakistan and Zambia) did not have adequate data for the direct head-to-head comparisons of the two GA methods. The adjusted mean differences for all three sites were small and negative suggesting that LMP may underestimate GA at the population level; however, future studies are needed to definitively establish the size and direction of any individual-level differences observed between the two methods in these locations.

Examining differences in preterm rates between LMP and USG is useful for evaluating the potential impact of GA measurement methods on population-level estimates. Rates of preterm birth in the Indian and Guatemalan

sites were similar, although rates in the two Indian sites were about 2% higher when USG dating was used. The African and Pakistani sites had significant differences in preterm rates by USG and LMP with LMP dating overestimating rates of preterm birth. Overall, the study results for each of the analyses revealed minimal differences between LMP and USG in the Guatemalan and Indian sites, suggesting that LMP may be an acceptable alternative for GA dating during the first trimester when USG is unavailable in these locations.

Our results are similar to those reported in other LMICs, although not all studies focused on first trimester USG and LMP dates. Rosenberg *et al*²¹ found that LMP underestimated GA in Bangladesh in the second and third trimesters by 1 day (± 11 days) compared with USG, while Unger *et al*² found the mean difference between USG and LMP was 2.4 days among women in a study of malaria in four sub-Saharan Africa countries during their second and third trimesters.²²¹ Taken together these non-population based studies in which LMP and USG GA estimates were directly compared suggest that LMP-estimated GA can be sufficiently accurate, in certain settings. Our study extends these data into a large population-based multisite study and its impact on estimates of preterm rates.

Maternal education plays an important role in site differences in GA estimates by LMP and USG—similar in the Guatemalan and two Indian sites but are less reliable in the three African sites and Pakistan. Others have reported that the date of the LMP is more reliable in women who have completed high school.^{22–24} This may explain results from the Pakistan site where almost 84% of women have no formal education. A recent qualitative study of 45 men and women in rural Western Kenya reported ‘high levels of misinformation about menstruation and fertility’ and misconceptions regarding the duration of pregnancy.²⁵ Calendar literacy appears to vary by site.²⁶ Facilitating tracking of LMPs (on paper or smart phones that are now possible in many locations globally) may improve estimation of GA using the date of the LMP where access to GA dating by USG is limited.²⁷

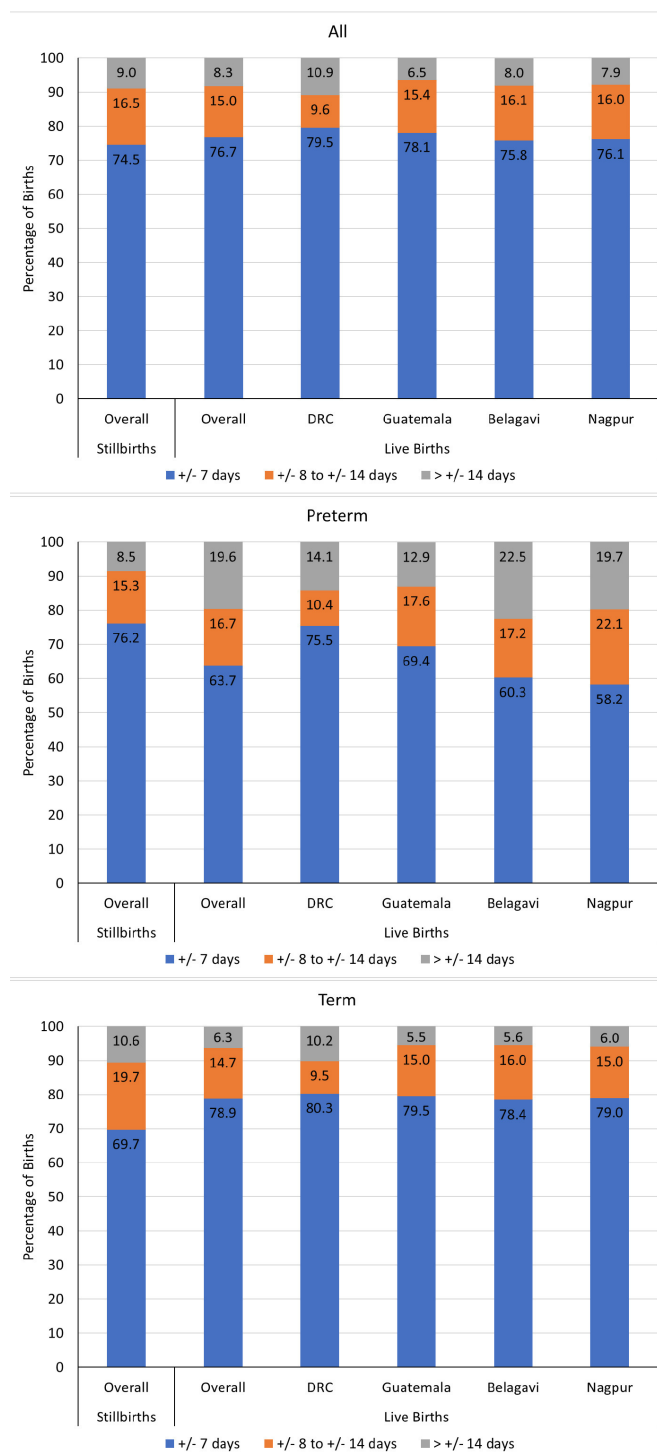


Figure 4 Difference between ultrasound and LMP gestational age measurements by preterm birth status and site among those with both first trimester USG and first trimester enrolment by LMP gestational age available for stillbirths and live births, 2017–2018. Includes all stillbirths and livebirths from DRC, Guatemala and India who have the following: gestational age available at enrolment by LMP for first trimester enrolees, gestational age available at ultrasound by first trimester US and gestational age available at delivery by LMP for first trimester enrolees and first trimester US and within range (ie, 20–42 weeks). Excludes Zambia, Kenya and Pakistan because of insufficient sample size. DRC, Democratic Republic of the Congo; LMP, last menstrual period; USG, ultrasonography.

Our study has a number of important strengths. First, the GN sites include a diverse range of locations and populations from different ethnic backgrounds so that our results are likely generalisable beyond the GN catchment

areas.^{15 28} Second, instead of evaluating individual-level concordance of LMP with USG, this study was unique in comparing population-level rates of GA when GA is estimated by LMP or USG at different LMIC sites. Third, the

MNHR uses common protocols and trainings for recruitment, prospective follow-up of the enrollees through pregnancy, labour and delivery through 6 weeks postpartum, standardised data collection instruments and constant monitoring and quality improvement processes,¹³ which results in high-quality data. A specific area targeted by the GN for quality improvement has been LMP dating.²¹ Fourth, retention and complete follow-up rates to 6 weeks postpartum are greater than 98%.²⁹ Finally, the GN has invested in site-specific training in the conduct of USG and interpretation of findings with follow-up quality control procedures to improve the accuracy of first trimester dating.²⁰

Limitations of our study include the following: first, we focused on first trimester registrations because there is an increasing trend for women to register for antenatal care early in pregnancy, and USG dating in the first trimester is more accurate than later in pregnancy. However, only about 45% of our MNHR enrollees registered in the first trimester, and we cannot generalise our data beyond 14 weeks of gestation at the time of registration. Those registering early in pregnancy are often different from those registering later,³⁰ and recall bias for LMP dating increases over the duration of pregnancy,²² so it is likely that later trimester registrations will worsen estimates of preterm birth rates compared with USG dating. Second, three of our sites still have limited access to USG and therefore, could not be included in the direct comparisons of LMP and USG, restricting the conclusions that could be made about those sites.

In conclusion, while USG remains as the gold standard for GA dating, our findings support the use of LMP for estimating preterm birth rates in the GN MNHR Guatemala and India sites when USG is not available during the first trimester. Future studies are needed to further examine the potential impact of LMP for first trimester GA dating in other locations to ensure accurate and reliable estimates of preterm birth rates and inform the global community about where resources need to be allocated to make a difference in reducing adverse outcomes for babies born prematurely. Furthermore, we recommend the further development of strategies to improve accuracy of the date of LMP as a less resource intensive and potentially faster approach to improving GA dating until the important, but more time consuming, endeavour of increasing access to USG in lower resource settings has been achieved.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were involved in the design, or conduct, or reporting or dissemination plans of this research. Refer to the Methods section for further details.

Patient consent for publication Not required.

Ethics approval This study involves human participants. The study was approved by the ethics review committees of all research sites, US partner universities and RTI International as follows: (1) Kinshasa School of Public Health, Kinshasa, Democratic Republic of the Congo (ESP/CE/04208/2017); (2) University of North Carolina at Chapel Hill, School of Medicine, Chapel Hill, North Carolina, USA (13-2099); (3) University of Zambia, Lusaka, Zambia (008-01-08); (4) University of Alabama, Birmingham, Alabama, USA (IRB-080521010); (5) Institute of Nutrition of Central American and Panama (INCAP), Guatemala City, Guatemala (19-13); (6) University of Colorado at Denver Anschutz Medical Campus, Aurora, Colorado (08-0511); (7) KLE University's JN Medical College, Belagavi, India (181219008); (8) Thomas Jefferson University, Philadelphia, Pennsylvania, USA (16F.349); (9) The Aga Khan University, Karachi, Pakistan (0581); (10) Columbia University, New York, New York, USA (IRB-AAAJ7651); (11) Lata Medical Research Foundation, Nagpur, India (RPC # 22E); (12) Boston University School of Medicine, Boston, Massachusetts, USA (H-35430); (13) Moi University School of Medicine, Eldoret, Kenya (00305) and (14) Indiana University School of Medicine, Bloomington, Indiana, USA (1011003646). Participants gave informed consent to participate in the study before taking part.

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