Title

Methods of gestational age assessment influence the observed association between antiretroviral therapy exposure, preterm delivery and small-for-gestational age infants: a prospective study in Cape Town, South Africa

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**ABSTRACT**

**Purpose**

Heterogeneous findings exist on antiretroviral treatment (ART) use in pregnancy and preterm delivery (PTD) or infants born small-for-gestational age (SGA).  Whether reported differences may be explained by methods used to ascertain gestational age(GA) has not been explored.

**Methods**

We enrolled consecutive pregnant women attending a large primary care antenatal clinic in South Africa. Public-sector midwives assessed GA by last menstrual period (LMP) and symphysis-fundal height (SFH). Separately, if clinical GA was <24weeks, ultrasound (US) was performed by a research sonographer blinded to midwife assessments. In analysis, the impact of measurement error on the association between HIV/ART status and birth outcome by GA method was assessed, and factors associated with clinical GA under- or over-estimation identified.

**Results**

In 1787 women included overall, estimated PTD incidence was 36% by LMP, 17% by SFH and 11% by US. PTD risk was higher for HIV-infected than HIV-uninfected women using US-GA (adjusted odds ratio (aOR) 1.95; 95% CI 1.10-3.46); for LMP/SFH-GA the associations were smaller and not significant. These findings persisted after adjustment for age, parity, height and previous PTD. PTD risk did not vary by timing of ART initiation (before or during pregnancy) for any method. Elevated BMI and older age were associated with decreased risk of under-estimation by both LMP and SFH; HIV status and obesity were associated with increased risk of over-estimation by SFH. There were no differences in SGA incidence across GA methods.

**Conclusions**

Findings for an association between HIV/ART and birth outcomes are substantially influenced by GA assessment method. With growing public health interest in this association, future research efforts should seek to standardize optimal measures of gestation.

**Background**

Increasing numbers of HIV-infected pregnant women use lifelong antiretroviral therapy (ART); in high prevalence HIV countries1 ART may be the most common drug exposure in pregnancy.2 While ART is invaluable for the health of HIV-infected women and their infants, some studies have reported associations between ART use in pregnancy and adverse birth outcomes such as preterm delivery (PTD)and small-for-gestational age (SGA) infants.3-5 PTD and SGA substantially increase the risk of infant mortality, with PTD accounting for the highest proportion of deaths in the critical neonatal period.6 Thus in high HIV prevalence settings it is essential to understand how ART use may influence the risk of preterm delivery. Findings on the association between ART use and adverse birth outcomes, including PTD, are conflicting 7,8 and possibly associated with ART regimen used, timing of initiation and ART eligibility criteria.

Studies investigating ART use in pregnancy are heterogeneous in their gestational age (GA) assessment method. GA assessment is challenging in resource-limited settings where ultrasonography is usually unavailable, and instead routinely based on clinical assessment via dating the last menstrual period (LMP) and/or measurement of the symphysis-fundal height (SFH). Although generally considered most reliable, ultrasound (US) is less accurate for pregnancy dating if carried out after 24 weeks, when biological variability of fetal biometry increases.9,10 The reliability of LMP-GA is limited by errors related to irregular menstrual cycles of varying duration or accurate recall of LMP dates, and variation in timing of ovulation.11,12 SFH-GA assessment is difficult early in pregnancy (<12 weeks); and even when measurable its accuracy can be diminished by multiple gestations, high body mass index (BMI), intrauterine growth restriction and other factors.13,14

Differences in GA assessment could provide an important methodological explanation for the observed heterogeneity in findings, but has not so far been evaluated. Measurement error of GA could lead to biased estimates of the association between HIV/ART and the GA-based outcomes PTD and SGA, with the severity and nature of this bias depending on the form of the error.

We set out to examine the impact of GA assessment method, and any measurement error therein, on the association between HIV/ART status and adverse birth outcomes among a population of pregnant women undergoing routine antenatal care at a large public sector primary care facility in Cape Town, South Africa.

**Methods**

Between April 2014 and October 2016, consecutive pregnant women (aged ≥18 years) were enrolled in a prospective cohort (n=3972). All women seeking antenatal care (ANC) at a large public sector primary care facility serving a low-income, high HIV prevalence sub-district of Cape Town South Africa16. Eligibility was regardless of their HIV status and included HIV-infected women who conceived while on ART continued their current regimen, and those newly diagnosed or not on ART were initiated on the WHO recommended first-line regimen. Women clinically assessed (LMP and/or SFH) during routine ANC services to be ≤24 weeks gestation were referred for a same day ultrasound by a research sonographer using standardized assessment protocols and blinded to the midwife GA assessment. The study was reviewed and approved by the University of Cape Town Faculty of Health Sciences Human Research Ethics Committee and the University of Southampton Faculty of Medicine Ethics Committee. Written informed consent was obtained for study participation, including data abstraction from routine clinical records through the pregnancy and post-partum period.

Following their first ANC visit, enrolled women had data abstracted on HIV status, pregnancy history (previous and index pregnancies), medications prescribed during pregnancy and any maternal diagnoses during pregnancy. Obstetric outcomes, including date of delivery and birthweight, were abstracted following delivery. HIV status was categorized as HIV-uninfected or HIV-infected; ART status was categorized as initiation before or during pregnancy. The primary outcome was GA at delivery based on completed weeks by LMP, SFH and US. PTD was defined as delivery at <37 weeks gestation, late to moderate preterm as delivery 32 to 37 weeks and post-term as delivery at >42 weeks.17 Using the gender-specific Intergrowth Standards-21st project standards,18 infants with birthweights <10th percentile for GA were classified as SGA, between 10th and 90th percentile as appropriate-for-gestational age (AGA), and >90th percentile as large-for-gestational age (LGA).

Statistical analyses were performed using STATA version 14.0 (Stata Corporation, College Station, TX, USA). Analyses focused on two comparisons: HIV-infected vs HIV-uninfected women; and among HIV-infected women ART initiation before pregnancy vs initiation during pregnancy. Outcome variables (PTD and SGA) were created for each assessment method (LMP, SFH and US), using GA at booking and date of delivery. Analyses compared GA estimated by each assessment method in live singleton births. Further analyses were restricted to women with GA estimated by all three assessment methods and to women with both a clinical GA estimate (LMP and/or SFH) and an ultrasound GA estimate. Comparisons of proportions were based on chi-squared tests and rank-sum tests.

In women with GA by all three assessment methods the associations between HIV/ART status and PTD were examined by each assessment method using logistic regression. Results were presented as odds ratios (OR) with 95% confidence intervals (CI), and adjusted for age, parity, BMI and previous PTD based on their association with adverse birth outcomes.3,5 BMI, assessed at the first ANC visit, was classified as underweight (≤18.5 kg/m2), normal (18.6-24.9 kg/m2), overweight (25.0-29.9 kg/m2), or obese (≥30kg/m2).19 To inform understanding of GA under- or over-estimation by LMP and SFH compared to US, multinomial logistic regression was conducted. Concordance was defined as <7 days difference which is deemed to be clinically relevant; however for US conducted in the second trimester LMP concordance was defined according to American College of Obstetricians and Gynaecologists recommendations: <7days between 14-15weeks GA, <10 days between 16-21weeks and <14days between 22-27weeks.20 Results were presented as risk ratios (RR) with 95% CI, and adjusted for age, parity, previous PTD, BMI and ART regimen (in HIV-infected only comparisons).

**Results**

A total of 1787 women with live singleton births were included: 1014 HIV-uninfected (57%) and 773 HIV-infected (43%), of whom 368 (48%) initiated ART before pregnancy and 405 (52%) during pregnancy. In line with local and WHO treatment guidelines, all enrolled HIV-infected women were on ART, and most (94%) were on a regimen of two NRTIs [tenofovir (TDF) and emtricitabine (FTC)], with the NNRTI efavirenz (EFV). HIV-infected women were older and less likely to be primigravid than HIV-uninfected women (Table 1). HIV-infected women who initiated ART before pregnancy were older and less likely to be primigravid than those who initiated during pregnancy. Overall 28% of women were overweight and 46% obese; there were no significant differences by HIV status or timing of ART initiation.

In total, 88% (n=1585) of women had an LMP-based GA , 68% (n=1220) SFH-based, and 67% (n=1204) US-based, with estimated median GA at enrolment varying by assessment method (Table 1). Of the ultrasounds 719 (60%) were conducted in the second trimester with 140 resulting in dating of >24 weeks GA.The estimated incidence of PTD using LMP-GA was 36% (95% CI 33-39%), 17% (95% CI 15-19%) by SFH and 11% (95% CI 9-13%) by US; with no significant difference by HIV status using LMP-GA (37% vs 36%) or SFH-GA (18% vs 17%). However, US-GA estimated PTD incidence was significantly higher in HIV-infected than HIV-uninfected women (14% vs 8%) (Table 2; Figure 1a). No significant differences in PTD incidence were observed between HIV-infected women initiating ART before or during pregnancy by LMP-GA (35% vs 39%), SFH-GA (18% vs 17%) and US-GA (12% vs 15%) (Table 2; Figure 1a).

*Women with GA assessed by all three methods (Restricted Group)*

Among the 35% (n=629) of women with GA assessed by all three methods, estimated median (IQR) GA at enrolment was 17 weeks (14-21) by LMP, 20 weeks (16-23) by SFH and 19 weeks (15-23) by US (Supp. Table 1a). HIV status was the only variable associated with having been assessed by all three methods, with increased odds for HIV-infected women (OR 1.54; 95% CI 1.22 - 1.84). In this group, estimated PTD incidence was 42% (95% CI 38-46%) using LMP-GA, 14% (95% CI 12-17%) using SFH-GA and 10% (95% CI 7-12%) using US-GA (Supp. Table 2).

As before for the whole group, estimated PTD incidence was significantly higher in HIV-infected than HIV-uninfected women using US-GA (12% vs 7%) but not significantly different using LMP-GA (40% vs 44%) or SFH-GA (16% vs 12%) (Supp. Table 2; Figure 1b). In HIV-infected women (n=320), no significant differences were observed between women initiating ART before or during pregnancy for LMP-GA (39% vs 41%), SFH-GA (17% vs 15%) or US-GA (10% vs 15%) (Supp. Table 2; Figure 1b).

Adjusting for age, parity, BMI and previous PTD, HIV-infected women had increased odds of US-GA PTD (aOR 1.98, 95% CI 1.12-3.52) compared to HIV-uninfected women. This was also seen with SFH-GA although not statistically significant (aOR 1.34, 95% CI 0.83-2.15); associations seen with LMP-GA were small and not significant (Table 3). In HIV-infected women, the odds of PTD did not vary appreciably by timing of ART initiation across the three assessment methods (Table 3). Similar results were seen in the overall cohort (Supp. Table 3).

*Factors associated with under- and over-estimation of GA*

In women with both an US estimate and a clinical estimate LMP (n=1057) or SFH (n=732), the estimated concordance with US-GA estimates was 29% (n=308) for LMP and 36% (n=261) for SFH. LMP under-estimated US-GA in 484 cases (46%), and over-estimated in 265 cases (25%). The percentages of under- and over-estimation for SFH were 21% (n=153) and 43% (n=318) respectively.

ComparingLMP-GA to US-GA, HIV status was not significantly associated with under-estimating (risk ratio (RR) 0.96, 95% CI 0.71-1.27), but was associated with over-estimating (RR 1.33, 95% CI 0.95-1.64) GA, although not statistically significant. These associations persisted in adjusted models (Table 4). No other factors were associated with under- or over-estimating by LMP-GA.

In HIV-infected women, timing of ART initiation was not significantly associated with either under- (RR 0.87, 95% CI 0.53-1.45) or over-estimating (RR 1.34, 95% CI 0.78-2.31) GA by LMP and this persisted in the adjusted models (Table 4).

Comparing SFH-GA to US-GA, there was no association with HIV status for either under- (RR 1.21, 95% CI 0.80-1.83) or over-estimating (RR 1.33, 95% CI 0.94-1.87) GA. In the adjusted model, obese women (aRR 1.72, 95% CI 1.09-2.73) were at increased risk of over-estimation by SFH; while being older (>30yrs) was associated with decreased risk of under-estimation by SFH (aRR 0.58, 95% CI 0.32-1.03).

Among HIV-infected women there was no association with timing of ART initiation for either under- or over-estimating GA by SFH and this persisted with adjustment (Table 4).

*Small for gestational age deliveries*

The incidence of SGA was similar according to LMP, SFH and US (10% vs 13% vs 11%); however differences were observed by assessment method for AGA and LGA, with much wider GA estimate variations when using LMP-GA and SFH-GA than using US-GA (Figure 2). The incidence of AGA was significantly higher with SFH-GA (73%) and US-GA (80%) than with LMP-GA (59%). Correspondingly, the incidence of LGA was significantly higher when LMP-GA was used (32%) than when SFH-GA (14%) and US-GA (9%) was used (Table 2; Figure 2).

When the incidence of SGA was compared according to HIV status, SGA incidence was higher for HIV-infected than HIV-uninfected women across all three assessment methods. Using LMP-GA or US-GA, SGA estimated did not differ by timing of ART initiation. However, using SFH-GA, women initiating before pregnancy appeared to have a lower SGA incidence than those initiating during pregnancy (11% vs 19%) (Table 2). In women with GA by all three assessment methods, overall SGA was slightly higher when SFH-GA (14%) was used compared to LMP-GA (9%) and US-GA (11%). Similar patterns to those seen overall were seen in this restricted group with the incidence of AGA and LGA.

By all three assessment measures, SGA incidence was higher in HIV-infected than HIV-uninfected women. No differences were observed according to timing of ART initiation for LMP-GA and US-GA, however a lower incidence of SGA was observed in women initiating before pregnancy for SFH-GA (Supp. Table 2).

**Discussion**

In this prospective cohort of pregnant women seeking ANC at a large public sector primary care facility in South Africa, we found that estimated GA varied by the measurement employed and that differences in GA resulted in significant differences in the occurrence of PTD. Where analysis of safety of ART use in pregnancy by timing of ART initiation is focused on outcomes such as PTD or SGA, our work highlights the need for standardized GA ascertainment methods.

Using US-GA, PTD risk was associated with maternal HIV infection, with HIV-infected women, all on ART, almost twice as likely to deliver preterm than HIV-uninfected women. A weaker association was observed with SFH-based GA ; and minimal differences with LMP-GA.. We did not find any appreciable differences in PTD risk for HIV-infected women by timing of ART initiation by all three assessment methods. Our findings (in both the overall cohort and in women with all three assessments) suggest that GA ascertainment methods could partially explain the heterogeneity of findings from previous studies on the association between ART use and adverse birth outcomes, suggesting that care should be taken when interpreting results from such studies.

Our finding for a higher PTD incidence in HIV-infected than HIV-uninfected women when using US-GA is consistent with several studies, using different assessment methods, across different settings with the incidence of any PTD (<37 weeks) ranging from 13% to 37%.3,7,8,21,22 Using US-GA we found an incidence of 11% (95% CI 9-13%) among HIV-infected women overall, similar to findings from a Nigerian study; 22 but significantly lower than findings from other studies. 3,7,8,21,22 In addition to differences in assessment methods, this could also be due to underlying differences in maternal (HIV disease stage and clinical characteristics) and behavioural risk factors (alcohol and substance abuse).

Using LMP-GA and SFH-GA, we found estimated PTD incidence to be higher than with US-GA, and similar to findings from previous studies, which primarily used LMP-based measures. However, in adjusted analyses the difference in PTD risk by HIV status was marginal and not significant using either method. The most plausible explanation for the discrepancy in findings between US and LMP/SFH measures is random measurement error. When stratified by HIV and BMI status, the proportions of PTD observed by each assessment method remained similar to the overall, pointing to non-differential misclassification of PTD status due to random measurement error in the GA estimated by LMP and SFH. In previous studies, GA measurement error resulted in an over-estimation of preterm and post-term delivery incidence,9, 12, 23 consistent with our findings of over-estimation by both LMP-GA and SFH-GA compared to US-GA. Our findings show that PTD over-estimation by LMP/SFH was driven primarily by the inflation of the tails of the GA distribution, where estimates were more extreme than the likely true-data estimates.

In our setting, LMP-GA estimates varied widely with measurement error resulting predominantly in under-estimated GA contributing to the substantially higher PTD incidence, in contrast over-estimation would have led to higher frequencies of post-term delivery classification. While we expected some differences between LMP-GA and US-GA,24 the degree of discrepancy was greater than previous reports from high-income countries and other resource-limited settings;24,25 it was, however, in keeping with previous South African data.26,27 These inconsistencies suggest the degree and type of error in LMP-GA may depend on demographic characteristics of the study population and data collection methods. For example, errors in GA estimates due to delayed ovulation usually lead to GA over-estimation, whereas GA errors due to recall issues can lead to errors in either direction. Additionally, women with non-normal BMI (<19 or >29kg/m2) may misreport their LMP due to menstrual irregularities.28,29 In our study population, numerous factors could have played a part in the under- and over-estimation of LMP-based PTD frequencies (relative to US). In addition to BMI, other socio-demographic factors, known to be associated with reduced accuracy of LMP-GA, are prevalent in our setting. This includes high level of injectable contraception use,30 long-term use of which affects return to menses and fertility, limiting LMP reliability.31 Additionally younger, primigravid women and women with lower educational levels are more likely to misreport LMP.12,28 Since HIV-uninfected women were more likely to be younger and primigravid it is possible they misreported their LMP more than HIV-infected women, which could explain why the expected association by HIV status was not observed with LMP-GA.

In contrast to LMP-GA, in our study SFH-GA was predominantly over-estimated, possibly a reflection of study participants BMI profile. Similar to a previous South African study, SFH-GA performed better than LMP-GA, with accuracy worsening with increasing BMI presumably due to increased maternal adiposity interfering with SFH measurement.26 This is consistent with reports that high BMI affects the accuracy of SFH-GA estimates;13,32 a Mozambican study demonstrated that among women of the same GA, the fundal height of obese women was higher than that of normal-weight women resulting in GAover-estimation.32 Accuracy in overweight and obese women is compromised because GA calculation is based on fundal height growth curves derived from normal weight women.33 SFH measurements are also subject to intra- and inter-observer errors, a particular problem with single measurements.33 Multiple SFH measurement models have been shown to have a higher level of accuracy when repeated measurements are used.34 While the association between HIV status and PTD was in the expected direction, attenuation by BMI-related measurement errors could have resulted in non-significant associations.

In HIV-infected women, we did not observe any significant association between timing of ART initiation and PTD by any of the assessment methods. It is possible that our sample size did not enable detection of small to moderate differences between these groups of women, however our results are consistent with findings from a previous South African cohort,21 and findings from other settings.35 This suggests that in our study population there might be minimal differences across the comparison group and that any GA measurement errors were equally distributed.

Overall, SGA incidence did not differ greatly by assessment method, which was surprising given GA differences observed according to assessment method. In contrast, LGA estimates differed by assessment methods, with larger than expected proportions of LGA by LMP-GA and SFH-GA, which would misclassification due to inaccurate GA. These results suggest that in our setting, in the absence of ultrasound facilities, SFH-GA is substantially more reliable than LMP-GA.

A major strength of our study was the prospective data collection and GA assessment by a research sonographer, who was blinded to other measures, ensuring a high-quality measure of gestation. Further, US was conducted in women ≤24 weeks, when US is highly reproducible and accurate to date pregnancy duration.36 While there is a possibility that our US-GA estimates may be biased due to systematic under-estimation of GA for smaller fetuses, these errors have been shown to be relatively small compared to the larger errors seen when using LMP- and SFH-based measures for GA estimation.12 While we did not directly measure LMP and/or SFH as part of study procedures and relied instead on data abstraction from routine records, we believe any measurement errors were random rather than systematic. Study limitations include the absence of data on pre-pregnancy BMI and other factors that can influence GA estimate accuracy, such as maternal education which is known to impact LMP-GA estimate accuracy. In addition to measurement error, selection bias is also likely to have contributed to the attenuation of associations in the group of women with three measures. Moreover, given that clinical estimates, which we showed are subject to substantial measurement error, were used to determine which women were referred for ultrasounds there is a possibility of selection bias which could have led to inaccurate estimate of the true association. Additionally, while it was intended that the sonographer be blinded, this was only partial since by study protocol only women with ≤24 weeks GA were referred for ultrasound assessment.

The relationship between ART and adverse birth outcomes is complex: it is well established that ART can improve birth and child health outcomes by improving maternal health, but ART may also contribute to worsening outcomes through mechanisms that have not been fully elucidated. With widespread use of ART in pregnancy and growing public health interest in this association, understanding and addressing adverse birth outcomes is vitally important. This study makes an important contribution to this emerging science by demonstrating that inaccurate GA measurement can distort associations between maternal characteristics, including HIV/ART, and adverse birth outcomes. In settings where LMP and SFH-based measures are widely used, bias due to GA ascertainment methods employed should be considered as an alternative explanation for observed associations or null findings. Additionally, given that there appear to be systematic differences in GA estimates according to BMI status, future research efforts should consider the BMI profile of study populations and seek to standardize optimal measures of GA. While early US should be encouraged whenever possible, simple and novel methods that accurately measure GA are required particularly in settings where ultrasound is unavailable; and this should include postnatal assessments to confirm GA based on antenatal determination.

Furthermore, quantitative bias analytic techniques that account for measurement error in GA and/or missing data should be considered in future analyses.

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**REFERENCES**

1. WHO. Guideline on when to start antiretroviral therapy and on pre-exposure prophylaxis for HIV. Geneva, Switzerland: 2015.

2. UNAIDS. On the fast-track to an AIDS-free generation. Geneva, Switzerland: 2016.

3. Chen JY, Ribaudo HJ, Souda S, Parekh N, Ogwu A, Lockman S, et al. Highly active antiretroviral therapy and adverse birth outcomes among HIV-infected women in Botswana. Journal of Infectious Diseases. 2012;206(11):1695-1705

4. Fowler MG, Qin M, Fiscus SA, Currier JS, Flynn PM, Chipato T, et al. Benefits and Risks of Antiretroviral Therapy for Perinatal HIV Prevention. New England Journal of Medicine. 2016;375(18):1726-1737.

5. Li N, Sando MM, Spiegelman D, Hertzmark E, Liu E, Sando D, et al. Antiretroviral therapy in relation to birth outcomes among HIV-infected women: a cohort study. Journal of Infectious Diseases. 2016;213:1057–1064.

6. UNICEF. Levels & Trends in Child Mortality. New York, USA: 2015.

7. Boer K, Nellen JF, Patel D, Timmermans S, Tempelman C, Wibaut M, et al. The AmRo study: pregnancy outcome in HIV‐1‐infected women under effective highly active antiretroviral therapy and a policy of vaginal delivery. BJOG: An International Journal of Obstetrics & Gynaecology. 2007;114(2):148-155.

8. Zash R, Souda S, Chen JY, Binda K, Dryden-Peterson S, Lockman S, et al. Reassuring Birth Outcomes with Tenofovir/Emtricitabine/Efavirenz used for Prevention of Mother to Child Transmission of HIV in Botswana. Journal of Acquired Immune Deficiency Syndromes. 2016;71(4):428-436.

9. Gardosi J, Geirsson RT. Routine ultrasound is the method of choice for dating pregnancy. BJOG: An International Journal of Obstetrics & Gynaecology. 1998;105(9):933-936.

10. Mongelli M, Wilcox M, Gardosi J. Estimating the date of confinement: ultrasonographic biometry versus certain menstrual dates. American Journal of Obstetrics and Gynecology. 1996;174(1):278-281.

11. Johnson SR, Miro F, Barrett S, Ellis JE. Levels of urinary human chorionic gonadotrophin (hCG) following conception and variability of menstrual cycle length in a cohort of women attempting to conceive. Current Medical Research and Opinion. 2009;25(3):741–748.

12. Dietz PM, England LJ, Callaghan WM, Pearl M, Wier ML, Kharrazi M. A comparison of LMP-based and ultrasound-based estimates of gestational age using linked California livebirth and prenatal screening records. Paediatric and Perinatal Epidemiology. 2007;21(Suppl 2):62-71.

13. Mongelli M, Gardosi J. Sympysis-fundus height and pregnancy characteristic in ultrasound-dated pregnancies. Obstetrics & Gynecology. 1999;94(4):591-594.

14. Gardosi J, Francis A. Controlled trial of fundal height measurement plotted on customized antenatal growth charts. BJOG: An International Journal of Obstetrics & Gynaecology. 1999;106(4):309-317.

15. WHO. Determinants of low birth weight: methodological assessment and meta-analysis. Bulletin of the World Health Organization. 1987;65(5):663-737.

16. Myer L, Phillips TK, Manuelli V, McIntyre J, Bekker LG, Abrams EJ. Evolution of antiretroviral therapy services for HIV-infected pregnant women in Cape Town, South Africa. Journal of Acquired Immune Deficiency Syndromes. 2015;69(2):e57–e65.

17. WHO. Fact Sheet: Preterm Birth. WHO Media Centre 2017.

18. Villar J, Ismail LC, Victora CG, Ohuma EO, Bertino E, Altman DG, et al. International standards for newborn weight, length, and head circumference by gestational age and sex: the Newborn Cross-Sectional Study of the INTERGROWTH-21st Project. The Lancet. 2014;384(9946):857-868.

19. WHO. Fact Sheet: HIV/AIDS. WHO Media Centre 2016.

20. American College of Obstetricians and Gynecologists. Methods for estimating the due date. Committee Opinion No. 700. Obstetrics & Gynecology. 2017;129(5):e150–e154.

21. Malaba TR, Phillips TK, Le Roux S, Brittain K, Zerbe A, Petro G, et al. Antiretroviral therapy use during pregnancy and adverse birth outcomes in South African women. International Journal of Epidemiology. 2017;46(5):1678-1689

22. Olagbuji BN, Ezeanochie MC, Ande AB, Oboro VO. Obstetric and perinatal outcome in HIV positive women receiving HAART in urban Nigeria. Archives of Gynecology and Obstetrics. 2010;281(6):991-994.

23. Haglund B. Birthweight distributions by gestational age: comparison of LMP‐based and ultrasound‐based estimates of gestational age using data from the Swedish Birth Registry. Paediatric and Perinatal Epidemiology. 2007;21(s2):72-78.

24. Tunón K, Eik-Nes SH, Grøttum P. A comparison between ultrasound and a reliable last menstrual period as predictors of the day of delivery in 15 000 examinations. Ultrasound in Obstetrics & Gynecology. 1996;8(3):178-185.

25. Jehan I, Zaidi S, Rizvi S, Mobeen N, McClure EM, Munoz B, et al. Dating gestational age by last menstrual period, symphysis-fundal height, and ultrasound in urban Pakistan. International Journal of Gynecology & Obstetrics. 2010;110(3):231-234.

26. Geerts LT, Poggenpoel E, Theron GB. A comparison of pregnancy dating methods commonly used in South Africa: a prospective study. South African Medical Journal. 2013;103(8):352-357.

27. Geerts LT, Theron AM, Grove D, Theron GB, Odendaal HJ. A community-based obstetric ultrasound service. International Journal of Gynecology & Obstetrics. 2004;8(1):23-31.

28. Lynch CD, Zhang J. The research implications of the selection of a gestational age estimation method. Paediatric and Perinatal Epidemiology. 2007;21(s2):86-96.

29. Kullinger M, Wesström J, Kieler H, Skalkidou A. Maternal and fetal characteristics affect discrepancies between pregnancy‐dating methods: a population‐based cross‐sectional register study. Acta Obstetricia et Gynecologica Scandinavica. 2017;96(1):86-95.

30. Chersich MF, Wabiri N, Risher K, Shisana O, Celentano D, Rehle T, et al. Contraception coverage and methods used among women in South Africa: A national household survey.South African Medical Journal. 2017;107(4): 307-314.

31. Waller DK, Spears WD, Gu Y, Cunningham GC. Assessing number-specific error in the recall of onset of last menstrual period. Paediatric and Perinatal Epidemiology. 2000;14(3):263–267.

32. Challis K, Osman NB, Nyström L, Nordahl G, Bergström S. Symphysis‐fundal height growth chart of an obstetric cohort of 817 Mozambican women with ultrasound‐dated singleton pregnancies. Tropical Medicine & International Health. 2002;7(8):678-684.

33. Deeluea J, Sirichotiyakul S, Weerakiet S, Arora R, Patumanond J. Fundal height growth curve for underweight and overweight and obese pregnant women in Thai population. ISRN Obstetrics and Gynecology. 2013;2013:1-8

34. White LJ, Lee SJ, Stepniewska K, Simpson JA, Dwell SLM, Arunjerdja R, et al. Estimation of gestational age from fundal height: a solution for resource-poor settings. Journal of the Royal Society Interface. 2012;9(68):503-510.

35. Njom Nlend A, Nga Motazé A, Moyo Tetang S, Zeudja C, Ngantcha M, Tejiokem M. Preterm Birth and Low Birth Weight after In Utero Exposure to Antiretrovirals Initiated during Pregnancy in Yaoundé, Cameroon. PLoS One. 2016;11(3):e0150565.

36. Butt K, Lim K, Bly S, Cargill Y, Davies G, Denis N, et al. Determination of gestational age by ultrasound. Journal of Obstetrics and Gynaecology. 2014;36(2):171-181.

Table 1: Description of enrolled women (n=1787) at 1st ANC Visit

|  |  | **Total****n=1787 (%)** | **HIV-uninfected****n=1014 (%)** | **HIV-****infected****n=773 (%)** | **HIV-infected** |
| --- | --- | --- | --- | --- | --- |
| **Initiated before pregnancy** **n=368 (%)** | **Initiated during pregnancy** **n=405 (%)** |
| **Maternal Characteristics**  |  |  |  |
| Age, years |  |  |  |  |  |
|  | <24 | 536 (30) | 398 (39) | 138 (18) | 43 (12) | 95 (23) |
|  | 25-29 | 500 (28) | 291 (29) | 209 (27) | 76 (21) | 133 (33) |
|  | >30 | 751 (42) | 325 (32) | 426 (55) | 249 (67) | 177 (44) |
|  | Median | 28 (24-32) | 26 (22-31) | 30 (26-33) | 32 (28-35) | 29 (25-32) |
| Height, cm |  |  |  |  |  |
|  | ≤155 | 500 (28) | 270 (27) | 230 (30) | 108 (29) | 122 (30) |
|  | 156-161 | 651 (36) | 375 (37) | 276 (36) | 143 (39) | 133 (33) |
|  | ≥162 | 636 (36) | 369 (36) | 267 (35) | 117 (32) | 150 (37) |
|  | Median | 158 (155-163) | 159 (155-163) | 158 (154-163) | 158 (154-162) | 158 (154-163) |
| Gestation at enrolment, weeks |  |  |  |  |  |
|  | Determined by LMP  | 1585 | 896 | 689 | 368 | 405 |
|  |  | Median | 17 (12-22) | 18 (13-24) | 15 (11-21) | 14 (11-19) | 16 (12-22) |
|  | Determined by SFH | 1219 | 709 | 510 | 215 | 295 |
|  |  | Median | 23 (18-28) | 24 (19-28) | 20 (16-26) | 19 (14-25) | 21 (17-27) |
|  | Determined by US | 1204 | 603 | 601 | 279 | 322 |
|  |  | Median | 16 (12-21) | 17 (12-22) | 15 (11-20) | 14 (11-18) | 16 (12-20) |
| Body Mass Index |  |  |  |  |  |
|  | Underweight | 16 (1) | 10 (1) | 6 (1) | 5 (1) | 1 (0.3) |
|  | Normal | 329 (18) | 188 (19) | 141 (18) | 66 (18) | 75 (19) |
|  | Overweight | 494 (28) | 282 (28) | 213 (18) | 104 (28) | 110 (27) |
|  | Obese | 823 (46) | 467 (46) | 356 (46) | 166 (45) | 189 (47) |
| Gravidity |  |  |  |  |  |
|  | 1 | 383 (21) | 263 (26) | 120 (16) | 41 (11) | 79 (20) |
|  | 2 | 639 (36) | 372 (37) | 267 (35) | 119 (32) | 148 (37) |
|  | ≥3 | 765 (43) | 379 (37) | 386 (50) | 208 (57) | 178 (44) |
|  | Median | 2 (2-3) | 2 (1-3) | 2 (2-3) | 2 (2-3) | 2 (2-3) |
| Parity |  |  |  |  |  |
|  | 0 | 472 (26) | 303 (30) | 169 (22) | 67 (18) | 102 (25) |
|  | 1 | 700 (39) | 397 (39) | 303 (39) | 135 (37) | 168 (42) |
|  | ≥2 | 615 (35) | 314 (31) | 301 (39) | 166 (45) | 135 (33) |
|  | Median | 1 (0-2) | 1 (0-2) | 1 (0-2) | 1 (1-2) | 1 (1-2) |
| Previous Preterm\* |  |  |  |  |  |
|  | Yes | 127 (9) | 58 (8) | 69 (11) | 37 (11) | 32 (10) |
| Haemoglobin g/dl |  |  |  |  |  |
|  | Normal (≥11.0) | 792 (44) | 496 (49) | 296 (46) | 171 (46) | 125 (31) |
|  | Mild Anaemia (9-10.9) | 613 (34) | 337 (33) | 276 (43) | 111 (30) | 165 (41) |
|  | Moderate Anaemia (7-8.9) | 122 (7) | 54 (5) | 68 (11) | 25 (7) | 43 (11) |
|  | Severe Anaemia (<7) | 3 (0.2) | 3 (0.3) | 0 | 0 | 0 |
| ART Regimen |  |  |  |  |  |
|  | TDF-3TC-EFV | 691 (39) | - | 691 (94) | 298 (89) | 393 (98) |
|  | TDF-3TC-NVP | 4 (0.2) | - | 4 (1) | 2 (1) | 2 (0.5) |
|  | Other NNRTI | 20 (1) | - | 20 (2) | 14 (4) | 6 (1) |
|  | PI regimen | 22 (1) | - | 22 (3) | 20 (6) | 2 (0.5) |

\*among women with a previous pregnancy

All variables, with the exception of height, BMI and haemoglobin had <1% missing data. For height, 6% (n=111) of data was missing and for BMI, 7% (n=126) was missing, for both there were similar proportions across all comparison groups. For Haemoglobin, 14% (n=57) of data was missing with similar proportions across all comparison groups. For ART status 2% (n=36) were missing regimen data

Figure 1: Incidence of preterm deliveries (<37weeks) by HIV/ART Status, **A** in all enrolled women (n=1787), and **B** in all HIV-infected women (n=773), **C** in women assessed by all three methods (n=629), and **D** in HIV-infected women assessed by all three methods (n=320)



Figure 2: Birthweight and gestational age according to GA assessment method (boys and girls separately)



\*

\*

SGA – small for gestational age; LGA – large for gestational age;

\* Superimposed smoothed 10th (SGA) and 90th (LGA) centile curves for birthweight according to gestational age and gender

Table 2: Birth outcomes in overall cohort (n=1787)

|  |  | **Total** | **HIV-uninfected** | **HIV-****infected** | **HIV-infected** |
| --- | --- | --- | --- | --- | --- |
| **Initiated before pregnancy**  | **Initiated during pregnancy**  |
| Gestational Age (weeks) LMP | n=1585 | n=896 | n=689 | n=336 | n=353 |
|  | Post-term >42 | 115 (7) | 57 (6) | 58 (8) | 33 (10) | 25 (7) |
|  | Term (37-42) | 896 (57) | 522 (58) | 374 (55) | 185 (55) | 189 (54) |
|  | Any Preterm (< 37)\* | 574 (36) | 317 (36) | 257 (37) | 118 (35) | 139 (39) |
|  |  | *Late to Mod Preterm (32-37)* | *418 (73)* | *238 (75)* | *180 (70)* | *86 (73)* | *94 (68)* |
|  |  | *Very Preterm (28-32)* | *156 (27)* | *79 (25)* | *77 (30)* | *32 (27)* | *45 (32)* |
| Gestational Age (weeks) SFH | n=1219 | n=709 | n=510 | n=215 | n=295 |
|  | Post-term >42 | 97 (8) | 59 (8) | 38 (7) | 15 (7) | 23 (8) |
|  | Term (37-42) | 914 (75) | 532 (75) | 382 (75) | 161 (75) | 221 (75) |
|  | Any Preterm (< 37)\* | 208 (17) | 118 (17) | 90 (18) | 39 (18) | 51 (17) |
|  |  | *Late to Mod Preterm (32-37)* | *171 (82)* | *98 (83)* | *73 (81)* | *33 (85)* | *40 (78)* |
|  |  | *Very Preterm (28-32)* | *37 (18)* | *20 (17)* | *17 (19)* | *6 (15)* | *11 (22)* |
| Gestational Age (weeks) US | n=1204 | n=603 | n=601 | n=279 | n=322 |
|  | Post-term >42 | 6 (1) | 6 (10) | 0 | 0 | 0 |
|  | Term (37-42) | 1065 (88) | 547 (91) | 518 (86) | 244 (88) | 274 (85) |
|  | Any Preterm (< 37)\* | 133 (11) | 50 (8) | 83 (14) | 35 (12) | 48 (15) |
|  |  | *Late to Mod Preterm (32-37)* | *114 (86)* | *47 (94)* | *67 (81)* | *28 (80)* | *39 (81)* |
|  |  | *Very Preterm (28-32)* | *19 (14)* | *3 (6)* | *16 (19)* | *7 (20)* | *9 (19)* |
| Size for Gestational Age LMP | n=1441 | n=825 | n=616 | n=294 | n=322 |
|  | Small (SGA) | 137 (10) | 61 (7) | 76 (12) | 36 (12) | 40 (13) |
|  | Appropriate (AGA) | 847 (59) | 498 (60) | 349 (57) | 181 (62) | 168 (52) |
|  | Large (LGA) | 457 (32) | 266 (32) | 191 (31) | 77 (26) | 114 (35) |
| Size for Gestational Age SFH | n=1113 | n=649 | n=464 | n=195 | n=269 |
|  | Small (SGA) | 145 (13) | 72 (11) | 73 (16) | 22 (11) | 51 (19) |
|  | Appropriate (AGA) | 814 (73) | 481 (74) | 333 (72) | 150 (77) | 183 (68) |
|  | Large (LGA) | 154 (14) | 96 (15) | 58 (13) | 23 (12) | 35 (13) |
| Size for Gestational Age US | n=1184 | n=596 | n=588 | n=273 | n=315 |
|  | Small (SGA) | 131 (11) | 49 (8) | 82 (14) | 39 (14) | 43 (14) |
|  | Appropriate (AGA) | 944 (80) | 492 (83) | 452 (77) | 213 (78) | 239 (76) |
|  | Large (LGA) | 109 (9) | 55 (9) | 54 (9) | 21 (8) | 33 (10) |

\* Any Preterm consists of the subset of Late Preterm, Moderately Preterm and Very Preterm deliveries

For Size for GA data missing for women whose GA is not within Intergrowth standard limits (<24w and >42w) and those missing either GA or birthweight

Table 3: Adjusted association between HIV/ART status and preterm delivery in women with all three assessment methods (n=629)

| **Assessment Method** | **HIV-infected vs** **HIV-uninfected (Ref)** | **Initiated before pregnancy (Ref) vs** **Initiated during pregnancy**  |
| --- | --- | --- |
|  | **aOR (95% CI)** | **P-value** |  | **aOR (95% CI)** | **P-value** |
| Last Menstrual Period (LMP) | HIV-infected | 0.89 (0.63-1.24) | 0.483 | During pregnancy | 0.94 (0.56-1.60) | 0.802 |
| Symphysis Fundal Height (SFH) | HIV-infected | 1.34 (0.83-2.15) | 0.225 | During pregnancy | 1.42 (0.76-2.84) | 0.290 |
| Ultrasound (US) | HIV-infected | 1.98 (1.12-3.53) | 0.020 | During pregnancy | 0.69 (0.33-1.47) | 0.335 |

\*Adjusted for age, parity, BMI and previous PTD

\*\*Adjusted for age, parity, BMI, previous PTD and ART regimen

Table 4: Adjusted association between HIV/ART status and concordance with US-based measures in women with both an US estimate and a clinical estimate

| **Assessment Method** | **HIV-infected vs HIV-uninfected (Ref)\*** | **Initiated before pregnancy (Ref) vs Initiated during pregnancy\*\***  |
| --- | --- | --- |
| **Ref category: No difference** | **ARR (95% CI)** | **P-value** | **Ref category: No difference** | **ARR (95% CI)** | **P-value** |
| Last menstrual period (LMP)(n=1057) | HIV-infected | Under-estimate | 0.96 (0.71-1.27) | 0.764 | During pregnancy | Under-estimate | 0.87 (0.53-1.45) | 0.609 |
| Over-estimate | 1.33 (0.95-1.64) | 0.098 | Over-estimate | 1.34 (0.78-2.31) | 0.288 |
| Symphysis Fundal Height (SFH)(n=732) | HIV-infected | Under-estimate | 1.21 (0.80-1.83) | 0.364 | During pregnancy | Under-estimate | 1.23 (0.66-2.30) | 0.518 |
| Over-estimate | 1.33 (0.94-1.87) | 0.102 | Over-estimate | 0.91 (0.54-1.52) | 0.711 |

\*Adjusted for age, parity, BMI

\*\*Adjusted for age, parity, BMI and ART regimen

**SUPPLEMENTARY MATERIAL**

Supplementary Table 1a: Description of women with all three GA assessment methods (n=629) at 1st ANC Visit

|  |  | **Total****n=629** | **HIV-uninfected****n=309** | **HIV-infected****n=320** | **HIV infected** |
| --- | --- | --- | --- | --- | --- |
| **Initiated before pregnancy** **n=134** | **Initiated during pregnancy** **n=186** |
| **Maternal Characteristics**  |  |  |
| Age, years |  |  |  |  |  |
|  | <24 | 173 (28) | 109 (35) | 64 (20) | 18 (13) | 46 (25) |
|  | 25-29 | 164 (26) | 94 (30) | 70 (22) | 18 (13) | 52 (28) |
|  | >30 | 292 (46) | 106 (34) | 186 (58) | 98 (74) | 88 (47) |
|  | Median | 29 (24-33) | 27 (23-31) | 31 (26-34) | 33 (29-36) | 29 (25-32) |
| Height, cm |  |  |  |  |  |
|  | ≤155 | 188 (30) | 92 (30) | 96 (30) | 34 (25) | 62 (33) |
|  | 156-161 | 232 (37) | 111 (36) | 121 (38) | 57 (43) | 64 (35) |
|  | ≥162 | 209 (33) | 106 (34) | 103 (32) | 43 (32) | 60 (32) |
|  | Median | 158 (154-163) | 158 (154-163) | 158 (154-162) | 158 (155-163) | 157 (153-162) |
| Gestation at enrolment, weeks |  |  |  |  |  |
|  | Determined by LMP  |  |  |  |  |  |
|  |  | Median | 17 (14-21) | 18 (15-21) | 17 (13-20) | 16 (12-20) | 17 (13-20) |
|  | Determined by SFH |  |  |  |  |  |
|  |  | Median | 20 (16-23) | 21 (17-24) | 18 (15-22) | 18 (14-21) | 19 (15-23) |
|  | Determined by US |  |  |  |  |  |
|  |  | Median | 19 (15-23) | 20 (17-24) | 18 (14-22) | 18 (14-21) | 19 (14-22) |
| Body Mass Index |  |  |  |  |  |
|  | Underweight | 6 (1) | 5 (2) | 1 (0.3) | 0 | 1 (0.5) |
|  | Normal | 122 (19) | 57 (18) | 65 (20) | 25 (19) | 40 (24) |
|  | Overweight | 178 (28) | 86 (28) | 92 (29) | 39 (31) | 53 (28) |
|  | Obese | 290 (46) | 146 (47) | 144 (45) | 63 (50) | 81 (44) |
| Gravidity |  |  |  |  |  |
|  | 1 | 127 (20) | 78 (25) | 49 (15) | 16 (12) | 33 (18) |
|  | 2 | 231 (37) | 115 (37) | 116 (36) | 43 (33) | 72 (39) |
|  | ≥3 | 271 (43) | 116 (38) | 155 (48) | 70 (54) | 81 (44) |
|  | Median | 2 (2-3) | 2 (1-3) | 2 (2-3) | 3 (2-3) | 2 (2-3) |
| Parity |  |  |  |  |  |
|  | 0 | 153 (24) | 88 (28) | 65 (20) | 23 (17) | 42 (23) |
|  | 1 | 247 (39) | 121 (39) | 126 (39) | 49 (37) | 77 (41) |
|  | ≥2 | 229 (37) | 100 (33) | 129 (40) | 62 (46) | 67 (36) |
|  | Median | 1 (1-2) | 1 (0-2) | 1 (1-2) | 1 (1-2) | 1 (1-2) |
| Previous Preterm\* |  |  |  |  |  |
|  | Yes | 44 (9) | 18 (8) | 26 (10) | 12 (10) | 14 (9) |
| Haemoglobin g/dl |  |  |  |  |  |
|  | Normal (≥11.0) | 260 (41) | 150 (49) | 110 (34) | 56 (43) | 51 (28) |
|  | Mild Anaemia (9-10.9) | 233 (37) | 104 (34) | 129 (40) | 44 (34) | 82 (46) |
|  | Moderate Anaemia (7-8.9) | 39 (6) | 11 (4) | 28 (9) | 10 (8) | 16 (9) |
|  | Severe Anaemia (<7g) | 1 (0.2) | 1 (0.3) | 0 | 0 | 0 |
| ART Regimen |  |  |  |  |  |
|  | TDF-3TC-EFV | 290 (46) | - | 290 (95) | 111 (93) | 179 (97) |
|  | TDF-3TC-NVP | 2 (0.3) | - | 2 (1) | 0 | 2 (1) |
|  | Other NNRTI | 6 (1) | - | 6 (2) | 4 (3) | 2 (1) |
|  | PI regimen | 7 (1) | - | 7 (2) | 5 (4) | 2 (1) |

\*among women with a previous pregnancy

All variables, with the exception of height, BMI and haemoglobin had <1% missing data. For height, 5% (n=30) of data was missing and for BMI, 5% (n=34) was missing, for both there were similar proportions across all comparison groups. For Haemoglobin, 15% (n=96) of data was missing with similar proportions across all comparison groups. For ART status 2% (n=15) were missing regimen data

Supplementary Table 1b: Description of women with all three GA assessment methods (n=629) vs women without all three GA assessment methods (n=1158) at 1st ANC Visit

|  |  | **Overall Cohort****n=1787** | **<3 measures****n=1158** | **3 measures****n=629** |
| --- | --- | --- | --- | --- |
|
| **Maternal Characteristics**  |  |
| Age, years |  |  |  |
|  | <24 | 536 (30) | 363 (31) | 173 (28) |
|  | 25-29 | 500 (28) | 336 (29) | 164 (26) |
|  | >30 | 751 (42) | 459 39) | 292 (46) |
|  | Median | 28 (24-32) | 28 (23-32) | 29 (24-33) |
| Height, cm |  |  |  |
|  | ≤155 | 500 (28) | 312 (27) | 188 (30) |
|  | 156-161 | 651 (36) | 419 (36) | 232 (37) |
|  | ≥162 | 636 (36) | 427 (37) | 103 (32) |
|  | Median | 158 (155-163) | 159 (155-163) | 158 (154-163) |
| Gestation at enrolment, weeks |  |  |  |
|  | Determined by LMP  | 1585 | 956 | 629 |
|  |  | 17 (12-22) | 17 (14-21) | 16 (11-25) | 17 (14-21) |
|  | Determined by SFH | 1219 | 590 | 629 |
|  |  | 23 (18-28) | 20 (16-23) | 27 (22-231) | 20 (16-23) |
|  | Determined by US | 1204 | 575 | 629 |
|  |  | 16 (12-21) | 19 (15-23) | 13 (9-17) | 19 (15-23) |
| Body Mass Index |  |  |  |
|  | Underweight | 16 (1) | 10 (1) | 6 (1) |
|  | Normal | 329 (18) | 207 (19) | 122 (20) |
|  | Overweight | 494 (28) | 317 (30) | 178 (30) |
|  | Obese | 823 (46) | 533 (50) | 290 (49) |
| Gravidity |  |  |  |
|  | 1 | 383 (21) | 256 (22) | 127 (20) |
|  | 2 | 639 (36) | 408 (35) | 231 (37) |
|  | ≥3 | 765 (43) | 494 (43) | 271 (43) |
|  | Median | 2 (2-3) | 2 (2-3) | 2 (2-3) |
| Parity |  |  |  |
|  | 0 | 472 (26) | 319 (28) | 153 (24) |
|  | 1 | 700 (39) | 453 (39) | 247 (39) |
|  | ≥2 | 615 (35) | 386 (33) | 229(36) |
|  | Median | 1 (0-2) | 1 (0-2) | 1 (1-2) |
| Previous Preterm\* |  |  |  |
|  | Yes | 127 (9) | 83 (7) | 44 (7) |
| Haemoglobin g/dl |  |  |  |
|  | Normal (≥11.0) | 792 (44) | 532 (53) | 260 (49) |
|  | Mild Anaemia (9-10.9) | 613 (34) | 380 (38) | 233 (44) |
|  | Moderate Anaemia (7-8.9) | 122 (7) | 83 (8) | 39 (7) |
|  | Severe Anaemia (<7g) | 3 (0.2) | 2 (0.2) | 1 (0.2) |
| HIV Status |  |  |  |
|  | HIV-uninfected | 1014 (57) | 705 (61) | 309 (49) |
|  | HIV-infected | 773 (43) | 453 (39) | 320 (51) |
| ART Status |  |  |  |
|  | Initiated before pregnancy | 368 (48) | 234 (52) | 134 (42) |
|  | Initiated during pregnancy | 405 (52) | 219 (19) | 186 (58) |
| ART Regimen |  |  |  |
|  | TDF-3TC-EFV | 691 (39) | 401 (35) | 290 (46) |
|  | TDF-3TC-NVP | 4 (0.2) | 2 (0.2) | 2 (0.3) |
|  | Other NNRTI | 20 (1) | 14 (1) | 6 (1) |
|  | PI regimen | 22 (1) | 15 (1) | 7 (1) |

\*among women with a previous pregnancy

All variables, with the exception of height, BMI and haemoglobin had <1% missing data. For height, 6% (n=111) of data was missing and for BMI, 7% (n=124) was missing, for both there were similar proportions across all comparison groups. For Haemoglobin, 14% (n=257) of data was missing with similar proportions across all comparison groups. For ART status 2% (n=36) were missing regimen data

Supplementary Table 2: Birth outcomes in women with all three GA assessment methods (n=629)

|  |  | **Total**n=629 | **HIV-uninfected**n=309 | **HIV-infected**n=320 | **HIV-infected** |
| --- | --- | --- | --- | --- | --- |
| **Initiated before pregnancy** n=134 | **Initiated during pregnancy** n=186 |
| Gestational Age (weeks) LMP |  |  |  |  |  |
|  | Post-term >42 | 36 (6) | 15 (5) | 21 (7) | 12 (9) | 9 (5) |
|  | Term (37-42) | 329 (52) | 159 (51) | 170 (53) | 70 (52) | 100 (54) |
|  | Any Preterm (<37)\* | 264 (42) | 135 (44) | 129 (40) | 52 (39) | 77 (41) |
|  |  | *Late to Mod Preterm (32-37)* | *186 (70)* | *101 (75)* | *85 (66)* | *32 (62)* | *53 (69)* |
|  |  | *Very Preterm (28-32)* | *78 (30)* | *34 (25)* | *44 (34)* | *20 (38)* | *24 (31)* |
| Gestational Age (weeks) SFH |  |  |  |  |  |
|  | Post-term >42 | 42 (7) | 24 (8) | 18 (6) | 6 (5) | 12 (7) |
|  | Term (37-42) | 498 (79) | 247 (80) | 251 (78) | 104 (83) | 147 (85) |
|  | Any Preterm (<37)\* | 89 (14) | 38 (12) | 51 16) | 24 (17) | 27 (15) |
|  |  | *Late to Mod Preterm (32-37)* | *68 (76)* | *32 (84)* | *36 (71)* | *20 (83)* | *16 (59)* |
|  |  | *Very Preterm (28-32)* | *21 (24)* | *6 (16)* | *15 (29)* | *4 (17)* | *11 (41)* |
| Gestational Age (weeks) US |  |  |  |  |  |
|  | Post-term >42 | 3 (0.5) | 3 (1) | 0 | 0 | 0 |
|  | Term (37-42) | 565 (90) | 285 (92) | 280 (88) | 121 (90) | 159 (85) |
|  | Any Preterm (<37)\* | 61 (10) | 21 (7) | 40 (12) | 13 (10) | 27 (15) |
|  |  | *Late to Mod Preterm (32-37)* | *48 (79)* | *19 (90)* | *29 (73)* | *9 (69)* | *20 (74)* |
|  |  | *Very Preterm (28-32)* | *13 (21)* | *2 (10)* | *11 (27)* | *4 (31)* | *7 (26)* |
| Size for Gestational Age LMP | n=578 | n=287 | n=291 | n=117 | n=174 |
| Small (SGA) | 51 (9) | 17 (6) | 34 (12) | 12 (10) | 22 (13) |
| Appropriate (AGA) | 320 (55) | 159 (55) | 161 (55) | 68 (58) | 93 (53) |
| Large (LGA) | 208 (36) | 111 (39) | 96 (33) | 37 (32) | 59 (34) |
| Size for Gestational Age SFH | n=581 | n=284 | n=297 | n=125 | n=172 |
| Small (SGA) | 84 (14) | 33 (11) | 51 (17) | 14 (12) | 37 (22) |
| Appropriate (AGA) | 440 (76) | 224 (79) | 216 (73) | 99 (80) | 117 (68) |
| Large (LGA) | 57 (10) | 27 (10) | 30 (10) | 12 (8) | 18 (10) |
| Size for Gestational Age US | n=618 | n=305 | n=313 | n=130 | n=183 |
| Small (SGA) | 65 (11) | 22 (7) | 43 (14) | 17(13) | 26 (14) |
| Appropriate (AGA) | 505 (82) | 258 (85) | 247 (79) | 105 (81) | 142 (78) |
| Large (LGA) | 48 (7) | 25 (8) | 23 (7) | 8 (6) | 15 (8) |

\* Any Preterm consists of the subset of Late Preterm, Moderately Preterm and Very Preterm deliveries

For Size for GA data missing for women whose GA is not within Intergrowth standard limits (<24w and >42w) and those missing either GA or birthweight

Supplementary Table 3: Adjusted association between HIV/ART status and preterm delivery in women with singleton deliveries (n=1787)

| **Assessment Method** | **HIV-infected vs** **HIV-uninfected (Ref)\*** | **Initiated before pregnancy (Ref) vs** **Initiated during pregnancy\*\*** |
| --- | --- | --- |
|  | **aOR (95% CI)** | **P-value** |  | **aOR (95% CI)** | **P-value** |
| Last menstrual period (LMP)(n=1470) | HIV-infected | 1.12 (0.90-1.39) | 0.325 | During pregnancy | 0.81 (0.57-1.13) | 0.252 |
| Symphysis Fundal Height (SFH)(n=1122) | HIV-infected | 1.07 (0.78-1.46) | 0.666 | During pregnancy | 1.15 (0.70-1.89) | 0.587 |
| Ultrasound (US)(n=1198) | HIV-infected | 1.53 (1.04-2.26) | 0.030 | During pregnancy | 0.79 (0.47-1.33) | 0.371 |

\*Adjusted for age, parity, BMI and previous PTD

\*\*Adjusted for age, parity, BMI, previous PTD and ART regimen

Supplementary Table 4a: Gestational Age at 1st ANC visit and delivery according to different assessment methods women with all three GA assessment methods (n=629): HIV & ART status

| **Assessment Method** | **Total**n=629 | **HIV-uninfected**n=309 | **HIV-****infected**n=320 | **HIV-infected**n= 320 |
| --- | --- | --- | --- | --- |
| **Initiated before pregnancy** n=134 | **Initiated during pregnancy** n=186 |
| **LMP** |  |  |  |  |  |
|  | Median GA\*  | 17 (14-21) | 18 (15-21) | 17 (13-20) | 16 (12-20) | 17 (13-20) |
|  | Median GA\*\*  | 38 (34-39) | 37 (34-39) | 38 (34-39) | 38 (34-40) | 37 (34-39) |
| **SFH** |  |  |  |  |  |
|  | Median GA\*  | 20 (16-23) | 21 (17-24) | 18 (15-22) | 18 (14-21) | 19 (15-23) |
|  | Median GA\*\*  | 39 (38-40) | 39 (38-40) | 39 (37-40) | 39 (37-40) | 39 (38-40) |
| **US** |  |  |  |  |  |
|  | Median GA\*  | 19 (15-23) | 20 (17-24) | 18 (14-22) | 18 (14-21) | 19 (14-22) |
|  | Median GA\*\*  | 39 (38-40) | 39 (38-40) | 39 (38-40) | 39 (38-40) | 39 (38-40) |

\*Gestation at 1st ANC Visit

\*\*Gestation at delivery

Supplementary Table 4b: Gestational Age at 1st ANC visit and delivery according to different assessment methods women with all three GA assessment methods (n=629): BMI status

| **Assessment Method** | **Underweight**n=6 | **Normal** n=122 | **Overweight** n=178 | **Obese** n=290 | **Unknown** n=33 |
| --- | --- | --- | --- | --- | --- |
| **LMP** |  |  |  |  |  |
|  | Median GA\*  | 19 (17-20) | 17 (14-19) | 18 (15-21) | 17 (14-21) | 17 (13-20) |
|  | Median GA\*\*  | 36 (33-38) | 36 (33-39) | 38 (34-40) | 38 (34-39) | 37 (35-39) |
| **SFH** |  |  |  |  |  |
|  | Median GA\*  | 22 (19-23) | 19 (15-23) | 20 (16-23) | 20 (15-23) | 21 (15-22) |
|  | Median GA\*\*  | 37 (36-38) | 39 (37-40) | 39 (38-40) | 39 (38-40) | 39 (38-40) |
| **US** |  |  |  |  |  |
|  | Median GA\*  | 23 (21-25) | 19 (16-22) | 19 (16-23) | 20 (15-23) | 19 (14-22) |
|  | Median GA\*\*  | 38 (37-39) | 38 (37-39) | 39 (38-40) | 39 (38-40) | 38 (38-39) |

\*Gestation at 1st ANC Visit

\*\*Gestation at delivery