Antenatal Syphilis Burden, Risk Factors and Pregnancy Outcomes in a High HIV Prevalence Setting

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ABSTRACT

*Syphilis in pregnancy remains an important preventable cause of perinatal mortality and morbidity, yet the respective modifiable factors remain inadequately described in resource-limited settings where syphilis and HIV infections have significant epidemic synergies. The study aimed to determine the burden of syphilis in HIV-uninfected and HIV-infected pregnant women during this era of lifelong antiretroviral therapy. This study was based on a prospective cohort of pregnant women ≥20 weeks' gestational age seeking antenatal care services at four primary healthcare facilities in Harare. A flocculation non-treponemal test (NTT) and rapid chromatographic treponemal test (TT) were run in parallel on fresh maternal sera. Confirmation was done using either the traditional algorithm (TA) or reverse algorithm (RA). TA is whereby a positive NTT result is confirmed with a TT, whilst in RA, a positive TT is followed by a NTT. Discordant test results were resolved using a second, but different TT. Adverse infant outcomes were assessed at delivery. Six hundred HIV-uninfected and 608 HIV-infected pregnant women mainly on Tenofovir/Lamivudine/Efavirenz were enrolled. The presence of syphilis antibodies, regardless of whether active or past infection, was 53/1208:**4.39%**, 95% confidence interval (CI) [3.2-5.6]; being 38/608:**6.25%**,95%CI[4.4-8.2] and 15/600:**2.50%**, 95%CI[1.3-3.7] in the HIV-infected and HIV-uninfected women, respectively. Prevalence of active syphilis (AS) was 25/1208:**2.07%**, 95%CI [1.3-2.9], disproportionately occurring in the HIV-infected; 19/25(76%) versus 6/25(24%) in their HIV-uninfected peers, **p=0.024**. In a regression model with variable elimination, the absence of anaemia was protective, odds ratio (CI):6.2(1.5-42.6), **p=0.027**. Infants exposed to AS in pregnancy had lower birth weights; median (interquartile range) 3000g (2700-3192) versus 3200g (2950-3400) in the unexposed, **p=0.014**. High and moderate syphilis burdens were observed in HIV-infected and HIV-uninfected pregnant women, respectively. This underscores the need to further strengthen the current sexually-transmitted infections prevention and care programmes, especially in HIV-infected women of reproductive age, if the 2030 Sustainable Development Goal target 3.2 is to be met* (www.clinicaltrials.gov,trialregistrationnumber:NCT04087239).

Keywords: sexually-transmitted infections; treponemal and non-treponemal testing in parallel, traditional and reverse algorithms; pregnancy during the era of lifelong antiretroviral therapy; syphilis and HIV coinfection synergies; resource-limited settings

Introduction

Syphilis is a sexually-transmitted infection (STI) caused by spirochete *Treponema pallidum.* Despite the availability of simple diagnostic tests and an effective first line treatment with long-acting penicillin, syphilis is re-emerging as a global public health challenge. Global incidence increased by 60% from 1990 to 2019 with >90% of infections occurring in resource-limited settings where the HIV burden is also high (Zhou *et al*., 2021; Gao *et al*., 2021; Kuznik *et al*., 2013; Tao *et al*., 2023). Seroconversion occurs ~six weeks following infection, with antibodies reaching

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peak levels in one to two years in the absence of any treatment (George *et al*., 1998). The spirochete is renowned for its immune-evasiveness, with the clinical manifestations often mimicking those of other diseases (Peeling & Hook, 2005). In addition, painless lesions of primary syphilis often occur in hidden anatomical sites like the vagina, pharynx or anus, making many patients ignore this infection. Often, primary syphilis and, always, latent syphilis, are asymptomatic (Marks & Mabey, 2017). Thus, syphilis remains largely undiagnosed and therefore untreated in resource-limited settings because routine screening for asymptomatic infections is rarely done.

The predominantly asymptomatic active syphilis (AS) is often missed in pregnancy, and this poses the risk of vertical transmission (Ng'wamkai *et al*., 2019). The progression to the secondary stage of the disease, together with pregnancy itself as a condition, are both associated with a shift to an anti-inflammatory Th2 cytokine profile that does not effectively clear off the spirochete, posing risks of congenital syphilis (Peeling & Hook, 2005). About 1.4 million pregnant women have untreated AS, potentially causing >500 000 adverse pregnancy outcomes, including >300 000 perinatal deaths (Gomez *et al*., 2013; Newman *et al*., 2013). Observational studies have linked maternal AS to stillbirths, non-immune hydrops, preterm labour, preterm births and premature rupture of membranes, including chorioamnionitis (Burnett *et al*., 2015; Dos Reis *et al*., 2015). Furthermore, infants exposed to AS during pregnancy are at high risk of neurodevelopmental impairment regardless of whether or not they have congenital syphilis (Verghese *et al*., 2018). In summary, syphilis in pregnancy remains an important preventable cause of perinatal mortality and morbidity. This is even more important in resource-limited settings where the combination of syphilis and HIV infections has significant epidemic synergies. Such coinfections have been associated with unusual clinical presentations and a more rapid progression of syphilis (Rekart *et al*., 2017).

There is a paucity of information on the burden of antenatal HIV/syphilis coinfections. Furthermore, how this duet impacts pregnancy outcomes remains poorly described, more so during this era of lifelong antiretroviral therapy (ART) for the treatment of HIV. The last published Zimbabwean gestational syphilis mono-infection studies were done mainly during the era of intrapartum single dose Nevirapine for the prevention of perinatal transmission of HIV. Back then, the prevalence of syphilis in pregnancy was ~1.2% (Kurewa *et al*., 2010; Munjoma *et al*., 2010), yet more recent studies have shown that ART has the potential to alter the innate and acquired immune responses in ways that may enhance host susceptibility to *T. pallidum* (Rekart *et al*., 2017). Understanding the syphilis/HIV coinfection dynamics and synergies during this era of lifelong ART is essential to effectively control STIs. Thus, the business-as-usual STIs mono-infections care and controls done in silos may not meet the Sustainable Development Goal (SGD) target 3.2, which aims to end preventable deaths of new-borns and children under five years of age by 2030.

Objectives

This study aimed to:

1. Determine the prevalence of the presence of syphilis antibodies in pregnancy.
2. Determine the burden of AS and describe the risk factors, predictors and adverse pregnancy outcomes (stillbirths, preterm births; <37 weeks, low birth weights; <2500 g) including impact on birth length (BL), and head circumferences (HC) in infants born of HIV-infected and HIV-uninfected women seeking antenatal care services at four primary healthcare facilities in Harare, Zimbabwe.

Methods and Materials

Study Setting and Design

The University of Zimbabwe Birth Cohort Study (UZBCS) is an ongoing prospective observational study of HIV-infected and uninfected pregnant women recruited from high-density suburbs of Harare, Zimbabwe. There were four study sites which included Glenview, Budiriro, Kuwadzana and Dzivaresekwa (Rujeko) municipal polyclinics. Study site selection was based on lack of competing research activities, prevalence of HIV, and higher rates of maternal and child health services (Duri *et al*., 2020). The aim of the UZBCS is to investigate the impact of maternal comorbidities such as coinfections with HIV, viral hepatitis, cytomegalovirus and some infectious diseases. The investigation includes some non-communicable diseases such as maternal malnutrition, hypertensive disorders as well as their effects on pregnancy outcomes, infant mortality and overall health (Duri *et al*., 2020). In all participants recruited into the study, 50% of the pregnant women were HIV-infected as described previously (Duri *et al*., 2020). In the HIV-infected women, non-suppression of plasma HIV-RNA load is a common phenomenon mainly driven by late pregnancy new HIV diagnosis, thus women will not be on ART or may have suboptimal exposure to ART (Duri *et al*., 2022). However, mother-to-child transmission (MTCT) rates of HIV and hepatitis B viruses are low as previously reported (Duri *et al*., 2022a; Duri *et al*., 2023).

Standard of Care in Pregnancy

Zimbabwe follows the World Health Organisation (WHO) STI guidelines which recommend syphilis screening in pregnant women at their initial antenatal care (ANC) visit (World Health Organization, 2017). A single on-site syphilis screening is the current standard of care using a treponemal test (TT) combined with HIV point-of-care tests (Abbott Bioline HIV/Syphilis Duo). A reactive TT is used as the sole indicator of syphilis infection, followed by immediate treatment recommendation to mitigate adverse pregnancy outcomes. The first line treatment option for syphilis in pregnancy is Benzathine penicillin G. In cases of penicillin allergy, a four times daily dose of oral erythromycin 500 mg is given for 30 days. However, the failure of erythromycin to completely cross the placental barrier exposes the foetus to infection risk (World Health Organization, 2016).

Inclusion and Exclusion Criteria

Pregnant women of at least 15 years of age and 20 weeks' gestation by the date of enrolment were included in the study. The women had to be planning to deliver at any of the four selected study sites. Any women indicating the presence of severe mental health disorders which rendered them incapacitated to provide informed consent or comply with study procedures were excluded from participating as described previously (Duri *et al*., 2020).

Study Procedures at Enrolment Late in Pregnancy

A structured questionnaire was administered to all pregnant women at enrolment, providing a comprehensive socio-demographic and clinical characterisation of the participants. Socio-economic information comprising maternal employment status, money set aside for food every month, average family monthly income, and food security, was assessed. Additional questions were asked regarding maternal lifestyle such as the presence of comorbidities and other STIs, sexual behaviour, alcohol use, concurrent medications use, and knowledge of spouse/ intimate partner's HIV status. Time since HIV diagnosis and duration of ART exposure were requested from the HIV-infected group. The study midwife also performed full physical examinations which included anthropometric indicators of maternal nutritional status such as mid-upper arm circumference (MUAC) and body mass index (BMI), as described previously (Duri *et al*., 2020).

Specimen Collection and Laboratory Analysis

Venous whole blood (10 ml) was collected in plain and ethylenediaminetetraacetic acid (EDTA) tubes, at enrolment. Maternal HIV diagnosis was carried out from whole EDTA blood using a qualitative SD Bioline HIV-1/2 3.0 kit (Standard Diagnostics Inc., Kyonggi-do, South Korea) and the Abbott's Determine® HIV-1/2 kit. In the event of indeterminate test results, the western blot was used as the tie breaker as previously described (Duri *et al*., 2020). Whole EDTA blood was used for the determination of full blood counts using a Mindray© 3-part Differential, BC-3600 Analyser (Shenzhen, China). The WHO definition of anaemia in pregnancy (haemoglobin <11.0 g/dL) was used to classify participants (World Health Organization, 2011).

For the HIV-infected, maternal absolute $CD4^+$ T-lymphocyte counts in whole blood EDTA samples were enumerated within a maximum of six hours after sample acquisition using a Partec Cyflow counter (Cyflow, Partec, Munster, Germany) (Duri *et al*., 2020). Plasma was extracted from EDTA blood for HIV-RNA load testing, and stored at -80°C as described previously (Duri *et al*., 2020).

Syphilis Screening and Diagnosis

Fresh maternal serum was tested for Treponemal and non-Treponemal antibodies. A flocculation non-treponemal test (NTT), rapid plasma reagin (RPR) (Fortress Diagnostics Limited, United Kingdom), and a TT (SD-Bioline 3.0), were run in parallel. Reactive samples were confirmed using the TA and RA approaches, where a NTT-First (RPR) was followed by a TT (SD Bioline 3.0),

and a TT-First (SD Bioline 3.0) followed by a NTT (RPR), respectively (Figure 1). Inconclusive results were evaluated using the second, but different TT (Qingdao Hightop Biotech Co. Ltd, China). Any pregnant woman with a reactive RPR and/or SD Bioline test results was considered syphilis antibody positive. Women were considered true seronegative if all tests were seronegative. If both the NTT and TT were positive this confirmed AS, provided the patient had not been recently treated. Two different reactive TTs and a non-reactive NTT also confirmed AS (Workowski, Berman and Centers for Disease Control and Prevention (CDC), 2010).

Study Follow-up Visit

Pregnancy and infant outcomes were assessed from delivery, which was the first follow-up visit. Demographic and anthropometric data such as birth length, birth weight, head circumference and date of birth, were recorded for each infant as previously described (Duri *et al*., 2020).

Data Collection, Management and Statistical Analysis

Socio-demographic and clinical data was collected and managed using the Research Electronic Data Capture (REDCap) database. Data quality and accuracy checks for entered data included independent double entries and verification.

Continuous data was checked for normalcy using the Shapiro-Wilk test. Summary statistics were determined for predictor and outcome variables of interest and reported as medians and interquartile ranges for continuous data, then frequencies and percentages for categorical variables. A p-value of <0.05 was considered significant. In univariate analyses, each syphilis antibody positive woman was compared with her four randomly selected negative counterparts with respect to sexual risk behaviour, presence of other STIs, and common invasive medical procedures. To compare differences and associations between groups, the Mann-Whitney U test, the Kruskal Wallis test, or the Fisher's Exact test were used where appropriate. A multivariable logistic regression analyses was performed at 95% confidence to predict factors associated with syphilis antibody seropositivity. Automated variable elimination was employed for the generation of simpler models with a higher predictive power. Elimination of variables was performed to maximise both the model fit and the number of parameters using the Akaike information criterion (Akaike, 1974). Statistical analyses were performed in R Studio, version 4.2.1.

Ethics and Participant Consent

The UZBCS was carried out in compliance with the ethical principles of the Declaration of Helsinki, the International Council for Harmonisation of Good Clinical and Laboratory Practice (GCLP) guidelines, and local regulatory requirements. Ethical approval was obtained from the Joint Research Ethics Committee of the University of Zimbabwe's Faculty of Medicine and Health Sciences and Parirenyatwa Group of Hospitals, reference number JREC/81/15, and the Medical Research Council of Zimbabwe, Ethics Approval Reference

Number: MRCZ/A/1968. Literacy is almost universal in Zimbabwe and participants enrolled in this study were able to read and comprehend the informed consent form. All participants provided written informed consent for participation in the study.

Results

Overall Syphilis Antibody Seropositivity Rates in Pregnancy

One thousand two hundred and eight pregnant women with ≥20 weeks' gestation were enrolled between 26 January 2016 and 31 August 2019. The frequency of the presence of syphilis antibody regardless of being active or past infection(s) as determined by reactive NTT-RPR and/or TT-SD Bioline run in parallel, was 53/1208: 4.39%, 95% CI [3.2-5.6] (Figure 1).

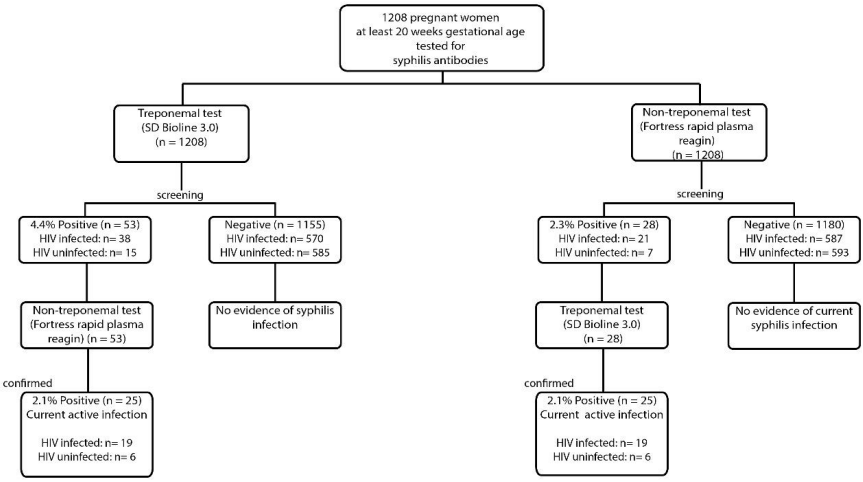


***Figure 1**:* Flow diagram showing the screening and confirmatory test kits and results

When stratified by study site, syphilis antibody seropositivity was not statistically different across all the four study sites. However, the prevalence was highest at Kuwadzana, at 30/596: **5%** 95% CI (3.3-6.7), followed by Rujeko, 13/284: **4.6%** 95% CI (2.2-7.0), and Budiriro, 4/100: **4.0%** 95% CI (3.9-4.1), with Glenview recording the least burden at 7/228: **3.1%** (0.9-5.3).

Age was not a significant factor in syphilis acquisition in this cohort. The detection rate for syphilis antibodies in women ≥20 years old was 52/1123: **4.63%** 95% CI [3.5-5.9]. None of the teen pregnant women (<20 years of age) presented with evidence of previous syphilis infection. Upon stratification by the adolescent girls and young women (AGYW) status (≤24 years) versus women >24 years, the rate of syphilis antibody positivity was comparable at 17/366: **4.64%** 95% CI [2.5-6.7] and 37/842: **4.39%** 95% CI [3.0-5.8], respectively (Table 1).

Risk Factors for Syphilis Antibody Positivity in Pregnancy

In a univariate analysis, we compared 53 women positive for syphilis antibody with their randomly selected syphilis antibody negative controls (N=216). Syphilis antibody positive women were more likely be HIV-infected; **p=0.02**. They also reported higher median numbers of life-time sex partners, median 2 (1-3) versus 1 (1-2) in the controls, **p=0.017** (Table 1). Furthermore, they were more likely to report younger ages of sexual début; median (IQR) 18 years (17-20), versus 19 years (18-21) reported by their seronegative counterparts, but with borderline significance at **p=0.057** (Table 1). Anthropometric indices assessing nutritional status in pregnancy differed between the syphilis antibody positive women versus their seronegative peers; MUAC was 26 cm (25-29) versus 28 cm (26-30.8), and BMI was 24.2 (22.6-27.1) versus 25.9 (23.6-29.7); p=**0.025** and **0.008**, respectively (Table 1). In addition, syphilis antibody positive women were more likely to be anaemic; median HGB (IQR) 10.6 (9.8-11.4) g/dL versus 11.2 (10.3-11.9) g/dL in the syphilis antibody negative peers, **p=0.013** (Table 1). Otherwise, there were no differences in the social demographic and lifestyle characteristics, including spouse/intimate partner factors between the two groups. It was concerning to note comparable rates of previously reported, but untreated STIs of up to 30% in the two groups.

Table 1: Socio-demographic, clinical data, and pregnancy/infant outcomes in pregnant women (N=265); stratified by syphilis antibody status: syphilis antibody positive (n=53) and syphilis antibody negative (n=212). Data is expressed as n (%) or median (IQR); min-max, unless stated otherwise.

Variables Median (IQR); min-max	**Syphilis antibody status in pregnancy: N=265**		
Maternal syphilis antibody status	**Syphilis antibody positive (n=53)**	**Syphilis antibody negative (n=212)**	**p-value**
HIV status, social demography and lifestyle			
Study site Budiriro Glenview Kuwadzana Rujeko	 4 (7.5%) 7 (13.2%) 29 (54.7%) 13 (24.5%)	 15 (7.1%) 49 (23.1%) 99 (46.7%) 49 (23.1%)	0.454
Maternal HIV Status Positive Negative	 38 (52.8%) 15 (47.2%)	 114 (53.8%) 98 (46.2%)	**0.020**
Maternal age (years) [Median (IQR); min-max]	29.0 (24.0-35.0); 19.0-43.0	29.0 (24.0-33.0); 19.0-43.0	0.687
Teenager No Yes	 52 (98.1%) 1 (1.9%)	 205 (96.7%) 7 (3.3%)	1

AGYW ≤ 24 years >24 years	16 (30.2%) 37 (69.8%)	57 (26.9%) 155 (73.1%)	0.611
Marital status Not married Married	1 (1.9%) 52 (98.1%)	11 (5.2%) 201 (94.8%)	0.470
Education status Primary school level At least some secondary school education	2 (3.8%) 51 (96.2%)	14 (6.6%) 198 (93.4%)	0.747
Employment status Employed Unemployed	17 (32.1%) 36 (67.9%)	62 (29.2%) 150 (70.8%)	0.738
Alcohol use in general (ever) Yes No	9 (17.0%) 44 (83.0%)	24 (11.3%) 188 (88.7%)	0.254
Alcohol use the index pregnancy Yes No	9 (17.0%) 44 (83.0%)	23 (10.8%) 189 (89.2%)	0.240
Average family income (US$) [Median (IQR); min-max]	193 (140-320); 0-1100	250 (150-350); 0-1190	0.109
STIs in pregnancy			
HBsAg Negative Positive	50 (94.3%) 3 (5.7%)	206 (97.2%) 6 (2.8%)	0.389
Pelvic inflammatory disease Yes No	7 (13.2%) 46 (86.8%)	12 (5.7%) 200 (94.3%)	0.216
Pain on urination Yes No	6 (11.3%) 47 (88.7%)	12 (5.7%) 200 (94.3%)	0.216
Genital warts Yes No	4 (7.5%) 49 (42.5%)	10 (4.7%) 201 (95.3%) (missing=1)	0.490
Self-reported previous STI treated (n=64) Yes No	12 (70.6%) 5 (29.4%)	31 (66.0%) 16 (34.0%)	1
Sexual behaviour			

Age at first sexual début [Median (IQR); min-max]	18.0 (17.0-20.0); 14.0-28.0	19.0 (18.0-21.0); 13.0-32.0	0.057
Total number of life sex partners [Median (IQR); min-max]	2.0 (1.0-3.0); 1.0-200.0	1.0 (1.0-2.0); 1.0-26.0	**0.017**
Presence of casual sex partners No Yes	 52 (98.1%) 1 (1.9%)	 210 (99.1%) 2 (0.9%)	0.490
Spouse/intimate partner factors			
Spouse age [Median (IQR); min-max]	34.5 (29.75-38.0); 19.0-48.0	34.0 (30.0-39.0); 20.0-62.0	0.822
Spouse tested for HIV Don't know No Yes	3 (5.7%) 11 (20.8%) 39 (73.5%)	17 (8.1%) 47 (22.2%) 147 (69.7%) (missing = 1)	0.903
Self-reported previous STI diagnosis spouse Don't know No Yes	1 (1.9%) 44 (83.0%) 8 (15.1%)	6 (2.8%) 186 (88.2%) 19 (9.0%) (missing=1)	0.404
Maternal clinical data			
MUAC (cm) [Median (IQR); min-max]	26.0 (25.0-29.0); 22.0-40.0	28.0 (26.0-30.75); 21.0-42.0	**0.025**
BMI [Median (IQR); min-max]	24.16 (22.55-27.07); 16.30-39.30	25.91 (23.62-29.74); 18.67-67.64	**0.008**
HGB (g/dL) [Median (IQR); min-max]	10.6 (9.83-11.40); 9.7-12.8	11.2 (10.3-11.9); 6.3-14.5	**0.013**

Abbreviations: AGYW: adolescent girls and young women, BMI: body mass index, HGB: haemoglobin, HBsAg: Hepatitis B surface antigen, MUAC: mid-upper arm circumference, IQR: interquartile range, STIs: sexually-transmitted infections, TB: tuberculosis, US$: United States dollar

Statistical analysis: Group comparisons using the Mann-Whitney U test, Kruskal Wallis test or Fisher's exact test where appropriate. Bold font p-values are significant (p< 0.05).

Burden of AS, Associated Risk Factors and Adverse Birth Outcomes

Of the 53 women testing positive for syphilis antibody, 25/1208: **2.07%** 95% CI [1.3-2.9] were AS cases (Figure 1). Rujeko study site recorded the highest burden of AS at 8/284: **2.82%** 95% CI [1.9-4.7], closely followed by Kuwadzana at 15/596: **2.52%** 95% CI [1.2-3.8], Budiriro at 1/100: **1%** 95% CI [-0.95-2.95], and

Glenview Polyclinic, once again, recorded the least burden at 1/228: **0.44%** 95% CI [-0.4-1.2] (data not shown). The prevalence of AS in women ≥20 years old was **2.14%** 95% CI [1.31-3.12] versus **1.18%** 95% CI [1.10-3.51] recorded in teen pregnant women (data not shown). Burdens of AS were comparable in AGYW ≤24 years at 7/366: **2.19%** 95% CI [0.7-3.7] and 18/842: **2.14%** 95% CI [1.1-3.1] in women >24 years old (not shown).

In a univariate analysis, we compared 25 women presenting with AS against their 100 randomly selected syphilis antibody negative peers. Women with AS were more likely to be HIV-infected, 19/26 (78%) versus 6/25 (24%) in controls, **p=0.024** (Table 2). The same trend was observed in the burden of HIV in the women's spouses/intimate partners, **p= 0.018** (Table 2). Women presenting with AS reported significantly higher median numbers of lifetime sexual partners; 2 (1-3) versus 1 (1-2) reported by their syphilis antibody negative peers; **p=0.022** (Table 2). In addition, AS cases reported higher frequencies of pelvic inflammatory diseases at 16% against 2% in their negative peers, **p=0.015** (Table 2). Otherwise, other demographics such as education level, marital status, employment status and lifestyle characteristics, including alcohol usage and frequencies of women experiencing any forms of domestic violence, were similar when comparing the two groups (Table 2). Up to 20% of women in both groups reported experiencing some form of domestic violence in the preceding six months.

Women presenting with AS were more likely to be anaemic (HBG <11g/dL); 78% versus 40.9% in the syphilis antibody negative controls, median; 10.1 (9.7-10.8) versus 11.2 (10.3-11.8) g/dL, respectively; **p=0.003** (Table 2).

Table 2: Socio-demographic, clinical data, and pregnancy/infant outcomes in pregnant women (N=125); stratified by syphilis status at enrolment: active syphilis infection (n=25) and syphilis uninfected (n=100). Data is expressed as n (%) or median (IQR); min-max unless stated otherwise

	Syphilis infection status in pregnancy; N=125		
Maternal syphilis infection status	**Active syphilis infection (n=25)**	**Syphilis uninfected (n=100)**	**p-value**
HIV status, social demography and lifestyle			
Study site			
Budiriro	1 (4%)	6 (6%)	
Glenview	1 (4%)	23 (23%)	0.127
Kuwadzana	15 (60%)	45 (45%)	
Rujeko	8 (32%)	26 (26%)	
Maternal HIV status	19 (76%)	49 (49%)	**0.024**
Positive	6 (24%)	51 (51%)	
Negative			

Maternal age (years) [Median (IQR); min-max]	29 (24-33); 19- 38	29 (26-33); 19-40	0.786
Teenager No Yes	 24 (96%) 1 (4%)	 98 (98%) 2 (2%)	0.491
AGYW ≤ 24 years >24 years	 7 (28%) 18 (72%)	 20 (20%) 80 (80%)	0.419
Marital status Not married Married	 1 (4%) 24 (96%)	 3 (3%) 97 (97%)	1.000
Education status Primary At least some secondary	 1 (4%) 24 (96%)	 6 (6%) 94 (94%)	1.000
Experienced any domestic violence No Yes	 20 (80%) 5 (20%)	 80 (80%) 20 (20%)	1.000
Employment status Employed Unemployed	 8 (32%) 17 (68%)	 30 (30%) 70 (70%)	0.813
Alcohol use in general (ever) Yes No	 3 (12%) 22 (88%)	 10 (10%) 90 (90%)	0.723
Average family income (US$) [Median (IQR); min-max]	173 (140-250); 20-600	222.5 (150-300); 0-900	0.100
STIs and other related factors in pregnancy			
HBsAg Negative Positive	 24 (96%) 1 (4%)	 96 (96%) 4 (4%)	1.000
Pelvic inflammatory disease Yes No	 4 (16%) 21 (84%)	 2 (2%) 98 (98%)	**0.015**
Self-reported presence of abnormal vaginal discharges Yes No	 3 (12%) 22 (88%)	 10 (10%) 90 (90%)	0.723

Self-reported previous STI diagnosis Yes No	5 (20%) 20 (80%)	24 (24%) 76 (76%)	0.795
Self-reported previous STI treated (n=29) Yes No	4 (80%) 1 (20%)	15 (62.5%) 9 (37.5%)	0.633
Sexual behaviour			
Age at first sexual debut (years) [Median (IQR); min-max]	18 (17-20); 14-24	19 (18-21); 14-28	0.191
Total life sex partners [Median (IQR); min-max]	2 (1-3); 1-15	1 (1-2); 1-6	**0.022**
Did you use condoms during your last sexual intercourse? No Yes	15 (68.2%) 7 (31.8%) (missing =3)	73 (73%) 27 (27%)	0.793
Presence of casual sex partners No Yes	24 (96%) 1 (4%)	98 (98%) 2 (2%)	0.491
Spouse/ intimate partner factors			
Spouse age (years) [Median (IQR); min-max]	35.5 (30-38); 24-48	34 (30-39); 22-62	0.790
Spouse HIV test result (n=94) Don't know Negative Positive	2 (10.5%) 5 (26.3%) 12 (63.2%) (missing = 1)	3 (4.2%) 43 (60.6%) 25 (35.2%) (missing= 3)	**0.018**
Maternal clinical data			
MUAC(cm) [Median (IQR); min-max]	26 (25-28); 22-40	27 (26-30.3); 22-40.5	0.070
BMI [Median (IQR); min-max]	24.7 (23.7-26.6); 18.5-37.4	25.3 (23.7-28.6); 19.6-67.6	0.303
HGB (g/dL) [Median (IQR); min-max]	10.1 (9.7-10.8); 6.7-12.8	11.2 (10.3-11.8); 7.9-13.3	**0.003**

Pregnancy outcomes			
Delivery <37 weeks' GA Yes No	4 (16.7%) 20 (83.3%)	10 (10%) 90 (90%)	0.470
Head circumference (cm) [Median (IQR); min-max]	35 (33-35); 31-36	35 (34-36); 31-45	0.159
Birth weight (g) [Median (IQR); min-max]	3000 (2700-3192); 2400-3440	3200 (2950-3400); 2100-4200	**0.014**
Birth length (cm) [Median (IQR); min-max]	49 (48-50); 45-51	50 (49-51); 40-55	**0.011**
Birth status Live birth Still birth	24 (96%) 1 (4%)	100 (100%) 0	0.200

Abbreviations: AGYW: adolescent girls and young women, BMI: body mass index, HGB: haemoglobin, HBsAg: hepatitis B surface antigen, MUAC: mid-upper arm circumference, IQR: interquartile range, STIs: sexually-transmitted infections, US$: United States dollar.

Statistical analysis: group comparisons using the Mann-Whitney U test, Kruskal Wallis test or Fisher's exact test where appropriate. Bold font p-values are significant (p< 0.05).

Adverse Birth Outcome

In this study, frequencies of premature deliveries, stillbirths and median head circumference were similar among infants born of women presenting with AS and the unexposed infants (Table 2). None of the infants were HIV-infected at birth**.** However, maternally AS exposed infants had significantly lower birth weights and shorter birth lengths; being 3 000 grams (2 700-3 192) versus 3 200 grams (2 950-3 400), and 49 cm (48-50) versus 50 cm (49-51) versus the unexposed infants, **p=0.014** and **p=0.011**, respectively (Table 2).

In a univariate analysis we compared the 25 women presenting with AS with those with evidence prior to syphilis infection **(n=20)** at enrolment (Supplementary Table 1). No differences were observed in the socio-demographic and lifestyle characteristics, frequencies of the presence of STIs, concurrent medications and adverse pregnancy/infant outcomes in women presenting with AS compared to their peers with past history of syphilis infection. However, women with AS were more likely to be anaemic, with median HBG levels of 10.1 g/dL (9.7-10.8) versus 11.3 g/dL (10.3-11.6), respectively; **p=0.031** (Supplementary Table 1).

Supplementary Table 1: Socio-demographic, clinical data, and pregnancy/infant outcomes in pregnant women (N=125); stratified by syphilis status at enrolment: active syphilis infection (n=25) and confirmed prior syphilis infection (n=20). Data is expressed as n (%) or median (IQR); min-max unless stated otherwise.

Variables Median (1st Quartile-3rd Quartile); min-max	**Presence of Syphilis Antibodies in Pregnancy; N= 45**		
Maternal syphilis infection status	**Active syphilis infection (n=25)**	**Confirmed prior syphilis infection (n=20)**	**p-value**
HIV status, social demography and lifestyle			
Study site Budiriro Glenview Kuwadzana Rujeko	 1 (4%) 1 (4%) 15 (60%) 8 (32%)	 2 (10%) 4 (20%) 10 (50%) 4 (20%)	 0.293
Maternal HIV status Positive Negative	 19 (76%) 6 (24%)	 16 (80%) 4 (20%)	 1.000
Maternal age (years) [Median (IQR); min-max]	29 (24-33); 19- 38	31 (24-36); 20-43	0.410
Teenager No Yes	 24 (96%) 1 (4%)	 20 (100 %) 0 (0%)	 1.000
AGYW ≤ 24 years >24 years	 7 (28%) 18 (72%)	 6 (30%) 14 (70%)	 1.000
Marital status Not married Married	 1 (4%) 24 (96%)	 0 (0%) 20 (100 %)	 1.000
Education status Primary At least some secondary	 1 (4%) 24 (96%)	 1 (5%) 19 (95%)	 1.000
Experienced any domestic violence No Yes	 20 (80%) 5 (20%)	 16 (80%) 4 (20%)	 1.000
Employment status Employed Unemployed	 8 (32%) 17 (68%)	 5 (25%) 15 (75%)	 0.745
Alcohol use in general (ever) Yes No	 3 (12%) 22 (88%)	 6 (30%) 14 (70%)	 0.157

Alcohol use the index pregnancy Yes No	3 (12%) 22 (88%)	6 (30%) 14 (70%)	0.157
Average family income (US$) [Median (IQR); min-max]	173 (140-250); 20-600	214 (149-315); 0-700	0.278
STIs and other infections in pregnancy			
HBsAg Negative Positive	24 (96%) 1 (4%)	19 (95%) 1 (5%)	1.000
Pelvic inflammatory disease Yes No	4 (16%) 21 (84%)	2 (10%) 18 (90%)	0.678
Self-reported presence of abnormal vaginal discharges Yes No	3 (12%) 22 (88%)	6 (30%) 14 (70%)	0.157
Self-reported previous STI diagnosis Yes No	5 (20%) 20 (80%)	10 (50%) 10 (50%)	0.056
Self-reported previous STI treated (n=17) Yes No	4 (80%) 1 (20%)	7 (70%) 3 (30%)	1.000
Sexual behaviour			
Age at first sexual debut (years) [Median (IQR); min-max]	18 (17-20); 14-24	18 (17-20); 14-26	0.746
Total number of life sex partners [Median (IQR); min-max]	2 (1-3); 1-15	2 (1-2); 1-200	0.630
Presence of casual sex partners No Yes	24 (96%) 1 (4%)	20 (100%) 0 (0%)	1.000
Spouse/ intimate partner factors			
Spouse age (years) [Median (IQR); min-max]	35.5 (30-38); 24-48	33 (28-38); 25-48	0.759

Spouse tested for HIV Don't know No Yes	2 (8%) 5 (20%) 18 (72%)	1 (5%) 6 (30%) 13 (65%)	0.790
Maternal clinical data			
Mid upper arm circumference (cm) [Median (IQR); min-max]	26 (25-28); 22-40	27 (24-29); 22-35	0.714
BMI [Median (IQR); min-max]	24.7 (23.7-26.6); 18.5-37.4	24 (22-27.8); 16.3-39.3	0.925
HGB (g/dL) [Median (IQR); min-max]	10.1 (9.7-10.8); 6.7-12.8	11 (10.3-11.6); 9-12.6	**0.031**
Reported current /previous TB treatment No Yes	24 (96%) 1 (4%)	19 (95%) 1 (5%)	1.000

Abbreviations: AGYW: adolescent girls and young women, BMI: body mass index, HGB: haemoglobin, HBsAg: Hepatitis B surface antigen, MUAC: mid-upper arm circumference, IQR: interquartile range, STIs: sexually-transmitted infections, TB: tuberculosis, US$: United States dollar, WBC: White blood cell.

Statistical analysis: Group comparisons using the Mann-Whitney U test, Kruskal Wallis test or Fisher's exact test where appropriate. Bold font p-values are significant (p< 0.05).

Presence of Syphilis by HIV Status

Of the 1 208 pregnant women recruited, 600 were HIV-uninfected. Six hundred and eight were HIV-infected and mainly on Tenofovir, Lamivudine and Efavirenz-regimen with a median ART use duration of 350 days (87-1477). The overall presence of syphilis antibodies, regardless of whether AS or past infection in the HIV-uninfected controls, was 15/600: **2.50%** 95% (CI) [1.3-3.7], and out of these 6/600: **1.0%** 95% CI [0.2-1.8] presented with AS infections.

The presence of syphilis antibodies was ~2.5 times higher in the HIV-infected group at 38/608: **6.25%** 95% (CI) [4.4-8.2] compared to the HIV-uninfected group. AS cases were also equally higher within the HIV-infected at 19/608: **3.13%** 95% (CI) [1.7-4.5].

Antenatal Syphilis Antibody Seropositivity by HIV Status

In a univariate analysis we compared syphilis antibody positive pregnant women (n=53) with randomly selected syphilis antibody negative counterparts (n=212). These two groups were further stratified by maternal HIV status at enrolment, resulting in the following four subgroups' comparisons:

1. Within the *syphilis antibody positive group* (n=53); the syphilis antibody positive-HIV-infected (n=38)-(co-infected) versus the syphilis antibody positive-HIV-uninfected- (syphilis mono-infected) (n=15), p-value=γ (Table 3).
2. Within the *syphilis antibody negative group* (n=212); the syphilis antibody negative-HIV-infected (n=114) versus the syphilis antibody negative-HIV-uninfected- (double negatives) (n=98), p-value=δ (Table 3).
3. Within the *HIV-infected group* (n=152); the HIV-infected-syphilis antibody positive (n=38) (co-infected) versus HIV-infected-syphilis antibody negative (HIV mono-infected) (n=114), p-value=α (Table 3) and,
4. Within the *HIV-uninfected group* (n=113); the HIV-uninfected-syphilis antibody positive- (syphilis antibody mono-infected) (n=15) versus HIV-uninfected-syphilis antibody negative- (double negatives) (n=98), p-value=β (Table 3).

Syphilis/HIV-co-infected Women Versus Syphilis Seronegative but HIV-infected Peers

In a univariate analysis of the HIV-infected group, we compared 38 pregnant women who were both syphilis antibody positive and HIV-infected, versus their 114 also HIV-infected, but syphilis antibody seronegative peers, **p=α** (Table 3). The co-infected pregnant women had significantly lower median (IQR) average family income at US$177 (128-299) versus US$250 (150-369), **p=0.01.** Actually, they earned the least compared to the rest of the other three groups (Table 3).

Furthermore, they were more likely to report higher frequencies of pelvic inflammatory diseases**, p=0.006**. Median age at sexual debut was significantly younger in the co-infected women, 18 years (17-20) versus 19 years (18-20) in their HIV-infected peers without syphilis, **p=0.041** (Table 3). Otherwise, there were no statistical significant differences in the demographic characteristics, alcohol usage and spouse/intimate partner related factors, frequencies of reported current/previous STI diagnoses or treatments (Table 3). No significant differences were observed with respect to the median numbers of lifetime sexual partners, or frequencies of condom use during previous sexual encounters. MUAC, BMI and HGB levels and rates of self-reported depression were all comparable between the two groups (Table 3). Interestingly, the HIV-infected-syphilis antibody seropositive women had been diagnosed with HIV for significantly longer durations. HIV diagnosis was 790 days (87-1365) versus 601 days (55-1402) in the HIV-infected-syphilis antibodies negatives, **p<0.001**. Interestingly, HIV/syphilis co-infected women reported significantly longer durations of ART exposure; median 422 days (71-1252) against 313 days (47-1269), in the HIV-mono-infected, **p<0.001** (Table 3).

Syphilis Seropositivity in the HIV-uninfected Women

In a univariate analysis within the HIV-uninfected group, we compared 15 pregnant women who were HIV-uninfected, but syphilis antibody positive, versus their 98 peers who were both HIV-uninfected and syphilis antibody seronegative, p=β; (Table 3). Socio-demographic characteristics, alcohol use, frequencies of

the presence of other STIs, and risky sexual behaviour including spouse related factors, were comparable between the two groups. However, syphilis antibody positive-HIV-uninfected women had significantly lower nutritional indices, MUAC was at 25 cm (24.5-27.5) compared to 28 cm (26-31) in the controls — both HIV-uninfected and syphilis-seronegative, **p=0.045**. A similar trend was observed with the BMI at 22.4 (21.1-36.4) versus 26.1 (22.9-30.1), **p=0.013**. Due to the small numbers of syphilis cases in the HIV-uninfected group, no further analyses were done.

Syphilis Antibody Seronegativity by HIV Status

In a univariate analysis within the syphilis seronegative group, we compared the HIV-infected women (n=114) with their HIV-uninfected peers (n=98), p=δ in Table 3. Both syphilis antibody negative and HIV-uninfected women were more likely to be younger, with a median age of 27 years (23-32) compared to their syphilis seronegative, but HIV-infected peers whose median age was at 32 years (27-34), **p<0.001** (Table 3). The same trend was also observed following stratification by AGYW status. HIV-uninfected AGYW were more likely to be syphilis antibody negative at 36.7%, versus 18.4% in their HIV-infected peers also without syphilis, **p=0.003** (Table 3).

Women who were both syphilis antibody negative and HIV-uninfected were more likely to have completed at least some secondary school education at 96.9%, versus 89.5% in their syphilis antibody negative, but HIV-infected counterparts, **p=0.013** (Table 3). However, other social demographic characteristics such as marital status, employment status, median family monthly income, alcohol use, the presence of HBsAg, including self-reported presence of STIs, were comparable between these two groups of women all without any syphilis antibodies detected, but differing by HIV status (Table 3). In addition, the median ages at sexual debut were comparable between the two groups, both without syphilis. However, the median spouse/intimate partner age was significantly older for the HIV-infected women without syphilis, at 36 years (31.5-40.5) relative to 33 years (28-36.5) for the spouses/intimate partners of the HIV-uninfected also without syphilis, **p<0.001.** Furthermore, the HIV-infected and syphilis negatives reported higher median numbers of lifetime sexual partners at 2 (1-3) versus 1 (1-1) in the HIV-uninfected women also without syphilis, **p<0.001.** Frequency of condom use during the previous sexual encounter was much lower in the HIV-uninfected without syphilis group at 5.1%, compared to 51.8% in their HIV-infected peers also without syphilis, **p<0.001**. Of concern was 2.8% of the HIV-uninfected women without syphilis reporting that their spouses were HIV-infected versus 63.9% of their HIV-infected counterparts reporting that their spouses were also HIV-infected, **p<0.001**. Worryingly was 5% of the women in both groups with no knowledge of their spouses'/intimate partners' HIV status.

In a univariate analysis of the syphilis antibody positive group, we compared the HIV-infected (n=38) versus their HIV-uninfected peers (N=15), p=γ in Table 3. Social demographic factors were comparable between the two groups except for

the median monthly family income earned. There were no significant differences in the presence of STIs or HBsAg in pregnancy in the two groups (Table 3).

***Table 3**:* Socio-demographic, sexually-transmitted infections, clinical data, concurrent medications, HIV and ART related factors and pregnancy/infant outcomes of pregnant women (**N=265)**; stratified first by maternal syphilis antibody status at enrolment, and further by HIV status into four groups — syphilis antibody positive-HIV-infected (**n=38**), syphilis positive-HIV-uninfected (**n=15**), syphilis antibody negative-HIV-infected (**n=114**), and syphilis negative-HIV-uninfected **(n=98**). Data is expressed as n (%) or median (IQR); min-max, unless stated otherwise.

Variables Median (1st Quartile-3rd Quartile); Min-max	**Syphilis antibody and HIV status in pregnancy; N= 265**				
Syphilis and HIV status	**Syphilis antibody positive (n=53)**		**Syphilis antibody negative (n=212)**		
	HIV-infected (n=38)	**HIV-uninfected (n=15)**	**HIV infected (n=114)**	**HIV-uninfected (n=98)**	**p-value**
HIV status, social demography and lifestyle					
Study site Budiriro Glenview Kuwadzana Rujeko	 4 (10.5%) 4 (10.5%) 19 (50%) 11 (28.9%)	 0 3 (20.0%) 10 (66.7%) 2 (13.3%)	 9 (7.9%) 33 (28.9%) 48 (42.1%) 24 (21.1%)	 6 (6.1%) 16 (16.3%) 51 (52%) 25 (25.5%)	 ᵞp= 0.309 ᵟp= 0.141 ᵅp= 0.115 ᵝp= 0.645
Maternal age (years) [Median (IQR); min-max]	31.00 (26.00-34.75); 20-43	25.0 (21.5-35.0); 19.0-38.0	32.0 (27.0-34.0); 19.0-43.0	27.0 (23.0-32.0); 19.0-38.0	ᵞp= 0.228 ᵟp= **<0.001** ᵅp= 0.830 ᵝp= 0.916
Teenager No Yes	 0 38 (100%)	 1 (6.7%) 14 (93.3%)	 1 (0.9%) 113 (99.1%)	 6 (6.1%) 92 (93.9%)	 ᵞp= 0.283 ᵟp= 0.051 ᵅp= 1 ᵝp= 1
AGYW ≤ 24 years >24 years	 9 (23.7%) 29 (76.3%)	 7 (46.7%) 8 (53.3%)	 21 (18.4%) 93 (81.6%)	 36 (36.7%) 62 (63.3%)	 ᵞp= 0.182 ᵟp= **0.003** ᵅp= 0.487 ᵝp=.0.57
Marital status Not married Married	 1 (2.6%) 37 (97.4%)	 0 15 (100%)	 7 (6.1%) 107 (93.9%)	 4 (4.1%) 94 (95.9%)	 ᵞp= 1 ᵟp= 0.551 ᵅp= 0.68 ᵝp= 1
Education status Primary At least some secondary	 2 (5.3%) 36 (94.7%)	 0 15 (100%)	 12 (10.5%) 102 (89.5%)	 3 (3.1%) 95 (96.9%)	 ᵞp= 1 ᵟp= **0.013** ᵅp= 0.519 ᵝp=.1
Employment status Employed Unemployed	 10 (26.3%) 28 (73.7%)	 7 (46.7%) 8 (53.3%)	 36 (31.6%) 78 (68.4%)	 26 (26.5%) 72 (73.5%)	 ᵞp= 0.197 ᵟp= 0.452 ᵅp= 0.684 ᵝp= 0.132
Alcohol use in general (ever) Yes No	 8 (21.1%) 30 (78.9%)	 1 (6.7%) 14 (93.3%)	 13 (11.4%) 101 (88.6%)	 11 (11.2%) 87 (88.8%)	 ᵞp= 0.418 ᵟp= 1 ᵅp= 0.174 ᵝp= 1

Alcohol use the index pregnancy Yes No	8 (21.1%) 30 (78.9%)	1 (6.7%) 14 (93.3%)	12 (10.5%) 102 (89.5%)	11 (11.2%) 87 (88.8%)	ᵞp= 0.418 ᵟp= 1 ᵅp= 0.104 ᵝp= 1
Average family income (US$) [Median (IQR); min-max]	177.0 (128.2-298.8); 0-700	250.0 (174.0-442.5); 80.0-1100.0	250.0 (150.0-368.8); 30.0-1190.0)	250.0 (148.8-348.5); 0-750.0	ᵞp= **0.027** ᵟp= 0.489 ᵅp= 0.010 ᵝp= 0.332
STIs in pregnancy					
H**BsAg** Negative Positive	36 (94.7%) 2 (5.3%)	14 (93.3%) 1 (6.7%)	110 (96.5%) 4 (3.5%)	96 (98%) 2 (2%)	ᵞp= 1 ᵟp= 0.689 ᵅp= 0.640 ᵝp= 0.350
Pelvic inflammatory disease Yes No	7 (18.4%) 31 (81.6%)	0 15 (100%)	4 (3.5%) 110 (96.5%)	3 (3.1%) 95 (96.9%)	ᵞp= 0.172 ᵟp= 1 ᵅp= **0.006** ᵝp= 1
Reported current/ previous STI diagnosis Yes No	13 (34.2%) 25 (65.8%)	4 (26.7%) 11 (73.3%)	26 (22.8%) 88 (77.2%)	21 (21.4%) 77 (78.6%)	ᵞp= 0.748 ᵟp= 0.869 ᵅp= 0.199 ᵝp= 0.739
Self-reported previous STI treated (n=64) Yes No	11 (84.6%) 2 (15.4%)	1 (25.0%) 3 (75.0%)	17 (65.4%) 9 (34.6%)	14 (66.7%) 7 (33.3%)	ᵞp= 0.053 ᵟp= 1 ᵅp= 0.276 ᵝp= 0.267
Sexual behaviour					
Age at first sexual debut [Median (IQR); min-max]	18.0 (17.00-20.00); 14-26	20.0 (18.0-21.5); 17.0-28.0	19.0 (18.0-20.0); 13.0-32.0	19.0 (18.0-21.0); 14.0-28.0	ᵞp= **0.033** ᵟp= 0.116 ᵅp= **0.041** ᵝp= 0.762
Number of life sex partners [Median (IQR); min-max]	2.00 (1.00-3.00); 1-200	1.0 (1.0-2.0); 1.0-2.0	2.0 (1.0-3.0); 1.0-26.0	1.0 (1.0-1.0); 1.0-5.0	ᵞp= **0.008** ᵟp= **<0.001** ᵅp= 0.150 ᵝp= 0.442
Did you use condoms during your last sexual intercourse? No Yes	21 (58.3%) 15 (41.7%) (missing=2)	13 (92.9%) 1 (7.1%) (missing=1)	54 (48.2%) 58 (51.8%) (missing=2)	93 (94.9%) 5 (5.1%)	ᵞp= **0.019** ᵟp= **<0.001** ᵅp= 0.439 ᵝp= 0.564
Spouse factors					
Spouse age (years) [Median (IQR); min-max]	35.00 (30.00-41.00); 26-48	30.0 (25.0-37.5); 19.0-40.0	36.0 (31.5-40.5); 22.0-62.0	33.0 (28.0-36.5); 20.0-50.0	ᵞp= **0.022** ᵟp= **<0.001** ᵅp= 0.673 ᵝp= 0.344
Living with spouse under the same roof No Yes	2 (5.3%) 36 (94.7%)	0 15 (100%)	7 (6.1%) 107 (93.9%)	4 (4.1%) 94 (95.9%)	ᵞp= 1 ᵟp= 0.551 ᵅp= 1 ᵝp= 1

Spouse tested for HIV Don't know No Yes	2 (5.3%) 10 (26.3%) 26 (68.4%)	1 (6.7%) 1 (6.7%) 13 (86.6%)	13 (11.4%) 24 (21.1%) 77 (67.5%)	4 (4.1%) 23 (23.7%) 70 (72.2%) (missing =1)	ᵞp= 0.212 ᵟp= 0.155 ᵅp= 0.536 ᵝp= 0.291
Spouse HIV test result (n=206) Negative Positive Don't know	6 (23.1%) 19 (73.1%) 1 (3.8%) (missing=2)	12 (85.7%) 1 (7.1%) 1 (7.1%)	25 (30.1%) 53 (63.9%) 5 (6%) (missing=7)	67 (93%) 2 (2.8%) 3 (4.2%) (missing=2)	ᵞp= **<0.001** ᵟp= **<0.001** ᵅp= 0.798 ᵝp= 0.319
Clinical data and lifestyle					
Mid upper arm circumference (cm) [Median (IQR); min-max]	27.00 (25.00-29.00); 22-40	25.0 (24.5-27.5); 23.0-34.0	28.0 (25.25-30.38); 21.0-40.0	28.0 (26.0-31.0); 21.0-42.0	ᵞp= 0.311 ᵟp= 0.986 ᵅp= 0.162 ᵝp= 0.045
BMI [Median (IQR); min-max]	24.24 (23.44); 18.5-39.3	22.41 (21.09-36.43); 16.30-33.30	25.89 (23.95-28.90); 18.67-67.64	26.07 (22.89-30.11); 19.31-39.25	ᵞp= 0.193 ᵟp= 0.863 ᵅp= 0.087 ᵝp= **0.013**
HGB (g/dL) [Median (IQR); min-max]	10.3 (9.8-11.4); 6.7-12.8	11.2 (40.6-11.4); 9.5-11.9	11 (10.2-11.7); 6.3-13.5	11.4 (10.3-12.1); 7.5-14.5	ᵞp= 0.261 ᵟp= **0.024** ᵅp= 0.073 ᵝp= 0.145
WBC count categorised < 4 × 10^9/L ≥ 4 x 10^9/L	4 (%) 32 (%) (missing=2)	0 14 (100%) (missing=1)	2 (2.1%) 95 (97.9%) (missing=17)	4 (4.4%) 86 (95.6%) (missing=8)	ᵞp= 0.566 ᵟp= 0.433 ᵅp= **0.046** ᵝp= 1
HIV related factors					
CD4⁺ T-lymphocyte count (cells/µL) [Median (IQR); min-max]	313 (251.8-467.8); 104-708	-	424.0 (256.5-532.0); 30.0-937.0	-	ᵅp= 0.182
Viral load (copies/mL) [Median (IQR); min-max]	42 (0-2050); 0-226116	-	0 (0-202.0); 0-200141	-	ᵅp= 0.107
Duration of ART use (days) [Median (IQR); min-max]	421.5 (71-1251.2); 0-3271	-	313.0 (46.5-1266.8); 0-4364)	-	ᵅp= **<0.001**
Duration of ART use ≤ 365 days > 365 days	18 (47.4%) 20 (52.6%)	-	59 (51.8%) 55 (48.2%)	-	ᵅp=0.709
Days since HIV diagnosis [Median (IQR); min-max]	789.5 (86.8-1364.5); 0-3332	-	600.5 (54.5-1401.8); 0-4364.0	-	ᵅp= **<0.001**

Days since HIV diagnosis ≤ 365 days > 365 days	14 (36.8%) 24 (63.2%)	-	50 (43.9%) 64 (56.1%)	-	$^{\alpha}$p=0.570

***Abbreviations**:* AGYW: adolescent girls and young women, ART: antiretroviral therapy, BMI: body mass index, CD: cluster of differentiation, HIV: human immunodeficiency virus, HGB: haemoglobin, IQR: interquartile range, MUAC: mid-upper arm circumference, STIs: sexually-transmitted infections, US$: United States dollar, WBC: white blood cell.

Statistical analysis: Group comparisons using the Mann-Whitney U test, Kruskal Wallis test or Fisher's exact test where appropriate. Bold font p-values are significant (p< 0.05).

p-values key:

$^{\gamma}$p= syp pos: hiv neg vs hiv pos
$^{\delta}$p=syp neg: hiv neg vs hiv pos
$^{\alpha}$p=hiv pos: syp pos vs syp neg
$^{\beta}$p=hiv neg: syp pos vs syp neg

Age at first sexual debut was significantly younger at median 18 years (17-20) in the syphilis antibody positives and HIV-infected women, versus 20 years (18-21.5) in their syphilis antibody positive and HIV-uninfected peers**, p=0.033**. Furthermore, they had significantly higher median numbers of lifetime sexual partners, 2 (1-3) compared to 1 (1-2) in their syphilis antibody positive HIV-uninfected peers, **p=0.008** (Table 3). The frequency of condom use at the previous sexual encounter was 41.7% in the syphilis antibody positives and HIV-infected women, versus 7.1% in those testing positive for syphilis antibody, but without HIV, **p=0.019.** Furthermore, their spouses/intimate partners were significantly older at 35 years (30-41) versus 30 years (25-37.5) for their syphilis antibody positives, but HIV-uninfected peers' partners, **p=0.022**. Notably, 3.8% of the syphilis antibody positives-HIV-infected women reported that they had no knowledge of their spouses'/intimate partners' HIV status compared to 7.1% in their syphilis antibody positives, but HIV-uninfected peers, **p<0.001** (Table 3).

Predictors of AS Late in Pregnancy in General

A multivariate logistic regression model was computed with and without variable elimination, and significant associations were observed in both models. In the regression model with variable elimination, absence of anaemia was protective, odds ratio (CI):4.33 (1.4-16.6), **p=0.017**. The presence of pelvic inflammatory diseases was also a significant factor, odds ratio (OR) of 6.2 (1.2-31.3), and so was HIV infection, OR of 4.1 (1.1-20), but with borderline significance (Table 4). Thus, women who reported having pelvic inflammatory diseases were 6.2 times more likely to have AS, relative to their counterparts without pelvic inflammatory diseases. HIV-infected women were 4.1 times more likely to have AS compared to their HIV-uninfected peers.

Table 4: Predictors of active syphilis infection in pregnant women without variable elimination (203 observations) and with variable elimination (204 observations)

	Predictors of active syphilis infection among pregnant women					
	Without variable elimination			**With variable elimination**		
	OR	**CI**	**p-value**	**OR**	**CI**	**p-value**
Intercept	0.103	0.018-0.416	0.003	0.056	0.013- 0.166	<0.001
Maternal HIV status (Positive)	4.012	1.008-20.428	0.062	4.078	1.073- 19.963	0.052
Total number of life sexual partners	1.125	0.834-1.423	0.378	1.060	0.835- 1.252	0.542
Anaemic (No)	0.253	0.066-0.789	0.027	0.231	0.060- 0.706	0.017
Use condom during last sexual intercourse (Yes)	0.853	0.260-2.725	0.788	0.764	0.239- 2.360	0.640
Pelvic inflammatory disease (Yes)	8.241	1.209-63.648	0.032	6.237	1.199- 31.322	0.024
AGYW (>24years)	0.967	0.282-3.905	0.959	-	-	-
Average family monthly income (US$)	0.998	0.994-1.000	0.163	-	-	-
Reported STI (Yes)	0.407	0.060-1.701	0.274	-	-	-

Abbreviations: AGYW: adolescent girls and young women, ART: antiretroviral therapy, CI: 95% confidence interval, HIV: human immunodeficiency virus, OR: odds ratio, STI: sexually-transmitted infections

Statistical analysis: Logistic regression with and without variable elimination

Predictors of AS Late in Pregnancy in the HIV-infected Women

In another multivariate logistic regression model, with and without variable elimination, significant associations were observed for the presence of anaemia, thus predicting AS in both models. In the without variable elimination model, the absence of anaemia was protective, **p=0.019**, and to some extent higher family income was protective, but with borderline significance, **p=0.078** (Table 5). For the regression model with variable elimination, the absence of anaemia remained protective, but with an even higher OR (CI): 6.2 (1.5-42.6), p=**0.027** (Table 5).

Table 5: Predictors of active syphilis infection among HIV-infected pregnant women of at least 20 weeks of gestational age without variable elimination (104 observations), and with variable elimination (104 observations)

	Predictors of active syphilis infection in HIV infected pregnant women					
	Without variable elimination			**With variable elimination**		
	OR	**CI**	**p-value**	**OR**	**CI**	**p-value**
Intercept	0.472	0.049- 3.767	0.485	0.870	0.211-3.754	0.847
Average family monthly income (US$)	0.995	0.990- 1.000	0.078	0.996	0.991-1.000	0.119
Anaemic (No)	0.104	0.011- 0.555	0.019	0.162	0.023-0.681	0.027
Use condom during last sexual intercourse (Yes)	1.189	0.297- 4.963	0.806	0.959	0.281-3.291	0.946
CD4+ T-lymphocyte count (cells/µL) (≥350)	0.840	0.219- 3.199	0.796	0.671	0.189-2.329	0.528
AGYW (>24years)	0.733	0.166- 3.784	0.690	-	-	-
Duration of ART use (≥ 126 days)	4.562	0.435- 47.762	0.191	-	-	-
Total number of life sexual partners	1.270	0.750- 1.767	0.185	-	-	-
Duration since HIV diagnosis (>365 days)	0.206	0.020- 2.146	0.169	-	-	-
Reported STI (Yes)	1.301	0.229- 6.167	0.747	-	-	-
Viral load (copies/mL) (>1000)	2.357	0.525- 10.532	0.253	-	-	-

Abbreviations: AGYW: adolescent girls and young women, ART: antiretroviral therapy, CD: cluster of differentiation, CI: 95% confidence interval, HIV: human immunodeficiency virus, OR: odds ratio, STI: sexually-transmitted infections

Statistical analysis: Logistic regression with and without variable elimination

Discussion

Summary of Main Findings

The study reports the results on the screening of syphilis antibodies late in pregnancy in a high HIV prevalence setting using the TT - SD Bioline Syphilis 3.0, and a NTT - RPR. The confirmation of the results was done using the RA and TA approach in 608 HIV-infected asymptomatic pregnant women mainly on Tenofovir, Lamivudine and Efavirenz regimen, with their 600 HIV-uninfected peers in the UZBCS. This is important since syphilis and HIV congenital infections

are being targeted for elimination through the SGD target 3.2. The key findings included the following:

1. In a study of 1 208 pregnant women, the overall prevalence of the presence of syphilis antibodies was 4.39%, whilst AS was at 2.07%.
2. In the HIV-uninfected group, the frequency of syphilis antibodies was much lower at 2.50%, whilst AS was even lower at 1.0%.
3. Syphilis antibodies were present in 6.25% of the HIV-infected pregnant women, with 3.13% presenting with AS.
4. HIV/Syphilis antibody coinfection was associated with a longer duration of ART exposure compared to the HIV-infected women without any syphilis antibodies detected.
5. The absence of anaemia was protective both in pregnancy in general, and also specifically in the HIV-infected women.
6. Single TT screening resulted in 112% overtreatments versus 4%, in one-step-NTT screening.
7. Adverse pregnancy outcomes associated with gestational syphilis were significantly lower infant birthweights and shorter birth lengths.

Syphilis Burden in Pregnancy

The overall frequency of the presence of syphilis antibodies regardless of HIV status or whether it was AS or past infection was 4.39%. This figure fell well below the cut-off of high prevalence classification of syphilis of ≥5%. In Zimbabwe, syphilis diagnosis in pregnancy is generally done in women seeking ANC services, and in men and women seeking healthcare for STIs. National syphilis estimates in Zimbabwe are based on diagnosed cases, with little information available on the burden of syphilis in the general population. Despite Zimbabwe falling in the low syphilis prevalence category, the on-site SD Bioline screening is offered to all pregnant women on their first antenatal visit. Those women testing positive for syphilis antibodies are immediately treated accordingly. Thus, the prevention and control of STIs, including syphilis, remain an integral part of comprehensive sexual and reproductive health services. Such services are necessary to ensure healthy lives and promote well-being for all ages (SDG No. 3), including ending preventable deaths of new-borns and children under five years of age (target 3.2), and the epidemics of AIDS and other communicable diseases (target 3.3).

The overall syphilis burden of 4.39%, observed in our study, was almost three times higher than the overall prevalence of 1.2% reported in a 2006 cross-sectional study of 691 pregnant women enrolled at 36 weeks gestational age, from a national prevention of MTCT programme of single dose Nevirapine offered intrapartum in three peri-urban clinics around Harare (15). However, the overall pregnancy syphilis burden observed in our study is lower than the 6.3% recorded in 1 000 pregnant women seeking ANC services in rural Eastern Cape Province, South Africa in 2007 (Blandford *et al*., 2007). In this South African study, the overall pregnancy burden is comparable to the frequency of 6.25% observed in the HIV-infected pregnant women in our study.

The overall syphilis burden of 2.5% in the HIV-uninfected observed in our study is much lower at 3.6% compared to the 2019 pooled syphilis prevalence in pregnant women for the whole of the Southern African region (Hussen & Tadesse, 2019). About 34% of women in our study reported the presence of previous, but untreated syphilis, a figure more than double the estimated global figure of 16% of women testing positive, but not treated (Korenromp *et al*., 2019). In addition, women older than 25 years were more likely to be syphilis positive, a finding contrary to a local finding by Chirenje *et al*. where, in a 2017 adult HIV cohort study, being younger than 25 years was associated with acquiring a new STI (Chirenje *et al*., 2017).

In our study, women presenting with AS were 4.1 times more likely to be HIV-infected, compared to the HIV-uninfected. This observation is comparable with a 2015 to 2016 HIV and syphilis ZIMPHIA population-based study of 22 501 adults, using POC NTT and TT of Chembio's Dual–Path Platform syphilis screen, that showed a 3.7 times greater likelihood of AS in HIV-infected adult females compared to their uninfected counterparts (Ruangtragool *et al*., 2022). Our results are also comparable to the 2008 HIV-infected Botswana pregnant women where the HIV-infected group was more likely to be infected with syphilis compared to the HIV-uninfected (Shava *et al*., 2019).

The AS prevalence of 2.07%, observed in our study, was a drastic decline relative to a 2005 urban hospital based cross-sectional study findings of pregnant women done before the era of lifelong ART. The 2005 study reported an RPR seroprevalence of 4.0%, 4.8% and 9.2% burden in a Murehwa rural study and at Harare Hospital (Pham *et al*., 2005; Kambarami, *et al*., 1998). In another urban ZVITAMBO study also done in the pre-ART era, the overall prevalence of maternal AS was assessed at delivery (Cowan *et al*., 2008). Evidence is suggestive of AS being generally on the downward trajectory in the HIV-uninfected, whilst the opposite trend is true for the HIV-infected. Syphilis/HIV co-infected women were on ART for significantly longer periods compared to the HIV-infected women without syphilis. This observation warrants further investigations on potential ART-associated factors such as sexual risk taking behaviours and/or host immune-dysregulation inadvertently fuelling the syphilis epidemic. However, other studies have shown that ART with suppressed viral load was protective in the unadjusted model, but not in the adjusted multivariate model (Gilbert *et al*., 2021).

Adverse Birth/Infant Outcomes

HIV/Syphilis co-infection in pregnant Botswana women declined in the past decade, but it was associated with adverse birth outcomes (Shava *et al*., 2019). Syphilis has been shown to cause stillbirths and premature deliveries. However, we did not observe differences in the frequencies of premature deliveries and stillbirths, including the median head circumferences of infants born to women with AS, compared to those infants born to women without AS in pregnancy. This was contrary to the finding of a sub-Sahara African study of 1 000 pregnant women done before the ART era that observed a risk of 1% and 11% of stillbirths

in women without syphilis and those with untreated syphilis, respectively (Owusu-Edusei *et al*., 2011). This is critical as infants exposed to AS during pregnancy are at high risk of neurodevelopmental impairment irrespective of whether or not they have congenital syphilis (Verghese *et al*., 2018). Similar to previous findings, birth weight and birth lengths were significantly reduced in AS exposed neonates (Owusu-Edusei *et al*., 2011). This observation underscores the need to timeously and accurately diagnose, and adequately treat the infection to reduce neonate/infant burden. Potential overtreatment rates observed in our study were high. However, the minor adverse effects of excessive antibiotic use are inconsequential as treatment mitigates the adverse effects of undiagnosed and untreated syphilis, leading to congenital syphilis and foetal loss. However, the overuse of antibiotics has generally been linked to the development of multidrug resistant bacterial strains.

Strengths and Limitations

The strength of our study includes the large number of pregnant women of whom ~50% were HIV-infected, and their HIV-uninfected peers, all sampled concurrently from the same community. Extensive data on nutrition and reproductive health behaviour, including spouse factors, was collected. However, the distinction of primary and secondary syphilis among women was not done, and so was an assessment of Treponemas in stillbirth autopsy material. Finally, because of the non-interventional nature of the study design, it could not be ascertained whether anaemia was the cause or the consequence of syphilis infection.

Conclusion

High and moderate syphilis endemics were observed in HIV-infected and HIV-uninfected pregnant women, respectively. AS was generally on the downward trajectory in the HIV-uninfected pregnant women, whilst the opposite was true for the HIV-infected. Syphilis antibodies were more prevalent in HIV-infected women with longer durations of ART usage mainly associated with viral suppression and wellness. This observation warrants further investigations around false beliefs that HIV could be cured by ART. This may result in high risk sexual behaviours and/or ART-associated immune-dysregulation, inadvertently fuelling the syphilis incidence. Evidence underscores the urgent need to further strengthen the current STIs prevention and care programmes, especially in HIV-infected women of reproductive age, if the 2030 SGD target 3.2 is to be met.

List of Abbreviations

ART:	Antiretroviral therapy
AS:	Active syphilis
BMI:	Body mass index
CD4:	Cluster of differentiation 4
CI:	Confidence interval
DNA:	Deoxyribonucleic acid
HBV:	Hepatitis B virus
HIV:	Human immunodeficiency virus

HBsAg:	Hepatitis B surface antigen
IQR:	Interquartile range
MUAC:	Mid-upper arm circumference
MTCT:	Mother-to-child transmission
NTT:	Non-Treponemal Test
POC:	Point of care
SGDs:	Sustainable Development Goals
STIs:	Sexually transmitted infections
TT:	Treponemal Test
UZBCS:	University of Zimbabwe Birth Cohort Study
WHO:	World Health Organisation

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