RESEARCH ARTICLE

Adverse birth outcomes and their clinical phenotypes in an urban Zambian cohort [version 1; peer review: 1 approved, 1 approved with reservations]

Joan T Price1-3, Bellington Vwalika2, Katelyn J Rittenhouse1, Humphrey Mwape3, Jennifer Winston1, Bethany L Freeman1, Ntazana Sindano13, Elizabeth M Stringer1, Margaret P Kasaro3, Benjamin H Chi1, Jeffrey SA Stringer1

1Division of Global Women’s Health, Department of Obstetrics and Gynecology, University of North Carolina, School of Medicine, Chapel Hill, NC, USA
2Department of Obstetrics and Gynaecology, University of Zambia School of Medicine, Lusaka, Zambia
3UNC Global Projects – Zambia, Lusaka, Zambia

Abstract

Background: Few cohort studies of pregnancy in sub-Saharan Africa use rigorous gestational age dating and clinical phenotyping. As a result, incidence and risk factors of adverse birth outcomes are inadequately characterized.

Methods: The Zambian Preterm Birth Prevention Study (ZAPPS) is a prospective observational cohort established to investigate adverse birth outcomes at a referral hospital in urban Lusaka. This report describes ZAPPS phase I, enrolled August 2015 to September 2017. Women were followed through pregnancy and 42 days postpartum. At delivery, study staff assessed neonatal vital status, birthweight, sex, and assigned a delivery phenotype. Primary outcomes were: (1) preterm birth (PTB; delivery <37 weeks), (2) small-for-gestational-age (SGA; <10th percentile weight-for-age at birth), and (3) stillbirth (SB; delivery of an infant without signs of life).

Results: ZAPPS phase I enrolled 1450 women with median age 27 years (IQR 23–32). Most participants (68%) were multiparous, of whom 41% reported a prior PTB and 14% reported a prior stillbirth. Twins were present in 3% of pregnancies, 3% of women had short cervix (<25mm), 24% of women were HIV seropositive, and 5% were syphilis seropositive. Of 1216 (84%) retained at delivery, 15% were preterm, 18% small-for-gestational-age, and 4% stillborn. PTB risk was higher with prior PTB (aRR 1.88; 95%CI 1.32–2.68), short cervix (aRR 2.62; 95%CI 1.68–4.09), twins (aRR 5.22; 95%CI 3.67–7.43), and antenatal hypertension (aRR 2.04; 95%CI 1.43–2.91). SGA risk was higher with twins (aRR 2.75; 95%CI 1.81–4.18) and antenatal hypertension (aRR 1.62; 95%CI 1.16–2.26). SB risk was higher with short cervix (aRR 6.42;
Conclusions: This study confirms high rates of PTB, SGA, and SB among pregnant women in Lusaka, Zambia. Accurate gestational age dating and careful ascertainment of delivery data are critical to understanding the scope of adverse birth outcomes in low-resource settings.

Keywords
 adverse birth outcomes, pregnancy, preterm birth, small for gestational age, stillbirth, sub-Saharan Africa, Zambia
**Introduction**

The often overlapping outcomes of preterm birth (PTB), small for gestational age (SGA), and stillbirth (SB), collectively called ‘adverse birth outcomes’, are responsible for most perinatal morbidity and mortality worldwide.\(^1\) Low- and middle-income countries bear the overwhelming burden of global PTB, SB, and SGA.\(^3\)-\(^5\) However, reliable classification and estimation of adverse birth outcomes in low-resources settings is challenging because of a number of interrelated factors, including (1) uncertain gestational age dating,\(^6\) (2) conflation of fetal growth restriction and PTB into the less useful metric of ‘low birthweight’,\(^3,5\) (3) inconsistent thresholds for fetal viability,\(^2\) and (4) misclassification of stillbirth and neonatal death.\(^7\) In many countries, including Zambia, data sources that adequately address these methodological challenges are lacking to the extent that national estimates of adverse birth outcomes must be modeled.\(^3,6,5,11\)

In sub-Saharan Africa, cohort studies in pregnancy rarely use reliable gestational age dating or clinical phenotyping to classify outcomes. Deliberate clinical phenotyping that characterizes the events that incite parturition (i.e., spontaneous vs. provider-initiated), quantifies maternal and fetal co-morbid conditions, and reliably distinguishes the timing of perinatal death is essential for rigorous classification of adverse birth outcomes.\(^12\) Accurate estimation of gestational age with fetal ultrasound is also critical. Other dating methods, such as maternal recall of last menstrual period (LMP),\(^14\)-\(^17\) symphysis-fundal height measurement,\(^18\) or newborn physical exam\(^19,20\) introduce error (and in some cases, bias\(^21\)).

We established a cohort of 1450 pregnant women and their infants at a tertiary care institution in Lusaka, Zambia, with the goal of better understanding the epidemiological factors and biological mechanisms leading to adverse birth outcomes. This report presents the outcomes of the first phase of this cohort.

**Methods**

The Zambian Preterm Birth Prevention Study (ZAPPS) is an ongoing prospective observational cohort study at the Women and Newborn Hospital of the University Teaching Hospitals (UTH-WNH), the primary referral hospital in Lusaka. Phase 1 of ZAPPS, the subject of this report, recruited and enrolled participants beginning in August 2015 and completed follow-up in June 2018. The sample size for this observational study was initially set at 2000 women, with a target of 250 preterm birth events based on published regional population estimates.\(^4\) For budgetary reasons and because the prematurity rate was higher than initially expected, enrollment was stopped in September 2017 after 1450 women had been enrolled. The ZAPPS protocol was developed to align with the Guidelines for Strengthening The Reporting of Observational Studies in Epidemiology (STROBE).\(^2\)

**Study population**

Pregnant women meeting the following criteria were eligible for enrollment in Phase 1 of the ZAPPS cohort: (1) 18 years of age or older; (2) viable intrauterine singleton or twin gestation; (3) presentation to antenatal care prior to 20 weeks of gestation if HIV-uninfected or 24 weeks if HIV-infected; (4) residing within Lusaka with no plans to relocate during the study follow-up period; (4) willing to provide written, informed consent; (5) willing to allow participation of their infant(s) in the study; (6) willing to be contacted and followed up at home if necessary.

The ZAPPS protocol was approved prior to study initiation and is subjected to annual review by the University of Zambia School of Medicine Biomedical Research Ethics Committee (reference number: 016-04-14) and the University of North Carolina School of Medicine Institutional Review Board (study number: 14-2113). The study also received approval from the Zambian Ministry of Health National Health Research Authority. Each participant provided written informed consent prior to enrollment.

**Procedures**

Full study procedures are described in detail elsewhere.\(^21\) Community educators identified potential participants at antenatal care clinics of UTH-WNH and five surrounding clinics in Lusaka, assessing basic eligibility criteria such as age and approximate gestational age. Interested volunteers underwent ultrasound examination per standard of care to determine pregnancy location, fetal viability, number of fetuses, and gestational age by standard biometry (Sonosite M-Turbo, Fuji Sonosite, Bothell, WA). Gestational age was calculated at enrollment by crown-rump length if <14 gestational weeks or by head circumference and femur length if ≥14 weeks. Fetal biometry structures were each measured twice and then averaged to calculate gestational age using INTERGROWTH-21st equations.\(^24,25\) Pregnancies below the lower threshold for INTERGROWTH-21st equations were dated by the Hadlock formula.\(^26\) Interested women who met preliminary ultrasound eligibility criteria completed an informed consent process in their preferred language of English, Nyanja, or Bemba.

At enrollment, study nurses collected demographic and behavioral information through medical record review and participant interview, and documented a thorough health history including prior pregnancy outcomes. As part of standard antenatal care, participants underwent a physical exam and rapid testing for hemoglobin, urinalysis, syphilis (SD Bioline Syphilis 3.0, Abbott Diagnostics), and HIV (SD Bioline 3.0, Abbott Diagnostics).

After enrollment, participants received routine antenatal care at follow-up visits scheduled at approximately 24 weeks, 32 weeks, and 36 weeks. All participants underwent cervical length measurement in the second trimester (i.e., 14–28 weeks) and fetal growth assessment by biometry in the third trimester.\(^27,28\) Cervical length measurements were performed by sonographers with certification in the Cervical Length Education and Review (CLEAR) program. Study nurses staffed the UTH-WNH labor ward full-time and collected detailed information about the clinical course and perinatal outcomes of participants and their infants, including gestational age at birth, neonatal vital
status, birthweight, and sex, and assigned a delivery phenotype. For participants who did not deliver at UTH-WNH or were not captured by study staff during their delivery admission, study staff collected perinatal outcomes either in person or by phone. Cohort retention in this analysis was defined as ascertainment of date of delivery.

**Exposures**

Primary exposures evaluated included maternal age (years), height (cm), and body mass index (BMI, kg/m²); reported prior preterm birth (nulliparous, parous with no prior PTB, or parous with one or more prior PTB); cervical length (mm) and short cervix (<25mm); gestation (single or twin); hypertension during pregnancy (≥140 systolic or ≥90mmHg diastolic at any antepartum study visit); anemia at enrollment (<10.5g/dL); bacteriuria during pregnancy (1+ leukocyte esterase and/or nitrites at any antepartum study visit); syphilis seropositivity (reactive at enrollment); and HIV seropositivity (reactive at enrollment).

**Outcomes**

Primary outcomes of this analysis were: PTB, defined as birth between 16 0/7 weeks and 36 6/7 weeks; gestational weeks, SGA (newborn weight-for-age <10th percentile by INTER-GROWTH-21st norms), and SB (delivery of an infant without signs of life ≥16 0/7 weeks). Secondary outcomes included very PTB (birth before 34 0/7 weeks) and very SGA (newborn weight-for-age <3rd percentile). Both PTB and very PTB were further characterized as either spontaneous (spontaneous labor or membrane rupture prior to labor) or provider-initiated (induction of labor or pre-labor cesarean). We differentiated antepartum stillbirth (i.e., fetal heart tones absent on admission or, if not assessed, maceration skin changes present at delivery) from intrapartum stillbirth (i.e., fetal heart tones present on admission or, if not assessed, absence of maceration skin changes at delivery).

**Statistical analysis**

We performed descriptive analyses of baseline characteristics and exposures of the cohort, reporting median and interquartile range (IQR) for continuous variables, and frequency and percent for categorical variables. Differences in baseline characteristics between women retained at delivery and those lost to follow-up were evaluated by univariate tests of association.

We summarized parturition phenotype among our retained participants by preterm versus term and spontaneous versus provider-initiated following a standard rubric. Among spontaneous PTB, we identified primary maternal, fetal, and/or placental conditions present at the time of delivery. Among provider-initiated PTB, we reported the primary indication for delivery as recorded by the provider. Finally, key individual conditions present and phenotypic clusters were used to classify all PTB, spontaneous PTB, and provider-initiated PTB.

We calculated the incidence of adverse birth outcomes: PTB, spontaneous PTB, very PTB, spontaneous very PTB, SGA, very SGA, and SB among all participants retained at delivery. Twin deliveries in which at least one neonate was SGA or still-born were classified as having met the respective outcome. Crude associations between key exposures and outcomes were analyzed as risk ratios estimated using Poisson regression analyses with a robust variance. Adjusted risk ratios were also estimated using Poisson regression accounting for other key exposures plus maternal age, BMI, estimated gestational age at enrollment, and HIV serostatus at enrollment.

Kaplan-Meier curves were plotted for time-to-delivery with and without a history of prior PTB, short cervix, and twin gestation. We accounted for loss to follow-up by right censoring women at their last study visit (if before delivery) and compared survival between exposure groups by log-rank tests. We also used Cox regression to calculate the hazard of delivery between participants with and without prior PTB, short cervix, and twin gestation, adjusting for maternal age. The proportional-hazards assumption was tested based on Schoenfeld residuals. Because of the inherent converging of survival curves in pregnancy at term, we restricted our models to the preterm period by administratively censoring all participants at 37 gestational weeks.

All statistical analyses were performed with Stata version 14 (College Station, TX, USA) and SAS version 9.4 (Cary, NC, USA).

**Results**

From August 2015 to September 2017, 1784 pregnant women were screened and 1450 (81%) enrolled (Figure 1). The median age of enrolled participants was 27 (IQR: 23–32) (Table 1). Median estimated gestational age (EGA) at enrollment was 16 weeks; 30% (n=427/1450) were enrolled before 14 completed gestational weeks. Of 1042 (72%) participants who had been pregnant at least once in the past; 19% (n=194/1042) reported a prior miscarriage. Of 992 (68%) with a prior delivery, 41% (n=411) reported a prior PTB. On ultrasound exam, 3% (n=35/1175) had short cervix <25mm, and 3% (n=38/1450) had twin gestation. The prevalence of HIV seropositivity at enrollment was 24% (n=350/1447). Syphilis seropositivity was detected in 5% (70/1342).

Of enrolled participants, 1216 (84%) were retained with delivery date ascertained. Compared to participants lost to follow-up, those retained at delivery were older (median: 27 versus 24 years, p<0.001), had more years of education (median: 12 versus 9 years, p<0.001), were more likely to have electricity (91% versus 85%, p=0.002) and flush or pour toilet facilities at home (55% versus 41%, p<0.001), had higher body mass index (23.9 versus 22.7 kg/m², p<0.001), and had higher gravidity (74% versus 63% multigravid, p=0.001) and parity (70% versus 60% parous, p=0.004).

Frequencies of our outcomes were as follows: 15% PTB (n=181/1216), 8% very PTB (n=92/1216), 18% SGA (n=207/1159), 7% very SGA (n=80/1159), and 4% SB (n=53/1209). Three participants (0.3%) experienced miscarriages before 16 weeks of gestation. Of the pregnancies that ended in
Figure 1. ZAPPS cohort participant flowchart. ANC, antenatal care; UTH-WNH, Women and Newborn Hospital of University Teaching Hospital.

Table 1. Baseline characteristics of ZAPPS cohort, N=1450.

| Characteristic                        | Total enrolled N=1450 | Retained at delivery visit N=1216 (83.9%) | Lost to follow-up N=234 (16.1%) | p     |
|--------------------------------------|-----------------------|------------------------------------------|--------------------------------|-------|
|                                      | N % or Median (IQR)*  | N % or Median (IQR)*                     | N % or Median (IQR)*           |       |
| Maternal age, years                  |                       |                                          |                               |       |
|                                      | 1409 27 (23–32)       | 1192 27 (23–32)                         | 217 24 (20–29)                 | <.001 |
| <20                                  | 111 7.9                | 72 6.0                                  | 39 18.0                       |       |
| 20–34                                | 1116 79.2              | 956 80.2                                | 160 73.7                      |       |
| ≥35                                  | 182 12.9               | 164 13.8                                | 18 8.3                        |       |
| Missing                              | 41 24                  | 17                                      |                               |       |
| Maternal education, years            |                       |                                          |                               |       |
|                                      | 1435 12 (9–12)         | 1204 12 (9–12)                         | 231 9 (7–12)                  | <.001 |
| None                                 | 26 1.8                 | 19 1.6                                  | 7 3.0                         |       |
| 0–12 years                           | 1225 85.4              | 1018 84.6                               | 207 89.6                      |       |
| ≥12 years                            | 184 12.8               | 167 13.9                                | 17 7.4                        |       |
| Missing                              | 15 12                  | 3                                       |                               |       |
| Married or cohabiting                | 1202 83.7              | 1014 84.1                               | 188 81.4                      | 0.310 |
| Missing                              | 13 10                  | 3                                       |                               |       |
| Electricity in home                  | 1302 90.6              | 1105 91.6                               | 197 85.3                      | 0.002 |
| Missing                              | 13 10                  | 3                                       |                               |       |
| Piped drinking water in home         | 1340 93.3              | 1123 93.2                               | 217 93.9                      | 0.678 |
| Missing                              | 14 11                  | 3                                       |                               |       |
| Toilet facilities in home            |                       |                                          |                               | <.001 |
| Flush or Pour                        | 762 53.0               | 667 55.3                                | 95 41.1                       |       |
| Pit / Latrine / Other                | 675 47.0               | 539 44.7                                | 136 58.9                      |       |
| Missing                              | 13 10                  | 3                                       |                               |       |
| Floor material in home               |                       |                                          |                               |       |
| Natural / rudimentary                | 138 9.6                | 119 9.9                                 | 19 8.2                        | 0.438 |
| Finished                             | 1299 90.4              | 1087 90.1                               | 212 91.8                      |       |
| Missing                              | 13 10                  | 3                                       |                               |       |
| Characteristic                        | Total enrolled | Retained at delivery visit N=1216 (83.9%) | Lost to follow-up N=234 (16.1%) | p    |
|--------------------------------------|----------------|------------------------------------------|---------------------------------|------|
|                                      | N = 1450       |                                          |                                 |      |
|                                      | N % or Median (IQR) | N % or Median (IQR) | N % or Median (IQR) |
| Domestic violence in past year       | 71             | 5.0                                      | 58                              | 4.9  | 13 | 5.6  | 0.641 |
| Missing                              | 28             |                                          | 2                               |      |    |      |      |
| Smoking in pregnancy                 | 8              | 0.6                                      | 7                               | 0.6  | 1  | 0.4  | 0.768 |
| Missing                              | 24             |                                          | 23                              |      |    |      |      |
| Alcohol use in pregnancy             | 124            | 8.7                                      | 106                             | 8.9  | 18 | 7.7  | 0.563 |
| Missing                              | 25             |                                          | 24                              |      |    |      |      |
| Maternal height at enrollment, cm    | 1368           | 156 (160–164)                            | 1151                            | 156 (160–165) | 217 | 156 (160–164) | 0.263 |
| BMI at enrollment, kg/m²             | 1366           | 23.6 (21.2–27.2)                         | 1149                            | 23.9 (21.4–27.6) | 217 | 22.7 (20.7–25.5) | <.001 |
| <18.5                                | 71             | 5.2                                      | 56                              | 4.9  | 15 | 6.9  |      |
| 18.5–30.0                            | 1003           | 80.8                                     | 919                             | 80.0 | 184 | 84.8 |      |
| >30.0                                | 192            | 14.1                                     | 174                             | 15.1 | 18 | 8.3  |      |
| Missing                              | 84             |                                          | 67                              |      |    | 17    |      |
| Gravity                              | 1450           | 2 (1–4)                                  | 1042                            | 2 (1–4) | 408 | 2 (1–3) | 0.003 |
| Primigravida                         | 408            | 28.1                                     | 321                             | 26.4 | 87 | 37.2 | 0.001 |
| Multigravida                         | 1042           | 71.9                                     | 895                             | 73.6 | 147 | 62.8 |      |
| Prior miscarriage, n=1042            |                |                                          |                                 |      |    |      | 0.933 |
| Multigravida, no prior miscarriage   | 848            | 81.4                                     | 728                             | 81.3 | 120 | 81.6 |      |
| Multigravida, ≥1 prior miscarriage   | 194            | 18.6                                     | 167                             | 18.7 | 27 | 18.4 |      |
| Parity                               | 1450           | 1 (0–2)                                  | 1216                            | 1 (0–2) | 234 | 1 (0–2) | 0.004 |
| Nulliparous                          | 458            | 31.6                                     | 365                             | 30.0 | 93 | 39.7 | 0.003 |
| Parous                               | 992            | 68.4                                     | 851                             | 70.0 | 141 | 60.3 |      |
| Prior PTB, n=992                     |                |                                          |                                 |      |    |      | 0.224 |
| Parous, no prior PTB                 | 581            | 58.6                                     | 505                             | 59.3 | 76 | 53.9 |      |
| Parous, ≥1 prior PTB                 | 411            | 41.4                                     | 346                             | 40.7 | 65 | 46.1 |      |
| Prior stillbirth, n=992              |                |                                          |                                 |      |    |      | 0.401 |
| Parous, no prior SB                  | 780            | 86.1                                     | 672                             | 86.5 | 108 | 83.7 |      |
| Parous, ≥1 prior SB                  | 126            | 13.9                                     | 105                             | 13.5 | 21 | 16.3 |      |
| Missing                              | 86             |                                          | 74                              |      |    | 12    |      |
| Short cervix < 2.5 cm                | 35             | 3.0                                      | 32                              | 3.0  | 3  | 3.2  | 0.899 |
| Missing                              | 275            |                                          | 135                             |      |    | 140   |      |
| Twin gestation                       | 38             | 2.6                                      | 31                              | 2.6  | 7  | 3.0  | 0.698 |
| HIV positive at enrollment           | 350            | 24.2                                     | 304                             | 25.0 | 46 | 19.7 | 0.084 |
| Missing                              | 3              |                                          | 2                               |      |    | 1     |      |
| Syphilis reactive                    | 70             | 5.2                                      | 63                              | 5.6  | 7  | 3.1  | 0.142 |
| Missing                              | 108            |                                          | 93                              |      |    | 15    |      |
| Hypertensive at enrollment†          | 52             | 3.7                                      | 46                              | 3.9  | 6  | 2.7  | 0.392 |
| Missing                              | 31             |                                          | 21                              |      |    | 10    |      |
| Hemoglobin at enrollment, g/dL       | 1025           | 12 (11–13)                               | 854                             | 12 (11–13) | 171 | 12 (11–13) | 0.274 |
| <10.5                                | 140            | 13.7                                     | 123                             | 14.4 | 17 | 9.9  |      |
| Missing                              | 425            |                                          | 362                             |      |    | 63    |      |
| Abnormal UA at enrollment†           | 69             | 5.0                                      | 55                              | 4.8  | 14 | 6.3  | 0.343 |
| Missing                              | 79             |                                          | 67                              |      |    | 12    |      |
| EGA at enrollment, weeks             | 1450           | 16.1 (13.3–18.3)                         | 1216                            | 16.0 (13.3–18.3) | 234 | 16.3 (13.3–18.6) | 0.421 |
| <14                                  | 427            | 29.4                                     | 362                             | 29.8 | 65 | 27.8 |      |

PTB, preterm birth; IQR, interquartile range; BMI, body mass index; EGA, estimated gestational age; UA, urinalysis; SB, stillbirth.

* Not all columns sum to 100% due to rounding.
† Defined as systolic blood pressure ≥ 140 and/or diastolic blood pressure ≥ 90.
‡ Defined as 1+ leukocyte esterase and/or + nitrites.

p values calculated by Wilcoxon rank sum or chi-square for continuous and categorical comparisons, respectively.
SB, 44 (83%) were antepartum and 9 (17%) occurred intrapartum. Among 1159 deliveries within the EGA range for SGA calculation and with birthweight recorded, 150 (13%) were PTB, 207 (18%) were SGA and 32 (3%) were stillborn (Figure 2).

Of 181 total PTB, 120 (66%) occurred spontaneously, 56 (31%) were provider-initiated, and 5 (3%) could not be definitively classified (Figure 3). The most common key conditions present in women with spontaneous PTB (n=120) were HIV infection (n=42, 35%), SB (n=26, 23%), hypertension alone (n=22; 18%), and twin gestation (n=18, 15%); 33 (28%) had no key condition identified. Most provider-initiated preterm deliveries were indicated for SB (n=16, 29%), preeclampsia or eclampsia (n=15, 27%) or hypertension alone (n=4, 7%), or both SB and preeclampsia (n=4, 7%). We identified major phenotypic clusters of PTB, spontaneous PTB, and provider-initiated PTB by presence of maternal, fetal, and/or placental conditions (Table 2).

Maternal age ≥35, prior PTB, short cervix, twin gestation, antenatal hypertension, and EGA at enrollment <14 weeks were associated with PTB (Table 3). Overall, these associations were stable or strengthened when restricting the outcome to spontaneous PTB and to very PTB (Table 4). Although associated with PTB, antenatal hypertension did not significantly predict spontaneous phenotypes of PTB. In multivariable regression models adjusting for maternal age, BMI, EGA at enrollment, and HIV status at enrollment, participants with prior PTB (aRR 1.88; 95% CI 1.32–2.68), short cervix (aRR 2.62; 95% CI 1.68–4.09), twin gestation (aRR 5.22; 95% CI 3.67–7.43), and antenatal hypertension (aRR 2.04; 95% CI 1.43–2.91) had increased risk of PTB (Table 2). The associations between the exposures of prior PTB, short cervix, and twin gestation with PTB were stable or strengthened when restricting the outcome to spontaneous phenotypes and very PTB (Table 4). The risk of PTB decreased with increasing cervical length (RR 0.58 per cm; 95% CI 0.46–0.73) (Figure 4).

Nulliparity, twin gestation, and antenatal hypertension were each associated with SGA in univariate analysis, and older age, low BMI, nulliparity, short cervix, twin gestation, and antenatal hypertension were associated with very SGA. In multivariable analysis, twin gestation (aRR 2.75; 95% CI 1.81–4.18) and antenatal hypertension (aRR 1.62; 95% CI 1.16–2.26) were associated with an increased risk of SGA; nulliparity was marginally associated with SGA (aRR 1.36; 95% CI 0.98–1.87). Nulliparity (aRR 1.92; 95% CI 1.12–3.32) and twin gestation (aRR 2.71, 95% CI 1.12–6.57) were associated with very SGA. Maternal height was not associated with SGA or very SGA in univariate analyses.

Finally, older maternal age, prior PTB, short cervix, syphilis seropositivity, and antenatal hypertension were individually associated with an elevated risk of SB (Table 3). In multivariable

---

Figure 2. Preterm birth <37 weeks, small for gestational age <10%, and stillbirth among participants retained at delivery in ZAPPS cohort. Among ZAPPS cohort participants retained at delivery, 15% (181/1216) were preterm (PTB), 18% (207/1159) were small for gestational age (SGA), and 4% (53/1209) were stillborn (SB). **11 preterm births, one term stillbirth, and 20 preterm stillbirths were either outside the gestational age threshold for INTERGROWTH-21 calculation of SGA, or were missing birthweight at delivery. Figure created with: EulerAPE.
Figure 3. Parturition phenotypes among ZAPPS participants with preterm delivery. Of participants who underwent preterm delivery (n=181) in the ZAPPS cohort, 120 of them were spontaneous and 56 were indicated. This figure presents the frequencies of primary conditions present in spontaneous preterm deliveries, primary indications for indicated preterm deliveries, and the overall frequency with 95% confidence intervals of key conditions in each group. Gray bars represent missing values. PTB, preterm birth; PEC, preeclampsia; EC, eclampsia; APH, antepartum hemorrhage; OB HX, obstetrical history.

Analysis, short cervix predicted SB (aRR 6.42; 95% CI 2.56–16.1), while syphilis was only marginally associated (aRR 2.34; 95% CI 0.91–6.04).

Elevated risks of PTB among women with prior PTB, short cervix, and twin gestation were supported by survival analyses, with log-rank tests of association demonstrating significant differences between groups of each variable (Figure 5; Table 5). In proportional hazards models adjusted for maternal age at enrollment, participants with prior PTB, short cervix, and twin gestation had significantly higher hazards of delivering before 37 gestational weeks compared to parous women with no prior PTB, women with cervical lengths ≥25mm, or with single gestations. Participants with increasing numbers of prior preterm births demonstrated increasing hazard ratios of delivering preterm.
Table 2. Phenotypes of preterm birth in ZAPPS cohort, N=181.

| All preterm birth | Spontaneous N (%)† | Provider-initiated N (%)† |
|-------------------|---------------------|--------------------------|
| N (%)             | 181 100             | 120 68                    | 56 32                     |
| Phsyiotypic clusters |                    |                          |                           |
| No significant clinical conditions | 41 23 | 33 87 | 5 13 |
| Maternal condition(s) only | 60 33 | 40 67 | 20 33 |
| Fetal condition(s) only | 27 15 | 21 81 | 5 19 |
| Placental condition(s) only | 4 2 | 1 25 | 3 75 |
| Maternal and fetal conditions | 37 20 | 17 47 | 19 53 |
| Maternal and placental conditions | 6 3 | 3 50 | 3 50 |
| Fetal and placental conditions | 1 1 | 1 100 | 0 0 |
| Maternal, fetal, and placental conditions | 5 3 | 4 80 | 1 20 |
| Significant maternal conditions | 108 60 | 64 60 | 43 40 |
| HIV infection | 53 29 | 42 81 | 10 19 |
| Urinary tract infection, n=41 | 9 22 | 7 78 | 2 22 |
| Clinical chorioamnionitis, n=120 | 1 1 | 1 100 | 0 0 |
| Diabetes (mellitus or gestational), n=179 | 5 3 | 1 20 | 4 80 |
| Hypertension | 34 19 | 19 56 | 15 44 |
| Preeclampsia, n=107 | 23 21 | 6 26 | 17 74 |
| Eclampsia, n=125 | 5 4 | 0 0 | 5 100 |
| Significant fetal conditions | 70 39 | 43 63 | 25 37 |
| Twin gestation | 21 12 | 18 90 | 2 10 |
| Stillbirth, n=176 | 48 27 | 26 55 | 21 45 |
| Fetal growth restriction | 2 1 | 0 0 | 2 100 |
| Fetal distress | 1 1 | 0 0 | 1 100 |
| Polyhydramnios | 1 1 | 0 0 | 1 100 |
| Oligohydramnios | 1 1 | 0 0 | 1 100 |
| Significant placental conditions | 16 9 | 9 56 | 7 44 |
| Placental abruption | 15 8 | 9 60 | 6 40 |
| Placenta previa | 4 2 | 0 0 | 4 100 |

* column percent.
† row percent.

Discussion
We present the primary results of the ZAPPS pregnancy cohort, established to evaluate the risk factors associated with adverse birth outcomes in Lusaka, Zambia. This study was notable for enrollment of pregnant women at early presentation to antenatal care, gestational age determination by early ultrasound, universal cervical length screening, comprehensive and uniform antenatal and postpartum care, and clinical phenotyping of birth outcomes. Our analyses revealed strong risks of prior preterm birth, short mid-trimester cervical length, and twin gestation on incident preterm birth, and these risks were supported by analyses of pregnancy ‘survival’ to term. We also report increased risks of small-for-gestational-age infants among nulliparous women and women with twin gestation, and of stillbirth among those with short cervix.

The proportion of gravidas who deliver before term varies significantly across individual studies and national estimates in sub-Saharan Africa. The most recent global report estimated a PTB rate of 12% in Zambia based on modeled regional estimates. In contrast, a census accounting of 237,219 public sector births over 6 years in Lusaka - where the vast majority of pregnancies are dated by last menstrual period - classified 46% of singleton deliveries as preterm. In Zambia, obstetrical ultrasound is rare and the reliance on maternal recall of LMP alone substantially over-estimates preterm birth rates, an inaccuracy that
Table 3. Risk of adverse birth outcomes among ZAPPS participants retained at delivery, n=1213.

| Exposure                        | Preterm birth 16 to <37 weeks | Spontaneous preterm birth 16 to <37 weeks | Small for gestational age | Stillbirth |
|---------------------------------|-------------------------------|--------------------------------------------|---------------------------|------------|
|                                 | n    | events | %    | RR  | 95% CI | aRR* | 95% CI |
|                                 | n    | events | %    | RR  | 95% CI |
|                                 | n    | events | %    | RR  | 95% CI | aRR* | 95% CI |
|                                 | n    | events | %    | RR  | 95% CI | aRR* | 95% CI |
|                                | n    | events | %    | RR  | 95% CI | aRR* | 95% CI |
| Age at enrollment, years       |         |        |      |     |        |      |        |
| <20                             | 72    | 5      | 6.9  | 1.00 |         |      |        |
| 20–34                           | 953   | 144    | 15.1 | 2.18 | 0.92–5.14 | 0.25 | 0.10–0.77 |
| ≥35                             | 164   | 31     | 18.9 | 2.72 | 1.10–6.72 | 0.54 | 0.21–2.29 |
| BMI at enrollment, kg/m²        |         |        |      |     |        |      |        |
| <18.5                           | 56    | 12     | 21.4 | 1.00 |         |      |        |
| 18.5–30.0                       | 916   | 136    | 14.9 | 0.69 | 0.41–1.17 | 0.30 | 0.16–0.62 |
| >30.0                           | 174   | 20     | 11.5 | 0.54 | 0.28–1.03 | 0.42 | 0.20–0.92 |
| Prior PTB                       |         |        |      |     |        |      |        |
| Nulliparous                     | 364   | 25     | 6.9  | 1.23 | 0.73–2.08 | 0.86 | 0.47–1.55 |
| Parous, no prior PTB            | 504   | 28     | 5.6  | 1.00 |         |      |        |
| Parous, ≥1 prior PTB            | 345   | 67     | 19.5 | 2.30 | 1.22–4.61 | 2.43 | 1.22–4.82 |
| Cervical length                 |         |        |      |     |        |      |        |
| ≥2.5cm                          | 1049  | 91     | 8.7  | 1.00 |         |      |        |
| <2.5cm                          | 32    | 16     | 50.0 | 3.83 | 2.62–5.60 | 2.62 | 1.68–4.09 |
| Gestation                       |         |        |      |     |        |      |        |
| Single                          | 1182  | 102    | 8.7  | 1.00 |         |      |        |
| Twin                            | 31    | 18     | 60.0 | 6.93 | 4.90–9.80 | 7.86 | 5.37–11.5 |
| HIV serostatus at enrollment    |         |        |      |     |        |      |        |
| Negative                        | 908   | 78     | 8.6  | 1.00 |         |      |        |
| Positive                        | 303   | 53     | 17.5 | 1.24 | 0.93–1.66 | 1.17 | 0.85–1.62 |

* aRR: adjusted relative risk; CI: confidence interval; PTB: preterm birth.
| Exposure                  | Preterm birth 16 to <37 weeks | Spontaneous preterm birth 16 to <37 weeks | Small for gestational age | Stilbirth |
|--------------------------|-------------------------------|--------------------------------------------|--------------------------|-----------|
|                          | n    | events | %   | RR  | 95% CI | aRR* | 95% CI | n    | events | %   | RR  | 95% CI | aRR* | 95% CI | n    | events | %   | RR  | 95% CI | aRR* | 95% CI | n    | events | %   | RR  | 95% CI | aRR* | 95% CI | n    | events | %   | RR  | 95% CI | aRR* | 95% CI |
| Syphilis                 |      |        |     |     |        |      |        |      |        |     |     |        |      |        |      |        |     |     |        |      |        |      |        |     |     |        |      |        |      |        |     |     |        |      |        |
| Non-reactive             | 1057 | 159    | 15.0 | 1.00 |        |      |        | 1053 | 109    | 10.4 | 1.00 |        |      |        | 1006 | 184    | 18.3 | 1.00 |        |      |        | 1053 | 41     | 3.9  | 1.00 |        |      |        | 1000 | 24     | 2.45 | 1.08 |        |      |        |
| Reactive                 | 63   | 9      | 14.3 | 0.95 | 0.51–1.77 |      |        | 63   | 3      | 4.8  | 0.46 | 0.15–1.41 |      |        | 63   | 13     | 20.6 | 1.13 | 0.68–1.86 |      |        | 63   | 6      | 9.5  | 2.45 | 0.91–6.04 |      |        |
| Blood pressure during pregnancy |     |        |     |     |        |      |        |      |        |     |     |        |      |        |      |        |     |     |        |      |        |      |        |     |     |        |      |        |
| Normotensive             | 1072 | 145    | 13.5 | 1.00 |        |      |        | 1067 | 106    | 9.9  | 1.00 |        |      |        | 1025 | 173    | 16.9 | 1.00 |        |      |        | 1068 | 42     | 3.9  | 1.00 |        |      |        | 1000 | 24     | 2.45 | 1.08 |        |      |        |
| Hypertensive‡            | 141  | 36     | 25.5 | 1.89 | 1.37–2.60 | 2.04 | 1.43–2.91 | 141  | 14     | 9.9  | 1.00 | 0.59–1.70 |      |        | 134  | 34     | 25.4 | 1.50 | 1.09–2.07 | 1.62 | 1.16–2.26 | 141  | 11     | 7.8  | 1.98 | 1.05–3.76 | 1.83 | 0.84–3.96 |      |        |
| Hemoglobin at enrollment |      |        |     |     |        |      |        |      |        |     |     |        |      |        |      |        |     |     |        |      |        |      |        |     |     |        |      |        |
| ≥10.5 g/dL               | 730  | 111    | 15.2 | 1.00 |        |      |        | 727  | 70     | 9.6  | 1.00 |        |      |        | 700  | 125    | 17.9 | 1.00 |        |      |        | 726  | 30     | 4.1  | 1.19 | 0.51–2.80 |      |        |
| <10.5 g/dL               | 121  | 21     | 17.4 | 1.14 | 0.75–1.75 |      |        | 121  | 15     | 12.4 | 1.29 | 0.76–2.17 |      |        | 113  | 20     | 17.7 | 0.99 | 0.65–1.52 |      |        | 122  | 6      | 4.9  | 1.00 |        |      |        |
| UA during pregnancy      |      |        |     |     |        |      |        |      |        |     |     |        |      |        |      |        |     |     |        |      |        |      |        |     |     |        |      |        |
| Normal                   | 907  | 125    | 13.8 | 1.00 |        |      |        | 903  | 82     | 9.1  | 1.00 |        |      |        | 880  | 168    | 19.1 | 1.00 |        |      |        | 903  | 32     | 3.5  | 1.00 |        |      |        | 900  | 24     | 2.45 | 1.08 |        |      |        |
| Abnormal†                | 189  | 31     | 16.4 | 1.19 | 0.83–1.71 |      |        | 189  | 20     | 10.6 | 1.17 | 0.73–1.85 |      |        | 182  | 26     | 14.3 | 0.75 | 0.51–1.10 |      |        | 191  | 6      | 3.1  | 0.89 | 0.38–2.09 |      |        |
| EGA at enrollment, weeks |      |        |     |     |        |      |        |      |        |     |     |        |      |        |      |        |     |     |        |      |        |      |        |     |     |        |      |        |
| <14                      | 360  | 72     | 20.0 | 1.00 |        |      |        | 358  | 45     | 12.6 | 1.00 |        |      |        | 333  | 62     | 18.6 | 1.00 |        |      |        | 357  | 20     | 5.6  | 1.00 |        |      |        | 350  | 19     | 5.4  | 1.00 |        |      |        |
| ≥14                      | 853  | 109    | 12.8 | 0.64 | 0.49–0.84 |      |        | 850  | 75     | 8.8  | 0.70 | 0.50–0.99 |      |        | 826  | 145    | 17.6 | 0.94 | 0.72–1.23 |      |        | 851  | 33     | 3.9  | 0.69 | 0.40–1.19 |      |        |

* Risk ratios calculated via Poisson regression with robust error variance. Multivariable model estimates of adjusted risk ratios include other exposure variables listed and all models adjusted for: maternal age, maternal BMI, and EGA at enrollment as continuous variables.
† Defined as systolic blood pressure ≥ 140 and/or diastolic blood pressure ≥ 90 at enrollment or at any follow-up ANC visit.
‡ Defined as 1+ leukocyte esterase and/or + nitrites.
Table 4. Risk of severe adverse birth outcomes in ZAPPS participants retained at delivery, n=1213.

| Exposure                  | Very preterm birth 16 to <34 weeks | Spontaneous very preterm birth 16 to <34 weeks | Very small for gestational age |
|---------------------------|-----------------------------------|-----------------------------------------------|--------------------------------|
|                           | n   | events | %     | RR | 95% CI | aRR | 95% CI | n   | events | %     | RR | 95% CI | aRR | 95% CI | n   | events | %     | RR | 95% CI | aRR | 95% CI |
| Age at enrollment, years  |      |        |       |    |        |     |        |      |        |       |    |        |     |        |      |        |       |    |        |     |        |
| <20                       | 72  | 3      | 4.2   | 1.00 |        |     |        | 71   | 2      | 2.8   | 1.00 |        |     |        | 70   | 7      | 10.0  | 1.00 |        |     |        |
| 20–34                     | 953 | 75     | 7.9   | 1.89 | 0.61–5.84 |   | 950 | 50 | 5.3 | 1.87 | 0.46–7.53 |   | 913 | 55 | 6.0 | 1.66 | 0.79–3.51 |   |
| ≥35                       | 164 | 13     | 7.9   | 1.90 | 0.56–6.48 |   | 164 | 6  | 3.7 | 1.30 | 0.27–6.28 |   | 155 | 17 | 11.0 | 1.82 | 1.09–3.05 |   |
| BMI at enrollment, kg/m²  |      |        |       |    |        |     |        |      |        |       |    |        |     |        |      |        |       |    |        |     |        |
| <18.5                     | 56  | 4      | 7.1   | 1.00 |        |     |        | 56   | 3      | 5.4   | 1.00 |        |     |        | 53   | 8      | 15.1  | 1.00 |        |     |        |
| 18.5–30.0                 | 916 | 72     | 7.9   | 1.10 | 0.42–2.90 |   | 913 | 46 | 5.0 | 0.94 | 0.30–2.93 |   | 879 | 60 | 6.8 | 0.45 | 0.23–0.90 |   |
| >30.0                     | 174 | 9      | 5.2   | 0.72 | 0.23–2.27 |   | 173 | 5  | 2.9 | 0.54 | 0.13–2.19 |   | 167 | 10 | 6.0 | 0.40 | 0.17–0.95 |   |
| Prior PTB                 |      |        |       |    |        |     |        |      |        |       |    |        |     |        |      |        |       |    |        |     |        |
| Nulliparous               | 364 | 22     | 6.0   | 1.45 | 0.81–2.60 | 1.10 | 0.55–2.21 | 364 | 14 | 3.9 | 1.61 | 0.75–3.44 | 0.88 | 0.34–2.31 | 345 | 32 | 9.3 | 1.98 | 1.18–3.32 | 1.92 | 1.12–3.32 |
| Parous, no prior PTB      | 504 | 21     | 4.2   | 1.00 |        | 1.00 |        | 502 | 12 | 2.4 | 1.00 |        | 1.00 |        | 491 | 23 | 4.7 | 1.00 |        | 1.00 |        |
| Parous, ≥1 prior PTB      | 345 | 49     | 14.2  | 3.41 | 2.08–5.58 | 2.27 | 1.28–4.04 | 343 | 33 | 9.6 | 4.02 | 2.11–7.68 | 2.89 | 1.39–5.98 | 323 | 25 | 7.7 | 1.65 | 0.95–2.86 | 1.39 | 0.76–2.53 |
| Cervical length           |      |        |       |    |        |     |        |      |        |       |    |        |     |        |      |        |       |    |        |     |        |
| ≥2.5cm                    | 1049 | 63 | 6.0 | 1.00 |        | 1.00 |        | 1046 | 39 | 3.7 | 1.00 |        | 1.00 |        | 1022 | 68 | 6.7 | 1.00 |        | 1.00 |        |
| <2.5cm                    | 32  | 12     | 37.5  | 6.24 | 3.76–10.4 | 3.97 | 2.15–7.33 | 32  | 7  | 21.9 | 5.87 | 2.84–12.1 | 3.19 | 1.35–7.55 | 31  | 5  | 16.1 | 2.42 | 1.05–5.59 | 2.06 | 0.88–4.82 |
| Gestation                 |      |        |       |    |        |     |        |      |        |       |    |        |     |        |      |        |       |    |        |     |        |
| Single                    | 1182 | 81 | 6.9 | 1.00 |        | 1.00 |        | 1179 | 51 | 4.3 | 1.00 |        | 1.00 |        | 1130 | 74 | 6.6 | 1.00 |        | 1.00 |        |
| Twin                      | 31  | 11     | 35.5  | 5.18 | 3.08–8.70 | 5.18 | 2.75–9.77 | 30  | 8  | 26.7 | 6.16 | 3.21–11.8 | 7.53 | 3.58–15.9 | 31  | 6  | 19.4 | 2.96 | 1.39–6.27 | 2.71 | 1.12–6.57 |
| HIV serostatus at enrollment |      |        |       |    |        |     |        |      |        |       |    |        |     |        |      |        |       |    |        |     |        |
| Negative                  | 908  | 67 | 7.4 | 1.00 |        | 1.00 |        | 905 | 41 | 4.5 | 1.00 |        | 1.00 |        | 871 | 61 | 7.0 | 1.00 |        | 1.00 |        |
| Positive                  | 303  | 25 | 8.3 | 1.12 | 0.72–1.74 | 1.20 | 0.71–2.01 | 302 | 18 | 6.0 | 1.32 | 0.77–2.26 | 1.35 | 0.68–2.66 | 286 | 19 | 6.6 | 0.95 | 0.58–1.56 | 0.86 | 0.48–1.53 |
| Syphilis                  |      |        |       |    |        |     |        |      |        |       |    |        |     |        |      |        |       |    |        |     |        |
| Non-reactive              | 1057 | 81 | 7.7 | 1.00 |        | 1.00 |        | 1054 | 53 | 5.0 | 1.00 |        | 1.00 |        | 1009 | 71 | 7.0 | 1.00 |        | 1.00 |        |
| Reactive                  | 63  | 5      | 7.9   | 1.04 | 0.44–2.46 |   | 63  | 2  | 3.2 | 0.63 | 0.16–2.53 |   | 63  | 6  | 9.5 | 1.35 | 0.61–2.99 |   |
### Exposure

| Blood pressure during pregnancy | Very preterm birth 16 to <34 weeks | Spontaneous very preterm birth 16 to <34 weeks | Very small for gestational age |
|---------------------------------|----------------------------------|------------------------------------------|-------------------------------|
|                                 | n  | events | %  | RR | 95% CI | aRR* | 95% CI | n  | events | %  | RR | 95% CI | aRR* | 95% CI | n  | events | %  | RR | 95% CI | aRR* | 95% CI |
| Normotensive                    | 1072 | 78 | 7.3 | 1.00 | | | | 1068 | 55 | 5.2 | 1.00 | | | | 1025 | 65 | 6.3 | 1.00 | | | 1.00 |
| Hypertensive‡                   | 141  | 14 | 9.9 | 1.36 | 0.79–2.34 | | | | 141  | 4 | 2.8 | 0.55 | 0.20–1.50 | | | | 134  | 15 | 11.2 | 1.77 | 1.04–3.00 | | | 1.68 | 0.92–3.06 |

#### Hemoglobin at enrollment

|                                 | n  | events | %  | RR | 95% CI | aRR* | 95% CI | n  | events | %  | RR | 95% CI | aRR* | 95% CI | n  | events | %  | RR | 95% CI | aRR* | 95% CI |
| ≥10.5 g/dL                      | 730 | 60 | 8.2 | 1.00 | | | | 727 | 35 | 4.8 | 1.00 | | | | 703 | 46 | 6.5 | 1.00 | | | |
| <10.5 g/dL                      | 121 | 7  | 5.8 | 0.70 | 0.33–1.50 | | | | 121 | 5 | 4.1 | 0.86 | 0.34–2.15 | | | | 113 | 5 | 4.4 | 0.68 | 0.27–1.67 | | | |

#### UA during pregnancy

|                                 | n  | events | %  | RR | 95% CI | aRR* | 95% CI | n  | events | %  | RR | 95% CI | aRR* | 95% CI | n  | events | %  | RR | 95% CI | aRR* | 95% CI |
| Normal                          | 907 | 59 | 6.5 | 1.00 | | | | 904 | 37 | 4.1 | 1.00 | | | | 881 | 68 | 7.7 | 1.00 | | | |
| Abnormal^                       | 189 | 14 | 7.4 | 1.14 | 0.65–2.00 | | | | 189 | 7 | 3.7 | 0.81 | 0.41–2.00 | | | | 183 | 10 | 5.5 | 0.71 | 0.37–1.35 | | | |

#### EGA at enrollment, weeks

|                                 | n  | events | %  | RR | 95% CI | aRR* | 95% CI | n  | events | %  | RR | 95% CI | aRR* | 95% CI | n  | events | %  | RR | 95% CI | aRR* | 95% CI |
| <14                             | 360 | 39 | 10.8 | 1.00 | | | | 358 | 26 | 7.3 | 1.00 | | | | 333 | 25 | 7.5 | 1.00 | | | |
| ≥14                             | 853 | 53 | 6.2 | 0.57 | 0.39–0.85 | | | | 851 | 33 | 3.9 | 0.92 | 0.86–0.99 | | | | 828 | 55 | 6.6 | 0.88 | 0.56–1.40 | | | |

* Risk ratios calculated via Poisson regression with robust error variance. Multivariable models include other exposure variables listed and adjusted for: maternal age, maternal BMI, and EGA at enrollment.

‡ Defined as systolic blood pressure ≥ 140 and/or diastolic blood pressure ≥ 90 at enrollment or at any follow-up visit.

^ Defined as 1+ leukocyte esterase and/or + nitrates.
Figure 4. Predicted probability of preterm birth <37 weeks by mid-trimester cervical length. Among ZAPPS cohort participants with a cervical length measured by ultrasound in the second trimester (n=1081), the probability of preterm birth <37 weeks decreased with increasing cervical length. PTB, preterm birth; RR, relative risk; CI, confidence interval.

worsens with later presentation to care. We report PTB based on ultrasound gestational age dating and prospectively ascertained delivery outcomes such that our data are likely more accurate than reports that rely on LMP recall or regional models.

The distinction of preterm parturition as spontaneously occurring versus provider-initiated is important but rarely reported from national surveillance or clinical research data in low-resource settings. Deliveries that are preceded by spontaneous labor or membrane rupture are phenotypically distinct from those that are induced medically or surgically for complications such as preeclampsia, fetal demise, or other maternal or fetal conditions. Further classification based on primary conditions present in spontaneous PTB and the primary indications for provider-initiated PTB is based on a standardized rubric proposed to elucidate phenotypic clusters of PTB. An understanding of prevailing phenotypes can direct research, policy, and preventive interventions towards regional and population-specific needs. While our cohort is limited by a small number of PTB events (n=181), we were able to classify nearly all (i.e., 97%) as either spontaneous or provider-initiated, and to identify the primary complications and phenotypic characteristics of each. Further granularity and generalizability requires a larger sample size, signaling a need for future high-quality obstetrical research on a greater scale.

As with PTB classification, identifying infants born SGA requires accurate gestational age estimation, which can be at best imprecise, and at worst biased, when based solely on LMP. The incidence of SGA in our cohort (18%) was modestly higher than a recent estimate of SGA in Zambia of 13%, again modeled from published rates in other neighboring countries because of scarcity of data from Zambia itself. In comparison to the ZAPPS cohort, in which older maternal age, low BMI, nulliparity, twin gestation, and antenatal hypertension predicted either SGA or very SGA, a study among over 19,000 singletons in Tanzania identified younger maternal age, height, and nulliparity as strong risk factors for SGA. The WHO Multi-country Survey on Maternal and Newborn Health found nulliparity and hypertensive disorders to indicate higher risk of preterm SGA and hypertensive disorders, sociodemographic factors, and anemia to predict term SGA. With a much smaller sample size and fewer outcomes compared to these two studies, we were not able to differentiate our outcome by preterm vs. term SGA due to low statistical precision for stratified associations with key risk factors. Due to this low precision, we are not able to discern whether or not SGA outcomes were modified by gestational age at delivery. However, both of these studies relied on reported LMP to estimate gestational age at delivery, which itself may have introduced error. Whether growth restriction is a distinct pathological process before 37 weeks compared to after 37 weeks is unclear. Finally, while the INTERGROWTH-21 gestational growth and newborn weight standards derived from an extensive multi-ethnic sample of women with adequate antenatal care and nutrition, its widespread use over ethnicity-specific or customized standards has been disputed. Despite this, we chose to define SGA in our cohort based on INTERGROWTH-21.
Figure 5. Kaplan-Meier survival curves by (a) prior preterm birth, (b) short cervix (<25mm), and (c) twin gestation. Survival curves are presented for participants with increasing numbers of prior preterm birth, those with cervical length <25mm compared to ≥25 mm, and those with twin compared to singleton gestation. The dashed vertical line represents a gestational age of 37 weeks, the threshold for preterm versus term delivery. EGA, estimated gestational age; PTB, preterm birth.
Table 5. Log-rank and Cox proportional hazards regression with test of proportionality assumption for prior preterm birth, short cervical length, and twin gestation.

|                      | Log-rank | Cox proportional hazards | Schoenfeld residual test | p   | HR* | 95% CI | p   | rho | χ² | p   |
|----------------------|----------|--------------------------|--------------------------|-----|-----|--------|-----|-----|----|-----|
| Prior preterm birth  | global   | 3.23                     | 0.66                     |     |     |        |     |     |    |     |
| Parous, no prior     |           | ref                      | ref                      |     |     |        |     |     |    |     |
| Parous, 1 prior      | <.001    | 2.21                     | 1.47–3.33                | <.001| -0.01| 0.01   | 0.92|     |    |     |
| Parous, 2 prior      |           | 2.79                     | 1.70–4.58                | <.001| 0.00 | 0.00   | 0.95|     |    |     |
| Parous, 3+ prior     |           | 4.70                     | 2.94–7.53                | <.001| -0.11| 2.33   | 0.13|     |    |     |
| Nulliparous          | -        | 1.16                     | 0.75–1.78                | 0.51 | -0.06| 0.80   | 0.37|     |    |     |
| Cervical length      | global   | 4.42                     | 0.11                     |     |     |        |     |     |    |     |
| ≥ 25mm               |           | ref                      | ref                      |     |     |        |     |     |    |     |
| <25mm                | <.001    | 5.19                     | 3.09–8.74                | <.001| -0.15| 3.26   | 0.07|     |    |     |
| Gestation            | global   | 3.19                     | 0.20                     |     |     |        |     |     |    |     |
| Singleton            |           | ref                      | ref                      |     |     |        |     |     |    |     |
| Twin                 | <.001    | 6.70                     | 4.25–10.60               | <.001| 0.13 | 3.19   | 0.07|     |    |     |

HR, hazards ratio; CI, confidence interval.

* Each proportional hazards model adjusted for maternal age at enrollment.

log-rank of trend, excluding nulliparas.

standards since local standards that include all pregnancies affected by undernutrition and/or pregnancy comorbidities tend to identify only the severest 10% of cases by definition.

Stillbirth, a composite outcome comprising antepartum and intrapartum fetal death, is particularly understudied in low-resource settings. The true global burden of stillbirth and its underlying causes are poorly classified due to inconsistent fetal viability limits and imperfect classification of neonatal death versus stillbirth, limited resources for case investigations, under-reporting of home births that result in perinatal death, and inadequate national and regional reporting of identified cases. Indeed, recent global and regional estimates of stillbirth included just 17% of its datapoints from sub-Saharan Africa and south Asia, regions that bear 77% of the global burden. Data from the recent Zambia Demographic and Health Survey reported a rate of stillbirth, defined as fetal death over 7 months’ gestation, as 1.3% among 13,563 births reported, with equal rates outside Lusaka province as within. This is similar to estimates from a Global Network study in Zambia, in which 2% of women enrolled delivered stillbirths. The slightly higher proportion of deliveries that resulted in stillbirth in the ZAPPS cohort, at least partly attributable to a broader gestational age range, was reflected in the ZEPRS database, in which 6% of 66,395 deliveries at UTH resulted in stillbirth. However, over half of stillbirths in ZEPRS and 67% in a Global Network study in Zambia were classified as intrapartum, compared to less than 20% in the ZAPPS cohort. These disparities may result from differential classification; it is standard practice outside of our study to classify stillbirths solely by neonatal skin maceration at delivery, particularly in the absence of but even despite the presence of documented fetal heart activity during labor.

Indeed, previous studies have demonstrated that reliance on observed skin maceration alone can over-estimate stillbirth proportions attributable to the intrapartum period. This study has several limitations, many of which have been noted previously. First, 16% of participants were lost to follow-up. While this is commensurate with other longitudinal pregnancy cohort studies in the region, error may be introduced if outcomes are not missing at random. Women lost to follow-up were younger, more likely to be primigravida and nulliparous, had lower BMIs, and had multiple lower measures of socioeconomic status; many of these characteristics were risk factors for at least one adverse outcome. Further, 250 (21%) of the retained participants either did not deliver at the study hospital or delivered at a time when ZAPPS staff were not present, requiring delivery outcomes to be ascertained by record review and/or participant report (it is worth noting that we found no difference in frequencies of outcomes between deliveries attended by ZAPPS staff versus those that were not; see Underlying data). Second, our data have noted missingness of key antenatal test results at baseline (i.e., hemoglobin, syphilis, and urinalysis) because tests were not routinely repeated nor results recorded in our database if performed at the recruitment clinic before enrollment. Of these test results, only syphilis was associated
with an outcome (stillbirth), but we cannot determine with certainty whether missingness introduced bias or simply reduced statistical power. Third, while the ZAPPS study recruits from several surrounding primary clinics, it is based at a tertiary referral hospital and many of our participants were drawn from this higher-risk pool. We note high prevalence of prior PTB, miscarriage, and stillbirth, and high HIV and syphilis seropositivity, which may have resulted from self-selection of high-risk women into a cohort study investigating adverse birth outcomes. It is likely that this resulted in an over-representation of outcomes, but less likely to have also introduced a biased association with identified risk factors.

In summary, the ZAPPS cohort study demonstrates high prevalence of antenatal comorbidities and identifies a number of factors associated with increased risks of preterm birth, small-for-gestational-age infants, and stillbirth. This is the first study of its kind to be conducted in Zambia, and one of the largest on the African continent. An understanding of the true global scope of adverse birth outcomes will require consistent definitions, meticulous ascertainment, and systematic reporting that has eluded those settings where the burden of these outcomes is highest. In the absence of sophisticated registry infrastructure, large pregnancy cohort studies may be able to approximate regional incidence estimates and can provide important data to identify, stratify, and direct care and resources for pregnancies at highest risk. Future sub-studies using data and stored biological specimens from the ZAPPS cohort will aim to identify underlying biological mechanisms, causal pathways, and appropriate interventions for the accurate prediction and prevention of adverse birth outcomes in Zambia and worldwide.

Data availability

Underlying data

Open Science Framework: Zambian Preterm Birth Prevention Study (ZAPPS) – Outcomes. https://doi.org/10.17605/OSF.IO/WT6Q8

This project contains the following underlying data:

- Z1A minimum dataset 2019-06-30.csv (underlying data for all participants)
- Z1A Codebook 2019-06-30.rtf (codebook for the variables within the dataset)

Data are available under the terms of the Creative Commons Attribution 4.0 International license (CC-BY 4.0).

Acknowledgments

The authors acknowledge the invaluable contributions to the study design and conduct by Eve Lackritz, James Litch, Marcela Castillo, Nancy Hancock, and Nurain Fuseini. The study protocol is registered at ClinicalTrials.gov, identifier: NCT02738892.

References

1. You D, Hug L, Ejdemry S, et al.: Global, regional, and national levels and trends in under-5 mortality between 1990 and 2015, with scenario-based projections to 2030: a systematic analysis by the UN Inter-agency Group for Child Mortality Estimation. Lancet. 2016; 386(10010): 2275-86. Published Abstract | Publisher Full Text

2. Hug L, Alexander M, You D, et al.: National, regional, and global levels and trends in neonatal mortality between 1990 and 2017, with scenario-based projections to 2030: a systematic analysis. Lancet Glob Health. 2019; 7(6): e710-e820. Published Abstract | Publisher Full Text | Free Full Text

3. Blencowe H, Cousins S, Jassir FB, et al.: National, regional, and worldwide estimates of stillbirth rates in 2015, with trends from 2000: a systematic analysis. Lancet Glob Health. 2016; 4(2): e88-e108. Published Abstract | Publisher Full Text

4. Blencowe H, Cousins S, Oestergaard MZ, et al.: National, regional, and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: a systematic analysis and implications. Lancet. 2012; 379(9832): 2162–72. Published Abstract | Publisher Full Text

5. Lee AC, Kozuki N, Cousins S, et al.: Estimates of burden and consequences of infants born small for gestational age in low and middle income countries with INTERGROWTH-21st standard: analysis of CHERG datasets. BMJ. 2017; 358: j3677. Published Abstract | Publisher Full Text | Free Full Text

6. Chawanpaiboon S, Vogel JP, Moller AB, et al.: Global, regional, and national estimates of levels of preterm birth in 2014: a systematic review and modelling analysis. Lancet Glob Health. 2019; 7(1): e37-e46. Published Abstract | Publisher Full Text | Free Full Text

7. Saleem S, Talib SS, McClure EM, et al.: Trends and determinants of stillbirth in developing countries: results from the Global Network’s Population-Based Birth Registry. Paediatr Perinat Epidemiol. 2018; 32(1): 6-15. Published Abstract | Publisher Full Text

8. Lawn JE, Gravett MG, Nunes TM, et al.: Global report on preterm birth and stillbirth (1 of 7): definitions, description of the burden and opportunities to improve data. BMC Pregnancy Childbirth. 2010; 10 (Suppl 1): S1. Published Abstract | Publisher Full Text | Free Full Text

9. McClure EM, Saleem S, Goudar SS, et al.: Stillbirth rates in low-middle income countries 2010 - 2013: a population-based, multi-country study from the Global Network. Reprod Health. 2015; 12 Suppl 2: S7. Published Abstract | Publisher Full Text | Free Full Text

10. Lawn JE, Blencowe H, Waiswa P, et al.: Stillbirths: rates, risk factors, and acceleration towards 2030. Lancet. 2016; 387(10018): 587–603. Published Abstract | Publisher Full Text

11. Blencowe H, Krasevec J, de Onis M, et al.: National, regional, and worldwide estimates of low birthweight in 2015, with trends from 2000: a systematic analysis. Lancet Glob Health. 2019; 7(7): e849–e60. Published Abstract | Publisher Full Text

12. Villar J, Papageorghiou AT, Knight HE, et al.: The preterm birth syndrome: a prototype phenotypic classification. Am J Obstet Gynecol. 2012; 206(2): 119–23. Published Abstract | Publisher Full Text

13. Manuck TA, Esplin MS, Biggio J, et al.: The phenotype of spontaneous preterm birth: application of a clinical phenotyping tool. Am J Obstet Gynecol. 2015; 212(4): 487.e1–e11. Published Abstract | Publisher Full Text | Free Full Text

14. Vwalika B, Price JT, Rosenbaum A, et al.: Reducing the global burden of preterm births. Lancet Glob Health. 2019; 7(6): e413. Published Abstract | Publisher Full Text

15. Lynch CD, Zhang J: The research implications of the selection of a gestational age estimation method. Paediatr Perinat Epidemiol. 2007; 21 Suppl 2: 86–96. Published Abstract | Publisher Full Text

16. Kramer MS, McLean FH, Boyd ME, et al.: The validity of gestational age estimation by menstrual dating in term, preterm, and postterm gestations. JAMA. 1988; 260(22): 3306-8. Published Abstract | Publisher Full Text

17. Savitz DA, Terry JW Jr, Dole N, et al.: Comparison of pregnancy dating by last menstrual period, ultrasound scanning, and their combination. Am J Obstet Gynecol. 2002; 187(6): 1660-6. Published Abstract | Publisher Full Text

18. Unger H, Thiermer K, Ley B, et al.: The assessment of gestational age: a comparison of different methods from a malaria pregnancy cohort in
sub-Saharan Africa. BMC Pregnancy Childbirth. 2019; 19(1): 12.
Publishd Abstract | Publisher Full Text | Free Full Text

19. Lee AC, Panchal P, Folger L, et al.: Diagnostic Accuracy of Neonatal Assessment for Gestational Age Determination: A Systematic Review. Pediatrics. 2010; 126(6): e1601-1603.
Published Abstract | Publisher Full Text | Free Full Text

20. Taylor RA, Denison FC, Beyal S, et al.: The external Ballard examination does not accurately assess the gestational age of infants born at home in a rural community of Zambia. Ann Trop Paediatr. 2010; 30(4): 197-204.
Published Abstract | Publisher Full Text | Free Full Text

21. Price JT, Winston J, Yawalka B, et al.: Quantifying bias between reported last menstrual period and ultrasonography estimates of gestational age in Lusaka, Zambia. Int J Gynaecol Obstet. 2015; 138(1): 9-15.
Published Abstract | Publisher Full Text | Free Full Text

22. von Elm E, Altman DG, Egger M, et al.: The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. Lancet. 2007; 370(9569): 1453-7.
Published Abstract | Publisher Full Text | Free Full Text

23. Castillo MC, Fuseni NM, Rittenhouse K, et al.: The Zambian Preterm Birth Prevention Study (ZAPPS): Cohort characteristics at enrollment (version 2; peer review: 2 approved). Gates Open Res. 2018; 2: 25.
Published Abstract | Publisher Full Text | Free Full Text

24. Papageorghiou AT, Kennedy SH, Salomon LJ, et al.: International standards for early fetal size and pregnancy dating based on ultrasound measurement of crown-rump length in the first trimester of pregnancy. Ultrasound Obstet Gynecol. 2014; 44(6): 641-8.
Published Abstract | Publisher Full Text | Free Full Text

25. Papageorghiou AT, Kemp B, Stones W, et al.: Ultrasound-based gestational-age estimation in late pregnancy. Ultrasound Obstet Gynecol. 2016; 48(6): 719-26.
Published Abstract | Publisher Full Text | Free Full Text

26. Hadlock FP, Shah YP, Kanon DJ, et al.: Fetal crown-rump length; reevaluation of relation to menstrual age (5-18 weeks) with high-resolution real-time US. Radiology. 1992; 182(2): 501-5.
Published Abstract | Publisher Full Text | Free Full Text

27. Romero R, Yeo L, Miranda J, et al.: A blueprint for the prevention of preterm birth: vaginal progesterone in women with a short cervix. J Perinat Med. 2013; 41(1): 27-44.
Published Abstract | Publisher Full Text | Free Full Text

28. Papageorghiou AT, Saris I, Ioannou C, et al.: Ultrasound methodology used to construct the fetal growth standards in the INTERGROWTH-21st Project. BJOG. 2013; 120 Suppl 2: 27–32, v.
Published Abstract | Publisher Full Text | Free Full Text

29. Papageorghiou AT, Kennedy SH, Salomon LJ, et al.: The INTERGROWTH-21st fetal growth standards: toward the global integration of pregnancy and pediatric care. Am J Obstet Gynecol. 2018; 218(2S): S630–S640.
Published Abstract | Publisher Full Text | Free Full Text

30. Zou G: A modified poisson regression approach to prospective studies with binary data. Am J Epidemiol. 2004; 159(7): 702-6.
Published Abstract | Publisher Full Text | Free Full Text

31. Schoenfeld D: Chi-squared goodness of fit test for the proportional hazards regression model. Biometrika. 1981; 68(1): 143-7.
Published Full Text

32. Thomeau TM, Grampsch PM: Modeling survival data: extending the Cox model. New York: Springer; 2000; xii, 350.
Published Full Text

33. Grunstein RU, Rose GP, et al.: Repression of normal corticosteroid androgen and estrogen action and glucocorticoid sensitivity by glucocorticoids. Science. 1994; 264(5157): 1247-50.
Published Abstract | Publisher Full Text | Free Full Text

34. Stringer JSA: Zambian Preterm Birth Prevention Study (ZAPPS) - Outcomes. 2019. http://www.doi.org/10.17605/OSF.IO/WWRQ8
Published Abstract | Publisher Full Text | Free Full Text

35. Walmrath D, Villar J, Salomon LJ, et al.: International estimated fetal weight standards for the INTERGROWTH-21st Project. Ultrasound Obstet Gynecol. 2017; 49(4): 478-86.
Published Abstract | Publisher Full Text | Free Full Text

36. Micallef L, Rodgers P: eulerAPE: drawing area-proportional 3-Venn diagrams using ellipses. PLoS One. 2014; 9(7): e101717.
Published Abstract | Publisher Full Text | Free Full Text

37. Ch BH, Yawalka B, Killam WP, et al.: Implementation of the Zambia electronic fetal biometry charts with the INTERGROWTH-21st standard. Ultrasound Obstet Gynecol. 2017; 49(4): 478-86.
Published Abstract | Publisher Full Text | Free Full Text

38. Vawala B, Stoner MC, Mwanahamuntu M, et al.: Maternal and newborn outcomes at a tertiary care hospital in Lusaka, Zambia, 2008-2012. Int J Gynaecol Obstet. 2017; 138(2): 180-7.
Published Abstract | Publisher Full Text | Free Full Text

39. Ambrose CS, Caspard H, Rizzo C, et al.: Standard methods based on last menstrual period dates misclassify and overestimate US preterm births. J Perinatol. 2015; 35(6): 411-7.
Published Abstract | Publisher Full Text | Free Full Text

40. Buck Louis GM, Grewal J, Albert PS, et al.: Racial/ethnic standards for fetal growth: the NICHD Fetal Growth Studies. Am J Obstet Gynecol. 2015; 213(4): 449 e1–e44.
Published Abstract | Publisher Full Text | Free Full Text

41. Cheng Y, Leung TV, Lao T, et al.: Impact of replacing Chinese ethnicity-specific fetal biometry charts with the INTERGROWTH-21st standard. BJOG. 2016; 123 Suppl 3: 68–55.
Published Abstract | Publisher Full Text | Free Full Text

42. Anderson NH, Sadler LC, McKinley CJD, et al.: INTERGROWTH-21st vs customized birthweight standards for identification of perinatal mortality and morbidity. Am J Obstet Gynecol. 2016; 214(4): 509 e1–e7.
Published Abstract | Publisher Full Text | Free Full Text

43. Kiserud T, Piaggio G, Carroll G, et al.: The World Health Organization Fetal Growth Charts: A Multinational Longitudinal Study of Ultrasound Biometric Measurements and Estimated Fetal Weight. Placenta. 2017; 14(1): e100220.
Published Abstract | Publisher Full Text | Free Full Text

44. Korevaar EN, Kandare-skawinska S, et al.: Realities and Challenges of a Five Year Follow Up of Mother and Child Pairs on a PMTCT Program in Zambia. Open AIDS J. 2011; 5: 51-6.
Published Abstract | Publisher Full Text | Free Full Text

45. Howie CJ, Cole SR, Lau B, et al.: Selection Bias Due to Loss to Follow Up in Cohort Studies. Epidemiology. 2016; 27(1): 91-7.
Published Abstract | Publisher Full Text | Free Full Text

Page 18 of 24
Open Peer Review

Current Peer Review Status: ✅ ✋

Version 1

Reviewer Report 06 November 2019

https://doi.org/10.21956/gatesopenres.14168.r28082

© 2019 Bhatnagar S. This is an open access peer review report distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Shinjini Bhatnagar
Maternal and Child Health program, Translational Health Science and Technology Institute, Faridabad, Haryana, India

In this manuscript, the authors report the rates of adverse birth outcomes such as preterm birth, stillbirth and small for gestational age from a well-characterized cohort of 1450 pregnant women from Zambia. This is a very important article, providing robust data from a low-middle income country like Zambia in the domain of maternal and child health.

The emphasis on accuracy and precision of gestational age estimation which is a cornerstone in preterm birth research is praise-worthy. The data is well represented and the statistical analyses are appropriate.

Comments:

1. Preterm birth has been defined as birth between 16 0/7 and 36 6/7 gestational weeks in this study. The definition seems to include stillbirths (the proportion of stillbirth among PTB is reported in paragraph 4 of the results section), which is in variance with the convention of reporting preterm birth among live-born babies. What is the rationale behind this choice? Does such a change in definition influence the preterm birth rate in this study population? If so, how much?

2. The lower limit of gestational age for defining preterm birth is taken as 16 0/7 weeks in this study. What was the rationale behind considering 16 weeks as the lower cut-off of preterm birth? Further, one of the inclusion criteria is “presentation to antenatal care prior to 20 weeks of gestation if HIV-uninfected or 24 weeks if HIV-infected”. Nearly 50% of the women first present to antenatal care after 16 weeks in this population and 25% above 18.3w (median EGA at enrolment is 16.1 (IQR: 13.3–18.3)w). This means there would be some pregnant women in the population who delivered preterm (between 16 & 24w) who couldn't be a part of the cohort because of the delay in seeking antenatal care. Would that create a bias? If so, how much would this influence the study’s estimate of the preterm birth rate in the population? Reporting preterm birth rate among the participants enrolled less than 16 weeks of gestation will add further information in this aspect.
The manuscript can be accepted for indexing after the resolution of the comments made above.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Partly

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Maternal and child health

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

---

**Author Response 14 Nov 2019**

**Joan Price**, University of North Carolina, School of Medicine, Chapel Hill, USA

We chose to include preterm stillbirths in our definition of preterm birth and demonstrate the overlapping adverse outcomes of preterm birth, stillbirth, and small for gestational age in Figure 2. We acknowledge significant limitations in differentiating intrapartum stillbirth from preterm birth with immediate neonatal death in our setting. We believe that to exclude stillbirths that occur in the process of parturition from spontaneous preterm birth would falsely lower the rate of spontaneous preterm delivery. As we note in the discussion, the categorization of antepartum vs. intrapartum stillbirth is also imperfect. Of 181 preterm deliveries in our cohort, 48 (27%) were stillborn. Excluding stillbirths from our preterm delivery definition reduced preterm delivery from 15% to 11% and spontaneous preterm delivery from 10% to 8%. Despite this overall reduction, excluding stillbirths from our regression models did not significantly alter our estimates of risk, which supports evidence that risk factors for live and stillborn preterm births demonstrate substantial overlap (Kramer 2012). Finally, our cohort was designed to evaluate the risk factors associated with adverse birth outcomes, and not necessarily to estimate population-level rates of these.
outcomes. We note in the discussion that our study population likely over-represents high-risk women and therefore overestimates the true population incidence of adverse birth outcomes.

Both lower and upper EGA boundaries for defining preterm birth vary widely in research and national statistics worldwide. Participants at the International Conference on Prematurity and Stillbirth of the Global Alliance to Prevent Prematurity and Stillbirth (GAPPS) have argued for including births occurring 16 weeks onward in the definition of preterm birth, citing studies that show similar etiological risk factors for births occurring as early as 16 weeks and those that occur later in the 2nd and 3rd trimester (Kramer 2012; Villar 2012). We acknowledge that the risk of preterm birth in any cohort increases with earlier gestational age at presentation for precisely the reason explained by the reviewer. There is a strong relationship between EGA at presentation and EGA at delivery, which therefore produces this effect regardless of relatively arbitrary EGA boundaries used to define outcomes. Indeed, the preterm birth rate among participants who enrolled <16 weeks was 18% in our cohort compared to 12% among those enrolled ≥16 weeks. Because of this, we include EGA at enrollment in all multivariable analyses. We also repeated analyses of preterm delivery outcomes restricting our sample to those participants who presented <20 weeks and again to those who presented <16 weeks to evaluate the potential for bias. These restricted analyses had no effect on our results (with the exception of short cervix as an exposure since it was only performed beyond 16 weeks).

**Competing Interests:** No competing interests were disclosed.
4. Distinction between spontaneous and provider-initiated PTB;

5. Comprehensive and reliable data analysis;

6. Results were well-organized clearly presented;

7. Limitations were well noted and discussed.

Minor comments:

1. The reviewer recognized that the authors tried to avoid using p-values in this report according to recent guidelines and recommendations. However, sometimes it could be helpful to the readers to comprehend the “substantiality” of the statistical evidence. The reviewer therefore would suggest including p-values in Figure 3, Table 2, etc. (as Table 1).

2. EGA at enrollment (<14) was shown to be associated with PTB. The authors might compare the baseline characteristics between the samples enrolled before and after 14wks to identify the possible reasons underlying this association (similar to the comparisons made in Table 1). In addition, as the gestational age was calculated differently in the samples enrolled before and after 14wks, the authors might also compare outcomes (e.g. gestation duration) between these two groups to examine whether the two methods could potentially cause systematic difference in the estimation of gestational age.

3. The authors presented the co-occurrence among PTB, SGA and SB using a Venn diagram (Figure 2). It is also interesting to learn whether the frequencies of the co-occurrence of these outcomes were higher than expected especially between the very PTB and very SGA group.

4. The authors dichotomized continuous exposures (e.g. maternal age, BMI) as well as the outcomes (e.g. PTB, SGA) in the association analysis (Table 3). It would be more informative if the authors could also show the results based on association tests of continuous variables.

5. Maternal height has been shown to be associated with gestational duration and birth weight (gestational age adjusted) in previous studies mainly in high-income countries. It is not known whether maternal height is associated with gestational duration (and PTB) in this study or not.

6. In the discussion section, the authors should compare the risk factors (e.g. their occurrence, frequencies and estimated effect sizes) and the frequencies of adverse birth outcomes reported in this study with those reported in high-income countries.

**Is the work clearly and accurately presented and does it cite the current literature?**

Yes

**Is the study design appropriate and is the work technically sound?**

Yes

**Are sufficient details of methods and analysis provided to allow replication by others?**
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Genetics of pregnancy outcomes

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 07 Feb 2020

Joan Price, University of North Carolina, School of Medicine, Chapel Hill, USA

The authors thank the reviewer for the thoughtful and constructive comments. Please find a point-by-point response to each comment here.

1. We have elected not to include p values in our tables beyond Table 1 because the we think point estimates and their 95% confidence intervals are the best estimates of association. (see Harrington et al NEJM 2019 https://www.nejm.org/doi/full/10.1056/NEJMe1906559).

2. Thank you for this suggestion. In response to the other reviewer’s comment regarding the increased risk of PTB with EGA at enrollment, we have repeated analyses of preterm delivery outcomes restricting our sample to those participants who presented <20 weeks and again those who presented <16 weeks to evaluate the potential for bias and our results proved stable. We also include EGA at enrollment in all multivariable analyses. We think that additional reporting of which baseline characteristics differ by EGA at enrollment and investigation of the effect of ultrasound algorithms used for EGA calculation is beyond the scope (and page limit) of this analysis but may be explored in future analyses.

3. Thank you for this suggestion. We have added a second Euler diagram to Figure 2 to illustrate the co-occurrence of very PTB, very SGA, and stillbirth.

4. We have added linear regression of continuous exposures and continuous outcomes (Table 6).

5. We have noted in the results section that maternal height was not associated with either PTB or SGA. It was similarly not associated with gestational duration.

6. We have added additional global context to our estimates of the frequencies of adverse birth outcomes in the discussion section. However, we acknowledge significant limitations to direct comparisons of risk estimates due to inconsistent definitions. Furthermore, as there is still no undisputed global standard by which to identify small for gestational age neonates, we have not directly compared the incidence of SGA in our cohort to estimates from high-income countries.
Competing Interests: No competing interests were disclosed.