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Complete List of Authors:	Malaba, Thokozile R; University of Cape Town Faculty of Health Sciences, Division of Epidemiology & Biostatistics, School of Public Health & Family Medicine Gray, Clive; University of Cape Town Faculty of Health Sciences, Division of Immunology, Institute of Infectious Disease and Molecular Medicine and Department of Pathology Myer, Landon; University of Cape Town Faculty of Health Sciences, Division of Epidemiology & Biostatistics, School of Public Health & Family Medicine Newell, Marie; University of Southampton Faculty of Medicine, Institute for Developmental Science
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Cohort Profile: Prematurity Immunology in HIV-infected Mothers and their infants Study (PIMS)

Author List: Thokozile R Malaba*[1], Clive Gray[2], Landon Myer[1,3] Marie-Louise Newell[4,5], for the PIMS Study Group

Affiliations:

1. Division of Epidemiology and Biostatistics, School of Public Health and Family Medicine, University of Cape Town, South Africa
2. Division of Immunology, Institute of Infectious Disease and Molecular Medicine and Department of Pathology, University of Cape Town, Cape Town, South Africa
3. Centre for Infectious Disease Epidemiology and Research, School of Public Health & Family Medicine, University of Cape Town, South Africa
4. Institute for Developmental Science, Faculty of Medicine, University of Southampton, Southampton, United Kingdom
5. School of Public Health, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

Corresponding Author (*)

Thokozile R Malaba, Division of Epidemiology and Biostatistics, School of Public Health and Family Medicine, Falmouth Building, University of Cape Town Faculty of Health Sciences, Anzio Road, Observatory, Cape Town, South Africa, 7925; Tel: +27 21 650 1424; Email: thoko.malaba@uct.ac.za

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Abstract

Purpose: PIMS, is a prospective cohort study in South Africa investigating the association between antiretroviral therapy (ART) use, preterm delivery (PTD)and small-for gestational age (SGA) live births. PIMS main hypotheses are that ART initiation in pregnancy and ART-induced hypertension are associated with PTD and SGA respectively and that reconstitution of cellular immune responses in women on ART from before pregnancy results in increases in PTD of appropriate-for-gestational age (AGA) infants.

Participants: Pregnant women (n=3972) aged ≥18 years regardless of HIV status recruited from 2015 to 2016 into the overall PIMS cohort (2517 HIV-uninfected, 1455 HIV-infected). A nested cohort contained 551 HIV-infected women who were ≤24 weeks' GA on ultrasound: 261 initiated ART before pregnancy, 290 initiated during pregnancy.

Findings to date: Women in the overall cohort were followed antenatally through to delivery using routine clinical records; further women in the nested cohort were actively followed up until 12 months postpartum, with data were collected on maternal health (HIV care and ART use, clinical care and inter-current clinical history). Other procedures conducted on the nested cohort included physical examinations (anthropometry, blood pressure measurement), assessment of fetal growth (ultrasound), maternal and infant phlebotomy for storage of plasma, RNA and peripheral blood mononuclear cells, collection of delivery specimens (placenta and cord blood), and infant 12 month developmental assessment. Preliminary findings have contributed to our understanding of risk factors for adverse birth outcomes, and the relationship between pregnancy immunology, HIV/ART and adverse birth outcomes.

Future plans: Using specimens collected from HIV-infected study participants throughout pregnancy and first year of life, the PIMS provides a valuable platform for answering a variety of research questions focused on temporal changes of immunology markers in women whose immune status is altered by HIV infection, and how ART initiated during pregnancy affects immune responses. The relationship between these immunological changes with adverse birth outcomes as well as possible longer-term impact of exposure to ART in fetal and early life will be explored. Additionally, further active and passive follow-up of mothers and their infants is planned at school-going age and beyond to chart growth, morbidity and development, as well as changes in family circumstances.

Strengths and limitations

- Robust measurement of gestational age early in pregnancy by research sonographer for the GA assessment in women ≤24 weeks when ultrasound is highly reproducible and accurate.
- Ability to track patients using the Western Cape unique identifier across different health and laboratory services, enables the passive long term follow-up of the Group 2 women and their infants; with available data including patient level data (administrative, demographic and clinical), visit level data (clinical observations and findings), laboratory tests and medication.
- Maternal biological specimens enable immunological, metabolomic and placental investigations will inform understanding of mechanisms underlying adverse birth outcomes in HIV-infected women.
- One of the first studies to combine metabolomic and immunologic assessments in infant biological specimens which will provide an integrated model of the immune-metabolism association in HIV-exposed infants and the consequences of maternal metabolic dysregulation for the immune responses of the infant.
- Absence of HIV-uninfected or ART-unexposed comparator groups for immunological investigations, which could hinder distinguishing ART exposure from HIV disease.

Introduction

Antiretroviral drugs in HIV-infected pregnant women prevent mother-to-child transmission (PMTCT) and delay HIV disease progression. WHO guidelines now recommend antiretroviral therapy (ART) for all, immediately upon HIV diagnosis, including for HIV-infected women during pregnancy and breastfeeding, to be continued lifelong [1]. However infants born to HIV-infected mothers would be exposed to multi-drug ART regimen for prolonged periods at a crucial time during their development [2], which could result in decreased health, developmental, and survival outcomes [3 4]. In high maternal HIV prevalence settings, the increasing population of ART-exposed infants could make the goal of under-five mortality reduction less likely.

Adverse birth outcomes contribute significantly to under-five mortality, as well as infant health and developmental problems [5]. There is an ongoing debate regarding the association between exposure to maternal ART during fetal life and adverse birth outcomes, following reports from Europe [6-9], USA [10] and Africa [11-14] of possible ART-associated increased risk of preterm delivery (PTD), small-for-gestational age (SGA) or low birthweight (LBW) infants. Furthermore, these exposed infants are also at increased risk of acquiring viral infections [15 16], as well as the negative impact of ART on fetal brain development and function [17]. Interpretation of findings from various studies, especially from African settings, is hindered by the reliability of gestational assessment, with ultrasound dating in early pregnancy usually unavailable.

There is limited understanding of general pregnancy-related immune changes in high HIV prevalence settings. Successful pregnancies require intricate fetal-maternal (FM) immune balances, to enable maternal tolerance of the semi-allogeneic fetus; this FM tolerance is primarily maintained by the placenta [18 19]. Consequently, adverse birth outcomes could be hypothesized to be due to placental interface FM tolerance disruption because of cytokine shifts associated with ART initiation causing early initiation of uterine contractions [20]. Additionally, there are suggestions that adverse birth outcomes could also be associated with ART-induced dysregulation of maternal and infant metabolism. In order to meet the specific needs during pregnancy and infancy, metabolism is tightly regulated, but ART is known to interfere with lipid metabolism [21]. The emerging field of immunometabolism has shown that alterations in the lipid profile increases susceptibility to viral infections by skewing immune responses towards an inflammatory profile. The complex interplay between pregnancy, HIV/ART, host immunity, adverse birth outcomes and long-term child health is poorly understood as detailed data are sparse and often related to drug combinations which are no longer be in use. Further research is required to examine epidemiologic and immunological associations and inform understandings of underlying biological mechanisms giving rise to adverse birth outcomes.

An increase in PTD rates, and especially of SGA infants, could impact on the long-term growth and development of children, and would have consequences for the health and wellbeing of their families and population more widely. We therefore aimed to improve understanding of maternal immune profiles during pregnancy in the context of ART use during gestation, adverse birth outcomes and long-term child health in Cape Town, South Africa, an area of high HIV prevalence. Our primary focus was to quantify the risk of preterm and SGA deliveries; with our underlying hypotheses that (i) timing of ART use (from before or during pregnancy) is associated with increased risk of PTD, (ii) ART-induced hypertension during pregnancy results in increased risk of SGA and (iii) reconstitution of cellular immune responses during ART in pregnancy results in increases in PTD of appropriate-for-gestational age (AGA) infants. Our secondary focus was to determine long-term (first five years of life) child health outcomes of PTD infants (by weight at gestational age and maternal HIV/ART status). Our hypotheses are that (i) throughout childhood SGA infants are disadvantaged in terms of growth and development compared to preterm AGA infants and (ii) ART use alters maternal and fetal lipid metabolism resulting in susceptibility to infections and alterations in vaccine responses in childhood.

Cohort Description

Setting

Between April 2015 and October 2016, we enrolled pregnant women (aged ≥18 years) at their first antenatal care (ANC) visit in a prospective cohort study, at a single large public sector primary care facility (Gugulethu Midwife Obstetric Unit (MOU)) in a low-income high HIV-prevalence sub-district of Cape Town, South Africa.

Study Design

The overall prospective, observational design includes two 'nested' groups of pregnant women:

- **Group 1:** the overall population of pregnant women (≥18 years) seeking ANC services at Gugulethu MOU during a 18-month period; within this group, a subset of women thought to be ≤24 weeks' gestation based on history or examination (clinical GA) underwent ultrasound scan by a research sonographer for more accurate gestation estimation; enrolled into observation group.
- **Group 2:** all HIV-infected pregnant women seeking ANC who are ≤24 weeks' gestation at US at their first ANC visit, regardless of current ART use at the first ANC visit (nested within Group 1). Enrolled into longitudinal cohort with data collection through questionnaires, clinical assessments and phlebotomy spanning pregnancy to early infancy.

This study design enables quantification of the risk of adverse birth outcomes in the overall cohort, as well as the more detailed Group 2 group also enabling investigation of the consequences of the immune response following ART initiation in pregnancy for onset of labour and preterm delivery.

Ethical Approval

The study was reviewed and approved by the University of Cape Town Faculty of Health Sciences Human Research Ethics Committee (UCT HREC 739/2014) and the University of Southampton Faculty of Medicine Ethics Committee (12542 PIMS). Public sector

Patient Public Involvement

No patient involvement.

Routine Care Services

As part of routine ANC services, gestational age (GA) was estimated based on date of last menstrual period (LMP) and symphysis-fundal height (SFH) by public sector midwives. All women without a previous HIV diagnosis underwent HIV testing, with universal ART eligibility. HIV-infected women conceiving while on ART continued their current regimen throughout pregnancy; regimens included NNRTIs such as efavirenz (EFV) or protease inhibitor (PI, predominantly used after failure of first-line therapy). For women initiating ART in pregnancy, a fixed-dose combination of tenofovir (TDF) + emtricitabine (FTC) + efavirenz (EFV) was used throughout.

Recruitment

Following screening of all women attending their first ANC visit, those ≥18 years were eligible and approached to participate in the study. Women who agreed to participate had their routinely collected LMP-based GA and SFH-based GA reviewed by the counsellor; women estimated to be ≤24 weeks were referred for a research ultrasound scan (US) for formal pregnancy dating by a research sonographer blinded to the midwife assessment. HIV-infected women who were ≤24 weeks' gestation on US were then recruited into a nested cohort (Group 2); half of these had initiated life-long ART prior to conception and half initiated ART during pregnancy.

All participants provided written informed consent prior to study participation, with re-consenting of mother-infant pairs at the first postpartum visit for paediatric follow-up. Consent for study participation included data abstraction from routine clinical records through the pregnancy and post-partum period.

Participant Baseline Characteristics

A total of 4431 women registered for ANC during the study recruitment period, of whom 4111 (93%) were screened for the study and 3972 (90%) enrolled; all delivered by May 2017 (Figure 1). Main reasons for being screened out were under-age, referrals from Basic Antenatal Clinics, or not interested. Of the enrolled women, 2517 (63.4%) were HIV-uninfected and 1455 (36.6%) HIV-infected (Table 1); 2199 (55.4%) were referred for ultrasound based on their clinical GA, 1327 (60.3%) were HIV-uninfected and 872 (39.7%) HIV-infected.

Median age at enrolment was 28 years (IQR 23-32), with HIV-uninfected women younger than HIV-infected women, and those initiating ART preconception older than HIV-infected women initiating ART during pregnancy. In line with age differences, HIV-infected women were of higher gravidity than HIV-uninfected women, but the difference in parity was small; women who initiated ART pre-pregnancy were of a higher gravidity than those initiating during pregnancy. A quarter (25%) of all women were overweight, while over half (55%) were obese, with little or no difference between groups. Having previously had a preterm delivery was more common among HIV-infected than uninfected women. Mild anaemia was relatively common in all groups, especially in women initiating ART during pregnancy (Table 1). Overall, 3479 (87.6%) women had gestation estimated by LMP, 2327 (58.6%) by SFH and 2334 (58.8%) by ultrasound; with estimated median GA at enrolment visit varying by assessment method (Table 1).

There were 1455 HIV-infected women in Group 1, of whom 718 (49.3%) were ≤24 weeks on ultrasound, and 551 were enrolled into Group 2 (Figure 1). The likelihood of inclusion into Group 2 did not differ by baseline characteristics (Table 2). In comparison to Group 1 participants, Group 2 participants differed in age, gravidity, parity and previous PTD, likely driven by the HIV-uninfected women (Table 3). In multivariable regression allowing for HIV infection, the only difference that persisted between these groups was age (Table 4). When Group 2 women were compared to other HIV-infected women (not enrolled in Group 2), they were slightly older, more likely to have normal haemoglobin levels (≥11g/dl) and lower gestational age (Table 3). In multivariable regression the only difference between these groups that persisted was gestational age, which was a Group 2 inclusion criterion (Table 4).

Of the 551 HIV-infected women in Group 2, 261 (47%) initiated before pregnancy and 290 (53%) during pregnancy (Table 5). Women who initiated ART during pregnancy were on average younger, and of lower gravidity. Overall, three-quarters of Group 2 women were overweight or obese, with little difference by ART status. Of the women who initiated ART during pregnancy, the majority (64%) had tested HIV positive in the index pregnancy; the rest had previously tested positive although were not on ART at conception. In line with local and WHO treatment guidelines, most women (91%) were on a regimen of two NRTIs (TDF +FTC), plus NNRTI EFV. PI usage in this cohort was low, at only 9% of women who initiated pre-pregnancy and 1% of women who initiated during pregnancy. Median CD4 count was 433 cells/µl overall, 527 cells/µl in women who initiated ART before pregnancy and 373 cells/µl in women initiating at the first antenatal visit. There were no differences in smoking or drug usage between these two groups of women, but women who initiated during pregnancy were more likely to have ever consumed alcohol or consumed in the last 30 days (Table 5).

Study Follow-up

At baseline, all women (Group 1) had clinical and medical history, routine first ANC visit physical examination, screening tests and GA assessment data collected via abstraction of the Maternity Case Record (MCR) booklet (Table 5), which is a standardised patient-held maternity record used by all facilities providing maternity services to record clinical data from the antenatal through to postpartum period, including labour. The MCR also serves as a referral letter, thus serving as a link between antenatal and labour care. In addition, the National Health Laboratory Services (NHLS) database was searched for CD4, Viral load results and other laboratory values not recorded in the MCR. Further follow-up for women in Group 1, not eligible for Group 2, was through data

abstraction of the MCR following discharge from the postnatal ward (MCR retained at delivery facility). Data was abstracted from follow-up ANC notes, clinical notes during labour and newborn assessments (Table 5).

Women in Group 2 participated in up to eight scheduled study visits, from the start of ANC through to 12 months postpartum. Women on ART from before pregnancy had three antenatal visits at <24 weeks, 28 and 34 weeks; women who initiated ART during pregnancy had an additional study visit two weeks after the ART initiation (which in most women took place on the same day, or close to, the first study visit). Following delivery, women were re-consented for infant participation, and study visits were conducted <7days, 10 weeks, 6 months and 12 months postpartum. At all study visits, data were collected on maternal health (HIV care and ART use, clinical care and inter-current clinical history). Other procedures included physical examinations (anthropometry, blood pressure measurement), phlebotomy (50ml) for storage of plasma, peripheral blood mononuclear cells (PBMC) and RNA; a follow-up ultrasound was conducted at 28 weeks to assess fetal growth (Table 5).

At postpartum study visits, additional data was collected on infant health (including infant feeding and inter-current clinical history) and physical examination of infants was conducted (basic anthropometry). At the 12 month visit, developmental assessment was conducted using the Ages and Stages questionnaire [22] – a general developmental screening tool testing five key areas: personal social, gross motor, fine motor, problem solving and communication skills. Infant specimen collection included Dried Blood Spot (DBS) sampling and storage at 10 weeks study visit, and phlebotomy (2ml) for measurement of immunological functioning and antibody responses to routine childhood immunisations (rotavirus and measles) at 12 month study visit. In addition, data on infant health status, including vaccinations, chemoprophylaxis use (including nevirapine and co-trimoxazole) and routine HIV PCR testing, was abstracted from Road-to-Health Booklets - patient-held booklet taken to all clinical and immunisation visits used to monitor infant growth and development until age 5 years (Table 6).

Further active follow-up of the women and their infants will occur at school-going age and beyond to chart growth, morbidity and development, as well as changes in family circumstances. It is envisaged that subsequent to this, longer term follow-up will be passive through the use of routinely collected data. The Western Cape Provincial Department of Health's public-sector patient administration systems all share unique health identifier [23]; data relating to participants health service contacts, health conditions and health outcomes for specific conditions will be obtained from the Provincial Health Data Centre, which consolidates person-level clinical data across government services [24].

Data Collection

An overview of the main included data collection instruments is presented in Table 6, covering self-reported information on clinical history, ART use and adherence, and medical events; as well as information obtained from routinely collected data.

Specimen Collection

To investigate the proposed hypothesis that immunological changes resulting from maternal ART exposure are associated with adverse birth outcomes, women enrolled into the follow-up cohort were intensively sampled, with repeated phlebotomy throughout pregnancy and the postpartum period for immunological investigations (Table 7). Using samples from all antenatal plasma, inflammatory markers (C-reactive Protein, Serum Amyloid A and CCL10 (IP-10) in women are being measured. Further, following a nested case-control design in Group 2, investigations compare women delivered preterm (PTD cases) or had small-for-gestational age infants (SGA cases) and those from appropriate controls (term AGA) (matched for GA and ART status). Investigations include longitudinal quantification of plasma cytokine profiles, phenotypic and functional characterisation of regulatory T cells (Tregs), antigen-presenting cells and metabolites associated with mitochondrial functioning and lysophospholipids (Figure 2). The combined studies of these immune parameters will inform understanding of ART use during pregnancy on the areas of the immune system that

have been shown to be critically involved in regulating maternal immune tolerance to the fetus, and their associations with onset of labour and preterm delivery.

At delivery, placentas and cord bloods were collected whenever possible, a scoring algorithm was developed which graded placentas and dictated specimen processing according to membrane completeness and time received in laboratory relative to delivery time (Table 8). Using flow cytometry and tissue immunostaining techniques the following investigations will be conducted: examination of the effect of HIV infection/ART exposure on the phenotypic characteristics and functionality of placental macrophages (Hofbauer cells and decidual macrophages) at the maternal-fetal interface and placental Tregs and their association with adverse birth outcomes. Characterisation of cord blood Tregs and correlation of their frequency, function and phenotype with placental Tregs and birth outcomes (Figure 2).

Study Retention

Loss to follow-up was categorised based on the last visit before the woman was lost; in total 158 (29%) women were lost to follow-up (LTFU) (Figure 3). Women lost before the post-delivery study visit (n=88), either experienced pregnancy losses (n=37), were no longer interested in participating (n=24) or relocated out of the study area (n=17). For women LTFU between delivery and the 6 month postpartum visit (n=24), reasons included relocation (n=8), not contactable (n=6), no longer interested in participating (n=5) and maternal/infant death (n=5) (Fig 2). For women LTFU between 6 month and 12 month postpartum (n=46), reasons included relocation (n=15), not contactable (n=26), no longer interested in participating (n=2) and maternal/infant death (n=3).
There were no appreciable differences by ART status in women LTFU before post-delivery visit (RR 0.81, 95% CI 0.55 – 1.19). However, women who initiated ART before pregnancy were less likely to be LTFU between delivery and 6 months postpartum (RR0.44, 95% 0.19 – 1.04) and between 6 month and 12 month postpartum (RR0.58, 95% 0.33 – 1.02). No baseline characteristics were associated with LTFU.

Findings to date

Gestational Age Assessment

In the overall cohort, 1787 women with live singleton births were included in the analysis of the association between HIV status and timing of ART initiation and PTD by GA assessment method used (last menstrual period (LMP), measurement of symphysis fundal height (SFH) and ultrasound (US). Using US-GA, PTD risk was associated with maternal HIV infection and ART use, with HIV-infected women, on ART from before or early pregnancy, almost twice as likely to deliver preterm than HIV-uninfected women [25]. A weaker association was observed when GA assessment was based on SFH; while with LMP-GA the difference by HIV status was minimal. We did not find any appreciable differences in the PTD risk for HIV-infected women by timing of ART initiation across all three assessment methods [25]. Our findings (in both the overall cohort and in women with all three assessments) suggest that methods of GA assessment explain at least partially the heterogeneity of findings from previous studies on the association between ART use and adverse birth outcomes, suggesting that care should be taken when interpreting results from such studies.

Obesity

In the overall cohort of HIV-infected and -uninfected women, 2921 women were included in the analysis of the association between maternal body mass index and adverse birth outcomes. In a subset cohort the association between gestational weight gain (GWG) and adverse birth outcomes was examined. Maternal obesity was associated with increased likelihood of having high birthweight and large size for gestational age infants. In the subset cohort, GWG was associated with increased likelihood of spontaneous PTD and high birth weight infants [26]. Obesity during pregnancy is prevalent in this setting and appears associated with increased risk of adverse birth outcomes in both HIV-infected and -uninfected women.

Placental Pathology

Preliminary analysis of placental histopathology from a sub-set of women enrolled in the prospective cohort showed significant associations between placental pathology and adverse birth outcomes: presence of focal infarction was associated with increased risk of low birthweight (LBW); the lower the weight of the basal plate weight lead to increased risk of LBW, PTD and SGA; and prolonged meconium exposure was associated with increased risk of SGA. These findings suggest that adverse birth outcomes are driven primarily by placental abnormalities which do not appear to be associated with the ART initiation timing [27]. Immunofluorescence and immunohistochemistry staining were performed on these wax blocks to identify regulatory T cells along with macrophages. Further analysis is ongoing.

Within the placenta, investigation of the distribution of pro-inflammatory (M1) and anti-inflammatory (M2) placental macrophages at the maternal-fetal interface showed no differences in the tissue density of these macrophages within the decidual membranes and villous tissue according to timing of ART initiation. Data suggest that the Hofbauer Cells (which are fetal macrophages) are not polarized into M1/M2 phenotypes but are rather "intermediate" types [28].

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Competing Interests Statement

There are no competing interests for any author

Data Availability Statement

The data collected in this study will be available to external investigators interested in collaboration upon submission and approval of a data analysis plan. The samples are being used by the named investigators, but remaining samples can be made available to external users. Requests for data and available samples within PIMS, or to submit a request for additional data collection, should be submitted to M.Newell@soton.ac.uk and Landon.Myer@uct.ac.za and will be reviewed by the study steering committee.

Contributorship Statement

TRM, LM, CG and MLN: Conceptualisation and design of the study

TRM: Study conduct, data collection and statistical analysis.

TRM and MLN: Interpretation of data and writing the manuscript

TRM, LM, CG and MLN review and editing

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Table 1: Baseline Characteristics of Group 1 women at 1st antenatal care visit (n=3972)

	Total N=3972	HIV-uninfected N=2517	HIV-infected N=1455	P value	HIV-infected N= 1455		P value
					Initiated before pregnancy N=722	Initiated during pregnancy N=733	
Age, years				<0.0001			<0.0001
<24	1273 (32)	987 (39)	286 (20)		112 (16)	174 (24)	
25-29	1108 (28)	702 (28)	406 (28)		157 (22)	249 (34)	
>30	1591 (40)	828 (33)	763 (52)		453 (63)	310 (42)	
Median	28 (23-32)	26 (22-31)	30 (25-34)		31 (27–35)	28 (25-32)	
Height, cm				0.221			0.562
≤155	1173 (30)	723 (29)	450 (31)		220 (30)	230 (31)	
156-161	1344 (34)	873 (35)	471 (32)		245 (34)	226 (31)	
≥162	1049 (26)	661 (26)	388 (27)		190 (27)	198 (27)	
Missing	*406 (10)*	*256 (10)*	*147 (10)*		*67 (9)*	*79 (11)*	
Median	158 (154-162)	158 (154-162)	158 (154-162)		158 (154-162)	158 (154-162)	
Body Mass Index, kg/m^2				0.666			0.535
Underweight (<18.5)	26 (0.7)	15 (0.6)	11 (0.7)		8 (1)	3 (0.4)	
Normal (18.5-24.9)	718 (18)	457 (18)	261 (18)		124 (17)	137 (19)	
Overweight (25.0-29.9)	1007 (25)	625 (25)	381 (26)		202 (28)	180 (25)	
Obese (>30.0)	1790 (55)	1148 (47)	801 (55)		313 (43)	329 (55)	
Missing	*431 (11)*	*272 (11)*	*159 (11)*		*75 (10)*	*84 (11)*	
Median	30 (26-35)	30 (26-35)	30 (26-35)		30 (26-35)	30 (25-35)	
Gravidity				<0.0001			0.002
1	967 (24)	726 (29)	241 (17)		100 (14)	141 (19)	
2	1347 (34)	849 (34)	498 (34)		238 (33)	260 (35)	
≥3	1567 (39)	887 (35)	680 (47)		368 (51)	312 (43)	
Missing	*91 (2)*	*55 (2)*	*36 (3)*		*16 (2)*	*20 (3)*	
Median	2 (1-3)	2 (1-3)	2 (2-3)		3 (2-3)	2 (2-3)	
Parity				<0.0001			0.011
0	1194 (30)	865 (34)	329 (23)		147 (20)	182 (25)	
1	1458 (37)	898 (36)	560 (38)		270 (37)	290 (40)	
≥2	1228 (31)	699 (28)	529 (36)		289 (40)	240 (33)	
Missing	*92 (2)*	*55 (0.1)*	*37 (3)*		*16 (2)*	*21 (3)*	
Median	1 (0-2)	1 (0-2)	1 (1-2)		1 (1-2)	1 (0-2)	
Previous Preterm*				0.013			0.617
Yes	280 (7)	159 (6)	121 (8)		63 (9)	58 (8)	
Haemoglobin g/dl				<0.0001			<0.0001
Normal (≥11.0)	1446 (36)	961 (38)	485 (33)		289 (40)	196 (27)	
Mild Anaemia (9-10.9)	1067 (27)	638 (25)	429 (29)		177 (25)	252 (34)	
Moderate Anaemia (7-8.9)	229 (6)	109 (4)	120 (8)		47 (7)	73 (10)	
Severe Anaemia (<7)	5 (0.1)	4 (0.2)	1 (0.1)		0	1 (0.1)	
Missing	*1225 (31)*	*805 (32)*	*420 (29)*		*209 (29)*	*211 (29)*	
Gestational Age Assessment*							
LMP	3479 (88)	2219 (88)	1260 (87)	0.150	642 (89)	618 (84)	0.010
Median (weeks)	17 (12-22)	17 (12–23)	16 (11-22)		15 (11-22)	17 (12-22)	
SFH	2327 (59)	1447 (57)	880 (60)	0.065	403 (56)	477 (65)	<0.0001
Median (weeks)	23 (18-28)	23 (19-28)	22 (17-27)		22 (16-27)	23 (18-28)	
US	2334 (59)	1411 (56)	923 (63)	<0.0001	433 (60)	490 (67)	0.006
Median (weeks)	16 (12-21)	16 (12-21)	15 (11-20)		14 (10-19)	17 (12-21)	

* LMP – Last menstrual period; SFH – Symphysis fundal height, US – Ultrasound

Table 2: Baseline Characteristics of Group 2 eligible but not enrolled vs Group 2 women at 1st antenatal care visit

	Group 2 Eligible N=718		P-value
	Not Enrolled N=167*	Enrolled N=551	
Age, years			0.274
<24	37 (22)	92 (17)	
25-29	44 (26)	156 (28)	
>30	86 (52)	303 (55)	
Median	30 (25-33)	30 (26-34)	
Height, cm			0.395
≤155	53 (32)	166 (33)	
156-161	54 (32)	191 (35)	
≥162	33 (20)	145 (26)	
Missing	*27 (16)*	*49 (9)*	
Median	157 (153-161)	158 (154-162)	
Body Mass Index, kg/m^2			0.920
Underweight (<18.5)	1 (0.01)	5 (0.01)	
Normal (18.5-24.9)	32 (19)	103 (19)	
Overweight (25.0-29.9)	35 (21)	134 (24)	
Obese (>30.0)	71 (43)	255 (46)	
Missing	*28 (17)*	*54 (10)*	
Median	30 (25-34)	30 (25-36)	
Gravidity			0.204
1	35 (21)	90 (16)	
2	57 (34)	184 (33)	
≥3	67 (40)	263 (48)	
Missing	*8 (5)*	*14 (3)*	
Median	2 (2-3)	2 (2-3)	
Parity			0.290
0	48 (29)	129 (23)	
1	62 (37)	222 (40)	
≥2	49 (29)	185 (34)	
Missing	*8 (5)*	*15 (3)*	
Median	1 (0-2)	1 (1-2)	
Previous Preterm**			0.100
Yes	9 (6)	52 (9)	
Haemoglobin g/dl			0.084
Normal (≥11.0)	46 (28)	232 (42)	
Mild Anaemia (9-10.9)	44 (26)	153 (28)	
Moderate Anaemia (7-8.9)	11 (7)	26 (5)	
Severe Anaemia (<7)	0	0	
Missing	*66 (40)*	*140 (25)*	
ART Status			0.577
Initiated before pregnancy	55 (92)	290 (53)	
Initiated during pregnancy	75 (45)	261 (47)	
Gestational Age Assessment***			
LMP	148 (89)	481 (87)	0.648
Median (weeks)	13 (10–17)	13 (9-17)	
SFH	87 (57)	258 (47)	0.232
Median (weeks)	17 (14-20)	17 (14-20)	
US	167 (100)	551 (100)	
Median (weeks)	14 (10-18)	13 (10-17)	

* Eligible for Group 2 based on US, but not enrolled into Group 2
** Among women with a previous pregnancy
*** LMP – Last menstrual period; SFH – Symphysis fundal height, US – Ultrasound

Table 3: Baseline Characteristics of Group 1 vs Group 2 women at 1st antenatal care visit

	Group 1		Group 2	P-value Grp1 vs Grp2	P-value HIV+ vs Grp2
	HIV-uninfected N=2517	HIV-infected* N=904	HIV-Infected N=551		
Age, years				<0.0001	0.078
<24	987 (39)	194 (21)	92 (17)		
25-29	702 (28)	250 (28)	156 (28)		
>30	828 (33)	460 (51)	303 (55)		
Median	26 (22-31)	30 (25-33)	30 (26-34)		
Height, cm				0.726	0.465
≤155	723 (29)	284 (31)	166 (30)		
156-161	873 (35)	280 (31)	191 (35)		
≥162	661 (26)	243 (27)	145 (26)		
Missing	*260 (10)*	*97 (11)*	*49 (9)*		
Median	158 (154-162)	158 (154-163)	158 (154-162)		
Body Mass Index, kg/m^2				0.756	0.456
Underweight (<18.5)	15 (0.6)	6 (0.7)	5 (1)		
Normal (18.5-24.9)	457 (18)	158 (17)	103 (19)		
Overweight (25.0-29.9)	625 (25)	248 (27)	134 (24)		
Obese (>30.0)	1148 (47)	387 (43)	255 (46)		
Missing	*272 (11)*	*105 (12)*	*54 (10)*		
Median	30 (26-35)	30 (26-35)	30 (25-34)		
Gravidity				<0.0001	0.820
1	726 (29)	151 (17)	90 (16)		
2	849 (34)	314 (35)	184 (33)		
≥3	887 (35)	417 (46)	263 (48)		
Missing	*55 (2)*	*22 (2)*	*14 (3)*		
Median	2 (1-3)	2 (2-3)	2 (2-3)		
Parity				0.004	0.236
0	865 (34)	200 (22)	129 (23)		
1	898 (36)	338 (37)	222 (40)		
≥2	699 (28)	344 (38)	185 (34)		
Missing	*55 (0.1)*	*22 (2)*	*15 (3)*		
Median	1 (0-2)	1 (1-2)	1 (1-2)		
Previous Preterm**				<0.0001	0.182
Yes	159 (6)	69 (8)	52 (9)		
Haemoglobin g/dl				0.007	<0.0001
Normal (≥11.0)	961 (38)	253 (28)	232 (42)		
Mild Anaemia (9-10.9)	638 (25)	276 (31)	153 (28)		
Moderate Anaemia (7-8.9)	109 (4)	94 (10)	26 (5)		
Severe Anaemia (<7)	4 (0.2)	1 (0.1)	0		
Missing	*805 (32)*	*280 (31)*	*140 (25)*		
Gestational Age Assessment***					
LMP	2219 (88)	779 (86)	481 (87)	0.823	0.542
Median (weeks)	17 (12–23)	19 (13-24)	13 (9-17)		
SFH	1447 (57)	622 (69)	258 (47)	<0.0001	<0.0001
Median (weeks)	23 (19-28)	25 (21-29)	17 (14-20)		
US	1411 (56)	376 (42)	551 (100)	<0.0001	<0.0001
Median (weeks)	16 (12-21)	21 (13-25)	13 (10-17)		

* All HIV-infected woman not included in Group 2

** Among women with a previous pregnancy

*** LMP – Last menstrual period; SFH – Symphysis fundal height, US – ultrasound

Table 4: Baseline characteristics associated with inclusion in Group 2 at 1st antenatal care visit

	OR Group 1 (Ref) vs Grp 2*				OR Group 1 HIV+ (Ref) vs Group 2**			
	OR	**P-values**	**aOR (95% CI)**	**P-values**	**OR**	**P-values**	**aOR (95% CI)**	**P-values**
Age, years								
<24	Reference		Reference		Reference		Reference	
25-29	2.10 (1.60-2.76)	<0.0001	2.23 (1.64-3.05)	<0.0001	1.32 (0.96-1.81)	0.091	1.09 (0.65-1.83)	0.758
>30	3.02 (2.36-3.86)	<0.0001	3.37 (2.45-4.63)	<0.0001	1.39 (1.04-1.85)	0.025	1.09 (0.66-1.81)	0.740
Body Mass Index, kg/m^2								
Underweight (<18.5)	Reference		Reference		Reference		Reference	
Normal (18.5-24.9)	0.70 (0.26-1.91)	0.489	0.75 (0.27-2.06)	0.573	0.78 (0.23-2.63)	0.691	1.02 (0.14-7.37)	0.988
Overweight (25.0-29.9)	0.64 (0.24-1.74)	0.386	0.60 (0.22-1.65)	0.324	0.65 (0.19-2.16)	0.481	1.34 (0.19-9.65)	0.773
Obese (>30.0)	0.70 (0.26-1.87)	0.474	0.56 (0.20-1.52)	0.255	0.79 (0.24-2.62)	0.701	1.10 (0.15-7.84)	0.923
Gravidity								
1	Reference		Reference		Reference		Reference	
2	1.54 (1.18-2.01)	0.001	0.99 (0.73-1.34)	0.929	0.98 (0.72-1.35)	0.917	1.08 (0.64-1.82)	0.765
≥3	1.97 (1.52-2.53)	<0.0001	0.94 (0.68-1.30)	0.716	1.05 (0.78-1.43)	0.715	1.09 (0.64-1.86)	0.746
Previous Preterm***								
Yes	1.50 (1.09-2.06)	0.012	1.19 (0.84-1.68)	0.318	1.29 (0.89-1.88)	0.183	1.25 (0.69-2.26)	0.468
ART Status	-		-	-				
Initiated during pregnancy	-		-	-	Reference		Reference	
Initiated before pregnancy	-		-	-	0.86 (0.70-1.07)	0.180	1.07 (0.76-1.51)	0.705
Gestational Age	-		-	-				
Weeks	-		-	-	0.86 (0.83-0.88)	<0.0001	0.84 (0.82-0.87)	<0.0001

* Comparison between all enrolled pregnant women (≥18 years) seeking ANC services at Gugulethu MOU (Group 1) (n=3421) vs HIV-infected pregnant women ≤24 weeks' gestation enrolled into cohort (Group 2) (n=551)

** Comparison between all enrolled HIV-infected pregnant women (≥18 years) seeking ANC services at Gugulethu MOU (Group 1) not enrolled in cohort (n=167) vs HIV-infected pregnant women ≤24 weeks' gestation enrolled into cohort (Group 2) (n=551)

*** Among women with a previous pregnancy

Table 5: Baseline Characteristics of Group 2 women at 1st antenatal care visit (n=551)

	Total N=551	HIV-infected		P-value
		Initiation before pregnancy N=261	Initiation during pregnancy N=290	
Maternal Characteristics				
Age, years				<0.0001
≤24	92 (17)	25 (10)	67 (23)	
25-29	156 (28)	58 (22)	98 (34)	
≥30	303 (55)	178 (68)	125 (43)	
Median (IQR)	30 (26-34)	32 (28-36)	29 (25-32)	
Education (Finished High School)	164 (30)	96 (33)	69 (26)	0.088
Employment Status				0.767
Employed	238 (46)	114 (44)	124 (43)	
Missing	*2 (0.4)*	*2 (1)*	*0*	
SES				0.694
Lowest	175 (32)	82 (31)	93 (32)	
Medium	175 (32)	88 (34)	87 (30)	
Highest	189 (34)	87 (33)	102 (35)	
Missing	*12 (2)*	*4 (3)*	*8 (3)*	
Obstetric Characteristics				
Gravidity				<0.0001
1	88 (16)	29 (11)	59 (20)	
2	187 (34)	78 (30)	109 (38)	
≥3	276 (50)	154 (59)	122 (42)	
Median (IQR)	2 (2-3)	3 (2-4)	2 (2-3)	
Parity				0.061
0	123 (22)	49 (18)	74 (26)	
1	220 (40)	99 (38)	121 (42)	
≥2	208 38)	113 (43)	95 (33)	
Median (IQR)	1 (1-2)	1 (1-2)	1 (0-2)	
Height, cm				0.858
≤155	130 (24)	64 (25)	66 (23)	
156-161	208 (38)	96 (37)	112 (39)	
≥162	208 (38)	99 (38)	109 (38)	
Missing	*5 (0.9)*	*2 (0.8)*	*3 (1)*	
Median (IQR)	160 (156-164)	159 (155-163)	160 (156-164)	
Body Mass Index, kg/m^2				0.591
Underweight (<18.5)	6 (1)	3 (1)	3 (1)	
Normal (18.5-24.9)	110 (20)	47 (18)	63 (22)	
Overweight (25.0-29.9)	148 (27)	76 (29)	72 (25)	
Obese (>30.0)	282 (51)	133 (51)	149 (51)	
Missing	*5 (0.9)*	2 (0.8)	1 (0.3)	
Median (IQR)	30 (26-35)	30 (26-34)	30 (25-35)	
Median Gestation (completed weeks)	14 (11-18)	13 (11-17)	14 (10-18)	0.054
HIV				
First Tested HIV positive				<0.0001
In this pregnancy	186 (34)	0	186 (64)	
Before this pregnancy	365 (66)	261 (100)	104 (36)	
ART Use History				<0.0001
Newly Diagnosed	186 (34)	0	186 (64)	
Known HIV+, No ART	104 (19)	0	104 (36)	
Known HIV+, On ART	261 (47)	261 (100)	0	

	Total N=551	HIV-infected		P-value
		Initiation before pregnancy N=261	Initiation during pregnancy N=290	
Current ART regimen, self-report				<0.0001
TDF-3TC-EFV	499 (91)	220 (84)	279 (96)	
TDF-3TC-NVP	4 (1)	2 (1)	2 (1)	
Other NNRTI-based regimen	23 (4)	16 (6)	7 (2)	
PI-based regimen	25 (4)	23 (9)	2 (1)	
CD4 cell count, cells/μL*				<0.0001
≤ 200	53 (10)	13 (5)	40 (14)	
201-350	111 (20)	37 (14)	74 (26)	
351-500	122 (22)	53 (20)	69 (24)	
>500	194 (34)	120 (46)	74 (26)	
Missing	*71 (13)*	*38 (15)*	*33 (11)*	
Median (IQR)	433 (298-600)	527 (368-638)	373 (246-519)	
VL, copies/mL*				0.015
<400	458 (83)	234 (90)	224 (77)	
401-1000	14 (3)	5 (2)	9 (3)	
>1000	64 (12)	21 (8)	43 (15)	
Missing	*15 (3)*	*1 (0.4)*	*14 (5)*	
Median (IQR)	20 (20-67)	20 (20-20)	20 (20-100)	
Substance Use				
Substance Use, ever				
Alcohol				0.014
Yes	357 (65)	155 (59)	202 (70)	
No	189 (34)	103 (39)	86 (29)	
Missing	*5 (1)*	*3 (1)*	*2 (1)*	
Smoking				0.123
Yes	56 (10)	21 (8)	35 (12)	
No	490 (89)	237 (91)	253 (87)	
Missing	*5 (1)*	*3 (1)*	*2 (1)*	
Drugs				0.146
Yes	11 (2)	2 (1)	9 (3)	
No	534 (97)	256 (98)	278 (96)	
Missing	*6 (1)*	*3 (1)*	*3 (1)*	
Substance Use, last 30 days				
Alcohol				0.061
Yes	105 (19)	41 (16)	64 (22)	
No	439 (80	216 (83)	223 (77)	
Missing	7 (1)	4 (1)	3 (1)	
Smoking				0.101
Yes	33 (6)	11 (4)	22 (7)	
No	512 (93)	246 (94)	266 (92	
Missing	6 (1)	4 (2)	2 (1)	
Drugs				0.101
Yes	3 (1)	0	3 (1)	
No	542 (98)	257 (98)	285 (98)	
Missing	6 (1)	4 (2)	2 (1)	

* CD4 and VL results abstracted from routine records and are the nearest in time to the first ANC visit

Table 6: Group 1 and Group 2 Measurements

Phase	Measurements Group 1	Measurements Group 2
Baseline	**Routine Care Clinical Record (MCR) Abstraction:** • Booking Visit - Obstetric and neonatal history - Medical and general history - Physical examinations (height, MUAC, weight, blood pressure) - Screening tests (syphilis, HIV, urine, Rhesus, haemoglobin) - Gestational age assessment	**Routine Care Clinical Record (MCR) Abstraction:** • Booking Visit - Obstetric and neonatal history - Medical and general history - Physical examinations (height, MUAC, weight, blood pressure) - Screening tests (syphilis, HIV, urine, Rhesus, haemoglobin) - Gestational age assessment **Study-specific Data Collection:** • Questionnaires - Demographics - Clinical (including obstetric) history - HIV care and ART use - TB care - Substance use. • Physical Examination (standardised measures) - Ultrasound - Anthropometry - Blood Pressure • Specimen Collection - Phlebotomy.
Follow-up	**Routine Care Clinical Record Abstraction:** *Maternity Case Record* • Follow-up antenatal visit notes including blood pressure readings • Obstetric notes - Initial labour assessment (general, abdominal and vaginal examinations) - Clinical notes during labour (2^{nd} – 4^{th} stage) - Newborn assessments (birth outcome, gender, birth anthropometry and delivery complications) - Postpartum notes	**Routine Care Clinical Record Abstraction:** *Maternity Case Record* • Follow-up antenatal visit notes including blood pressure readings • Obstetric notes - Initial labour assessment (general, abdominal and vaginal examinations) - Clinical notes during labour (2^{nd} – 4^{th} stage) - Newborn assessment (birth outcome, gender, birth anthropometry and delivery complications) - Postpartum notes *Infant Road-to-Health Booklet* • Vaccinations • Chemoprophylaxis use • HIV PCR testing

Study-specific Data Collection:

Maternal

- Questionnaires
  - ART use and Adherence, Medical and Obstetric Events
  - Labour and Delivery (at <7days only)
- Physical Examination (standardised measures)
  - Anthropometry (height, weight and MUAC)
  - Blood Pressure
  - Ultrasound (at 28 week visit only)
- Specimen Collection
  - Phlebotomy for storage of plasma and PBMCs
  - Placenta and cord blood (at delivery)
    - Storage of cord blood PBMCs
    - Isolation of PBMCs from decidua membrane for T cells and macrophage subsets identification
    - Tissue section formalin fixing and paraffin embedding for histopathology

Infant

- Questionnaires
  - Medical Events
  - Feeding Practices
  - Development Assessment (at 12 months only)
- Physical Examination (standardised measures)
  - Anthropometry (weight, length, head circumference and MUAC)
- Specimen Collection
  - DBS (at 10 weeks only)
  - Phlebotomy for storage of plasma and PBMCs (at 12 months only)

MUAC Mid-Upper Arm Circumference
PCR Polymerase chain reaction
PBMC Peripheral blood mononuclear cell
DBS Dried blood spots

Table 7: Number of available specimens per study visit

Specimen type	Specimen storage	Visits*								
		A1	A1.5	A2	A3	Del	P1	P2	P3	P4
Maternal										
PBMC**	Sodium Heparin	463	227	445	419		405	412	403	364
	EDTA†	466	-	-	-		344	-	-	-
Plasma	Sodium Heparin	483	236	452	424		407	413	404	366
	EDTA†	499	-	-	-		345	-	-	-
	PAXGene	493	-	-	-		-	-	-	-
Delivery										
Placenta	Block					229				
	OCT††					190				
	RNA Sequencing					176				
	RNA later					146				
Cord Blood										
PBMC**	Sodium Heparin					161				
Plasma	Sodium Heparin					161				
Infant										
DBS***	-						-	228	67	18
PBMC**	Sodium Heparin						-	-	-	225
Plasma	Sodium Heparin						-	-	-	228

* Study Visits - **A1** Enrolment; **A1.5** ~2 weeks post ART initiation; **A2** ~28 weeks gestation; **A3** ~34 weeks gestation; **P1** ~7 days postpartum, **P2** ~ 10 weeks postpartum; **P3** ~ 6 months postpartum; **P4** ~12 months postpartum

** PBMC - Peripheral blood mononuclear cell

*** DBS - Dried blood spots

† EDTA - Ethylenediaminetetraacetic acid

†† OCT - Optimal cutting temperature

Table 8: Placenta scoring algorithm for histopathology and laboratory analyses.

Score	Description	Membranes	Time Received*	Lab Action
1a	Good	Complete	< 7 hours	Process ○ Isolate cells ○ Preserve dissected sections ○ Fix for pathological analysis
1b	Good	Incomplete		
2a	Good	Complete	7 - 12 hours	
2b	Good	Incomplete		
3	Variable	Complete/Incomplete	12 – 24 hours	Process ○ Preserve dissected sections ○ Fix for pathological analysis
4	Variable	Complete/Incomplete	24 – 36 hours	Variable ○ Preserve dissected sections ○ Fix for pathological analysis
5	Variable	Complete/Incomplete	> 36 hours	Do not process ○ Fix for pathological analysis
6	Bad	Complete/Incomplete		Do not process ○ Fix in formalin and discard

* relative to delivery time

Figure Legend

Figure 1: Cohort Profile

Figure 2: Maternal and Infant Specimens

Figure 3: Loss to follow up in Group 2 cohort

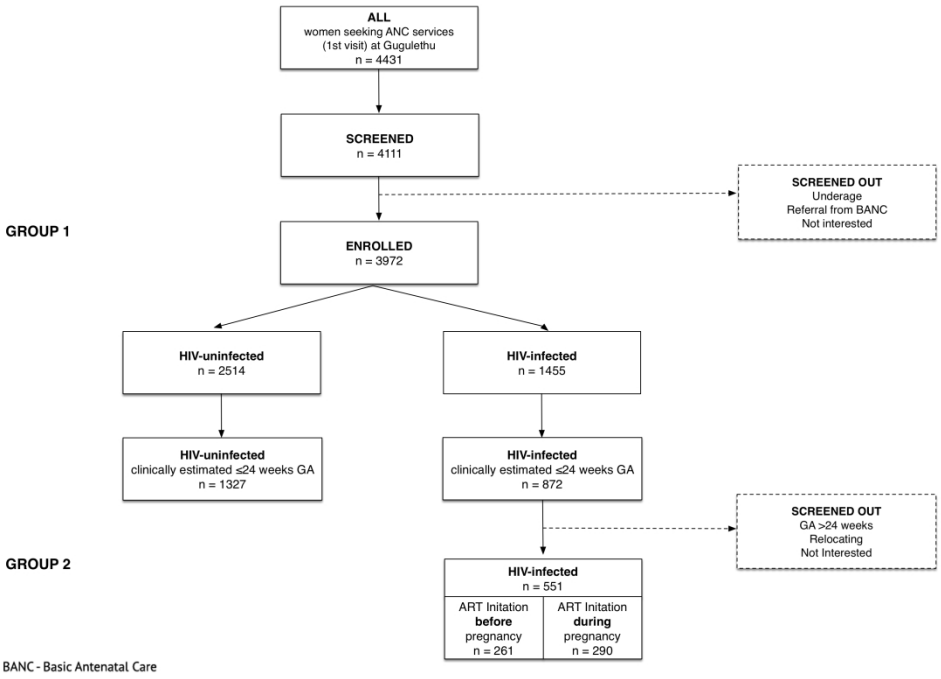

Figure 1: Cohort Profile

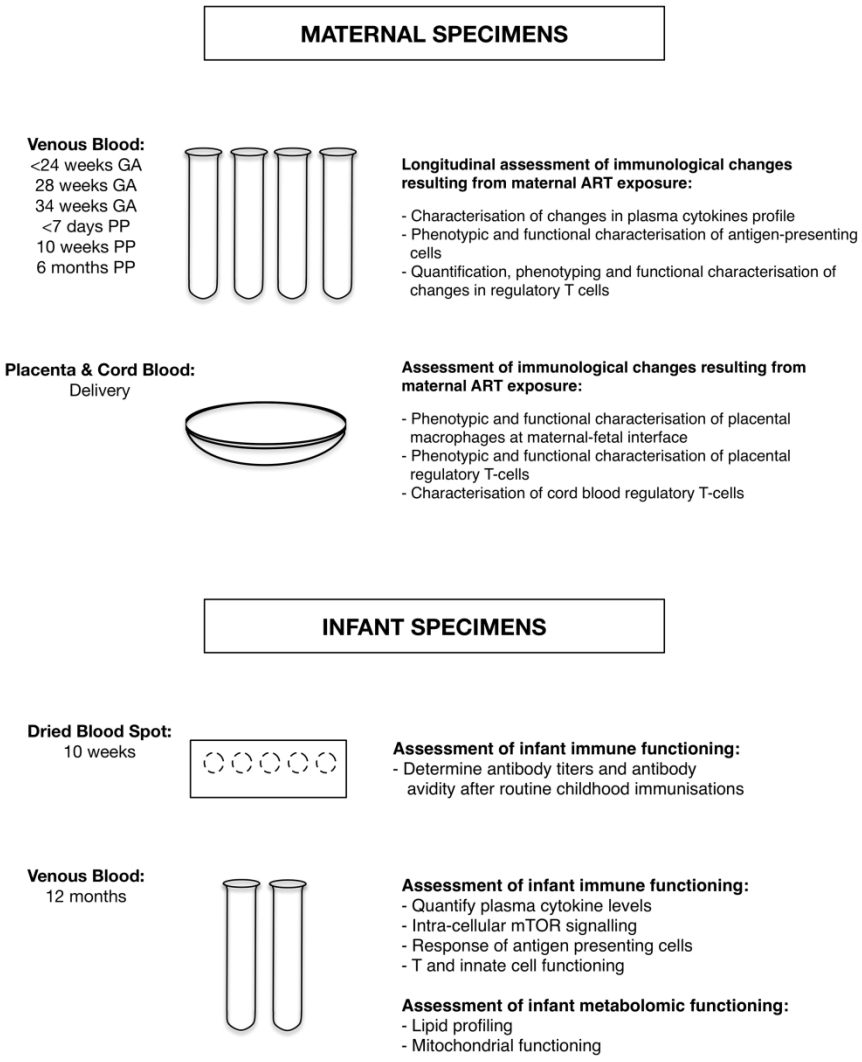

Figure 2: Maternal and Infant Specimens

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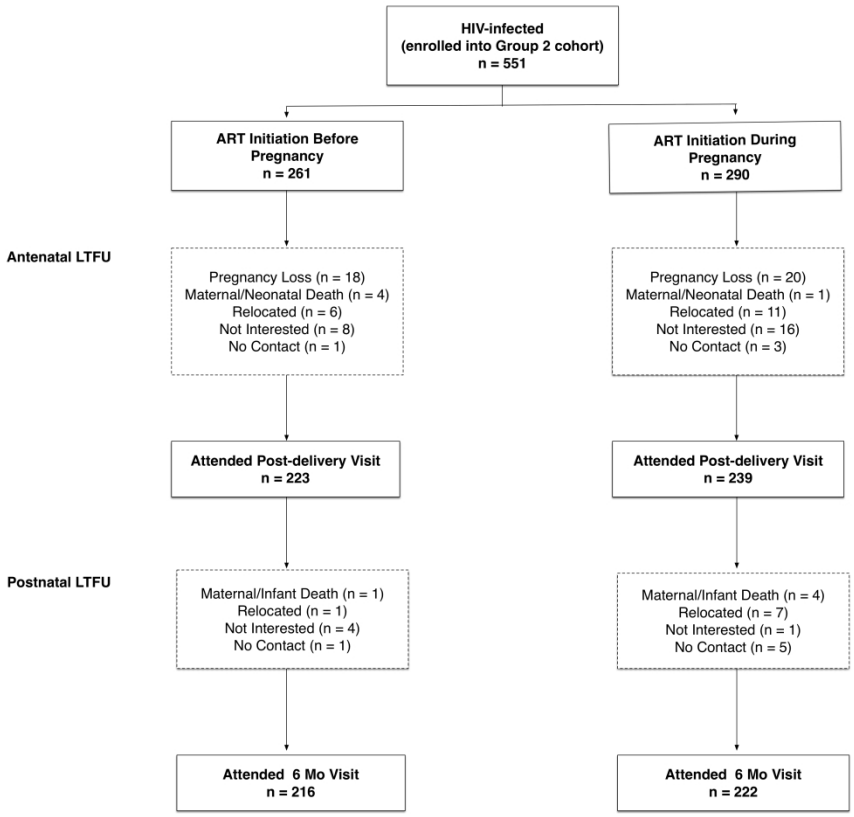

Figure 3: Loss to follow up in Group 2 cohort

591x552mm (200 x 200 DPI)

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Cohort Profile: Prematurity Immunology in Mothers living with HIV and their infants Study (PIMS)

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Cohort Profile: Prematurity Immunology in Mothers living with HIV and their infants Study (PIMS)

Author List: Thokozile R Malaba*[1], Landon Myer[1,2] ,Clive Gray[3], Marie-Louise Newell[4,5], for the PIMS Study Group

Affiliations:

1. Division of Epidemiology and Biostatistics, School of Public Health and Family Medicine, University of Cape Town, South Africa
2. Centre for Infectious Disease Epidemiology and Research, School of Public Health & Family Medicine, University of Cape Town, South Africa
3. Division of Immunology, Institute of Infectious Disease and Molecular Medicine and Department of Pathology, University of Cape Town, Cape Town, South Africa
4. Institute for Developmental Science, Faculty of Medicine, University of Southampton, Southampton, United Kingdom
5. School of Public Health, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

Corresponding Author (*)

Thokozile R Malaba, Division of Epidemiology and Biostatistics, School of Public Health and Family Medicine, Falmouth Building, University of Cape Town Faculty of Health Sciences, Anzio Road, Observatory, Cape Town, South Africa, 7925; Tel: +27 21 650 1424; Email: thoko.malaba@uct.ac.za

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Abstract

Purpose: PIMS is a prospective cohort study in South Africa investigating the association between antiretroviral therapy (ART) use, preterm delivery (PTD)and small-for gestational age (SGA) live births. PIMS main hypotheses are that ART initiation in pregnancy and ART-induced hypertension are associated with PTD and SGA respectively and that reconstitution of cellular immune responses in women on ART from before pregnancy results in increases in PTD of appropriate-for-gestational age (AGA) infants.

Participants: Pregnant women (n=3972) aged ≥18 years regardless of HIV status recruited from 2015 to 2016 into the overall PIMS cohort (2517 HIV-uninfected, 1455 HIV-infected). A nested cohort contained 551 women living with HIV who were ≤24 weeks' GA on ultrasound: 261 initiated ART before pregnancy, 290 initiated during pregnancy.

Findings to date: Women in the overall cohort were followed antenatally through to delivery using routine clinical records; further women in the nested cohort were actively followed up until 12 months postpartum, with data collected on maternal health (HIV care and ART use, clinical care and inter-current clinical history). Other procedures conducted on the nested cohort included physical examinations (anthropometry, blood pressure measurement), assessment of fetal growth (ultrasound), maternal and infant phlebotomy for storage of plasma, RNA and peripheral blood mononuclear cells, collection of delivery specimens (placenta and cord blood), and infant 12 month developmental assessment. Preliminary findings have contributed to our understanding of risk factors for adverse birth outcomes, and the relationship between pregnancy immunology, HIV/ART and adverse birth outcomes.

Future plans: Using specimens collected from study participants living with HIV throughout pregnancy and first year of life, the PIMS provides a valuable platform for answering a variety of research questions focused on temporal changes of immunology markers in women whose immune status is altered by HIV infection, and how ART initiated during pregnancy affects immune responses. The relationship between these immunological changes with adverse birth outcomes as well as possible longer-term impact of exposure to ART in fetal and early life will be explored. Additionally, further active and passive follow-up of mothers and their infants is planned at school-going age and beyond to chart growth, morbidity and development, as well as changes in family circumstances.

Strengths and Limitations

- Robust measurement of gestational age early in pregnancy by research sonographer for the GA assessment in women ≤24 weeks when ultrasound is highly reproducible and accurate.
- Ability to track patients using the Western Cape unique identifier across different health and laboratory services, enables passive long term follow-up of women and their infants; with available data including patient level data (administrative, demographic and clinical), visit level data (clinical observations and findings), laboratory tests and medication.
- Maternal biological specimens, collected three or four times over pregnancy for the cohort of women living with HIV enrolled before 20 weeks gestation, enable immunological, metabolomic and placental investigations will inform understanding of mechanisms underlying adverse birth outcomes in women living with HIV.
- One of the first studies to combine metabolomic and immunologic assessments in infant biological specimens which will provide an integrated model of the immune-metabolism association in HIV-exposed infants and the consequences of maternal metabolic dysregulation for the immune responses of the infant.
- Absence of HIV-uninfected or ART-unexposed comparator groups for immunological investigations, which could hinder distinguishing ART exposure from HIV disease.

Introduction

Antiretroviral drugs in pregnant women living with HIV prevent mother-to-child transmission (PMTCT) and delay HIV disease progression. WHO guidelines now recommend antiretroviral therapy (ART) for all, immediately upon HIV diagnosis, including for women living with HIV (WLHIV) during pregnancy and breastfeeding, to be continued lifelong [1]. However infants born to WLHIV would be exposed to multi-drug ART regimen for prolonged periods at a crucial time during their development [2], which could result in decreased health, developmental, and survival outcomes [3 4]. In high maternal HIV prevalence settings, the increasing population of ART-exposed infants could make the goal of under-five mortality reduction less likely.

Adverse birth outcomes contribute significantly to under-five mortality, as well as infant health and developmental problems [5]. There is an ongoing debate regarding the association between exposure to maternal ART during fetal life and adverse birth outcomes, following reports from Europe [6-9], USA [10] and Africa [11-14] of possible ART-associated increased risk of preterm delivery (PTD), small-for-gestational age (SGA) or low birthweight (LBW) infants. Furthermore, these exposed infants are also at increased risk of acquiring viral infections [15 16], as well as the negative impact of ART on fetal brain development and function [17]. Interpretation of findings from various studies, especially from African settings, is hindered by the reliability of gestational assessment, with ultrasound dating in early pregnancy usually unavailable.

There is limited understanding of general pregnancy-related immune changes in high HIV prevalence settings. Successful pregnancies require intricate fetal-maternal (FM) immune balances, to enable maternal tolerance of the semi-allogeneic fetus; this FM tolerance is primarily maintained by the placenta [18 19]. Consequently, adverse birth outcomes could be hypothesized to be due to placental interface FM tolerance disruption because of cytokine shifts associated with ART initiation causing early initiation of uterine contractions [20]. Additionally, there are suggestions that adverse birth outcomes could also be associated with ART-induced dysregulation of maternal and infant metabolism. In order to meet the specific needs during pregnancy and infancy, metabolism is tightly regulated, but ART is known to interfere with lipid metabolism [21]. The emerging field of immunometabolism has shown that alterations in the lipid profile increases susceptibility to viral infections by skewing immune responses towards an inflammatory profile. The complex interplay between pregnancy, HIV/ART, host immunity, adverse birth outcomes and long-term child health is poorly understood as detailed data are sparse and often related to drug combinations which are no longer be in use. Further research is required to examine epidemiologic and immunological associations and inform understandings of underlying biological mechanisms giving rise to adverse birth outcomes.

An increase in PTD rates, and especially of SGA infants, could impact on the long-term growth and development of children, and would have consequences for the health and wellbeing of their families and population more widely. We therefore aimed to improve understanding of maternal immune profiles during pregnancy in the context of ART use during gestation, adverse birth outcomes and long-term child health in Cape Town, South Africa, an area of high HIV prevalence. This manuscript presents the details of the setting up of the cohort, including aims and objectives and a description of baseline findings along with other preliminary findings.

Aim and Objectives

The primary focus of the PIMS study was to investigate and quantify the risk of preterm and SGA deliveries; with underlying hypotheses that (i) timing of ART use (from before or during pregnancy) is associated with increased risk of PTD, (ii) ART-induced hypertension during pregnancy results in increased risk of SGA and (iii) reconstitution of cellular immune responses during ART in pregnancy results in increases in PTD of appropriate-for-gestational age (AGA) infants. Our secondary focus was to determine long-term (first five years of life) child health outcomes of PTD infants (by weight at gestational age and maternal HIV/ART status). Our hypotheses are that (i) throughout childhood SGA infants are disadvantaged in terms of growth and development compared to preterm AGA infants and (ii) ART use alters maternal and fetal lipid metabolism resulting in susceptibility to infections and alterations in vaccine responses in childhood.

Cohort Description

Setting

Between April 2015 and October 2016, we enrolled pregnant women (aged ≥18 years) at their first antenatal care (ANC) visit in a prospective cohort study, at a single large public sector primary care facility (Gugulethu Midwife Obstetric Unit (MOU)) in a low-income high HIV-prevalence sub-district of Cape Town, South Africa.

Study Design

The overall prospective, observational design includes two 'nested' groups of pregnant women:

- **Group 1:** the overall population of pregnant women (≥18 years) seeking ANC services at Gugulethu MOU during a 18-month period; within this group, a subset of women thought to be ≤24 weeks' gestation based on history or examination (clinical GA) underwent ultrasound scan by a research sonographer for more accurate gestation estimation; enrolled into observation group.
- **Group 2:** all pregnant WLHIV seeking ANC who are ≤24 weeks' gestation at US at their first ANC visit, regardless of current ART use at the first ANC visit (nested within Group 1). Enrolled into longitudinal cohort with data collection through questionnaires, clinical assessments and phlebotomy spanning pregnancy to early infancy.

This study design enables quantification of the risk of adverse birth outcomes in the overall cohort, as well as the more detailed Group 2 group also enabling investigation of the consequences of the immune response following ART initiation in pregnancy for onset of labour and preterm delivery.

Ethical Approval

The study was reviewed and approved by the University of Cape Town Faculty of Health Sciences Human Research Ethics Committee (UCT HREC 739/2014) and the University of Southampton Faculty of Medicine Ethics Committee (12542 PIMS).

Patient Public Involvement

No patient involvement.

Routine Care Services

As part of routine ANC services, gestational age (GA) was estimated based on date of last menstrual period (LMP) and symphysis-fundal height (SFH) by public sector midwives. All women without a previous HIV diagnosis underwent HIV testing, with universal ART eligibility. Women living with HIV conceiving while on ART continued their current regimen throughout pregnancy; regimens included NNRTIs such as efavirenz (EFV) or protease inhibitor (PI, predominantly used after failure of first-line therapy). For women initiating ART in pregnancy, a fixed-dose combination of tenofovir (TDF) + emtricitabine (FTC) + efavirenz (EFV) was used throughout.

Recruitment

Following screening of all women attending their first ANC visit, those ≥18 years were eligible and approached to participate in the study. Following screening, ineligible women were referred back to their ANC clinics in line with the Western Cape Department of Health's health care model. Women who agreed to participate had their routinely collected LMP-based GA and SFH-based GA reviewed by the counsellor; women estimated to be ≤24 weeks were referred for a research ultrasound scan (US) for formal pregnancy dating by a research sonographer blinded to the midwife assessment. Women living with HIV who were ≤24 weeks' gestation on US were then recruited into a nested cohort (Group 2); half of these had initiated life-long ART prior to conception and half initiated ART during pregnancy.

All participants provided written informed consent prior to study participation, with re-consenting of mother-infant pairs at the first postpartum visit for paediatric follow-up. Consent for study participation included data abstraction from routine clinical records through the pregnancy and post-partum period.

Participant Baseline Characteristics

A total of 4431 women registered for ANC during the study recruitment period, of whom 4111 (93%) were screened for the study and 3972 (90%) enrolled; all delivered by May 2017 (Figure 1). Main reasons for being screened out were under-age, referrals from Basic Antenatal Clinics (BANC) or not being interested. Of the enrolled women, 2517 (63.4%) were HIV-uninfected and 1455 (36.6 %) WLHIV (Table 1); 2199 (55.4%) were referred for ultrasound based on their clinical GA, 1327 (60.3%) were HIV-uninfected and 872 (39.7%) living with HIV.

Median age at enrolment was 28 years (IQR 23-32), with HIV-uninfected women younger than WLHIV women, and those initiating ART preconception older than WLHIV initiating ART during pregnancy. In line with age differences, WLHIV were of higher gravidity than HIV-uninfected women, but the difference in parity was small; women who initiated ART pre-pregnancy were of a higher gravidity than those initiating during pregnancy. A quarter (25%) of all women were overweight, while over half (55%) were obese, with little or no difference between groups. Having previously had a preterm delivery was more common among WLHIV than HIV-uninfected women. Mild anaemia was relatively common in all groups, especially in women initiating ART during pregnancy (Table 1). Overall, 3479 (87.6%) women had gestation estimated by LMP, 2327 (58.6%) by SFH and 2334 (58.8%) by ultrasound; with estimated median GA at enrolment visit varying by assessment method (Table 1).

There were 1455 WLHIV in Group 1; 718 (49.3%) were ≤24 weeks on ultrasound, of whom 551 were enrolled into Group 2 (Figure 1). The likelihood of inclusion into Group 2 did not differ by baseline characteristics (Table 2). In comparison to Group 1 participants, Group 2 participants differed in age, gravidity, parity and previous PTD, likely driven by the HIV-uninfected women (Table 3). In multivariable regression allowing for HIV infection, the only difference that persisted between these groups was age (Table 4). When Group 2 women were compared to other WLHIV (not enrolled in Group 2), they were slightly older, more likely to have normal haemoglobin levels (≥11g/dl) and lower gestational age (Table 3). In multivariable regression the only difference between these groups that persisted was gestational age, which was a Group 2 inclusion criterion (Table 4).

Of the 551 WLHIV in Group 2, 261 (47%) initiated before pregnancy and 290 (53%) initiated during pregnancy (Table 5). Women who initiated ART during pregnancy were on average younger, and of lower gravidity. Overall, three-quarters of Group 2 women were overweight or obese, with little difference by ART status. Of the women who initiated ART during pregnancy, the majority (64%) had tested HIV positive in the index pregnancy; the rest had previously tested positive although were not on ART at conception. In line with local and WHO treatment guidelines, most women (91%) were on a regimen of two NRTIs (TDF +FTC), plus NNRTI EFV. PI usage in this cohort was low, at only 9% of women who initiated pre-pregnancy and 1% of women who initiated during pregnancy. Median CD4 count was 433 cells/µl overall, 527 cells/µl in women who initiated ART before pregnancy and 373 cells/µl in women initiating at the first antenatal visit. There were no differences in smoking or

drug usage between these two groups of women, but women who initiated during pregnancy were more likely to have ever consumed alcohol or consumed in the last 30 days (Table 5).

Study Follow-up

At baseline, all women (Group 1) had clinical and medical history, routine first ANC visit physical examination, screening tests and GA assessment data collected via abstraction of the Maternity Case Record (MCR) booklet (Table 5), which is a standardised patient-held maternity record used by all facilities providing maternity services to record clinical data from the antenatal through to postpartum period, including labour. The MCR also serves as a referral letter, thus serving as a link between antenatal and labour care. In addition, the National Health Laboratory Services (NHLS) database was searched for CD4, Viral load results and other laboratory values not recorded in the MCR. Further follow-up for women in Group 1, not eligible for Group 2, was through data abstraction of the MCR following discharge from the postnatal ward (MCR retained at delivery facility). Data was abstracted from follow-up ANC notes, clinical notes during labour and newborn assessments (Table 5).

Women in Group 2 participated in up to eight scheduled study visits, from the start of ANC through to 12 months postpartum. Women on ART from before pregnancy had three antenatal visits at <24 weeks, 28 and 34 weeks; women who initiated ART during pregnancy had an additional study visit two weeks after the ART initiation (which in most women took place on the same day, or close to, the first study visit). Following delivery, women were re-consented for infant participation, and study visits were conducted <7days, 10 weeks, 6 months and 12 months postpartum. At all study visits, data were collected on maternal health (HIV care and ART use, clinical care and inter-current clinical history). Other procedures included physical examinations (anthropometry, blood pressure measurement), phlebotomy (50ml) for storage of plasma, peripheral blood mononuclear cells (PBMC) and RNA; a follow-up ultrasound was conducted at 28 weeks to assess fetal growth (Table 5).

At postpartum study visits, additional data was collected on infant health (including infant feeding and inter-current clinical history) and physical examination of infants was conducted (basic anthropometry). At the 12 month visit, developmental assessment was conducted using the Ages and Stages questionnaire [22] – a general developmental screening tool testing five key areas: personal social, gross motor, fine motor, problem solving and communication skills. Infant specimen collection included Dried Blood Spot (DBS) sampling and storage at 10 weeks study visit, and phlebotomy (2ml) for measurement of immunological functioning and antibody responses to routine childhood immunisations (rotavirus and measles) at 12 month study visit. In addition, data on infant health status, including vaccinations, chemoprophylaxis use (including nevirapine and co-trimoxazole) and routine HIV PCR testing, was abstracted from Road-to-Health Booklets - patient-held booklet taken to all clinical and immunisation visits used to monitor infant growth and development until age 5 years (Table 6).

Further active follow-up of the women and their infants will occur at school-going age and beyond to chart growth, morbidity and development, as well as changes in family circumstances. It is envisaged that subsequent to this, longer term follow-up will be passive through the use of routinely collected data. The Western Cape Provincial Department of Health's public-sector patient administration systems all share unique health identifier [23]; data relating to participants health service contacts, health conditions and health outcomes for specific conditions will be obtained from the Provincial Health Data Centre, which consolidates person-level clinical data across government services [24].

Data Collection

An overview of the main included data collection instruments is presented in Table 6, covering self-reported information on clinical history, ART use and adherence, and medical events; as well as information obtained from routinely collected data.

Specimen Collection

To investigate the proposed hypothesis that immunological changes resulting from maternal ART exposure are associated with adverse birth outcomes, women enrolled into the follow-up cohort were intensively sampled, with repeated phlebotomy throughout pregnancy and the postpartum period for immunological investigations (Table 7). Using samples from all antenatal plasma, inflammatory markers (C-reactive Protein, Serum Amyloid A and CCL10 (IP-10) in women are being measured. Further, following a nested case-control design in Group 2 (n=90), investigations compare women who delivered preterm (PTD cases) or had small-for-gestational age infants (SGA cases) and those from appropriate controls (term AGA) (matched for GA and ART status) (Figure 1). Investigations include longitudinal quantification of plasma cytokine profiles, phenotypic and functional characterisation of regulatory T cells (Tregs), antigen-presenting cells and metabolites associated with mitochondrial functioning and lysophospholipids (Figure 2). The combined studies of these immune parameters will inform understanding of ART use during pregnancy on the areas of the immune system that have been shown to be critically involved in regulating maternal immune tolerance to the fetus, and their associations with onset of labour and preterm delivery.

At delivery, placentas and cord bloods were collected whenever possible, a scoring algorithm was developed which graded placentas and dictated specimen processing according to membrane completeness and time received in laboratory relative to delivery time (Table 8). Using flow cytometry and tissue immunostaining techniques the following investigations will be conducted: examination of the effect of HIV infection/ART exposure on the phenotypic characteristics and functionality of placental macrophages (Hofbauer cells and decidual macrophages) at the maternal-fetal interface and placental Tregs and their association with adverse birth outcomes. Characterization of cord blood Tregs and correlation of their frequency, function and phenotype with placental Tregs and birth outcomes (Figure 2).

Study Retention

Loss to follow-up was categorised based on the last visit before the woman was lost; in total 158 (29%) women were lost to follow-up (LTFU) (Figure 3). Women lost before the post-delivery study visit (n=88), either experienced pregnancy losses (n=37), were no longer interested in participating (n=24) or relocated out of the study area (n=17). For women LTFU between delivery and the 6 month postpartum visit (n=24), reasons included relocation (n=8), not contactable (n=6), no longer interested in participating (n=5) and maternal/infant death (n=5) (Fig 2). For women LTFU between 6 month and 12 month postpartum (n=46), reasons included relocation (n=15), not contactable (n=26), no longer interested in participating (n=2) and maternal/infant death (n=3).
There were no appreciable differences by ART status in women LTFU before post-delivery visit (RR 0.81, 95% CI 0.55 – 1.19). However, women who initiated ART before pregnancy were less likely to be LTFU between delivery and 6 months postpartum (RR0.44, 95% 0.19 – 1.04) and between 6 month and 12 month postpartum (RR0.58, 95% 0.33 – 1.02). No baseline characteristics were associated with LTFU.

Findings to date

Gestational Age Assessment

In the overall cohort, 1787 women with live singleton births were included in the analysis of the association between HIV status and timing of ART initiation and PTD by GA assessment method used (last menstrual period (LMP), measurement of symphysis fundal height (SFH) and ultrasound (US). Using US-GA, PTD risk was associated with maternal HIV infection and ART use, with WLHIV, on ART from before or early pregnancy, almost twice as likely to deliver preterm than HIV-uninfected women [25]. A weaker association was observed when GA assessment was based on SFH; while with

LMP-GA the difference by HIV status was minimal. We did not find any appreciable differences in the PTD risk for WLHIV by timing of ART initiation across all three assessment methods [25]. Our findings (in both the overall cohort and in women with all three assessments) suggest that methods of GA assessment explain at least partially the heterogeneity of findings from previous studies on the association between ART use and adverse birth outcomes, suggesting that care should be taken when interpreting results from such studies.

Obesity

In the overall cohort, 2921 women with live singleton births were included in the analysis of the association between maternal body mass index and adverse birth outcomes. In a subset cohort the association between gestational weight gain (GWG) and adverse birth outcomes was examined. Maternal obesity was associated with increased likelihood of having high birthweight and large size for gestational age infants. In the subset cohort, GWG was associated with increased likelihood of spontaneous PTD and high birth weight infants [26]. Obesity during pregnancy is prevalent in this setting and appears associated with increased risk of adverse birth outcomes in both WLHIV and HIV-uninfected women.

Placental Pathology

Preliminary analysis of placental histopathology from a sub-set of women enrolled in the prospective cohort showed significant associations between placental pathology and adverse birth outcomes: presence of focal infarction was associated with increased risk of low birthweight (LBW); the lower the weight of the basal plate weight lead to increased risk of LBW, PTD and SGA; and prolonged meconium exposure was associated with increased risk of SGA. These findings suggest that adverse birth outcomes are driven primarily by placental abnormalities which do not appear to be associated with the ART initiation timing [27]. Immunofluorescence and immunohistochemistry staining were performed on these wax blocks to identify regulatory T cells along with macrophages. Further analysis is ongoing.

Within the placenta, investigation of the distribution of pro-inflammatory (M1) and anti-inflammatory (M2) placental macrophages at the maternal-fetal interface showed no differences in the tissue density of these macrophages within the decidual membranes and villous tissue according to timing of ART initiation. Data suggest that the Hofbauer Cells (which are fetal macrophages) are not polarized into M1/M2 phenotypes but are rather “intermediate” types [28].

Strengths and limitations

Key strengths of the PIMS study include the recruitment of a large community-based cohort in an area of high HIV prevalence; the observational nature of the study provides good external validity of experiences of a public sector primary care population over pregnancy. A further strength lies in the use of a research sonographer for the GA assessment in women ≤24 weeks when US is highly reproducible and accurate (while routinely used clinical assessments are less reliable) which is particularly important when studying associations with adverse birth outcomes, as compromises in outcome ascertainment methods can affect the detection of the magnitude of associations.

Additionally, the maternal biological specimen from Group 2 at three or four times (depending on ART status group) throughout pregnancy and at delivery is an important strength because it enables immunological, metabolomic and placental investigations to inform understanding of mechanisms underlying adverse birth outcomes in in women living with HIV. As pregnancy is a state of immunoregulation requiring tolerance of a semi-allogeneic fetus, the assessment of placentas of enrolled women provides a unique opportunity to investigate the link between HIV, ART and adverse birth outcomes. Collection of infant specimens further strengthens the study as it is one of the first studies combining metabolomic and immunologic assessments. This will provide an integrated model of the immune-metabolism association in HIV-exposed infants and the consequences of maternal metabolic dysregulation for the immune responses of the infant. Furthermore, the developmental assessments carried out in the infants provides the opportunity to consider the

association between maternal immune function during pregnancy and early childhood immunological and developmental outcomes.

PIMS has valuable sub-designs in addition to the observational study design with the overall cohort stratified by maternal HIV status, ART use, and related risk factors, with all details to be analysed by timing of ART initiation in line with the main PIMS objectives. One sub-design is the cohort study in HIV-infected women, who have data collection through questionnaires, clinical assessments and phlebotomy spanning pregnancy to early infancy. This study design enables quantification of the risk of adverse birth outcomes in the overall cohort, as well as the more detailed Group 2 group also enabling investigation of the consequences of the immune response following ART initiation in pregnancy for onset of labour and preterm delivery. Another sub-design is the nested case–control study which will enable immunological investigations in women who did and did not delivery preterm/SGA infant. The ability to track patients using the Western Cape unique identifier across different health and laboratory services, enables the passive long term follow-up of the Group 2 women and their infants; with available data including patient level data (administrative, demographic and clinical), visit level data (clinical observations and findings), laboratory tests and medication.

A limitation of the study is while HIV-uninfected women are included in Group 1, providing a comparison group for birth outcome and various maternal characteristics, Group 2 did not include HIV-uninfected or ART-unexposed comparator groups. As such the detailed immunological analyses over pregnancy are limited to WLHIV, with timing of ART initiation a main explanatory variable. Additionally, we do not have full detailed information on all Group 1 women and were instead limited by routinely collected data in medical notes some information relating to maternal characteristics was based on self-report and thus subject to potential biases. In order to address this limitation, routinely collected data was also collected to confirm data on birth outcomes.

Findings to date

Using data collected from study participants living with HIV throughout pregnancy and first year of life, PIMS provides a valuable platform for answering a variety of research questions related to maternal and child health. In particular, PIMS well equipped to investigate temporal changes of immunology markers in women whose immune status is altered by HIV infection, and how ART initiated during pregnancy affects immune responses. The relationship between these immunological changes with adverse birth outcomes as well as possible longer-term impact of exposure to ART in fetal and early life will be explored. Additionally, through use of the Western Cape Department of Health unique identifier further active and passive follow-up of mothers and their infants is planned at school-going age and beyond to chart growth, morbidity and development, as well as changes in family circumstances.

The PIMS Study Investigators welcome new collaborations with other investigators interested in using study data and stored specimens. Interested investigators should contact Professors Marie-Louise Newell (M.Newell@soton.ac.uk) and Landon Myer (Landon.Myer@uct.ac.za) to obtain additional information and discuss collaborative opportunities. Proposed projects and data analyses plans will be reviewed to ensure no overlap with planned projects and efficient use of data and specimens.

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Competing Interests Statement

There are no competing interests for any author

Data Availability Statement

The data collected in this study will be available to external investigators interested in collaboration upon submission and approval of a data analysis plan. The samples are being used by the named investigators, but remaining samples can be made available to external users. Requests for data and available samples within PIMS, or to submit a request for additional data collection, should be submitted to M.Newell@soton.ac.uk and Landon.Myer@uct.ac.za and will be reviewed by the study steering committee.

Contributorship Statement

TRM, LM, CG and MLN: Conceptualization and design of the study
TRM: Study conduct, data collection and statistical analysis.
TRM and MLN: Interpretation of data and writing the manuscript
TRM, LM, CG and MLN review and editing

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Table 1: Baseline Characteristics of Group 1 women at 1st antenatal care visit (n=3972)

	Total N=3972	HIV-uninfected N=2517	HIV-infected N=1455	P value	HIV-infected N= 1455		P value
					Initiated before pregnancy N=722	Initiated during pregnancy N=733	
Age, years				<0.0001			<0.0001
<24	1273 (32)	987 (39)	286 (20)		112 (16)	174 (24)	
25-29	1108 (28)	702 (28)	406 (28)		157 (22)	249 (34)	
>30	1591 (40)	828 (33)	763 (52)		453 (63)	310 (42)	
Median	28 (23-32)	26 (22-31)	30 (25-34)		31 (27–35)	28 (25-32)	
Height, cm				0.221			0.562
≤155	1173 (30)	723 (29)	450 (31)		220 (30)	230 (31)	
156-161	1344 (34)	873 (35)	471 (32)		245 (34)	226 (31)	
≥162	1049 (26)	661 (26)	388 (27)		190 (27)	198 (27)	
Missing	*406 (10)*	*256 (10)*	*147 (10)*		*67 (9)*	*79 (11)*	
Median	158 (154-162)	158 (154-162)	158 (154-162)		158 (154-162)	158 (154-162)	
Body Mass Index, kg/m^2				0.666			0.535
Underweight (<18.5)	26 (0.7)	15 (0.6)	11 (0.7)		8 (1)	3 (0.4)	
Normal (18.5-24.9)	718 (18)	457 (18)	261 (18)		124 (17)	137 (19)	
Overweight (25.0-29.9)	1007 (25)	625 (25)	381 (26)		202 (28)	180 (25)	
Obese (>30.0)	1790 (55)	1148 (47)	801 (55)		313 (43)	329 (55)	
Missing	*431 (11)*	*272 (11)*	*159 (11)*		*75 (10)*	*84 (11)*	
Median	30 (26-35)	30 (26-35)	30 (26-35)		30 (26-35)	30 (25-35)	
Gravidity				<0.0001			0.002
1	967 (24)	726 (29)	241 (17)		100 (14)	141 (19)	
2	1347 (34)	849 (34)	498 (34)		238 (33)	260 (35)	
≥3	1567 (39)	887 (35)	680 (47)		368 (51)	312 (43)	
Missing	*91 (2)*	*55 (2)*	*36 (3)*		*16 (2)*	*20 (3)*	
Median	2 (1-3)	2 (1-3)	2 (2-3)		3 (2-3)	2 (2-3)	
Parity				<0.0001			0.011
0	1194 (30)	865 (34)	329 (23)		147 (20)	182 (25)	
1	1458 (37)	898 (36)	560 (38)		270 (37)	290 (40)	
≥2	1228 (31)	699 (28)	529 (36)		289 (40)	240 (33)	
Missing	*92 (2)*	*55 (0.1)*	*37 (3)*		*16 (2)*	*21 (3)*	
Median	1 (0-2)	1 (0-2)	1 (1-2)		1 (1-2)	1 (0-2)	
Previous Preterm*				0.013			0.617
Yes	280 (7)	159 (6)	121 (8)		63 (9)	58 (8)	
Haemoglobin g/dl				<0.0001			<0.0001
Normal (≥11.0)	1446 (36)	961 (38)	485 (33)		289 (40)	196 (27)	
Mild Anaemia (9-10.9)	1067 (27)	638 (25)	429 (29)		177 (25)	252 (34)	
Moderate Anaemia (7-8.9)	229 (6)	109 (4)	120 (8)		47 (7)	73 (10)	
Severe Anaemia (<7)	5 (0.1)	4 (0.2)	1 (0.1)		0	1 (0.1)	
Missing	*1225 (31)*	*805 (32)*	*420 (29)*		*209 (29)*	*211 (29)*	
Gestational Age Assessment*							
LMP	3479 (88)	2219 (88)	1260 (87)	0.150	642 (89)	618 (84)	0.010
Median (weeks)	17 (12-22)	17 (12–23)	16 (11-22)		15 (11-22)	17 (12-22)	
SFH	2327 (59)	1447 (57)	880 (60)	0.065	403 (56)	477 (65)	<0.0001
Median (weeks)	23 (18-28)	23 (19-28)	22 (17-27)		22 (16-27)	23 (18-28)	
US	2334 (59)	1411 (56)	923 (63)	<0.0001	433 (60)	490 (67)	0.006
Median (weeks)	16 (12-21)	16 (12-21)	15 (11-20)		14 (10-19)	17 (12-21)	

n (%)

* LMP – Last menstrual period; SFH – Symphysis fundal height, US – Ultrasound

Table 2: Baseline Characteristics of Group 2 eligible but not enrolled vs Group 2 women at 1st antenatal care visit

	Group 2 Eligible N=718		P-value
	Not Enrolled N=167*	Enrolled N=551	
Age, years			0.274
<24	37 (22)	92 (17)	
25-29	44 (26)	156 (28)	
>30	86 (52)	303 (55)	
Median	30 (25-33)	30 (26-34)	
Height, cm			0.395
≤155	53 (32)	166 (33)	
156-161	54 (32)	191 (35)	
≥162	33 (20)	145 (26)	
Missing	*27 (16)*	*49 (9)*	
Median	157 (153-161)	158 (154-162)	
Body Mass Index, kg/m^2			0.920
Underweight (<18.5)	1 (0.01)	5 (0.01)	
Normal (18.5-24.9)	32 (19)	103 (19)	
Overweight (25.0-29.9)	35 (21)	134 (24)	
Obese (>30.0)	71 (43)	255 (46)	
Missing	*28 (17)*	*54 (10)*	
Median	30 (25-34)	30 (25-36)	
Gravidity			0.204
1	35 (21)	90 (16)	
2	57 (34)	184 (33)	
≥3	67 (40)	263 (48)	
Missing	*8 (5)*	*14 (3)*	
Median	2 (2-3)	2 (2-3)	
Parity			0.290
0	48 (29)	129 (23)	
1	62 (37)	222 (40)	
≥2	49 (29)	185 (34)	
Missing	*8 (5)*	*15 (3)*	
Median	1 (0-2)	1 (1-2)	
Previous Preterm**			0.100
Yes	9 (6)	52 (9)	
Haemoglobin g/dl			0.084
Normal (≥11.0)	46 (28)	232 (42)	
Mild Anaemia (9-10.9)	44 (26)	153 (28)	
Moderate Anaemia (7-8.9)	11 (7)	26 (5)	
Severe Anaemia (<7)	0	0	
Missing	*66 (40)*	*140 (25)*	
ART Status			0.577
Initiated before pregnancy	55 (92)	290 (53)	
Initiated during pregnancy	75 (45)	261 (47)	
Gestational Age Assessment***			
LMP	148 (89)	481 (87)	0.648
Median (weeks)	13 (10–17)	13 (9-17)	
SFH	87 (57)	258 (47)	0.232
Median (weeks)	17 (14-20)	17 (14-20)	
US	167 (100)	551 (100)	
Median (weeks)	14 (10-18)	13 (10-17)	

n (%)

* Eligible for Group 2 based on US, but not enrolled into Group 2

** Among women with a previous pregnancy

*** LMP – Last menstrual period; SFH – Symphysis fundal height, US – Ultrasound

Table 3: Baseline Characteristics of Group 1 vs Group 2 women at 1st antenatal care visit

	Group 1		Group 2	P-value Grp1 vs Grp2	P-value HIV+ vs Grp2
	HIV-uninfected N=2517	HIV-infected* N=904	HIV-Infected N=551		
Age, years				<0.0001	0.078
<24	987 (39)	194 (21)	92 (17)		
25-29	702 (28)	250 (28)	156 (28)		
>30	828 (33)	460 (51)	303 (55)		
Median	26 (22-31)	30 (25-33)	30 (26-34)		
Height, cm				0.726	0.465
≤155	723 (29)	284 (31)	166 (30)		
156-161	873 (35)	280 (31)	191 (35)		
≥162	661 (26)	243 (27)	145 (26)		
Missing	*260 (10)*	*97 (11)*	*49 (9)*		
Median	158 (154-162)	158 (154-163)	158 (154-162)		
Body Mass Index, kg/m^2				0.756	0.456
Underweight (<18.5)	15 (0.6)	6 (0.7)	5 (1)		
Normal (18.5-24.9)	457 (18)	158 (17)	103 (19)		
Overweight (25.0-29.9)	625 (25)	248 (27)	134 (24)		
Obese (>30.0)	1148 (47)	387 (43)	255 (46)		
Missing	*272 (11)*	*105 (12)*	*54 (10)*		
Median	30 (26-35)	30 (26-35)	30 (25-34)		
Gravidity				<0.0001	0.820
1	726 (29)	151 (17)	90 (16)		
2	849 (34)	314 (35)	184 (33)		
≥3	887 (35)	417 (46)	263 (48)		
Missing	*55 (2)*	*22 (2)*	*14 (3)*		
Median	2 (1-3)	2 (2-3)	2 (2-3)		
Parity				0.004	0.236
0	865 (34)	200 (22)	129 (23)		
1	898 (36)	338 (37)	222 (40)		
≥2	699 (28)	344 (38)	185 (34)		
Missing	*55 (0.1)*	*22 (2)*	*15 (3)*		
Median	1 (0-2)	1 (1-2)	1 (1-2)		
Previous Preterm**				<0.0001	0.182
Yes	159 (6)	69 (8)	52 (9)		
Haemoglobin g/dl				0.007	<0.0001
Normal (≥11.0)	961 (38)	253 (28)	232 (42)		
Mild Anaemia (9-10.9)	638 (25)	276 (31)	153 (28)		
Moderate Anaemia (7-8.9)	109 (4)	94 (10)	26 (5)		
Severe Anaemia (<7)	4 (0.2)	1 (0.1)	0		
Missing	*805 (32)*	*280 (31)*	*140 (25)*		
Gestational Age Assessment***					
LMP	2219 (88)	779 (86)	481 (87)	0.823	0.542
Median (weeks)	17 (12–23)	19 (13-24)	13 (9-17)		
SFH	1447 (57)	622 (69)	258 (47)	<0.0001	<0.0001
Median (weeks)	23 (19-28)	25 (21-29)	17 (14-20)		
US	1411 (56)	376 (42)	551 (100)	<0.0001	<0.0001
Median (weeks)	16 (12-21)	21 (13-25)	13 (10-17)		

n (%)

* All HIV-infected woman not included in Group 2

** Among women with a previous pregnancy

*** LMP – Last menstrual period; SFH – Symphysis fundal height, US – ultrasound

Table 4: Baseline characteristics associated with inclusion in Group 2 at 1st antenatal care visit

	OR Group 1 (Ref) vs Grp 2*				OR Group 1 HIV+ (Ref) vs Group 2**			
	OR	**P-values**	**aOR (95% CI)**	**P-values**	**OR**	**P-values**	**aOR (95% CI)**	**P-values**
Age, years								
<24	Reference		Reference		Reference		Reference	
25-29	2.10 (1.60-2.76)	<0.0001	2.23 (1.64-3.05)	<0.0001	1.32 (0.96-1.81)	0.091	1.09 (0.65-1.83)	0.758
>30	3.02 (2.36-3.86)	<0.0001	3.37 (2.45-4.63)	<0.0001	1.39 (1.04-1.85)	0.025	1.09 (0.66-1.81)	0.740
Body Mass Index, kg/m^2								
Underweight (<18.5)	Reference		Reference		Reference		Reference	
Normal (18.5-24.9)	0.70 (0.26-1.91)	0.489	0.75 (0.27-2.06)	0.573	0.78 (0.23-2.63)	0.691	1.02 (0.14-7.37)	0.988
Overweight (25.0-29.9)	0.64 (0.24-1.74)	0.386	0.60 (0.22-1.65)	0.324	0.65 (0.19-2.16)	0.481	1.34 (0.19-9.65)	0.773
Obese (>30.0)	0.70 (0.26-1.87)	0.474	0.56 (0.20-1.52)	0.255	0.79 (0.24-2.62)	0.701	1.10 (0.15-7.84)	0.923
Gravidity								
1	Reference		Reference		Reference		Reference	
2	1.54 (1.18-2.01)	0.001	0.99 (0.73-1.34)	0.929	0.98 (0.72-1.35)	0.917	1.08 (0.64-1.82)	0.765
≥3	1.97 (1.52-2.53)	<0.0001	0.94 (0.68-1.30)	0.716	1.05 (0.78-1.43)	0.715	1.09 (0.64-1.86)	0.746
Previous Preterm***								
Yes	1.50 (1.09-2.06)	0.012	1.19 (0.84-1.68)	0.318	1.29 (0.89-1.88)	0.183	1.25 (0.69-2.26)	0.468
ART Status	-		-	-				
Initiated during pregnancy	-		-	-	Reference		Reference	
Initiated before pregnancy	-		-	-	0.86 (0.70-1.07)	0.180	1.07 (0.76-1.51)	0.705
Gestational Age	-		-	-				
Weeks	-		-	-	0.86 (0.83-0.88)	<0.0001	0.84 (0.82-0.87)	<0.0001

* Comparison between all enrolled pregnant women (≥18 years) seeking ANC services at Gugulethu MOU (Group 1) (n=3421) vs HIV-infected pregnant women ≤24 weeks' gestation enrolled into cohort (Group 2) (n=551)

** Comparison between all enrolled HIV-infected pregnant women (≥18 years) seeking ANC services at Gugulethu MOU (Group 1) not enrolled in cohort (n=167) vs HIV-infected pregnant women ≤24 weeks' gestation enrolled into cohort (Group 2) (n=551)

*** Among women with a previous pregnancy

Table 5: Baseline Characteristics of Group 2 women at 1st antenatal care visit (n=551)

	Total N=551	HIV-infected		P-value
		Initiation before pregnancy N=261	Initiation during pregnancy N=290	
Maternal Characteristics				
Age, years				<0.0001
≤24	92 (17)	25 (10)	67 (23)	
25-29	156 (28)	58 (22)	98 (34)	
≥30	303 (55)	178 (68)	125 (43)	
Median (IQR)	30 (26-34)	32 (28-36)	29 (25-32)	
Education (Finished High School)	164 (30)	96 (33)	69 (26)	0.088
Employment Status				0.767
Employed	238 (46)	114 (44)	124 (43)	
Missing	*2 (0.4)*	*2 (1)*	*0*	
SES				0.694
Lowest	175 (32)	82 (31)	93 (32)	
Medium	175 (32)	88 (34)	87 (30)	
Highest	189 (34)	87 (33)	102 (35)	
Missing	*12 (2)*	*4 (3)*	*8 (3)*	
Obstetric Characteristics				
Gravidity				<0.0001
1	88 (16)	29 (11)	59 (20)	
2	187 (34)	78 (30)	109 (38)	
≥3	276 (50)	154 (59)	122 (42)	
Median (IQR)	2 (2-3)	3 (2-4)	2 (2-3)	
Parity				0.061
0	123 (22)	49 (18)	74 (26)	
1	220 (40)	99 (38)	121 (42)	
≥2	208 38)	113 (43)	95 (33)	
Median (IQR)	1 (1-2)	1 (1-2)	1 (0-2)	
Height, cm				0.858
≤155	130 (24)	64 (25)	66 (23)	
156-161	208 (38)	96 (37)	112 (39)	
≥162	208 (38)	99 (38)	109 (38)	
Missing	*5 (0.9)*	*2 (0.8)*	*3 (1)*	
Median (IQR)	160 (156-164)	159 (155-163)	160 (156-164)	
Body Mass Index, kg/m^2				0.591
Underweight (<18.5)	6 (1)	3 (1)	3 (1)	
Normal (18.5-24.9)	110 (20)	47 (18)	63 (22)	
Overweight (25.0-29.9)	148 (27)	76 (29)	72 (25)	
Obese (>30.0)	282 (51)	133 (51)	149 (51)	
Missing	*5 (0.9)*	2 (0.8)	1 (0.3)	
Median (IQR)	30 (26-35)	30 (26-34)	30 (25-35)	
Median Gestation (completed weeks)	14 (11-18)	13 (11-17)	14 (10-18)	0.054
HIV				
First Tested HIV positive				<0.0001
In this pregnancy	186 (34)	0	186 (64)	
Before this pregnancy	365 (66)	261 (100)	104 (36)	
ART Use History				<0.0001
Newly Diagnosed	186 (34)	0	186 (64)	
Known HIV+, No ART	104 (19)	0	104 (36)	
Known HIV+, On ART	261 (47)	261 (100)	0	

	Total N=551	HIV-infected		P-value
		Initiation before pregnancy N=261	Initiation during pregnancy N=290	
Current ART regimen, self-report				<0.0001
TDF-3TC-EFV	499 (91)	220 (84)	279 (96)	
TDF-3TC-NVP	4 (1)	2 (1)	2 (1)	
Other NNRTI-based regimen	23 (4)	16 (6)	7 (2)	
PI-based regimen	25 (4)	23 (9)	2 (1)	
CD4 cell count, cells/μL*				<0.0001
≤ 200	53 (10)	13 (5)	40 (14)	
201-350	111 (20)	37 (14)	74 (26)	
351-500	122 (22)	53 (20)	69 (24)	
>500	194 (34)	120 (46)	74 (26)	
Missing	*71 (13)*	*38 (15)*	*33 (11)*	
Median (IQR)	433 (298-600)	527 (368-638)	373 (246-519)	
VL, copies/mL*				0.015
<400	458 (83)	234 (90)	224 (77)	
401-1000	14 (3)	5 (2)	9 (3)	
>1000	64 (12)	21 (8)	43 (15)	
Missing	*15 (3)*	*1 (0.4)*	*14 (5)*	
Median (IQR)	20 (20-67)	20 (20-20)	20 (20-100)	
Substance Use				
Substance Use, ever				
Alcohol				0.014
Yes	357 (65)	155 (59)	202 (70)	
No	189 (34)	103 (39)	86 (29)	
Missing	*5 (1)*	*3 (1)*	*2 (1)*	
Smoking				0.123
Yes	56 (10)	21 (8)	35 (12)	
No	490 (89)	237 (91)	253 (87)	
Missing	*5 (1)*	*3 (1)*	*2 (1)*	
Drugs				0.146
Yes	11 (2)	2 (1)	9 (3)	
No	534 (97)	256 (98)	278 (96)	
Missing	*6 (1)*	*3 (1)*	*3 (1)*	
Substance Use, last 30 days				
Alcohol				0.061
Yes	105 (19)	41 (16)	64 (22)	
No	439 (80	216 (83)	223 (77)	
Missing	7 (1)	4 (1)	3 (1)	
Smoking				0.101
Yes	33 (6)	11 (4)	22 (7)	
No	512 (93)	246 (94)	266 (92	
Missing	6 (1)	4 (2)	2 (1)	
Drugs				0.101
Yes	3 (1)	0	3 (1)	
No	542 (98)	257 (98)	285 (98)	
Missing	6 (1)	4 (2)	2 (1)	

n (%)

* CD4 and VL results abstracted from routine records and are the nearest in time to the first ANC visit

Table 6: Group 1 and Group 2 Measurements

Phase	Measurements Group 1	Measurements Group 2
Baseline	**Routine Care Clinical Record (MCR) Abstraction:** • Booking Visit - Obstetric and neonatal history - Medical and general history - Physical examinations (height, MUAC, weight, blood pressure) - Screening tests (syphilis, HIV, urine, Rhesus, haemoglobin) - Gestational age assessment	**Routine Care Clinical Record (MCR) Abstraction:** • Booking Visit - Obstetric and neonatal history - Medical and general history - Physical examinations (height, MUAC, weight, blood pressure) - Screening tests (syphilis, HIV, urine, Rhesus, haemoglobin) - Gestational age assessment **Study-specific Data Collection:** • Questionnaires - Demographics - Clinical (including obstetric) history - HIV care and ART use - TB care - Substance use. • Physical Examination (standardised measures) - Ultrasound - Anthropometry - Blood Pressure • Specimen Collection - Phlebotomy.
Follow-up	**Routine Care Clinical Record Abstraction:** *Maternity Case Record* • Follow-up antenatal visit notes including blood pressure readings • Obstetric notes - Initial labour assessment (general, abdominal and vaginal examinations) - Clinical notes during labour (2^{nd} – 4^{th} stage) - Newborn assessments (birth outcome, gender, birth anthropometry and delivery complications) - Postpartum notes	**Routine Care Clinical Record Abstraction:** *Maternity Case Record* • Follow-up antenatal visit notes including blood pressure readings • Obstetric notes - Initial labour assessment (general, abdominal and vaginal examinations) - Clinical notes during labour (2^{nd} – 4^{th} stage) - Newborn assessment (birth outcome, gender, birth anthropometry and delivery complications) - Postpartum notes *Infant Road-to-Health Booklet* • Vaccinations • Chemoprophylaxis use • HIV PCR testing

Study-specific Data Collection:

Maternal

- Questionnaires
  - ART use and Adherence, Medical and Obstetric Events
  - Labour and Delivery (at <7days only)
- Physical Examination (standardised measures)
  - Anthropometry (height, weight and MUAC)
  - Blood Pressure
  - Ultrasound (at 28 week visit only)
- Specimen Collection
  - Phlebotomy for storage of plasma and PBMCs
  - Placenta and cord blood (at delivery)
    - Storage of cord blood PBMCs
    - Isolation of PBMCs from decidua membrane for T cells and macrophage subsets identification
    - Tissue section formalin fixing and paraffin embedding for histopathology

Infant

- Questionnaires
  - Medical Events
  - Feeding Practices
  - Development Assessment (at 12 months only)
- Physical Examination (standardised measures)
  - Anthropometry (weight, length, head circumference and MUAC)
- Specimen Collection
  - DBS (at 10 weeks only)
  - Phlebotomy for storage of plasma and PBMCs (at 12 months only)

MUAC Mid-Upper Arm Circumference
PCR Polymerase chain reaction
PBMC Peripheral blood mononuclear cell
DBS Dried blood spots

Table 7: Number of available specimens per study visit

Specimen type	Specimen storage	Visits*								
		A1	A1.5	A2	A3	Del	P1	P2	P3	P4
Maternal										
PBMC**	Sodium Heparin	463	227	445	419		405	412	403	364
	EDTA†	466	-	-	-		344	-	-	-
Plasma	Sodium Heparin	483	236	452	424		407	413	404	366
	EDTA†	499	-	-	-		345	-	-	-
	PAXGene	493	-	-	-		-	-	-	-
Delivery										
Placenta	Block					229				
	OCT††					190				
	RNA Sequencing					176				
	RNA later					146				
Cord Blood										
PBMC**	Sodium Heparin					161				
Plasma	Sodium Heparin					161				
Infant										
DBS***	-						-	228	67	18
PBMC**	Sodium Heparin						-	-	-	225
Plasma	Sodium Heparin						-	-	-	228

* Study Visits - **A1** Enrolment; **A1.5** ~2 weeks post ART initiation; **A2** ~28 weeks gestation; **A3** ~34 weeks gestation; **P1** ~7 days postpartum, **P2** ~ 10 weeks postpartum; **P3** ~ 6 months postpartum; **P4** ~12 months postpartum

** PBMC - Peripheral blood mononuclear cell

*** DBS - Dried blood spots

† EDTA - Ethylenediaminetetraacetic acid

†† OCT - Optimal cutting temperature

Table 8: Placenta scoring algorithm for histopathology and laboratory analyses.

Score	Description	Membranes	Time Received*	Lab Action
1a	Good	Complete	< 7 hours	Process ○ Isolate cells ○ Preserve dissected sections ○ Fix for pathological analysis
1b	Good	Incomplete		
2a	Good	Complete	7 - 12 hours	
2b	Good	Incomplete		
3	Variable	Complete/Incomplete	12 – 24 hours	Process ○ Preserve dissected sections ○ Fix for pathological analysis
4	Variable	Complete/Incomplete	24 – 36 hours	Variable ○ Preserve dissected sections ○ Fix for pathological analysis
5	Variable	Complete/Incomplete	> 36 hours	Do not process ○ Fix for pathological analysis
6	Bad	Complete/Incomplete		Do not process ○ Fix in formalin and discard

* relative to delivery time

Figure Legend

Figure 1: Cohort Profile

Figure 2: Maternal and Infant Specimens

Figure 3: Loss to follow up in Group 2 cohort

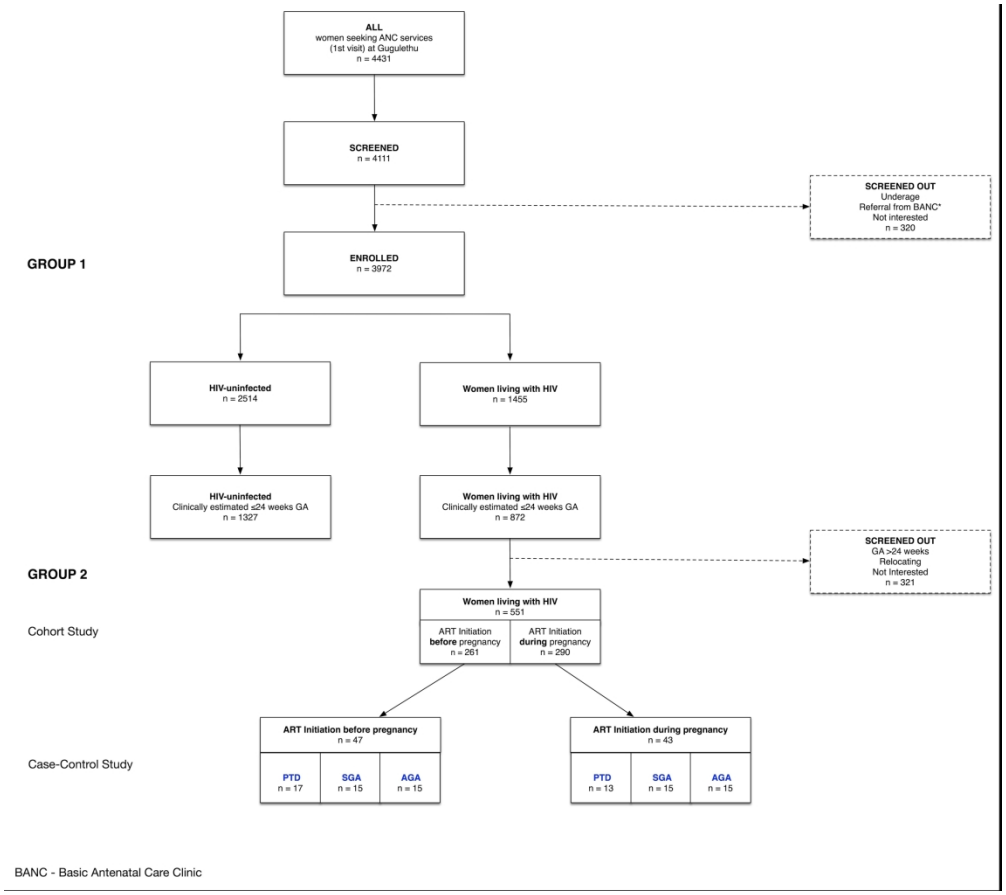

Figure 1: Cohort Profile

238x211mm (300 x 300 DPI)

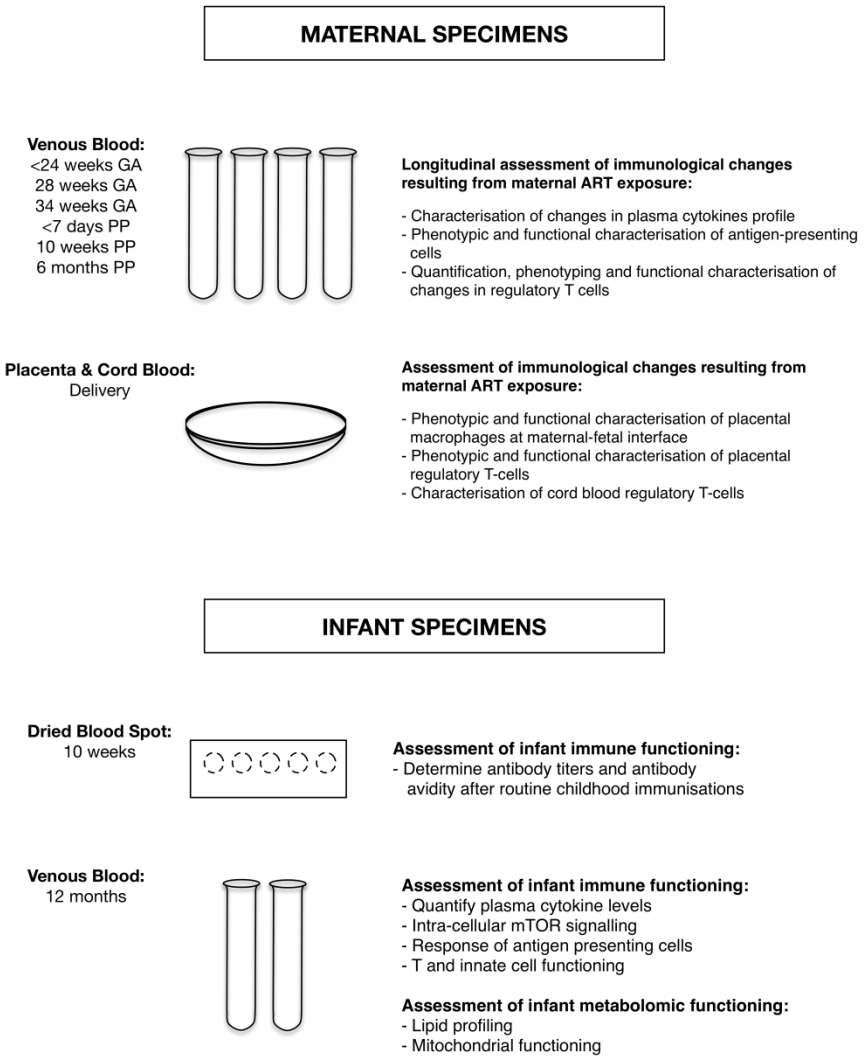

Figure 2: Maternal and Infant Specimens

247x287mm (500 x 500 DPI)

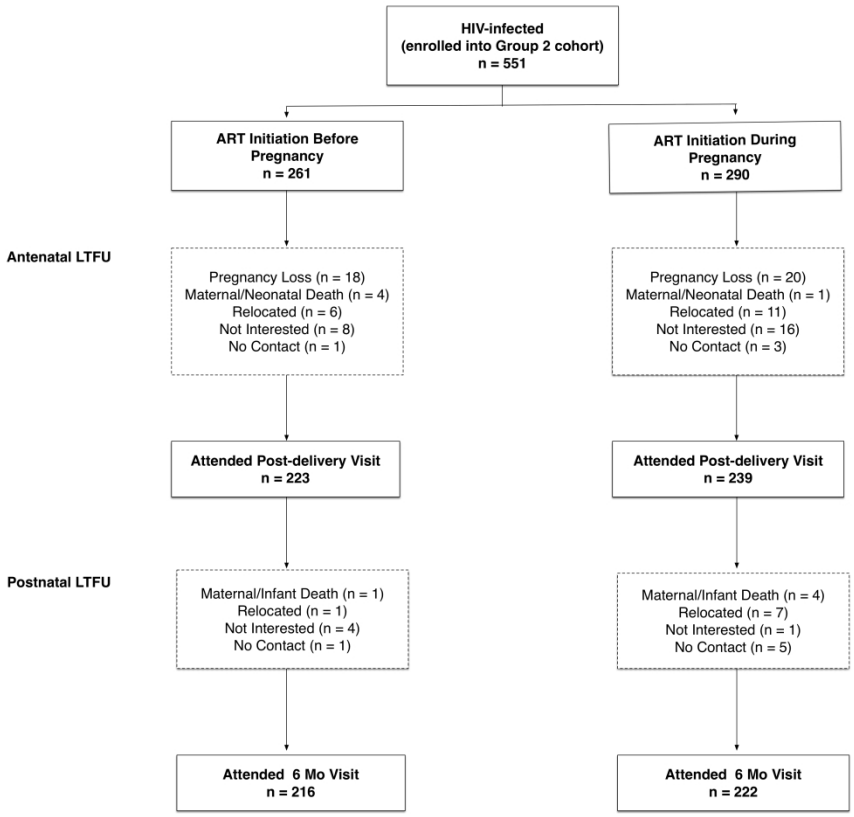

Figure 3: Loss to follow up in Group 2 cohort

591x552mm (200 x 200 DPI)