Perinatal outcomes in HIV-positive women

Thesis submitted in partial fulfilment of the requirements for the degree
Doctor of Philosophy

By

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Abstract

In 2015, the majority (80%) of the 1.2 million HIV-positive pregnant women globally, most of whom reside in sub-Saharan Africa (91%), received antiretroviral treatment (ART) for the prevention-of-mother-to-child-transmission (PMTCT). The use of Highly Active Antiretroviral Treatment (HAART) during pregnancy improves maternal health, reduces mother-to-child-transmission to <1%, and reduces horizontal HIV-transmission to serodiscordant couples. Consequently, in 2016, WHO recommended that all HIV-positive women of reproductive age initiate lifelong HAART. Despite the benefits of ART, an increasing body of conflicting evidence continue to report high rates of adverse perinatal outcomes in HIV-positive pregnant women, with no clarity on whether this is attributable to HIV-infection, ART or underlying confounding. This thesis explored the association between maternal HIV-infection, ART and perinatal outcomes, using 3 rigorous methods.

A systematic review and pairwise meta-analysis showed that ART-naïve maternal HIV-infection significantly increased the risk of preterm birth (PTB), low birthweight (LBW), small for gestational age (SGA), and stillbirth. This effect was most prominent in sub-Saharan Africa, it persisted after adjustment for confounders and was directly correlated with the clinical stage of disease. Secondly, a systematic review and network meta-analysis of randomised control trials showed PI-based HAART and NNRTI-based HAART, both of which are recommended for PMTCT in developed and developing countries, to be the most efficacious ART for PMTCT but also associated with the highest risks of PTB, spontaneous PTB, LBW and very LBW compared to other commonly used ART. Lastly, prospectively collected data on a South African cohort of HIV-negative and HIV-positive women on a predominantly NNRTI-based HAART regimen, with pregnancies dated by early ultrasound (<14 weeks gestation), was used to determine the association between HIV/ART and perinatal outcomes in a "real-world" context. The limited power of the study, and high background incidence of adverse perinatal outcomes in HIV-negative women prevented multivariate analyses from detecting an independent association between maternal HIV/ART and PTB, LBW or SGA.

The findings of this thesis highlight the importance of recent efforts by national governments and international stakeholders (WHO, USAID, UNAIDS) to accelerate ART coverage in HIV-positive women of reproductive age; however, it also shows that an unintended negative consequence of the current HAART regimens recommended for PMTCT will be a significant increase in the burden of adverse perinatal outcomes that directly contribute to neonatal and under-5 mortality, particularly in sub-Saharan Africa.

Acknowledgements

The traditional African saying, “it takes a village to raise a child” perfectly describes my experience during this DPhil. More people than I can name have contributed to my growth – both personally and academically – and to the completion of this work; however, there are a few that need a special mention. First and foremost, thank you to my Lord and saviour Jesus Christ. You went before me, guided me, and carried me throughout this DPhil journey, just as you promised. Your word has never ever failed me. Secondly, thank you to my lovely husband Jean Marc. I would have never commenced or completed this DPhil without his unwavering love and support. Thank you, Jean Marc, for always exceeding my expectations; 10 years later and you remain the kindest, and most wonderful man I know. You truly are more than I could have ever, hoped and prayed for, and I look forward to spending the rest of my days with you.

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"So the wall was completed on the twenty-fifth of Elul, in fifty-two days. When all our enemies heard about this all the surrounding nations were afraid and lost confidence, because they realised that this work had been done with the help of our God" Nehemiah 6:15-16.

To God be the glory.

"It always seems impossible until it is done."
President Nelson Mandela

Contribution to this thesis and future work

- **Chapter 3: HIV systematic review and Chapter 4: ART systematic review**

I took the lead on every stage of the conduct of both systematic reviews under the supervision of Dr. Joris Hemelaar. I wrote both systematic review protocols, compiled the list of search terms, and conducted the search of the trial databases. The searches of the general databases were conducted by a specialist librarian. I reviewed the citations retrieved from the initial search with help from one reviewer (RC) for the HIV systematic review (Chapter 3) and two reviewers for the ART systematic review (Chapter 4). I identified all full text articles and abstracts, assessed whether each study met the eligibility criteria, conducted the quality and risk of bias assessments, extracted all the data, and went through the references of all included papers and previous reviews. All the extracted data, and the quality and risk of bias assessments that I assigned to each study was checked by second reviewers (HIV review = JH, ART review = JH, RC, SN). I conducted all the pairwise and network meta-analyses, under the guidance of Dr. Sally Hopewell and Dr. Joris Hemelaar. I wrote the thesis chapters under the supervision of Dr. Joris Hemelaar, Professor Shane Norris and Professor Stephen Kennedy. I am the first author on one manuscript based on the work presented in Chapter 3, and presented the findings of the HIV systematic review at the South African HIV Clinicians Society Conference (Johannesburg, 2016, oral presentation) and the DOHaD conference (Cape Town, 2014, oral presentation).

- **Chapter 5 and 6: SHAPOSSA study**

The INTERBIO-21st Fetal study is an ongoing prospective cohort study that I joined in 2012, and which provided a platform for the SHAPOSSA study. The SHAPOSSA study aims to assess the association between maternal HIV infection/ART and perinatal outcomes using prospectively collected data on all women included in the INTERBIO-21st Fetal Study, as well as additional HIV and ART data collected specifically for this analysis. I was the lead researcher on the SHAPOSSA study, which I conceptualised, implemented and managed over an 18-month period with guidance from Dr. Joris Hemelaar, Professor Stephen Kennedy and Professor Shane Norris. I wrote the study

protocol, designed the SHAPOSSA data collection sheet, applied for and received ethical approval for the project, implemented the study at the study site, trained research nurses, research assistants and data capturers on the study SOPs, collected a proportion of the data from HIV-positive pregnant participants personally, and the rest was collected by the trained local staff. I captured a proportion of the data and the rest was captured by a trained data capturer. I conducted data quality assessments at several stages during the study and cleaned the final dataset included in this thesis, which was reviewed by a second researcher (WS) for quality purposes. I conducted all statistical analyses under the guidance of Professor Shane Norris and wrote the thesis Chapters under the supervision of Professor Stephen Kennedy and Professor Shane Norris. I presented preliminary results of this work at the DOHaD Conference (Cape Town, 2014, oral presentation) and the International AIDS Conference (Durban, 2016, poster presentation).

The publication and list of conference abstracts and presentations arising from this work is presented in Appendix 1.

- **Future work**

I initiated two additional cohort studies under the supervision of Dr. Joris Hemelaar, Professor Stephen Kennedy and Professor Shane Norris, which I managed over an 18-month period; the Maternal Immunology Study and the HIV Immunology Study. The Maternal Immunology Study (N = 210 HIV-positive and HIV-negative pregnant women) is an immunological study that aims to investigate whether HIV/ART is associated with aberrations in the maternal immunological profiles that contribute to the development of PTB and SGA. The HIV Immunology Study (N = 27 HIV-positive pregnant women) is also an immunological study that aims to investigate the HIV-specific T-lymphocyte response in pregnancy. I was responsible for all administrative and project management activities on these studies, I wrote the study protocol, designed all data collection sheets, applied for and received ethical approval for the studies, implemented the study at the study site in Soweto, South Africa, trained research nurses, research assistants and data capturers on the study SOPs, set up a laboratory at the Chris Hani Baragwanath Academic Hospital (CHBAH), and

was involved in the processing of plasma, PBMCs and DNA from fresh samples collected by the research staff, and shipping of these samples to Oxford for further analysis. The analysis of the biological samples collected will contribute to future research work.

Dedication

Pour toi Jean Marc, Matala Tshivuila
mon amour, ma force, mon soutien, mon cadeau de Dieu.

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List of abbreviations

3TC	Lamivudine
ABC	Abacavir
AGA	Appropriate weight for gestational age
AIDS	Acquired Immuno-Deficiency Syndrome
ANC	Antenatal care
ART	Antiretroviral treatment
ATV	Atazanavir
AZT	Zidovudine
BMI	Body Mass Index
BPP	Biophysical profile
CCR5	C-C chemokine receptor type 5
CD4	Cluster of differentiation 4
CDC	Centre for Disease Control and Prevention
CHBH	Chris Hani Baragwanath Hospital
CRL	Crown-rump length
CXCR4	C-X-C chemokine receptor type 4
D4T	Stavudine
ddl	Didanosine
ddNTP	Dideoxynucleoside analogue triphosphate
DLV	Delavirdine
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide
DRV	Darunavir
EFV	Efavirenz
Env	Envelope protein
ETR	Etravirine
EU	European Union
FHR	Foetal heart rate
FTC	Emtricitabine
FPV	Fosamprenavir
GA	Gestational age
Gag	Group-specific antigen
GALT	Gut-associated-lymphoid-tissue
GDP	Gross Domestic Product
GP120	120kDA Envelope Glycoprotein Surface Subunit
GP41	41kDA Envelope Glycoprotein Transmembrane Subunit

HAART	Highly Active Antiretroviral Therapy
Hb	Haemoglobin
HELLP	Haemolysis, elevated liver enzymes, low platelet count
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
HR	Hazard Ratio
IDV	Indinavir
InSTI	Integrase strand transfer inhibitor
IQR	Interquartile rate
IUFD	Intrauterine foetal death
IUGR	Intrauterine growth restriction
IVDU	Intravenous Drug Use
kb	kilobase
LBW	Low birthweight
LGA	Large for gestational age
LMICs	Low-to-middle-income countries
LNMP	Last normal menstrual period
LPV	Lopinavir
LTR	Long terminal repeat
MC	Miscarriage
MDG	Millennium Development Goal
MIHS	Maternal Immunology and HIV study
mRNA	Messenger RNA
MSM	Men who have sex with men
MTCT	Mother to child transmission
MUAC	Mid-upper arm circumference
MVC	Maraviroc
Nef	Negative regulatory factor
NFV	Nelfinavir
NHLS	National Health Laboratory Services
NICU	Neonatal Intensive Care Unit
NMR	Neonatal Mortality Rate
NND	Neonatal death
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NRTI	Nucleoside reverse transcriptase inhibitor
NtRTI	Nucleotide reverse transcriptase inhibitor
NVP	Nevirapine
OXTREC	Oxford Tropical Research Ethics Committee

p24	Protein 24
PACTG076	Paediatric AIDS Clinical Trials Group protocol 076
PI	Protease inhibitors
PIC	Pre-integration complex
PMTCT	Prevention-of-mother-to-child-transmission
POH	Pat obstetric history
Pol	Polymerase
PrEP	Pre-exposure prophylaxis
PTB	Preterm birth
RCT	Randomised controlled trials
Rev	Regulator of Expression of Virion Proteins
RNA	Ribonucleic acid
RNase H	Ribonuclease H
RPV	Rilpivirine
RT	Reverse transcriptase
RTV	Ritonavir
SB	Stillbirth
SD	Standard deviation
SES	Socioeconomic status
SFH	Symphysio-fundal height
SGA	Small for gestational age
SHAPOSSA	Soweto HIV and ART pregnancy study: The influence of HIV and Antiretroviral Treatment on Pregnancy Outcome in Soweto, South Africa.
SIV	Simian Immunodeficiency Virus
SOP	Standard Operating Procedure
SQV	Saquinavir mesylate
STD	Sexually Transmitted Disease
TAT	Transactivator of transcription
TDF	Tenofovir Disoproxil Fumarate
TPV	Tipranavir
UK	United Kingdom
UN	United Nations
UNAIDS	The Joint United Nations Programme on HIV and AIDS
UR	Uncertainty range
USA	United States of America
V3	Third variable loop
Vif	Viral infectivity factor
VLBW	Very low birthweight

Vpr	Viral protein R
VPTB	Very preterm birth
Vpu	Viral protein unique
VSGA	Very small for gestational age
WHO	World Health Organisation

Chapter 1: Introduction

The Human Immunodeficiency Virus (HIV) is a single-stranded retrovirus of the Lentivirus family. It was first isolated and characterized in the early 1980s, and is the causative agent for the Acquired Immuno-Deficiency Syndrome (AIDS). Despite substantial advances made in both preventative and therapeutic interventions, HIV remains one of the leading infectious causes of mortality and morbidity worldwide, particularly in developing countries where 90% of the global HIV burden is found. Since the start of the epidemic, an estimated 78 (69.5 – 87.6) million people have been infected with HIV, and almost half [35 (29.6 – 40.8) million people] of those infected have died from AIDS-related illnesses[1].

In 2015, more than 70% of the estimated 38.8 (37.6 – 40.4) million people living with HIV resided in sub-Saharan Africa[2], a region where women are disproportionately affected by the virus. Women in this region account for 58% of all HIV-infections and 91% of pregnant HIV-positive women globally[2,3]. In the absence of antiretroviral treatment (ART), pregnant HIV-positive women are eight times more likely to die during pregnancy or in the postpartum period compared to their HIV-negative pregnant counterparts[4]. Furthermore, up to 48% of mothers transmit the virus to their babies during the antenatal, intra-partum, or postnatal period whilst breastfeeding[5]. Conflicting reports from the USA and Europe suggest an association between maternal HIV infection and adverse perinatal outcomes, including preterm birth (PTB), the leading cause of neonatal and under-five mortality globally[6]. However, before the relationship with PTB could be determined, antiretroviral drugs were discovered and HIV-positive women were swiftly initiated on ART for the prevention-of-mother-to child-transmission (PMTCT). Therefore, the question of the association between "untreated" maternal HIV-infection and adverse perinatal outcome remained unanswered.

The initiation of ART during pregnancy results in improved maternal health, reduced maternal mortality, and reduced heterosexual transmission of genetically-linked viruses in serodiscordant couples[7]. When used in combination with other prevention methods, ART reduces the risk of mother

to child transmission (MTCT) to <1%. Despite the benefits of effective ART, conflicting reports continue to emerge regarding the association between maternal HIV-infection and perinatal outcomes. Furthermore, recent evidence from Europe and sub-Saharan Africa suggest that ART itself may be associated with increased rates of adverse perinatal outcomes[8-10], depending on when treatment is initiated, and what ART drug, class or regimen is used during pregnancy. This, however, has been disputed by some US researchers, who have attributed this effect to the maternal stage of disease[11]. Therefore, the association between ART and adverse perinatal outcomes also remains uncertain.

The question of the safety of ART in pregnancy is more pertinent now as countries accelerate their response to HIV, and rapidly adopt recommendations to initiate all HIV-positive women of reproductive age on lifelong ART irrespective of $CD4^+$ T-lymphocyte count and HIV disease stage[12]. In addition to HIV-positive women, HIV-negative women in geographic hotspots are also initiating ART through combination prevention programmes using conventional ART (oral tenofovir or tenofovir-emtricitabine combination) or newer drugs and methods of drug administration, i.e. dipivarine ring[12,13]. As a result of these policies, a large proportion of women of reproductive age, particularly in HIV geographic hotspots, will be exposed to ART at conception and throughout pregnancy.

This thesis explores the association between maternal HIV infection, ART and 11 perinatal outcomes, which are grouped into four categories based on the following characteristics: (1) **gestational age (GA) at delivery** [(PTB, very preterm birth (VPTB)], (2) **birthweight** [low birthweight (LBW), very low birthweight (VLBW)], (3) **GA at delivery and birthweight** (term-LBW, preterm-LBW, small for gestational age (SGA), very small for gestational age (VSGA)] and (4) **fetal and neonatal mortality** [(miscarriage, stillbirth, neonatal death (NND)]. These associations have been investigated using a prospective cohort study conducted at the largest public sector hospital in South Africa; a country with the largest HIV-epidemic and ART programme in the world. Secondly, two systematic reviews have been conducted with pairwise and network meta-analyses.

This thesis is divided into four sections (Figure 1.1). Section one provides a background (Chapter 2) to the interaction between maternal health status and perinatal health outcomes, and discusses the global burden and consequences of the perinatal outcomes of interest. A background on the HIV epidemic and ART is then provided, and the research questions are introduced.

Section two assesses the association between "untreated" maternal HIV infection and perinatal outcomes (Chapter 3) using a systematic review and pairwise meta-analysis of observational studies, followed by the association between ART and perinatal outcomes (Chapter 4), focusing on the effects of different ART drugs, classes and regimens using a systematic review, pairwise and network meta-analyses of randomised control trials (RCTs).

Section three assesses the association between maternal HIV infection/ART (Chapter 5) and perinatal outcomes using prospectively collected data from HIV-positive and HIV-negative pregnant South African women who participated in the Soweto HIV and ART pregnancy study (SHAPOSSA Study). Multivariate regression analyses are used to control for potential confounders identified *a priori* (Chapter 6).

Section four (Chapter 7) summarises the overall findings of this thesis, and discusses the policy implications and recommendations that have emerged from this work, as well as directions for future research.

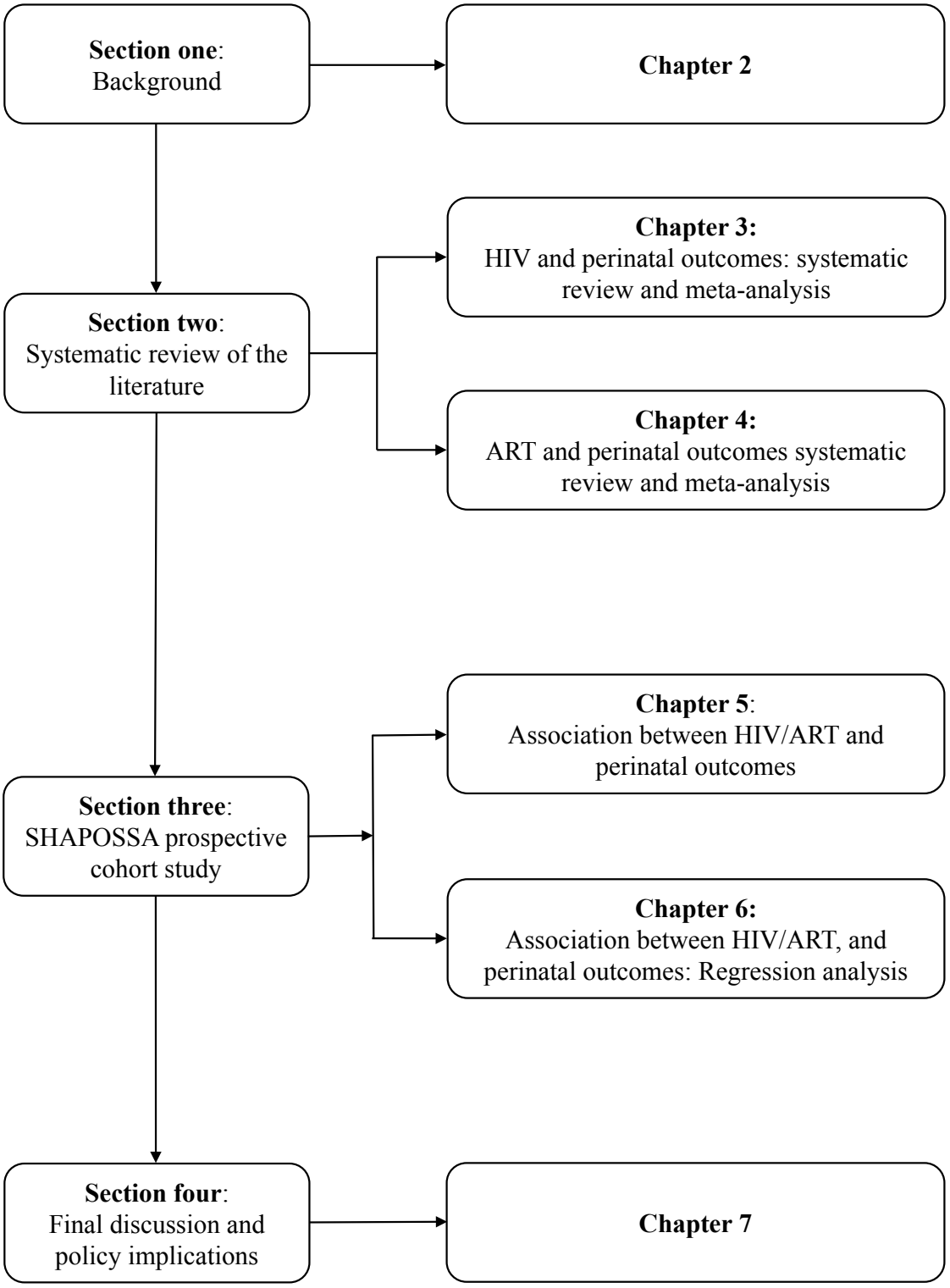


Figure 1.1: Flow diagram of thesis structure.

Section 1

Chapter 2: Background

2.1 Maternal health and impact on perinatal outcome

An estimated 210 million women conceive annually, and give birth to 140 million babies[14]. The health of these women during pregnancy and the consequent in-utero environment they provide have a direct impact on the fetus' perinatal outcome[15], the health and wellbeing of the child throughout his or her life course, and future generations[16]. The in-utero period presents a critical stage for the development and growth of the fetus, and poor maternal health during this period exposes the fetus to a hazardous intrauterine environment that inflicts more damage on the short and long-term health potential of the child than at any other stage of development[16,17].

The causes of poor maternal health during pregnancy are multifactorial, and include many influences from long before conception (Figure 2.1)[14,18]. The perinatal health framework adapted from Misra et al (2003)[18] illustrates the distal and proximal determinants of maternal and perinatal health (Figure 2.1). The distal determinants include the woman's genetic composition, physical environment (e.g. air pollution) and social context (e.g. socioeconomic status) (Figure 2.1), that exert influence throughout a woman's reproductive life[18]. These factors have the potential to influence the woman's health status directly, however, more importantly, they increase or decrease her susceptibility towards developing proximal determinants[18].

The proximal determinants are classified into biomedical or behavioural factors, and directly impact a woman's health status[18] (Figure 2.1). The behavioural factors include both high-risk and protective behaviours exhibited by the mother[18], while the biomedical factors focus on the maternal health status, including any compromising health condition she may have[18].

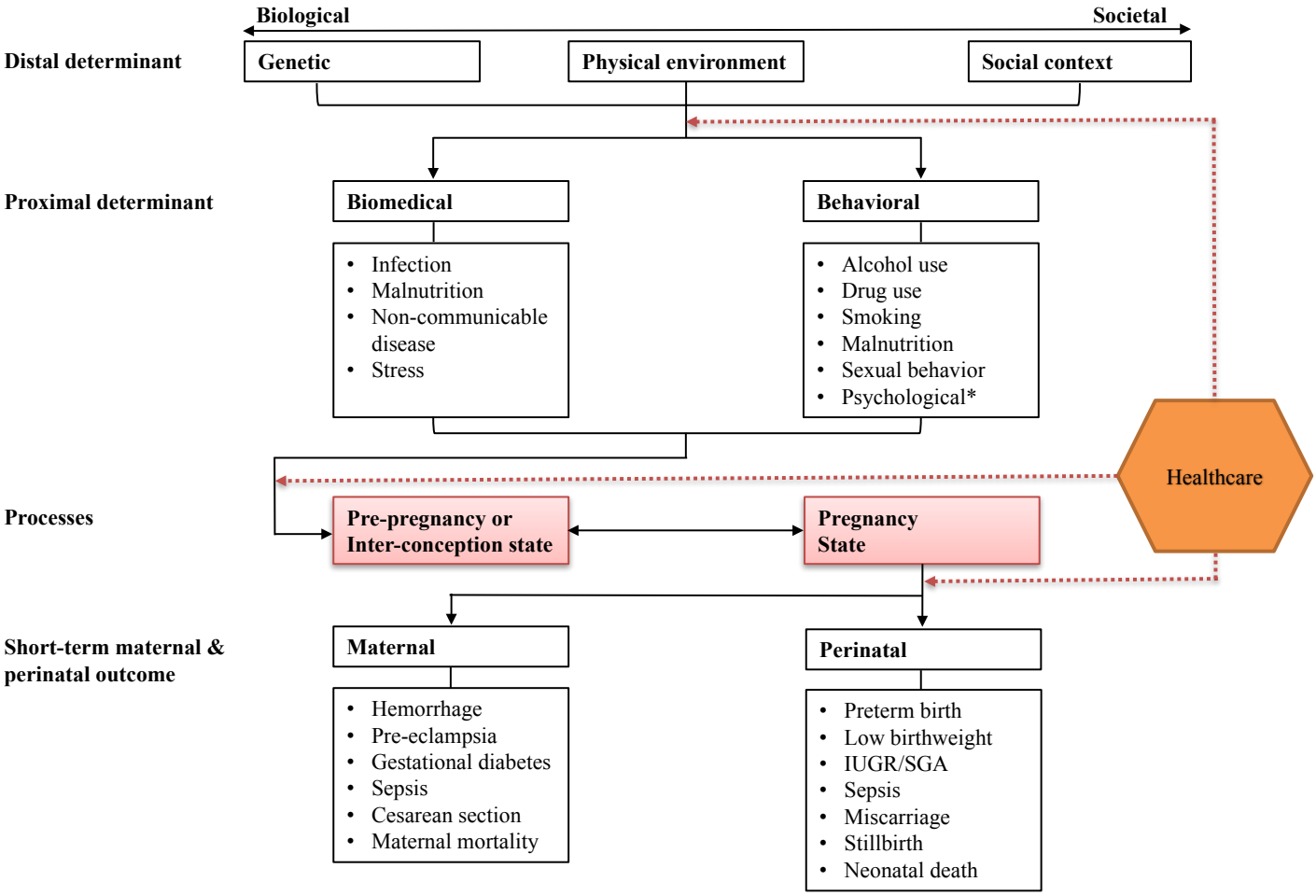


Figure 2.1: Multiple determinant framework for poor maternal and perinatal health.

* psychological factors: stress, self-efficacy, depression and anxiety.

Image adapted from Misra et al 2003[18]

While maternal and perinatal health is influenced by biomedical and behavioural factors independently, the interaction between these factors and how they influence each other also contribute substantially to the health outcomes of the mother and child[18]. For example, women with high-risk sexual behaviour are also more likely to smoke and use alcohol and illicit drugs. These behaviours influence the risks of acquiring a sexually transmitted disease (STD), e.g. HIV-infection, and also increase the risk of developing a non-communicable disease (e.g. obesity or hypertension). Consequently, the compounded effect of the risky behavioural practices in the context of an infectious and non-communicable disease, means that the individual will have a significantly compromised health status during the pre-conception or inter-conception (between pregnancies)

period. The demands of pregnancy will exacerbate the poor maternal health status, and without proper interventions, the fetus will be exposed to a hazardous intrauterine environment that will have detrimental effects on the infant's short-term (e.g. PTB or stillbirth), medium-term (e.g. cerebral palsy, poor cognitive development) and long-term outcomes (e.g. poor school performance, reduced earning potential and loss of productivity). This illustrates the importance of the proximal determinants particularly as they are targets for interventions[18] .

Strong health systems and access to timely, high-quality, evidence-based healthcare services modify the relationships between elements within the framework, and improve maternal and neonatal outcomes[15,18]. This is particularly effective when preventative and therapeutic interventions are introduced along the continuum of care, which connects care throughout the life-course using the three main service delivery levels, i.e. community level, primary healthcare centres and referral hospital[19,20]. However, structural health system deficiencies are ubiquitous, and contribute substantially to the direct and indirect causes of poor maternal and perinatal health outcomes.

2.1.1 Epidemiology

While the underlying causes of poor maternal health occur in all countries across all regions, certain groups of women have an increased vulnerability for these conditions, and consequently have higher burdens of adverse maternal and perinatal outcomes. These include women from low-to-middle-income countries (LMICs), particularly in sub-Saharan Africa and Southern Asia, which together account for 88% of maternal deaths globally[14]. The improving socioeconomic status of LMICs and consequent change in the behaviour and lifestyle of these populations have resulted in an increase in the prevalence of non-communicable diseases and what is characterised as the "double burden of malnutrition", i.e. stunted growth with nutrient deficiencies and obesity[21,22]. This socioeconomic and epidemiological transition means that LMICs now experience a high burden of both communicable and non-communicable diseases[23]. Consequently, a large proportion of women of reproductive age in these settings have a combination of these underlying factors (e.g. HIV-infection in obese women),

which further exacerbates the impact on perinatal health. Subgroups of vulnerable populations in LMICs also exhibit extremely high burdens of poor maternal health and consequently poor perinatal outcomes[14]. These include adolescent and unmarried women, women residing in fragile states or conflict zones, refugees, internally displaced and migrant women, indigenous women within non-indigenous communities, ethnic or religious minorities, and women living within poverty or informal urban settlements[22,24].

While there has been a great deal of attention on women residing in LMICs, these disparities also extend to residents of high-income countries[24-26]. For example, Black women in New York, USA, are twice as likely to die during childbirth than women in middle-income countries[24], and three times more likely to deliver a preterm infant compared to Caucasian women in the same setting[27]. Furthermore, in comparison to Caucasian women in the UK, Black women are almost twice as likely to have a stillbirth or NND[28].

Take home points:

1. The health of the mother and the intrauterine environment have a direct impact on perinatal health.
2. Maternal health status is a product of distal and proximal determinants, and while many distal determinants are difficult to alter, proximal factors have a direct impact on the fetus' health status, and are targets for interventions.
3. Poor maternal health remains rampant because of the continued and widespread inequities in global health. This is particularly true for women in LMICs, vulnerable groups of women in fragile states and conflict zones and marginalized women in high-income countries.

2.2 Perinatal outcomes

The term 'perinatal' is defined as the period between 22 completed weeks' gestation until 7 days post-delivery[29]. While there are multiple perinatal outcomes during this period, this thesis focuses on the 11 most common globally. These outcomes are categorised into four groups depending on whether they are characterised by the birthweight, GA at birth or fetal or neonatal mortality (Figure 2.2). The four categories include: Perinatal outcomes according to (1) birthweight (2) GA, (3) birthweight and GA combined (4) fetal and neonatal mortality.

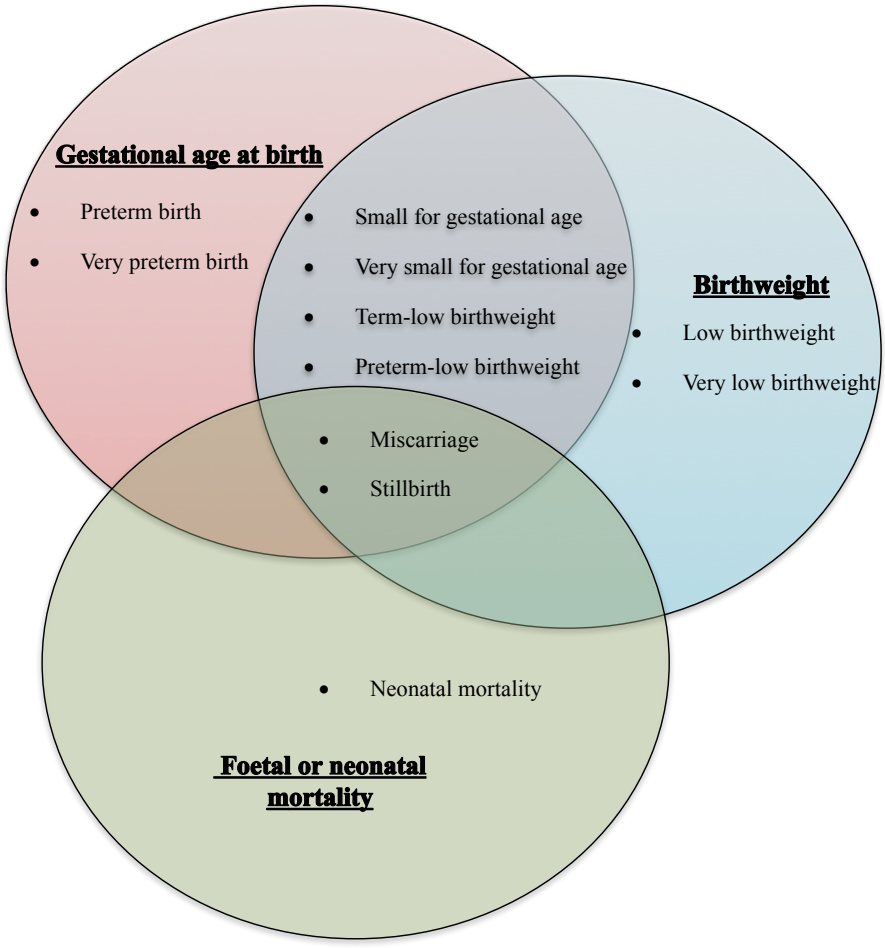


Figure 2.2: Venn diagram illustrating the 11 perinatal outcomes of interest

2.2.1 Perinatal outcomes according to birthweight

2.2.1.1 Low birthweight and very low birthweight

- Definition

Perinatal, neonatal and infant health are heavily dependent on an infant's birthweight and GA at delivery[30,31]. Numerous methods have been developed to assess GA (Table 2.1). However, the most accurate method remains the "best obstetric estimate" which determines GA by comparing estimates generated from a first trimester (<14 weeks' gestation) ultrasound scan, with that generated using the first day of the woman's last normal menstrual period (LNMP)[32]. In most developing country settings access to an early ultrasound scan is limited and costly[32]. Consequently, other less accurate measures of GA determination are used (Table 2.1), which leads to an over or under-estimation of perinatal outcomes that are dependent on an accurate assessment of GA, e.g. PTB[32].

Unlike GA, a birthweight measurement is more readily available in most LMICs, and for a long time was considered as the most important determinant of perinatal morbidity and mortality[33,34]. Birthweight is a product of two major processes, the duration of gestation and the rate of intrauterine growth [31]. Therefore, LBW, defined as a birthweight <2500g[34], and VLBW, defined as a birthweight <1500g, occur either due to a short gestation, restricted intrauterine growth or a combination of these two events[35].

Table 2.1: Gestational age methods, accuracy and limitations

	Accuracy	Details	Availability/ feasibility	Limitations
1. Early ultrasound	± 5 days if first trimester ± 7 days if conducted after the first trimester	Estimation of fetal crown-rump length ± biparietal diameter/femur length between 6 – 18 weeks	Ultrasound not always available in low-income settings and rarely done in the first trimester	May be less accurate in the presence of fetal malformation, or maternal obesity
2. Symphysio - fundal height	± 3 weeks	Distance from symphysis pubis to fundus measured with a tape measure	Feasible and low cost	In some studies, similar accuracy to LNMP. Potential use with other variables to estimate GA when no other information available
3. LNMP	± 2 weeks	Woman's recall of the date of the first day of her last menstrual period	Most widely used	Lower accuracy in settings with low literacy. Affected by variation in ovulation and also by breastfeeding.
4. Birthweight as a surrogate of GA	More sensitive/specific at lower gestational age e.g. <1500g most babies are preterm	-	Birthweight measured for around half of the world's births	Requires scale and skill.
5. Newborn examination	± 13 days for Dubowitz, higher range for all others	Validated scores using external and/or neurological examination of the neonate e.g. Parkin, Finnström, Ballard and Dubowitz score	Mainly specialist use. Most accurate with neurological criteria which require considerable skill. Potential wider use for simple scoring systems.	Accuracy is dependent on complexity of score and skill of examiner. Training and ongoing quality control required to maintain accuracy.
6. Best obstetric estimate	± 13 days	Uses an algorithm to estimate gestational age based on best information available	Commonly used in high-income settings	Various algorithms in use, not standardised

GA = gestational age, LNMP = last normal menstrual period
Source: adapted from Blencowe et al 2013[32]

This suggests that infants who are classified as LBW should be stratified into three groups: infants born preterm, those born at term whose intrauterine growth rate was below normal as a consequence of aberrations occurring during the antenatal period [intrauterine growth restriction (IUGR)], resulting in them being born SGA, and those who are both preterm and SGA (Figure 2.3)[36]. The deficiencies associated with the use of LBW as an index of population health in developing countries are known; however, the inability to determine gestational age accurately and monitor fetal growth with serial fetal ultrasound scans prevent the use of more appropriate measures of fetal outcome, e.g. PTB or SGA[37].

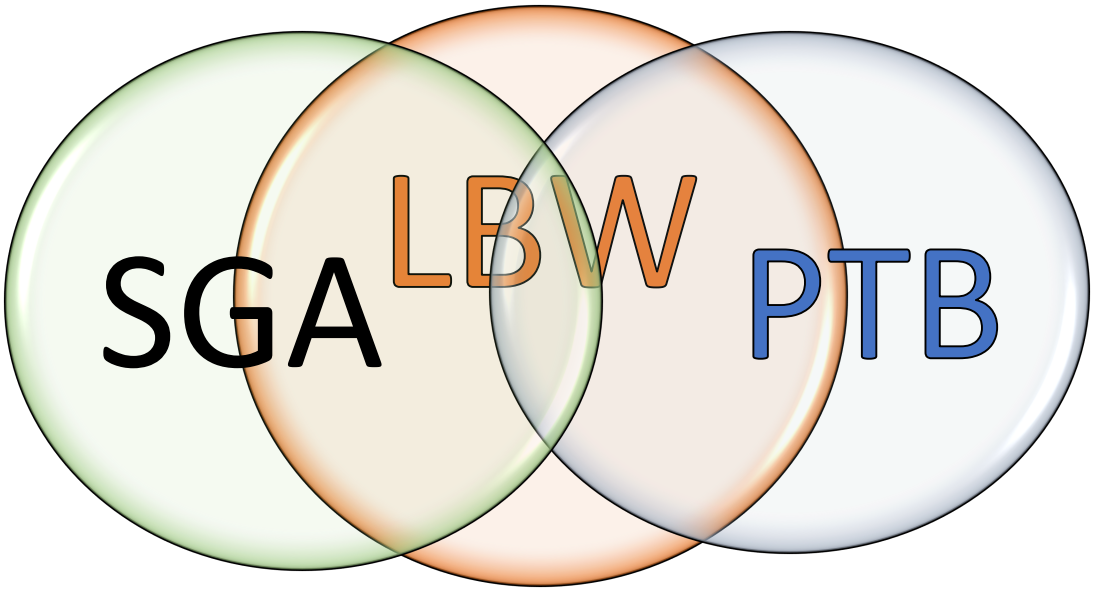


Figure 2.3: Venn diagram illustrating the relationship between LBW, SGA and PTB.
Image adapted from Semba et al. 2008 [36]

- Epidemiology

In 2010, an estimated 18 million infants were born LBW in LMICs (Figure 2.4)[38]. The majority of these LBW infants were born at term (59%) (≥37 weeks 0 days' gestation) (term-LBW) and 41% were born preterm (<37 weeks' gestation) (i.e. preterm-LBW)[38] (Figure 2.4). While it was previously believed that the definition of LBW included all high-risk neonates, including those who are born preterm and SGA, figure 2.4 clearly demonstrates that the definition of LBW only includes a small proportion of preterm and SGA neonates, with the burden of neonates born preterm and SGA far exceeding that of infants born LBW. Therefore, in order to identify all high-risk infants at birth it is important to identify neonates born preterm and SGA, in addition to those classified as LBW.

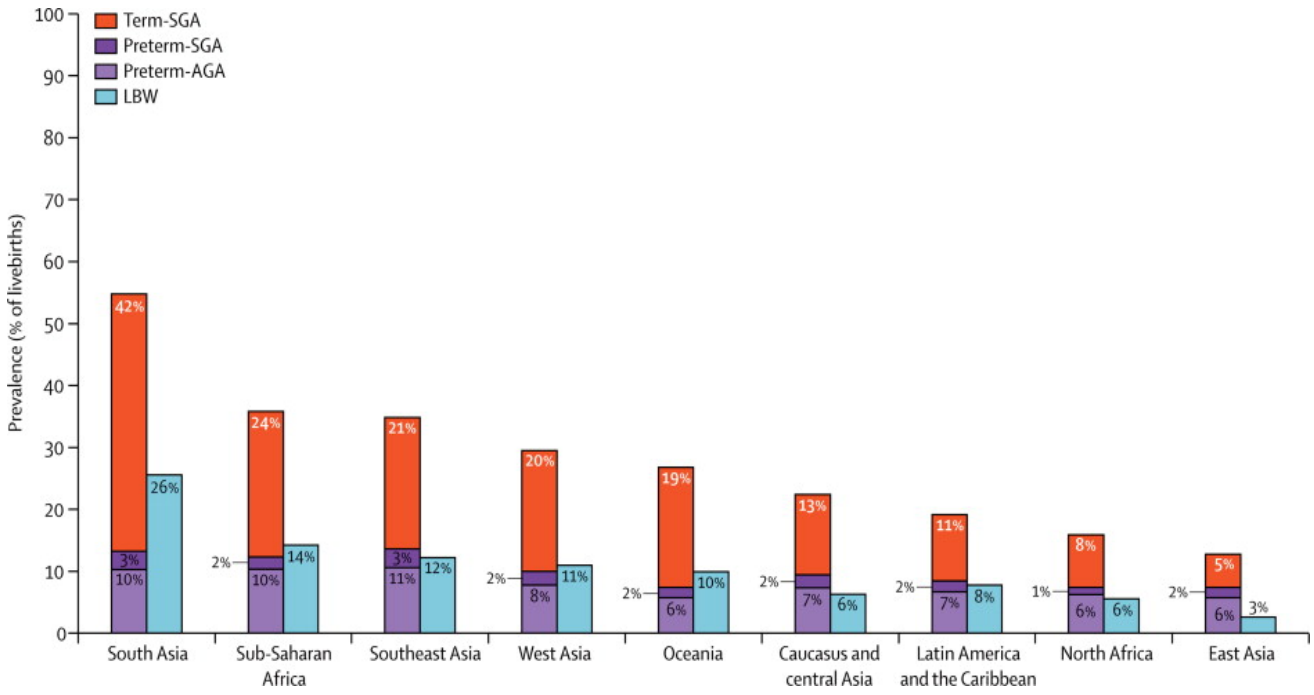


Figure 2.4: The prevalence of LBW, PTB and SGA according, to UN-MDG regions in 2010.
Source: Lee et al 2013[38].

In 2010, an estimated 14.2% of neonates born in sub-Saharan Africa and 25.6% of neonates born in Southern Asia were classified as being LBW (Figure 2.4) [38]. These two regions alone accounted for more than a third (39.8%) of the global burden of LBW neonates (Figure 2.4) [38], which can be attributed to the increased burden of maternal morbidity in these regions, coupled with weak health systems, disparities in healthcare access and the poor socioeconomic status of the population.

- Short and long-term complications

LBW neonates are 40 times more likely to die within the first 28 days of life, and account for 60-80% of neonatal deaths[33,39]. They are five times more likely to die during infancy, and have a fourfold higher rate of hospitalization compared to infants who are born with a birthweight ≥ 2500g[33,40].

LBW infants who survive the neonatal and post-neonatal period experience significant short and long term morbidity including neurodevelopmental complications[33,41,42]. Furthermore, the consequences of being born LBW extend beyond infancy, and has been associated with an increased risk of infectious and non-communicable diseases in adolescence and adulthood[16,43].

2.2.2 Perinatal outcomes according to gestational age at delivery

2.2.2.1 Preterm birth and very preterm birth

- Definition

PTB is most commonly defined as birth before 37 complete weeks of gestation since the first day of a woman's LNMP[44]. Traditionally, PTB was classified into two groups; spontaneous PTB (sPTB), which implies that a woman laboured spontaneously, and provider-initiated PTB, which occurs when labour is induced or a woman undergoes a caesarean section before 37 weeks' gestation following an indication related to the maternal or fetal health status, or other non-medical reason[45]. While this simplistic approach is easy to understand and use, it fails to incorporate the fact that PTB is a multifaceted syndrome that develops as a consequence of multiple overlapping aetiological factors related to the maternal health status, fetal or placental condition (Figure 2.5)[45-47].

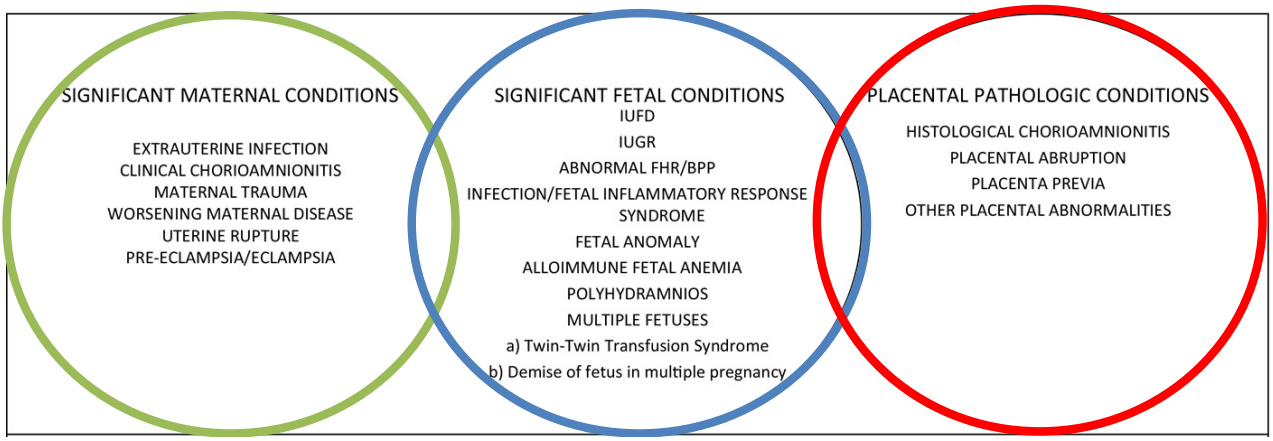


Figure 2.5: PTB aetiological factors.
Source: Villar et al 2012.[47]

Most recently, these aetiological factors (maternal, fetal and placental condition) have been used in conjunction with clinical signs at hospital admission to classify PTB into 12 phenotypes with differential rates of neonatal morbidity and mortality (Table 2.2)[48] . The poorest neonatal outcomes not related to multiple pregnancy (group 3) occurred in preterm infants characterised by phenotype clusters 4, 5, 8, 9 11 and 12, even after adjusting for birthweight and GA (Table 2.2) [48] .

The identification of these 12 phenotypes confirm that PTB is not a single disease and the most appropriate approach for the future management of PTB requires a wide range of preventative and therapeutic interventions targeting the underlying causes of specific PTB phenotypes[48].

Table 2.2: Neonatal mortality and morbidity according to phenotype clusters

Phenotype cluster	Neonatal morbidity	Neonatal death	
	NICU ≥ 7 days %	**NMR/ 1000 live births**	**Adjusted odds ratio***
1. No severe maternal, fetal or placental condition detected	13.9	5	Reference
2. Mostly pre-eclampsia or eclampsia	45.8	36	2.5 (0.9 – 7.2)
3. Mostly multiples (e.g. twins)	31.2	24	3.4 (1.1 – 10.2)
4. Mostly extra-uterine infections	26.3	36	3.9 (1.2 – 12.2)
5. Mostly chorioamnionitis	37.3	43	3.4 (1.1 – 10.5)
6. Mostly mid- or late- bleeding	38.9	27	1.7 (0.5 – 5.8)
7. Mostly suspected IUGR	42.4	28	3.2 (0.9 – 11.5)
8. Mostly mixed conditions	66.8	99	4.5 (1.6 – 12.6)
9. Mostly early bleeding	31.8	68	3.5 (1.1 – 11.6)
10. Mostly stillbirths	-	-	-
11. Mostly fetal distress	26.6	45	10.9 (3.1 – 37.9)
12. Mostly severe maternal disease	22.4	37	7.0 (1.9 – 25.8)

* Odds ratio adjusted for country, gestational age, and z-score of birthweight-for-gestational-age, NICU = neonatal intensive care unit, NMR = neonatal mortality rate. Source: Barros, et al. 2015[48]

- Epidemiology

In 2010, an estimated 11.1% (14.9 million) of all livebirths were born preterm[49]. Similar to LBW, the majority (9.1 million; 60%) of PTBs occurred in sub-Saharan Africa and Southern Asia (Figure 2.6)[49]. Developed countries have also reported an increasing trend in the burden of PTB (8.6%) (Figure 2.6); with the highest estimates reported in Cyprus (14.7%) and Bahrain (14.0%)[26]. The USA, which only accounts for 30% of livebirths in developed countries, contributes almost half (42%) of all PTBs in this region with 1 in 10 women delivering a PTB infant (12%)[26,49].

While the burden of PTB in developing countries have mostly been attributed to poor maternal health, the increasing trend in developed countries have mostly been attributed to an increase in provider initiated PTB, and the improved survival and registration of PTBs that occur at lower thresholds (<28 weeks' gestation) [26,49].

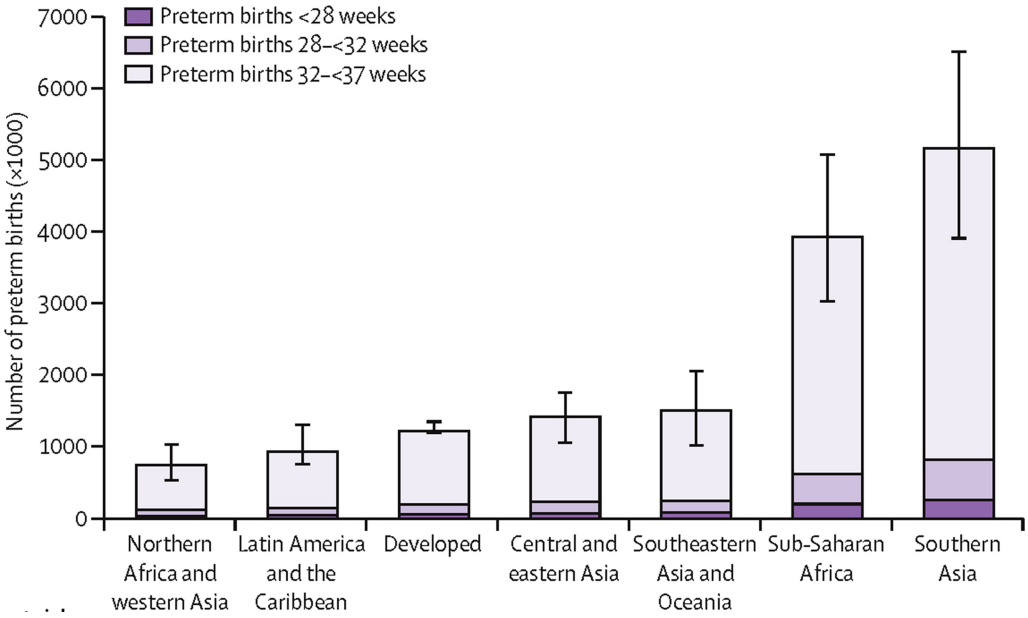


Figure 2.6: PTB rates according to region and gestational age grouping in 2010.
Source: Blencowe et al 2012[49]

- Short and long term complications

Preterm neonates are born with immature organ systems that are prone to complications, and struggle to support life in the extra-uterine environment[50]. Consequently, 15.9% of neonatal and 17.8% of under-5 deaths occur in infants who are born preterm (Figure 2.7) [6].

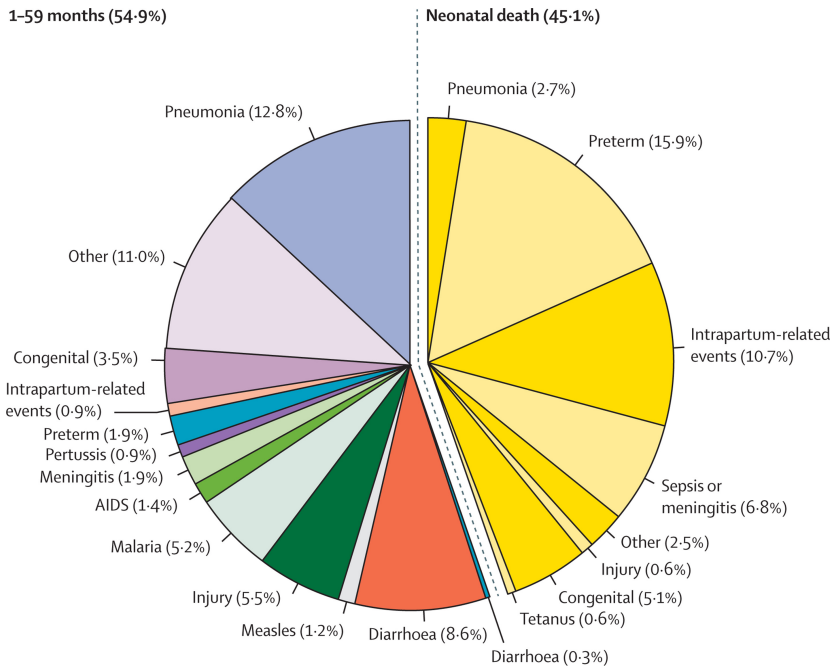


Figure 2.7. Global causes of neonatal and under-5 deaths in 2015.
Source: Liu et al 2016[6]

The majority of these infants die during the first week of life, with a large proportion of deaths during the neonatal period being attributed to their increased susceptibility to infections[35,39,50]. This is especially true in LMICs where increasing levels of poverty and health inequity mean that basic interventions (e.g. antenatal steroids[51], Kangaroo Mother Care[52] or treatment for neonatal infections) and neonatal intensive care services are unavailable or extremely costly (Figure 2.8)[35,49]. Consequently, in low-income countries only 10% of infants born before 28 weeks' gestation survive, while more than 90% survive in high-income countries[49].

The rate of mortality and disability increases proportionally with a decline in gestational age, with infants who are born very preterm – defined as birth before 32 weeks' gestation – exhibiting a much higher rate of disability and mortality than infants born <37 weeks' gestation (Figure 2.8) [35,50]. Substantial advancements made in supportive and neonatal intensive care interventions have significantly improved the survival of these infants in developed countries, where 90% of infants born before 28 weeks' gestation and 50% of those born before 25 weeks' gestation survive beyond the neonatal period[49,53]. Unfortunately, the majority who survive exhibit lifelong disabilities[54,55] and are at an increased risk of developing non-communicable diseases in adulthood[56,57].

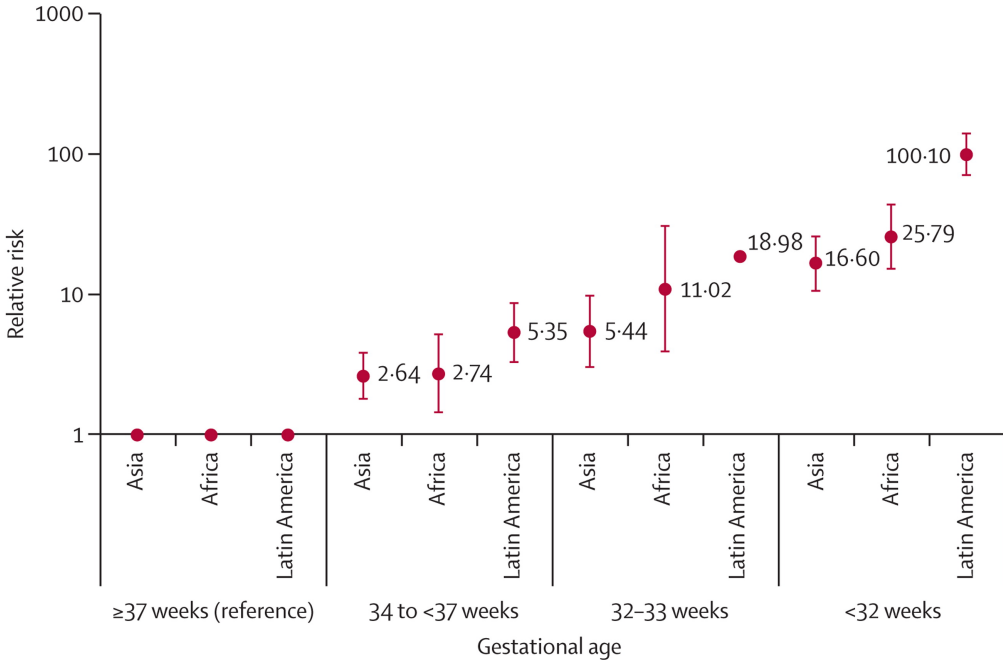


Figure 2.8. **Relative risk of neonatal mortality associated with gestational age**.
Source: Katz et al 2013[35]

2.2.3 Perinatal outcomes according to gestational age and birthweight combined

2.2.3.1 Small for gestational age, very small for gestational age

- Definition

Within each birthweight category, the risk of morbidity and mortality varies with GA at birth[33,58]. The neonates size at birth is a direct consequence of the maternal health status during pregnancy, the intrauterine environment and the resultant pattern of intrauterine growth[30]. A fetus who experiences a normal intrauterine growth pattern is born with an appropriate weight for GA (AGA), defined as a birthweight for GA between the 10th and 90th percentile. However, a fetus who experiences an abnormal intrauterine growth pattern, can either be born SGA, defined as a birthweight <10th percentile for GA, VSGA, defined as a birthweight <3rd percentile for GA or large for GA (LGA), defined as a birthweight > 90th percentile for GA [30].

- Epidemiology

In 2010, 32.4 million neonates in LMICs were born SGA (Figure 2.9)[38]. Similar to LBW and PTB, the majority (87%; 28.2 million) of this burden occurs in Southern Asia and sub-Saharan Africa (Figure 2.9)[38]. Furthermore, 23% (3.9 million) of neonates in Southern Asia are born VSGA[38] .

- Short and long term complication

In comparison to AGA neonates, SGA neonates present with a high rate of clinical complications and almost double the risk of death during the neonatal period and infancy (Figure 2.10)[35,58] This risk is particularly elevated in those born VSGA, who are four times more likely to die during the neonatal period compared to AGA neonates (Figure 2.10), as well as neonates who are both preterm and SGA, who have a 10 to 40 times increased risk of NND than AGA infants, irrespective of the region of assessment (Figure 2.11 - Figure 2.12)[35].

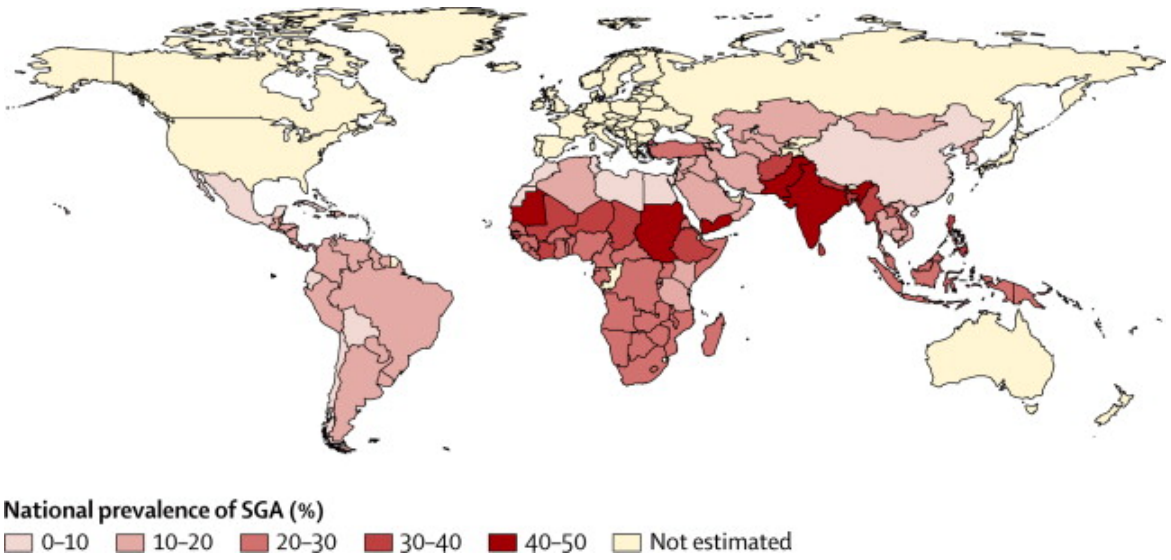


Figure 2.9. **Global burden of SGA in 2010**.

Source: Lee et al 2013[38]

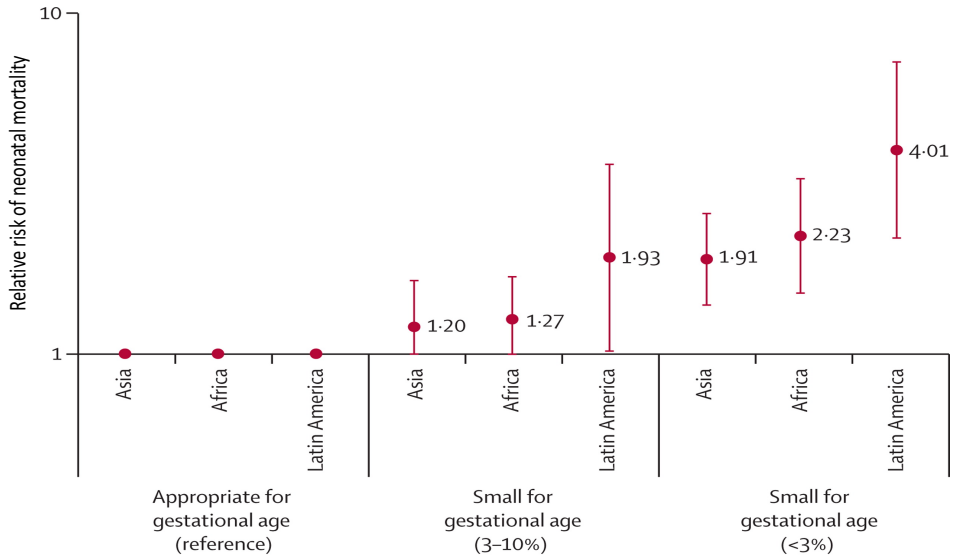


Figure 2.10. Relative risk of neonatal mortality associated with SGA and VSGA neonates. Source: Katz et al 2013[35].

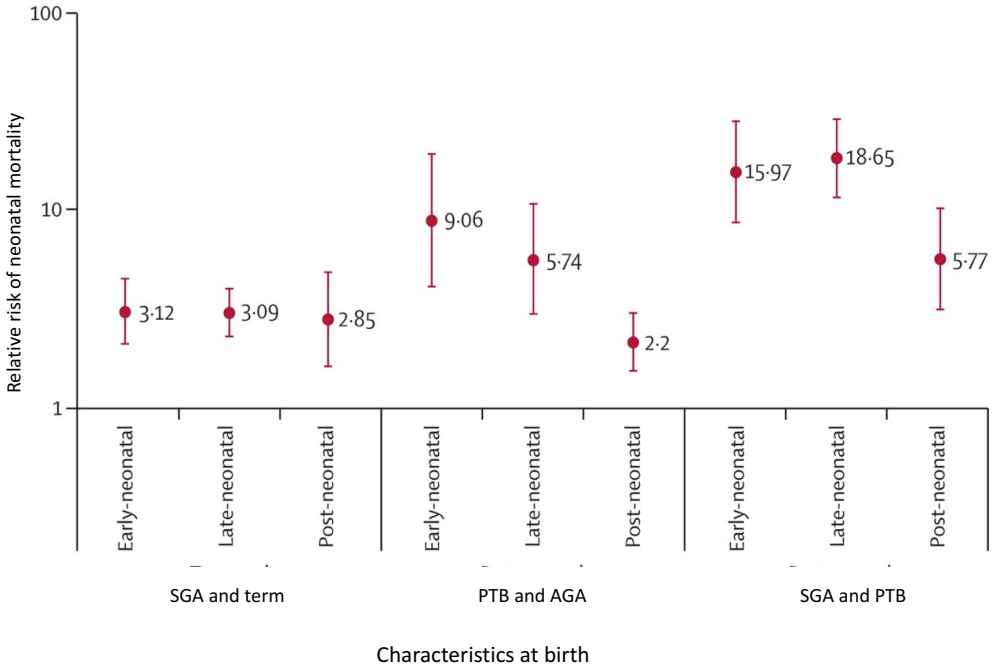


Figure 2.11. **Relative risk of neonatal mortality associated with AGA, SGA, and PTB neonates**. Source: Katz et al 2013[35].

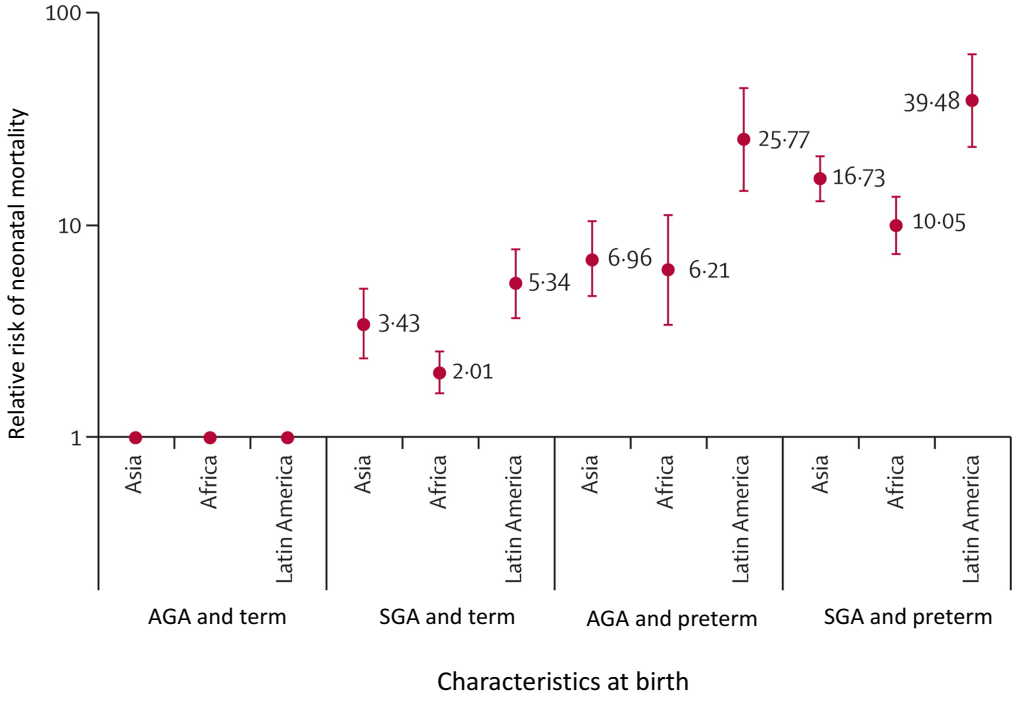


Figure 2.12. Relative risk of neonatal mortality associated with AGA, SGA and PTB neonates, according to regions. Source: Katz et al 2013[35].

The overlapping nature of these outcomes (PTB, LBW and SGA) means that they share similar short and long term complications, with varying degrees, particularly in infants who survive to childhood and adulthood. These include minor and major physical and neurodevelopmental disabilities, which impair the child's learning ability and school performance, and reduces the likelihood of these infants completing high school, and receiving a bachelor or postgraduate degree (Figure 2.13)[54,59]. Consequently, these children have a significantly reduced earning potential and economic productivity in adulthood[49,54].

These outcomes also have significant economic consequences for families who have to pay exorbitant fees for neonatal intensive care, ongoing healthcare costs during childhood, and additional fees for social and educational support[49]. The majority of these outcomes occur in sub-Saharan Africa and Southern Asia, both of which have a low level of development, with weak health systems and minimal or no access to universal health coverage or social assistance programs[60]. These exorbitant healthcare costs - the majority of which will need to be paid out-of-pocket - puts these families at risk of catastrophic health expenditure and of being driven into poverty[61].

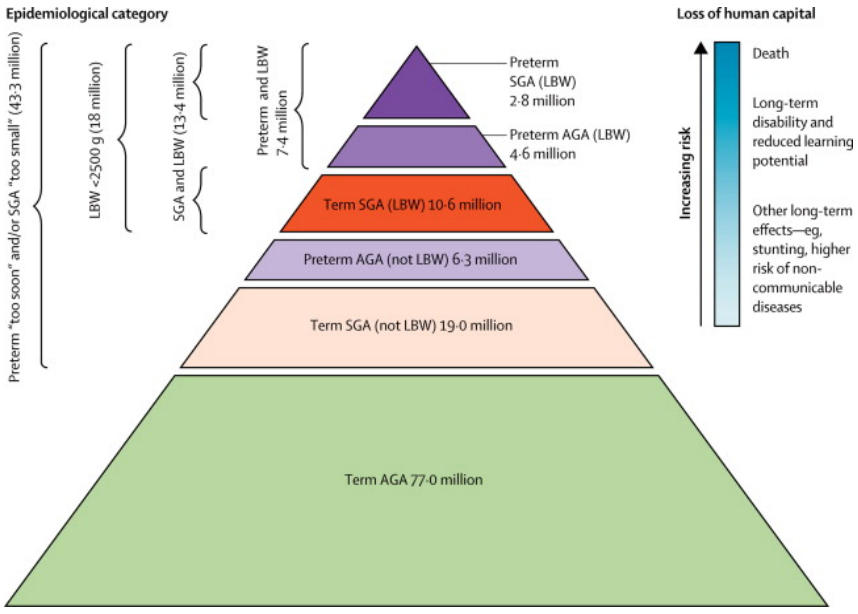


Figure 2.13: Public health implications of the burden of LBW, PTB and SGA in LMICs.
Source: Katz et al 2013[35]

The loss of life as a consequence of these outcomes, and the increasing burden of infants who survive with disabilities and are predisposed to developing non-communicable diseases in adulthood means that these LMICs in these regions lose a large proportion of its future labour capacity. Furthermore, a greater proportion of the Gross Domestic Product (GDP) of these economies will be shifted from activities that promote economic development to those needed to manage the health and social consequences of these outcomes. This has significant implications for the future growth and development of these economies, and significantly impedes progress towards the sustainable development goals (Figure 2.13)[35,62].

2.2.4 Perinatal outcomes according to fetal and neonatal mortality

2.2.4.1 Miscarriage and stillbirth

- Definition

There is substantial variation in the definition of stillbirth, which is generally considered as any third trimester delivery of a demised fetus[63]. The simplest definition classifies stillbirths into those that occurred during the antepartum (before the onset of labour) or intrapartum (during labour) period[15]; however, depending on the settings and resources available, stillbirth may be classified according to the birthweight (≥1000g), GA (≥24^{+0} or ≥ 28^{+0} completed weeks) or length at birth (≥35cm)[15,63]. Similarly, miscarriage can also be defined according to the birthweight (<500g), GA (<22 weeks) or length at birth (<25cm)[63]. For both miscarriage and stillbirth, the GA threshold - preferably determined using early ultrasound - is considered as the most appropriate method of identifying these outcomes[63], because unlike birthweight or length at birth, the GA takes into account the fact that a large proportion of fetuses at risk of these outcomes are also growth restricted[63].

- Epidemiology

The incidence of stillbirth in any setting is directly correlated with the nation's wealth and development and inversely associated with indices of sex equality, e.g. girl's education and contraception use[64]. Consequently, in 2015, almost all (98%) of the world's 2.62 million (2.36 – 2.98

million) stillbirths occurred in LMICs (Figure 2.14)[63]. Similar to what has been shown with LBW, PTB and SGA, the majority of stillbirths occurred in sub-Saharan Africa (stillbirth rate: 28.7 per 1000 total births) and Southern Asia (stillbirth rate: 25.5 per 1000 total births), which together account for more than two-thirds (77%) of the global burden of stillbirths[63] (Figure 2.14).

The stillbirth rate within a setting also accurately reflects weaknesses within the health system and the level and quality of care provided during the antenatal and particularly the intrapartum periods[15,63]. In sub-Saharan Africa and Southern Asia, more than 50% of all stillbirths occur during the intrapartum period, and two-thirds of stillbirths in these regions occur in rural communities[15]. As the majority of stillbirths occur intrapartum, this outcome is more likely a reflection of the scarcity of intrapartum monitoring and delays in the rapid delivery of a compromised fetus[15]. As such, looking at the effect of maternal HIV status on stillbirth rates may be an unreliable metric.

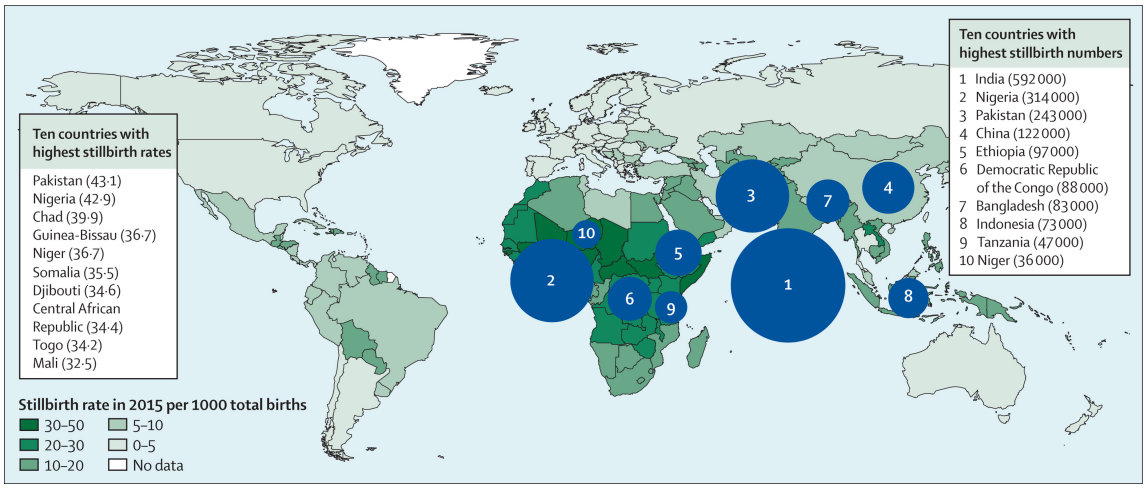


Figure 2.14. Variation between countries in stillbirth rates in 2015 showing the ten countries with the highest rates, and those with the largest numbers. Source: Blencowe et al 2016[63].

2.2.4.2 Neonatal deaths

In 2015, almost half (45.1%) of all under-5 deaths (5.94 million children) occurred during the neonatal period (28 days)[6]. This translates to 2.68 million children[6], a minimal decline from estimates reported in 2013 (2.76 million)[65]. Complications of PTB remain the leading cause of NND (15.9%), followed by complications from intrapartum related events (10.7%), and sepsis or meningitis (6.8%)[6]. As LBW, SGA and PTB contribute significantly to the global burden of NNDs and occur most commonly in LMICs, the majority of NNDs occur in LMICs[6]. These deaths have been attributed to the fact that women and neonates in these regions have poor access to healthcare services during the antenatal, intrapartum and neonatal period, with 98% of all neonatal deaths occurring in countries where neonatal intensive care services are minimal or non-existent[39,66,67].

Take home points:

1. All adverse perinatal outcomes are most prevalent in LMICs, particularly in sub-Saharan Africa and Southern Asia.
2. The use of LBW as a proxy for all high-risk babies including those born preterm and SGA should be avoided as it excludes a large number of high-risk babies from receiving essential interventions during the neonatal period.
3. Babies born preterm, LBW or SGA are at high-risk of mortality in the neonatal period and during infancy, with the risk of mortality increasing exponentially when these outcomes occur in combination, e.g. preterm-SGA.
4. In the short- and long-term, LBW, PTB and SGA significantly increase the burden of disease in LMICs, which needs to be managed by already weak health systems. This is compounded by significant social and economic costs at an individual, community and country level, with far reaching consequences for the economic growth and development of these countries.

2.3 HIV-infection

2.3.1 Origin of HIV-1

The Acquired Immuno-Deficiency Syndrome (AIDS) was first described in the USA in 1981 when five previously healthy males were diagnosed with opportunistic infections in the presence of severe immune suppression[68,69]. The infectious agent, HIV-1, was identified and characterised in 1983; however, a comparative evolutionary genetic study of HIV-1 from a human blood sample obtained in Kinshasa, Democratic Republic of Congo in 1960, suggests that the first human HIV-1 infection occurred in 1908[70-72].

HIV-1 emerged in humans following zoonotic transmission of Simian Immunodeficiency Virus (SIV) from naturally infected African primates (chimpanzees and gorillas)[73-75]. It is phylogenetically classified into four groups: major (M), non-major and non-outlier (N), outlier (O) and putative (P), each of which represents a unique zoonotic transmission event[74-77]. Infections as a result of groups N, O and P are infrequent, contributing to ±2% of the global HIV-1 prevalence, and is restricted to individuals from West African countries, e.g. Cameroon[76]. Group M represents the pandemic form of HIV-1, and accounts for more than 76 million infections globally[76].

2.3.2 Molecular structure of HIV-1

The HIV-1 virion is a Lentivirus, within the *Retroviridae* family. It is approximately 120nm in diameter and consists of an outer lipoprotein membrane, an inner protein capsid or core, and a layer of matrix protein between the membrane and the core (Figure 2.15)[78]. The outer lipid membrane contains viral glycoprotein complexes consisting of a surface envelope (Env) glycoprotein (gp120) and a transmembrane Env glycoprotein (gp41) (Figure 2.15)[79,80]. Furthermore, it contains Host proteins, including HLA class I and II, which are derived from the host cell membrane as a consequence of budding during viral replication (Figure 2.15)[81,82].

The inner protein capsid (p24 antigen) contains two copies of a single stranded, RNA genome of approximately 9.8kb in length (Figure 2.15), which encodes for nine genes (gag, env, pol, vif, vpu, vpr, tat, rev and nef) (Figure 2.16)[83].

The three major coding regions (gag, pol and env) encode for the structural and enzymatic proteins of the HIV-1 virus[84]:

- Gag: Encodes for a precursor polyprotein ($Pr56^{gag}$), which is processed by viral protease to generate three structural proteins, including matrix (p17), capsid (p24) and nucleocapsid (p7) (Figure 2.15 and Figure 2.16)[85,86].
- Pol: The 3' end of the gag genome overlaps with the 5' end of the Pol gene on a different reading frame, which when translated - through ribosomal frameshifting - produces a gag-pol polyprotein ($PR160^{gag\text{-}pol}$)[87]. This polypeptide is proteolytically processed and yields all the enzymes needed for viral replication, including integrase (endonuclease)(P32), reverse transcriptase (RT) and protease (p11) (Figure 2.15 and Figure 2.16)[86].
- Env: Encodes for the envelope glycoprotein gp160, which is cleaved to form gp120 and gp41[88].

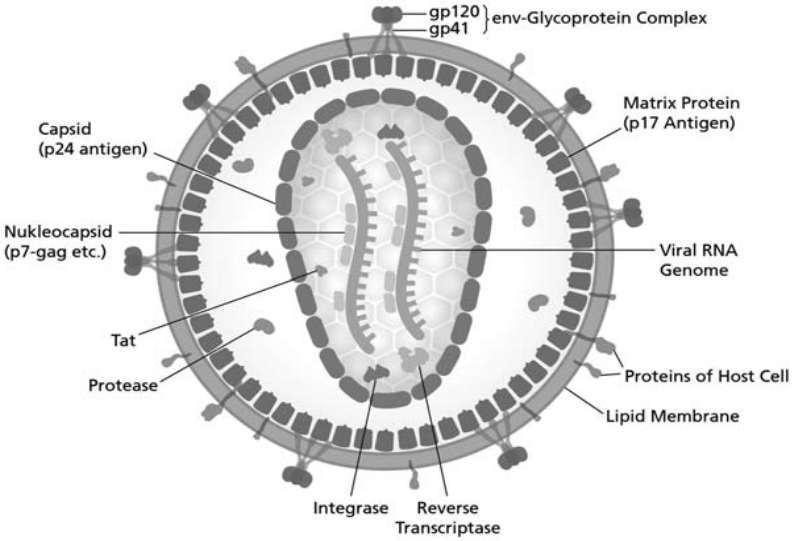


Figure 2.15 A schematic representation of the HIV-1 virion.
Source: Hoffman, et al. 2015[89]

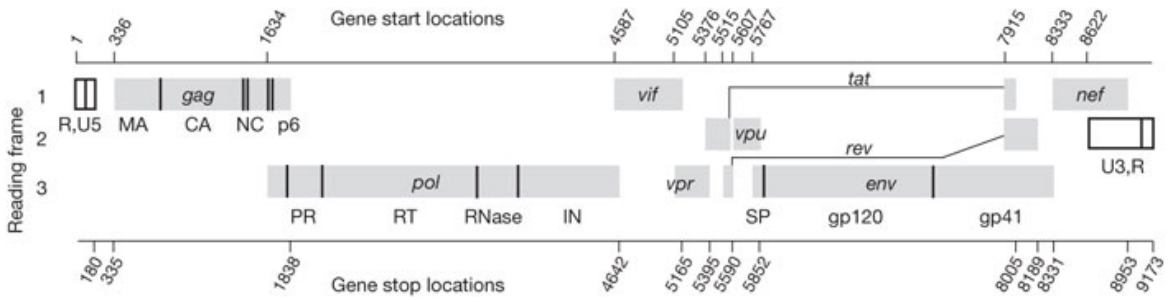


Figure 2.16 HIV-1 genome organization.
Source: Watts, et al. 2009[90]

The remaining six genes encode for regulatory (rev, tat) and accessory proteins (nef, vif, vpr, vpu), which regulate viral replication, enhance viral infectivity and facilitate viral escape[91]. The 5' and 3' ends of the HIV-1 genome are flanked by a long terminal repeat region (LTR), which facilitates the integration of the viral genome into the host's DNA and is responsible for the chronicity of the infection[84].

2.3.3 HIV-1 transmission, replication cycle and clinical course.

2.3.3.1 HIV-1 transmission

Unprotected sexual intercourse exposes mucosal surfaces to HIV-1 infected bodily fluids, and remains the most frequent (80%) route of transmission in adults (Table 2.3)[92]. The risk of sexual transmission varies depending on biological, behavioural and structural factors (reviewed by Shaw et al 2012[93]); however, the HIV-1 circulating viral load (VL) is the most important predictor of sexual transmission[94]. For each log increment in the VL, the risk of transmission increases by 2.45 (95%CI 1.85 – 3.26), conversely a 0.74 $\log_{10}$ decline in the circulating VL reduces the risk of transmission by 50%[95,96].

Other, less frequent routes of transmission include percutaneous or intravenous inoculation with HIV-1 infected bodily fluid, and mother-to-child-transmission (MTCT) of the virus during pregnancy, the intrapartum period or postnatal period though breastfeeding (Table 2.3)[92].

Table 2.3: Transmission routes and risks for HIV-1 infection

HIV invasion site	Anatomical sublocation	Transmission medium	Transmission probability per exposure	Estimated contribution of HIV cases world wide
Female genital tract	Vagina, cervix	Semen, blood	1 in 200 to 1 in 2000	12.6 million
Male genital tract	Inner foreskin, penile urethra	Cervicovaginal and rectal secretions, blood	1 in 700 to 1 in 3000	10.2 million [a]
Intestinal tract	Rectum	Semen, blood	1 in 20 to 1 in 300	3.9 million [b]
	Upper gastrointestinal tract	Semen, blood	1 in 2500	1.5 million
		Intrapartum: maternal blood, genital secretions	1 in 5 to 1 in 10	960,000 [c]
		Breast milk	1 in 5 to 1 in 10	960,000 [c]
Placenta	Chorionic villi	Intrauterine: Maternal blood	1 in 10 to 1 in 20	480,000 [c]
Blood stream	-	Blood products, sharps	95 in 100 to 1 in 150	2.6 million [d]

[a] Includes: men who have sex with men (MSM), bisexual men, and heterosexual men.

[b] Includes: MSM, bisexual men, and women infected via anal receptive intercourse.

[c] Mother-to-child transmission (MTCT)

[d] Mostly intravenous drug use, but includes infections by transfusions and healthcare related accidents

Adapted from Shaw, et al. 2012[93]

2.3.3.2 HIV-1 viral replication

Following exposure, HIV-1 crosses the mucosal membrane and primarily targets $CD4^+$ T-lymphocytes, macrophages and dendritic cells[91,95]. It binds its surface glycoprotein (gp120) to the extracellular component of the host's $CD4^+$ T-lymphocyte receptor, while gp41 glycoprotein anchors gp120 to the viral membrane (Figure 2.17: step 1) (Figure 2.18)[97,98].

The binding of gp120 to the $CD4^+$ T-lymphocyte receptor induces a conformational change to gp120 that exposes the third variable region (V3) loop on the gp120 glycoprotein (Figure 2.18)[98,99]. Depending on the viral strain, the V3 loop segment recognizes and binds to one of two clinically important chemokine receptors on the target cell: (1) CCR5 receptor, expressed on T-lymphocytes, macrophages and dendritic cells or (2) CXCR4 receptor, expressed on naïve $CD4^+$ T-lymphocytes only (Figure 2.18)[100,101]. The CCR5 (monocytotropic) viral strain is preferentially transmitted to the host and represents the majority of transmitted (founder) viruses[92]. However, an individual may contain CCR5 and CXCR4 HIV-1 isolates, or HIV-1 isolates that are able to use both CCR5 or CXCR4 for viral entry (dual tropic)[102]. Once the V3 loop of the gp120 glycoprotein binds to the target cell co-receptor (CCR5 or CXCR4), the gp41 glycoprotein penetrates the target cell membrane and instigates the fusion of the viral and target cell membranes (Figure 2.17: step 2)[103] .

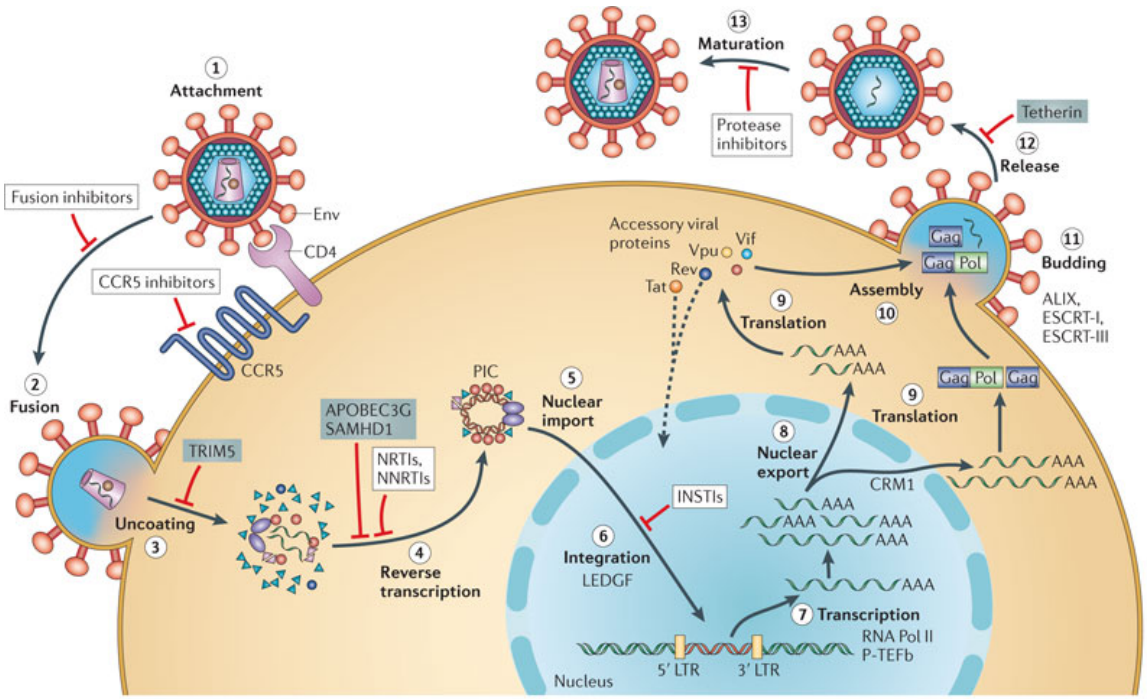


Figure 2.17 Schematic overview of the HIV-1 replication cycle.

Source: Engelman et al 2012[104]

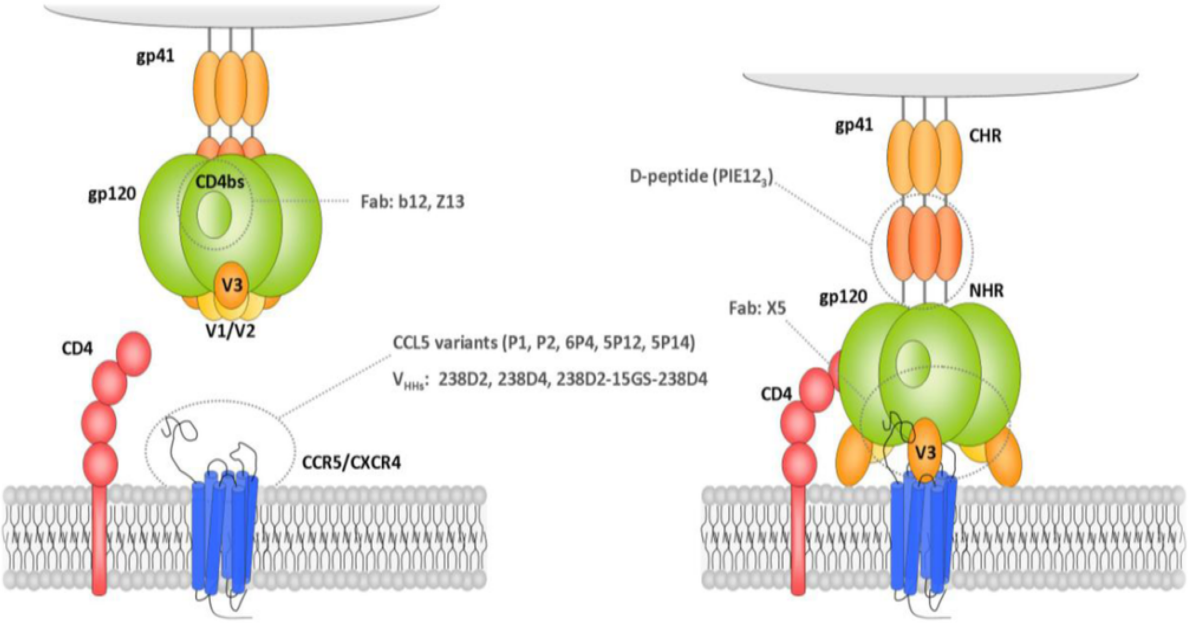


Figure 2.18: Binding of CD4 T-lymphocyte to gp120.

Source: Delhalle et al 2012[105]

Though an "un-coating" process, the viral core - containing the RNA strands and three replication enzymes, RT, integrase and protease - is inserted into the cytoplasm of the target cell (Figure 2.17: step 3)[104]. Once inside the cytoplasm of the target cell, viral RT enzyme generates a double-stranded linear viral DNA[106]. Viral RT has two enzymatic activities:

- DNA polymerase is used to generate a DNA strand using the viral RNA as a template, resulting in a RNA/DNA double helix[106,107].
- RNase H cleaves the RNA component of the RNA/DNA double helix, exposing the DNA strand, which is reverse transcribed to form a double-stranded viral DNA with LTR regions at each end[106].

The process of reverse transcription produces the viral pre-integration complex (PIC), consisting of the viral DNA in association with viral (e.g. integrase) and host proteins[108]. Following T-lymphocyte activation, the PIC is transported through the nuclear pores to the nucleus of the target cell (Figure 2.17: step 5)[104]. Within the nucleus of the target cell, viral integrase catalyses the processing of the 3' end of the viral genome in preparation for integration with the host genome; furthermore, it catalyses the integration of the viral genome into the host genome – through a process called DNA strand transfer – to yield proviral DNA (Figure 2.17: step 6)[109]. Integrase then binds to the viral RNA, which is crucial for the generation of mature and infectious viral particles[110].

The proviral DNA is transcribed by the host RNA polymerase into messenger RNA (mRNA) and is exported from the nucleus to the cytoplasm where it is translated to produce viral polyprotein precursors (Figure 2.17: step 7-9)[104,111]. These polyprotein precursors include gag, gag-pol, env glycoprotein, as well as the regulatory and accessory proteins[111]. The viral proteins and two copies of RNA assemble on the inner surface of the host cell membrane to form a new immature virion, which buds off the cell and is released (Figure 2. 17: step 11 and 12)[111]. During the budding process or as soon as the virus is released, viral protease enzyme cleaves the polyprotein precursors to yield its structural components (see section 2.3.2). This event triggers the maturation of the immature viral particle into an infectious virus (Figure 2.17: step 13)[104,111].

2.3.3.3 HIV-1 clinical course

The clinical course of HIV can be divided into three stages: acute HIV-syndrome, asymptomatic chronic infection and symptomatic infection, also known as AIDS (Figure 2.19[112], Figure 2.20[113]).

- Acute HIV-syndrome

The acute stage of HIV encompasses the period between HIV-1 acquisition and seroconversion, and manifests with nonspecific "flu-like symptoms" including myalgia, fatigue and fever[114,115]. Following exposure, HIV-1 crosses the mucosal membrane to infect $CD4^+$ T-lymphocytes, macrophages, dendritic cells and Langerhans cells, and for the next 7-21 days (eclipse phase) it replicates within the local mucosa, submucosa and draining lymphoreticular tissues (Figure 2.19)[92,114]. Viral replication migrates to the gut-associated-lymphoid-tissue (GALT) within the small intestine where it destroys and depletes the memory and activated $CD4^+$ T-lymphocytes[116]. Thereafter, viral replication spreads systemically and is associated with a peak in the viral RNA circulating in the plasma (i.e. VL) at 10 million copies per ml (Figure 2.19), rendering these individuals highly infective[117,118]. Primary HIV infection can also be categorised into six clinical stages based on the sequential detection of HIV-1 antigens and antibodies (Figure 2.19)[119].

- Asymptomatic chronic HIV-1 infection

The period of clinical latency is characterised by the establishment of a viral set-point 6 weeks after infection, persistent viral replication, generalised immune activation and a progressive decline in the circulating $CD4^+$ T-lymphocyte count, in the absence of clinical symptoms (Figure 2.20)[113,120]. In the absence of ART, the duration at which one progresses to AIDS varies depending on the age at seroconversion, virological and host factors[121,122]. It ranges from 6-10 years in the general population[122] to up to 20 years in long-term non-progressors, i.e. HIV-positive individuals with stable $CD4^+$ T-lymphocyte counts and no disease progression without the use of ART[121].

- Symptomatic Acquired Immunodeficiency Syndrome (AIDS)

This stage is characterised by a rapid decline in the circulating $CD4^+$ T-lymphocyte count to <200 cells/µl, the development of opportunistic infections (e.g. *pneumocystis jirovecii* pneumonia) or malignancies (e.g. Kaposi's sarcoma)[123].

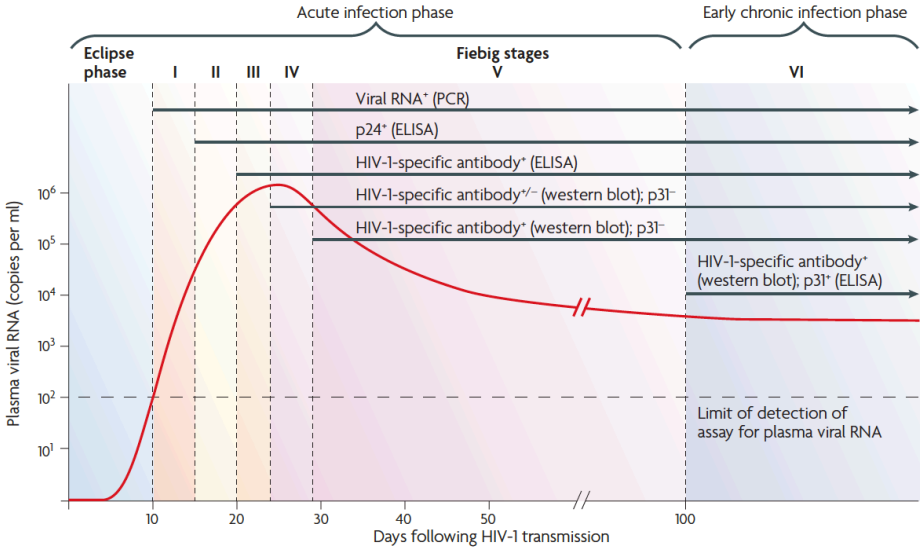


Figure 2.19 Early HIV-1 infection and Fiebig stages. Source: McMichael, et al. 2010[112]. Stage I: viral RNA detected by PCR; Stage II: In addition to RNA, p24 (*Gag*) antigen is detected by enzyme-linked immunosorbent assay (ELISA); Stage III: In addition to RNA and p24 (*Gag*) antigen, HIV-1 specific antibodies detected by ELISA; Stage IV-VI: As stage III, but p31 (*Pol)* antigen detected by ELISA and HIV-1 specific antibodies detected by western blot[119].

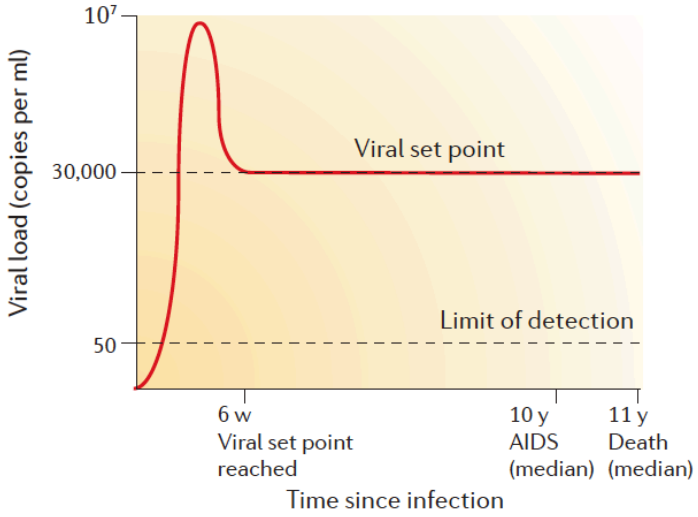


Figure 2.20 Typical (progressing) adult HIV infection, from acquisition to the development of AIDS. Source: Goulder, et al. 2016[113]

2.3.4 Global HIV epidemic

In 2015, an estimated 38.8 [95% Uncertainty Interval (UI) 37.6 – 40.4] million people were living with HIV-1 (Figure 1.5A); 52.8% of whom were female[2]. Between 2000 and 2015, the global HIV prevalence increased by 0.8% per year (figure 2.20 A) and the annual incidence has remained stable (2.5-2.6 million cases per year), barring 74 countries where the incidence rate increased (Figure 2.20 B)[2]. Since the start of the epidemic, an estimated 78 (69.5 - 87.6) million people have been infected with the virus, and almost half [35 (29.6 – 40.8) million people] of those infected have died from AIDS-related illnesses [1].

The majority of HIV-1 infected individuals reside in sub-Saharan Africa (75.8%; 29.4 million people) (Figure 2.20 A, Figure 2.21)[2]. This includes 83.1% (17.0 million; 95%UI 16.5 -17.6 million) of all HIV-positive females, and 91% of all HIV-positive pregnant women[2,3]. Furthermore, sub-Saharan Africa accounts for the majority of all new HIV-infections (75.4%) (Figure 2.20 B) and AIDS-related deaths (72.1%)[2]. Ten African countries account for 82% of all HIV-positive individuals living in sub-Saharan Africa: Ethiopia, Kenya, Malawi, Mozambique, Nigeria, South Africa, Uganda, Tanzania, Zambia and Zimbabwe (Figure 2.21)[124]. South Africa, which only represents 0.7% of the global population, currently has the largest epidemic in the world, accounting for 19% of the global HIV burden (Figure 2.21)[2,125].

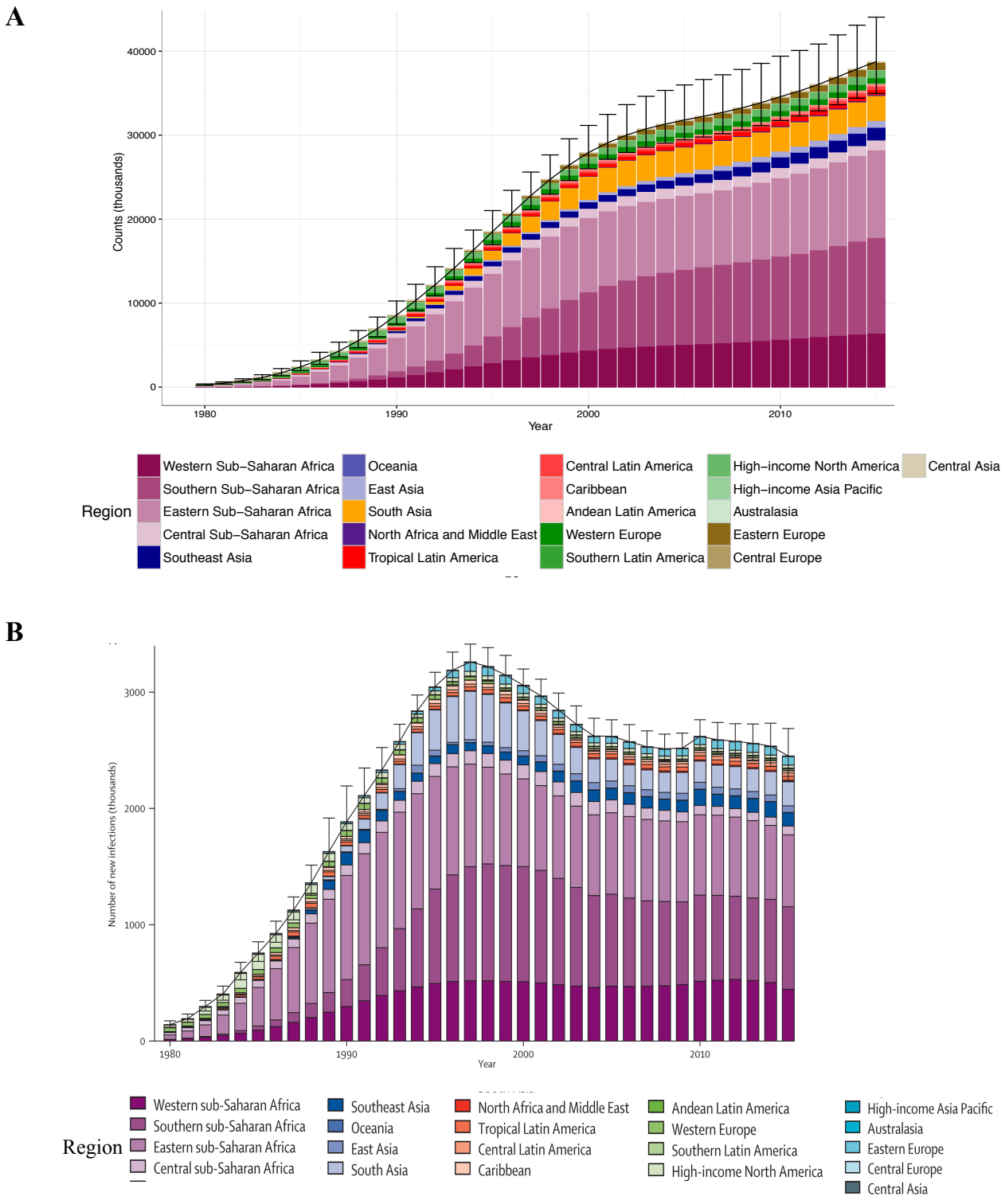


Figure 2.21: A: Global HIV-1 prevalence from 1980 – 2015 stratified according to regions, with 95% uncertainty intervals (UI), B: Global HIV-1 incidence from 1980 – 2015, stratified according to regions with 95%UI.
Source: Wang et al 2016[2]

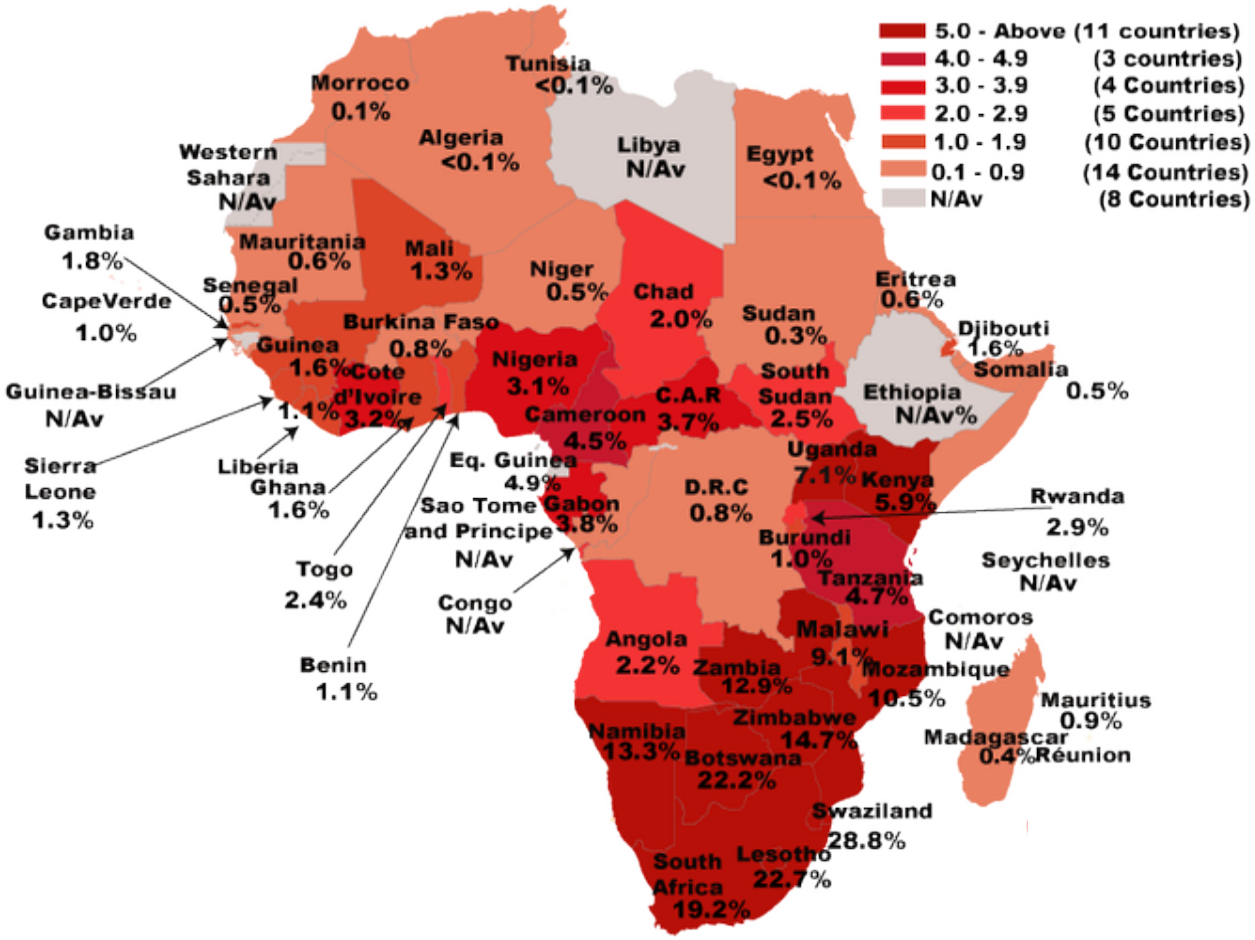


Figure 2.22 Adult HIV-1 prevalence in African countries in 2016.
Source: Afri-Dev.Info 2006[126]

2.3.5 HIV infection in women of reproductive age

Compared to men, women have a disproportionately higher risk of contracting HIV because of physiological, behavioural, economic, cultural, social and structural factors[127-129]. This is particularly true for adolescent girls and young women (15-24 years) in sub-Saharan Africa, who represent 15% of all HIV-positive women, and acquire the virus 5-7 years before their male counterparts[124].

HIV-infection remains the leading cause of death for women of reproductive age (15-44 years), (19%) particularly in developing countries[130-132]. In the absence of ART, HIV-positive women have a twenty-fold (rate ratio: 20.5; 95%CI 18.9 – 22.4) increased risk of dying compared to HIV-negative women[133]. The risk of mortality declines during pregnancy because of the "healthy pregnant woman effect", a selection effect where women with advanced disease do not conceive due to reduced fertility[134]. Despite this effect, HIV-positive women are still eight times (rate ratio: 8.2; 95%CI 5.7 – 11.8) more likely to die during pregnancy or in the postnatal period, compared to HIV-negative pregnant women[4]. HIV-infection contributes to 5.5% of maternal deaths globally, and between 6.5% to 26% of maternal deaths in sub-Saharan African countries[135,136]. This has been attributed to the increased susceptibility of HIV-positive women to non-pregnancy related infections (e.g. tuberculosis, pneumonia) and pregnancy-related sepsis[137].

Untreated HIV-infection – with its persistent state of immune activation and inflammation – is also associated with severe non-AIDS related diseases, including malignancies, cardiovascular events (e.g. myocardial infarction), end stage renal disease, liver cirrhosis and neurocognitive disorders[138-140]. These non-AIDS related diseases are the primary cause of death in HIV-positive individuals residing in high-income settings[141]. In addition to the increased risks of morbidity and mortality, HIV can be transmitted from the mother to her child during the antenatal, intrapartum and post-natal periods[5]. The rate of MTCT varies from 15-30% in non-breastfeeding populations, to 20-45% depending on the duration of breastfeeding[5]. A third of infected children die before their 1st birthday, and 50% of infected infants die before 2 years of age[142,143].

2.4 Antiretroviral treatment

The discovery and wide dissemination of ART marked the turning point of the HIV epidemic[144]. The initiation of ART does not cure HIV/AIDS, but improves the health and quality of life of those infected and significantly reduces the risk [adjusted Hazard Ratio (HR) 0.23; 95%CI 0.08 – 0.61) and rate (5.5% decline per year) of HIV-related mortality with HIV-positive individuals now having an almost normal life expectancy[2,145,146] (Figure 22.2 A).

The scale-up of ART in LMICs has led to a significant decline in the infection rate, particularly as a result of heterosexual and perinatal routes of transmission (Figure 2.22 A)[147,148]. In 2015, an estimated 17 million people living with HIV received ART, including 38.6% of HIV-positive men and 42.2% of HIV-positive women (Figure 2.22 B)[2,149].

2.4.1 Antiretroviral class, regimens and drugs

In the absence of ART, HIV-positive individuals experience rapid viral replication (rate of 10^{10} virions per day) in the presence of a high mutation (3.4×10^{-5} million mutations per base pairs) and recombination rate, resulting in the production of millions of genetically variable HIV virions within the infected individual[150-153]. Antiretroviral drugs target and inhibit different stages of viral replication in actively replicating virions, and are classified (classed) according to the stage of viral replication they inhibit or their chemical structure (Figure 2.22)[154]. The aim of treatment is to suppress viral replication and reduce the circulating VL to levels where the virus is unable to evolve or develop resistance to ART[154]. The decline in the VL allows for the reconstitution of the immune system, which is associated with a rapid increase in the circulating $CD4^+$ T-lymphocyte count, thereby reducing the individual's susceptibility to developing opportunistic infections and dying[155,156].

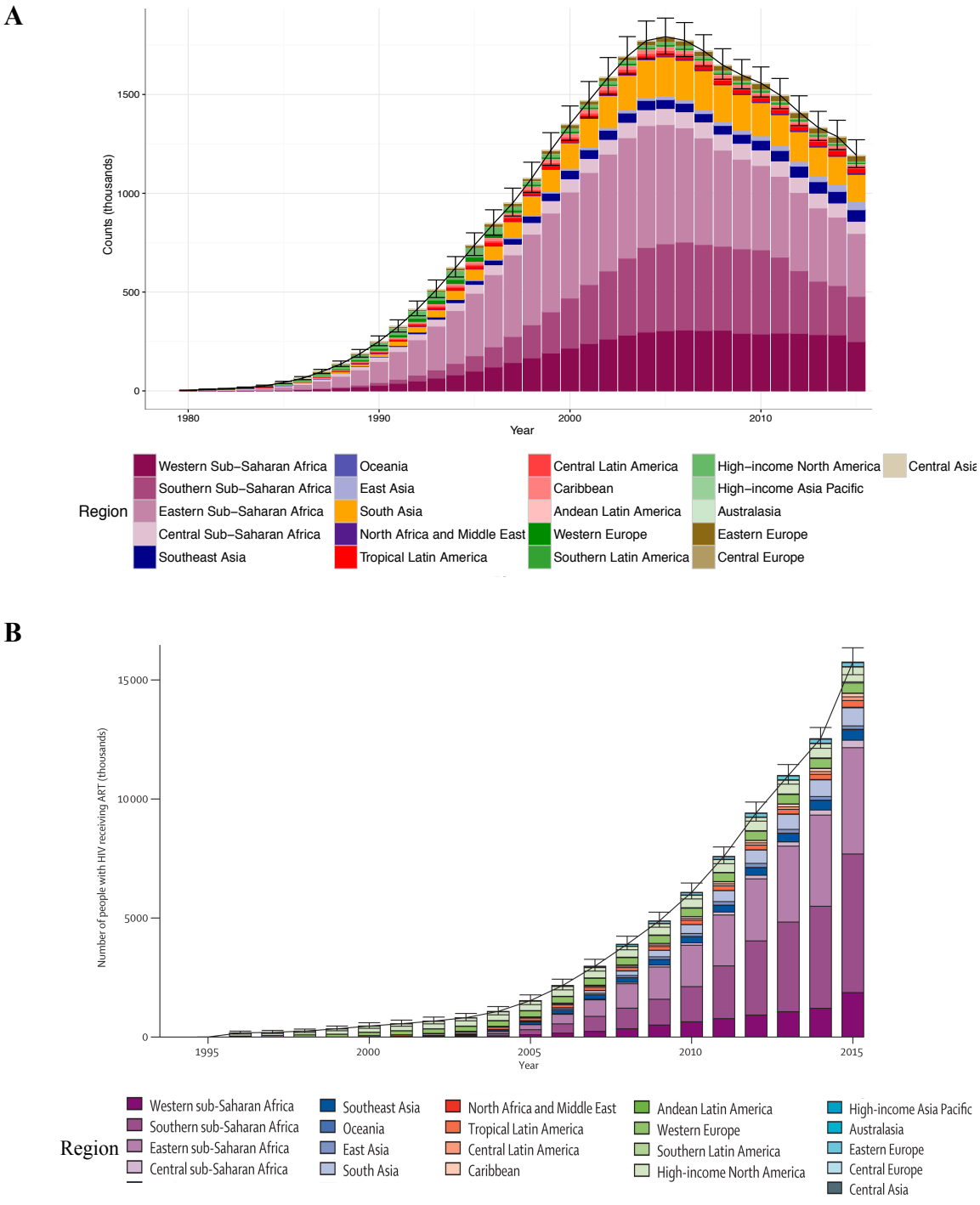


Figure 2.23: A: Number of AIDS-related deaths from 1980 – 2015, stratified according to regions, with 95%UI, B: Mean number of HIV-positive individuals who receive ART per year, from 1995 – 2015, stratified according to regions, with 95%UI. Source: Wang et al 2016[2]

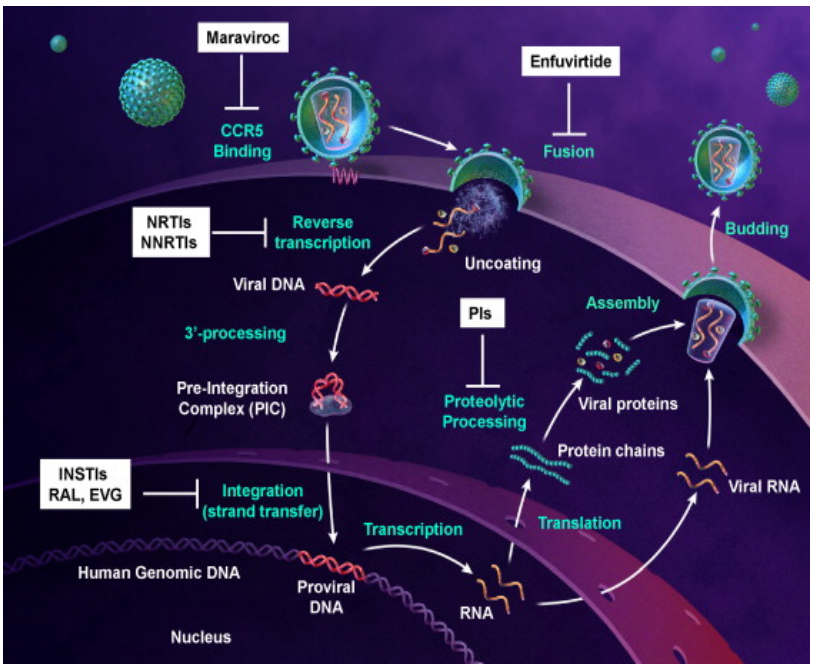


Figure 2.24: HIV replication cycle and antiretroviral drugs.

Source: McColl et al 2010[157]

Currently, five classes of antiretrovirals, including more than 25 antiretroviral drugs are commonly used in clinical practice (Table 2.4). These are generally initiated as one of three ART regimens, including monotherapy (only one antiretroviral drug), dual therapy (two antiretroviral drugs) or Highly Active Antiretroviral Treatment (HAART) (three or more antiretroviral drugs, two of which target different viral structures)[158]. The genetic variability of the virus reduces its susceptibility to certain ARTs; therefore, effective viral suppression can only be achieved through the initiation of dual therapy with drugs containing a high barrier to genetic resistance, e.g. (protease inhibitors)[159] or HAART with each drug included in the regimen inhibiting a different stage of HIV replication[160,161].

Table 2.4: Antiretroviral class and drugs in clinical use

	Class	Class abbreviation	Target site during viral replication	Drug (abbreviation)
1.	Nucleoside/ nucleotide reverse transcriptase inhibitor	NRTI/NtRTI	Reverse transcriptase enzyme	Tenofovir Disproxil Fumarate (TDF)* abacavir (ABC) zidovudine (AZT) stavudine (D4T) lamivudine (3TC) emtricitabine (FTC) didanosine (ddl)
2.	Non-nucleoside reverse transcriptase inhibitors	NNRTIs	Reverse transcriptase enzyme	Efavirenz (EFV) nevirapine (NVP) etravirine (ETR) delavirdine (DLV) rilpivirine (RPV)
3.	Protease inhibitors	PIs	Protease inhibitor enzyme	Fosamprenavir (FPV) atazanavir (ATV darunavir (DRV) indinavir (IDV) lopinavir (LPV) nelfinavir (NFV) saquinavir mesylate (SQV) tipranavir (TPV) ritonavir (RTV)
4.	Integrase strand transfer inhibitor	InSTIs	Integrase enzyme	Raltegravir (RAL) elvitegravir (EVG) dolutegravir (DTG)
5.	CCR5 inhibitor		CCR5 receptor binding	Maraviroc (MVC)

* Nucleotide reverse transcriptase inhibitors (NtRTI)

Source: adapted from Volberding et al 2010, Art et al 2012, McColl et al 2010[154,157,160]

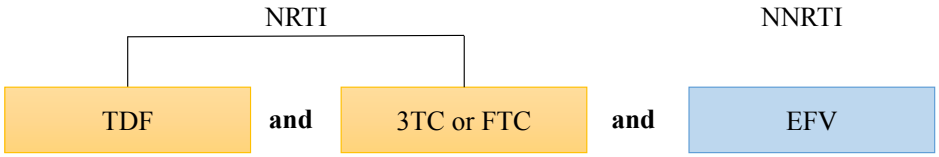
2.4.1.1 Nucleoside (nucleotide) reverse transcriptase inhibitor

In March 1987, zidovudine (AZT) became the first antiretroviral drug approved for the palliative treatment of HIV-positive patients with AIDS[162]. Nucleoside reverse transcriptase inhibitors (NRTI) and nucleotide reverse transcriptase inhibitor (NtRTI) are analogues of naturally occurring deoxynucleotides (dNTPs), which are administered as prodrugs and exert their antiviral activities once metabolised intracellularly by the host-cell enzymes[163]. The metabolism of the nucleoside analogue generates an active 5'-triphosphorylated compound, while the metabolism of the nucleotide prodrug generates an active 5'-diphosphate compound[163,164].

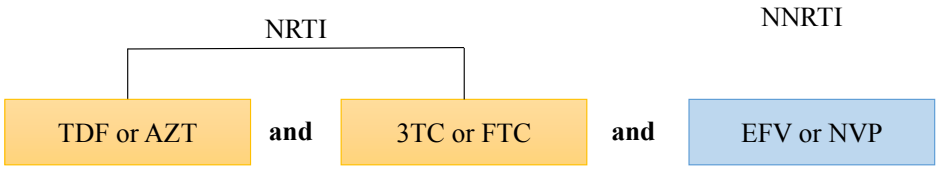
The 5'-triphosphate compound, called dideoxynucleoside analogue triphosphate (ddNTPs), lacks a 3'-hydroxyl group at the sugar moiety and competes with the host dNTPs for substrate binding to viral reverse transcriptase enzyme[164]. The absent 3'-hydroxyl group prevents the formation of a 3'-5' phosphodiester bond between the elongating proviral DNA chain and the incoming 5' nucleoside triphosphate[163]. Consequently, the elongating proviral DNA chain is terminated, preventing viral replication[163].

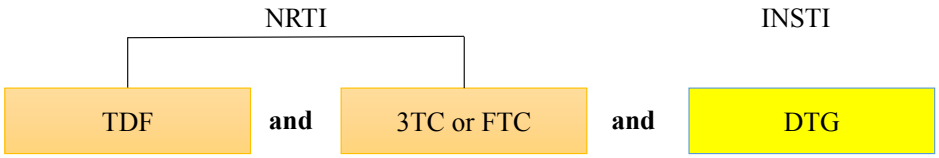
While NRTIs are associated with numerous side effects (e.g. mitochondrial toxicity, lipoatrophy, anaemia and peripheral neuropathy)[165], they remain crucial in the management of HIV-positive individuals and are the most common antiretroviral drug in clinical use, particularly in sub-Saharan Africa[166]. They constitute the backbone of all recommended first line HAART regimens and are the only class of drugs approved for pre-exposure prophylaxis (PrEP) (Figure 2.24)[167].

Preferred first-line regimen:



Alternative first-line regimen





Second-line regimen*

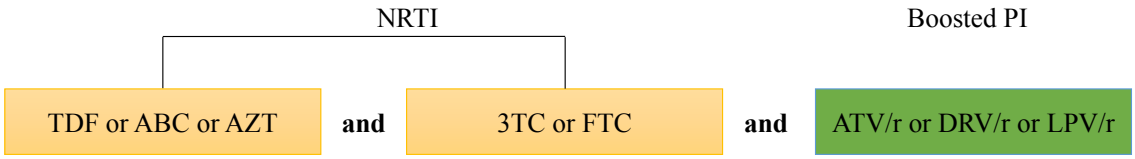


Figure 2.25: **WHO 2016 consolidated guidelines on first and second line regimens for adults and pregnant women.** *Raltegravir and lopinavir/ritonavir can be used as an alternative second-line treatment. Abbreviations: AZT = zidovudine, d4t = stavudine, 3TC = lamivudine, TDF = tenofovir, ABC = abacavir, EFV = efavirenz, NVP = Nevirapine
Source: WHO et al 2016[12], image adapted from Gilks et al 2006[168].

2.4.1.2 Non-nucleoside reverse transcriptase inhibitor

The success of NRTIs was short-lived as limitations quickly became evident. Numerous reports emerged of increased rates of NRTI-related side effects, an inability of the drug to inhibit viral replication completely and the rapid development of drug resistance[169]. By chance, Merluzzi et al (1990) discovered non-nucleoside reverse transcriptase inhibitors (NNRTIs), a non-competitive inhibitor of RT enzyme that binds to a hydrophobic pocket adjacent to the polymerase catalytic site of RT[169]. The binding of NNRTI to RT induces a conformational change of the substrate-binding site and reduces the polymerase activity; consequently, RT is unable to produce proviral DNA[170,171]. The most common NNRTIs in clinical use are nevirapine (NVP) and efavirenz (EFV), both of which are potent, have a long half-life and effectively suppress viral replication (Figure 2.24)[166,172]

Despite its low cost, NVP has been associated with numerous challenges, including the development of hypersensitivity reactions, hepatotoxicity, and the emergence of resistance mutations, even after a single-dose[154,173]. These adverse effects occur more frequently when NVP is initiated in women who present with a $CD4^+$ T-lymphocyte count >250 cell/µl[154,174,175]. EFV, which has less frequent and milder side-effects – including central nervous system effects and inconsistent reports of teratogenicity – is therefore the preferred NNRTI and is recommended by WHO as part of its preferred first-line regimen for adults (Figure 2.24)[12].

2.4.1.3 Protease inhibitors

Protease inhibitors (PIs) are potent antiretroviral drugs with a long half-life and high genetic barrier to resistance resulting in sustained viral suppression even in HIV-positive individuals with lower levels of adherence (70-80%)[176]. PIs consist of large peptide-like compounds that require co-administration with a "boosting" agent to inhibit their metabolism and enhance drug levels[160]. Ritonavir is the only boosting agent available for use with PIs, and is a potent inducer and inhibitor of several P450 cytochromes and CYP3A4 enzyme[177].

In 2016, Kanters et al (2016)[178] conducted a systematic review and network meta-analysis of 71 RCTs in order to investigate the comparative effectiveness of common ARTs recommended for clinical use in ART-naïve patients (Figure 2.25). PI-based regimens were the least effective at suppressing viral replication compared to all other ART classes, with boosted lopinavir-based HAART having the lowest virological efficacy (Figure 2.26)[178]. Furthermore, PI's are expensive, have a high pill burden and have numerous side-effects including gastrointestinal disturbances, lipodystrophy, dyslipidaemia and an increased risk of cardiovascular disease in patients exposed to lopinavir (LPV) and indinavir (IDV)[154,172,179,180]. Darunavir (DRV) and atazanavir (ATV) have reduced side-effect profiles, with no cardiovascular effects following exposure to atazanavir (ATV)[181], and minimal discontinuations in individuals receiving DRV[178]. PIs are currently reserved for special circumstances or for HIV-positive individuals who require a second-line regimen (Figure 2.24)[12].

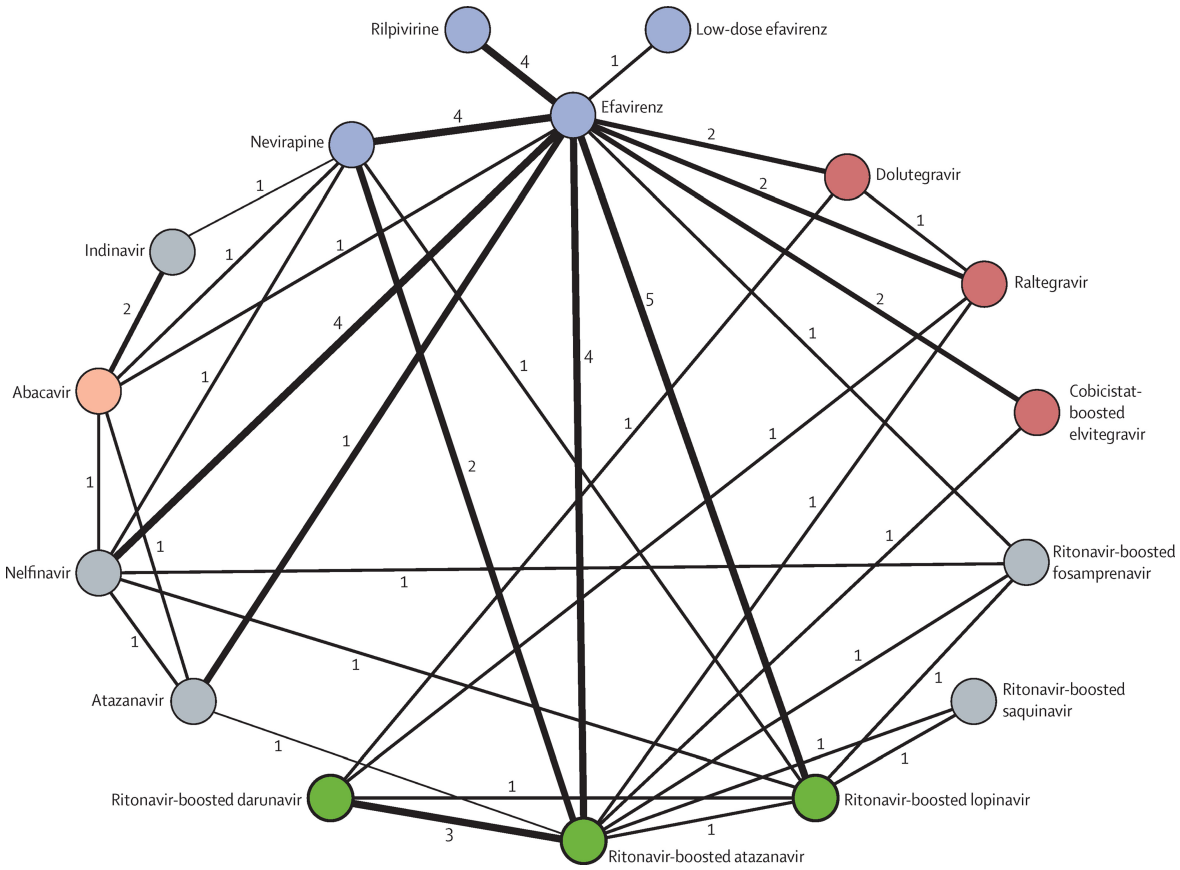


Figure 2.26: **Network graph illustrating eligible comparisons between antiretroviral drugs**
Source: Kanters et al 2016[178]

EFV	**1·90 (1·40–2·59)**	**1·45 (1·07–1·95)**	1·10 (0·77–1·59)	0·69 (0·48–1·03)	0·93 (0·74–1·18)	0·99 (0·71–1·40)	**0·49 (0·30–0·82)**	1·19 (0·73–1·95)	1·34 (0·92–1·94)
1·87 (1·34–2·64)	DTG	0·76 (0·56–1·03)	**0·58 (0·37–0·92)**	**0·36 (0·24–0·56)**	**0·49 (0·35–0·69)**	**0·52 (0·37–0·74)**	**0·26 (0·14–0·47)**	0·63 (0·35–1·11)	0·71 (0·44–1·14)
1·40 (1·02–2·59)	0·75 (0·53–1·05)	RAL	0·76 (0·49–1·18)	**0·48 (0·32–0·73)**	**0·64 (0·47–0·88)**	**0·68 (0·48–0·97)**	**0·34 (0·19–0·61)**	0·82 (0·46–1·44)	0·93 (0·57–1·49)
1·28 (0·87–1·89)	0·68 (0·41–1·14)	0·91 (0·56–1·50)	EVG/c	0·63 (0·39–1·03)	0·84 (0·59–1·22)	0·90 (0·57–1·44)	**0·45 (0·24–0·83)**	1·09 (0·58–1·98)	1·22 (0·72–2·03)
0·76 (0·59–0·98)	**0·40 (0·27–0·60)**	**0·54 (0·37–0·78)**	**0·59 (0·38–0·92)**	LPV/r	1·34 (0·96–1·85)	1·43 (1·00–2·00)	0·72 (0·39–1·27)	1·73 (0·91–3·11)	**1·94 (1·12–3·21)**
0·90 (0·74–1·10)	**0·48 (0·33–0·69)**	**0·64 (0·46–0·89)**	0·70 (0·48–1·04)	1·18 (0·92–1·54)	ATV/r	1·07 (0·78–1·48)	**0·54 (0·31–0·92)**	1·28 (0·73–2·20)	1·45 (0·92–2·22)
0·91 (0·66–1·28)	**0·49 (0·33–0·72)**	**0·65 (0·45–0·94)**	0·71 (0·44–1·16)	1·21 (0·87–1·69)	1·02 (0·74–1·40)	DRV/r	**0·50 (0·27–0·90)**	1·20 (0·65–2·17)	1·36 (0·80–2·21)
0·87 (0·70–1·07)	**0·46 (0·32–0·68)**	**0·62 (0·43–0·89)**	0·68 (0·44–1·04)	1·15 (0·85–1·54)	0·97 (0·76–1·23)	0·95 (0·65–1·37)	NVP	**2·42 (1·18–4·88)**	**2·72 (1·46–5·11)**
1·16 (0·67–2·02)	0·62 (0·33–1·17)	0·82 (0·44–1·55)	0·90 (0·46–1·77)	1·52 (0·83–2·59)	1·29 (0·72–2·31)	1·26 (0·67–2·39)	1·33 (0·74–2·40)	low EFV	1·13 (0·61–2·13)
1·18 (0·90–1·55)	**0·63 (0·41–0·98)**	0·85 (0·55–1·28)	0·92 (0·57–1·48)	**1·57 (1·07–2·25)**	1·32 (0·93–1·83)	1·29 (0·83–1·98)	1·36 (0·96–1·92)	1·02 (0·56–1·87)	RPV

Treatment | 48 week network results, OR (95% CI) | 96 week network results, OR (95% CI)

Figure 2.27: **Random-effect network meta-analysis of the relative efficacy of antiretroviral drugs for viral suppression.**

Source: Kanters et al 2016[178]

2.4.1.4 Integrase strand transfer inhibitors

Integrase strand transfer inhibitors (InSTIs) are a relatively new and exciting class of antiretrovirals that have already replaced more conventional antiretrovirals in the management of HIV-positive individuals in resource-rich countries[182]. They target the DNA strand transfer process activity of integrase enzyme and prevent the integration of the viral genome into the host genome, thereby effectively suppressing the replication of wild-type and multidrug-resistant HIV-1 virions in vitro and in vivo[183-185].

When used by ART-naïve HIV-positive individuals, raltegravir (RAL) is more effective, safer and associated with fewer side-effects than standard NNRTI or PI-based HAART regimens[186-188]; however, it is currently restricted for use in ART-experienced individuals with virological failure on first-line treatment or ART-naïve individuals who present with HIV-1 isolates resistant to first-line treatment (Figure 2.24)[12]. Similarly, DTG[189,190] and elvitegravir (EVG)[191], have recently both been shown to be more potent and effective, with a more favourable side-effect profile than standard NNRTI or PI-based HAART regimens in ART-naïve HIV-positive women.

Dolutegravir (DTG) is currently the most efficacious antiretroviral drug with regards to its viral suppressive effects[178]; furthermore, it has a lower side-effect profile, results in a higher mean increase in the circulating $CD4^+$ T-lymphocyte count and is associated with a lower discontinuation rate than current first line NNRTI-based regimens, (Figure 2.26)[178]. It is also more effective, safer and has a higher genetic barrier to resistance than other InSTIs[192]. Consequently, WHO has recommended the incorporation of DTG into first-line treatment regimens for ART-naïve HIV-positive adults (Figure 2.24)[12].

2.4.1.5 Chemokine receptor antagonists

CCR5 antagonists are allosteric inhibitors that target the host CCR5 receptors where they induce a conformational change of the receptor[193]. This prevents the interaction between the host CCR5 receptor and the viral V3 loop of gp120 glycoprotein (Figure 2.18), thus prohibiting HIV-1 entry into the target cell[193]. Maraviroc (MVC) is the only CCR5 agonist approved for clinical use in humans with CCR5 tropic viruses[158]. It is safe, potent with minimal side-effects and contains anti-inflammatory properties[158]. It has a favourable renal and lipid profile, which are crucial factors for the aging HIV population who are inherently at increased risk of cardiovascular disease and renal dysfunction[158]. The limited use of MVC in routine clinical practice has been attributed to the need for viral tropism testing before initiation of treatment[158].

2.4.2 Antiretroviral treatment in women of reproductive age

In women of reproductive age, ART is used both as a therapeutic and a preventative intervention.

2.4.2.1 Antiretroviral therapy as treatment

The initiation of ART in HIV-positive women has similar effects to that observed in the general population; it rapidly reduces the VL, increases the $CD4^+$ T-lymphocyte count, reduces the risk of developing opportunistic infection, and increases the woman's quality of life and long-term survival[145,189,191,194]. For pregnant women initiating ART for their own health, it has the added benefit of also preventing the transmission of the virus to the fetus during the antenatal, intrapartum and post-natal periods[5].

While the benefits of ART cannot be disputed, the timing of treatment initiation has long been debated, particularly when considering the toxicity, potency, convenience and cost of ART[5,154]. Developed countries have for a long time used the "universal test and treat" approach to manage HIV-positive individuals (Figure 2.27)[195], which allows for the initiation of ART at diagnosis irrespective of the immunological, virological or clinical status (Figure 2.27); however, a public

health approach was needed to scale up treatment in resource poor settings, many of which have weak health systems[168].

In 2002, WHO produced a comprehensive guideline on the management of HIV-positive individuals in developing countries[144]. It recommended that HAART only be initiated in HIV-positive women with symptomatic disease or those presenting with a $CD4^+$ T-lymphocyte count <200 cells/µl (Figure 2.27)[144]. In 2010, treatment was expanded to include HIV-positive women with a $CD4^+$ T-lymphocyte count ≤350 cells/ µl[196] following evidence that early HAART initiation ($CD4^+$ T-lymphocyte count between 200 cells/µl – 350 cells/µl) significantly reduces mortality (75% reduction), and the development of tuberculosis (50% reduction)[197].

In 2013, the recommendations were altered to include HIV-positive women with a $CD4^+$ T-lymphocyte count between 350 cells/µl and 500 cells/µl (Figure 2.27)[167], and then to a universal test and treat strategy in 2016[12]. This policy shift was informed by strong evidence from RCTs[139,140,198,199] that showed a significant survival benefit associated with early HAART initiation and a significant reduction in the incidence of opportunistic diseases (e.g. tuberculosis, malignancy) and non-AIDS related disease.

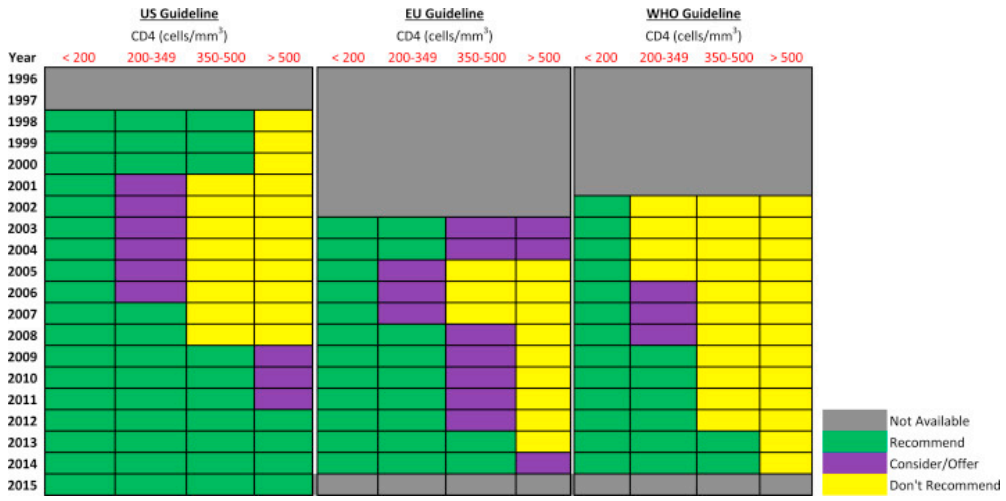


Figure 2.28: Evolution of $CD4^+$ T-lymphocyte threshold for initiation of ART in HIV-positive individuals residing in the USA, European Union (EU), and developing countries (WHO).
Source: Maina et al 2015[200]

2.4.2.2 Antiretroviral therapy as prevention

ART is used for the prevention of HIV-infection in two scenarios, the prevention of vertical transmission from a mother to her fetus or infant i.e. PMTCT, and the prevention of sexual transmission from an HIV-positive individual to an HIV-negative partner.

2.4.2.2.1 Prevention of Mother-To-Child-Transmission (PMTCT)

In 2015, an estimated 1.2 million (1.1 – 1.4 million) HIV-positive women required ART for PMTCT[130]. The maternal VL during the antenatal and intrapartum periods is the best predictor of vertical transmission[201]. This is particularly true during acute HIV infection when the VL is highest in the blood and genital tract[117], and also in the intrapartum period – when the majority (one- to two-thirds) of vertical infections occur – as the baby is directly exposed to the circulating HIV virions in the maternal birthing canal[202]. ART prevents vertical HIV transmission by reducing the maternal VL to an undetectable level, and by reaching the fetus transplacentally where it provides pre-exposure and post-exposure prophylaxis[202].

Numerous trials have assessed the effectiveness of different classes, regimens and timing of treatment initiation (first, second or third trimester, intrapartum, postnatal) for PMTCT (reviewed in Siegfried et al 2011[203]). HAART is the most effective regimen, reducing the rate of vertical transmission to less than 1%[9], particularly when initiated early during pregnancy (first trimester). Furthermore, populations that continued HAART until the cessation of breastfeeding also reported very low rates of MTCT (1.1%; 95%CI 0.5 – 2.2)[204].

NRTIs such as AZT and NNRTIs cross the placenta thus providing pre- and post-exposure prophylaxis[205-207]; however, when administered in isolation (i.e. monotherapy) they do not supress viral replication[208], thereby reducing their efficacy for PMTCT. Therefore, NRTI-based HAART regimens and NNRTI-HAART regimens - which generally contain an NRTI backbone - effectively suppress the maternal VL and provide pre- and post-exposure prophylaxis, providing maximal protection against vertical transmission[202]. Boosted PI's effectively reduce the circulating maternal

VL, even when initiated as monotherapy[209]; however, they do not cross the placental barrier and therefore need to be administered as part of a regimen incorporating either NRTI or NNRTI to achieve maximal reduction in MTCT[202].

Similar to policies on ART, policies on PMTCT regimens have evolved over time (Table 2.5). In developing countries, pregnant women who required treatment for their own health (Table 2.5 "Treatment") [i.e. $CD4^+$ T-lymphocyte dropped below initiation threshold (Figure 2.27) or development of AIDS-related disease) were immediately initiated on life-long, standard, first-line HAART regimen at HIV diagnosis (Figure 2.24). Despite the efficacy of EFV, many were concerned about possible teratogenicity when used in pregnancy; therefore, it was avoided in women of reproductive age who planned to conceive and those who were already pregnant with some restricting its use to the second and third trimesters of pregnancy[144,196,202]. In such instances, EFV is replaced with another NNRTI or a PI[144,196,202].

Women who only require ART for PMTCT (Table 2.5 "PMTCT") initially (2004-09) received short-course monotherapy or dual therapy during the antenatal period, with dual therapy provided during the intrapartum period ± a 7-day course of dual therapy postnatal (Table 2.5). In 2010, WHO recommended two regimens for PMTCT, which at the time were believed to be of similar efficacy, option A and B (Table 2.5), and countries were advised to adopt a national approach that was feasible and cost-effective for their setting[210]. Similar to previous guidelines, option A recommended the initiation of monotherapy during the antenatal period; however, unlike previous guidelines, treatment was to be initiated in the second trimester of pregnancy (≥14 weeks' gestation)[210]. Option B recommended the initiation of HAART during the period of highest risk for MTCT (antenatal, intrapartum and postnatal periods), and treatment was stopped one week after the cessation of breastfeeding[210].

Table 2.5: Evolution of maternal component of PMTCT program according to WHO guidelines 2002 - 2016

Year	Timing	Indication		
		Treatment	**PMTCT**	
2002 - 2003				
	Antenatal	- AZT or d4t + 3TC + NVP	Not specified	
2004				
	Antenatal	Preferred first-line - AZT or d4t + 3TC + NVP Alternative: - AZT + 3TC + SQV/r or NFV or NVP	Choice based on feasibility: - AZT (> 28 weeks') - AZT + 3TC (>36 weeks')	
	Intrapartum	Same as antenatal	- sd-NVP +AZT	
	Postnatal	Same as antenatal	No maternal component	
2006				
	Antenatal	- AZT + 3TC + NVP	- AZT (>28 weeks')	
	Intrapartum	Same as antenatal	- sd-NVP + AZT + 3TC	
	Postnatal	Same as antenatal	- AZT + 3TC (7 days)	
2010				
	Antenatal	- AZT + 3TC + NVP or EFV [a] - TDF + 3TC or FTC + NVP or EFV	Option A - AZT (≥14 weeks')	Option B [bc] (≥14 weeks') - AZT + 3TC + LPV/r or ABC or EFV - TDF + 3TC or FTC + EFV
	Intrapartum	Same as antenatal	- sd-NVP + AZT + 3TC	Same as antenatal
	Postnatal	Same as antenatal	- AZT + 3TC (7 days)	Same as antenatal
2013				
	Antenatal	Preferred first-line: - TDF + 3TC or FTC + EFV Alternatives: - AZT + 3TC + EFV or NVP TDF + 3TC or FTC + NVP	Option B [bc] Preferred first-line: - TDF + 3TC or FTC + EFV Alternatives: - AZT + 3TC + EFV or NVP - TDF + 3TC or FTC + NVP	Option B+ [cd] Preferred first-line: - TDF + 3TC or FTC + EFV Alternatives: - AZT + 3TC + EFV or NVP TDF + 3TC or FTC + NVP
	Intrapartum	Same as antenatal	Same as antenatal	Same as antenatal
	Postnatal	Same as antenatal	Same as antenatal	Same as antenatal
2016				
	Antenatal	Option B+ [cde]	Option B+ [cde]	
	Intrapartum	Same as antenatal	Same as antenatal	
	Postnatal	Same as antenatal	Same as antenatal	

[a] Efavirenz to be avoided in the first trimester, [b] ART is stopped one week after the cessation of breastfeeding, [c] HAART initiated at diagnosis, [d] Life-long HAART, [e] Same HAART regimens as those recommended in 2013.
Source: WHO, et al. 2002, 2003, 2004, 2006, 2010, 2013, 2016[12,144,167,196,202,211,212].

While these recommendations improved substantially on previous guidelines in developing country settings, the initiation of treatment was still impeded by limited or non-existent laboratory facilities for $CD4^+$ T-lymphocyte count testing[213]. Consequently, the government of Malawi pioneered the Option B+ strategy, which proposed the initiation of life-long, standard, first-line HAART regimen (tenofovir, lamivudine and efavirenz) as a fixed-dose combination in all pregnant women, irrespective of $CD4^+$ T-lymphocyte[213]. This simple, feasible and cost-effective approach had both clinical and programmatic benefits, it increased PMTCT coverage per quarter in Malawi by 748% (Figure 2.28), improved maternal health, prevented heterosexual transmission in serodiscordant couples and provided effective protection for the woman's next pregnancy[214].

Option B+ was subsequently recommended by WHO in 2013, and rapidly adopted by most developing countries including 20 of the 21 priority countries where almost all HIV-positive pregnant women reside (>90%)[130]. Consequently, the proportion of pregnant HIV-positive women receiving HAART for PMTCT has doubled (Figure 2.29) (36% in 2009 to 80% in 2015), with 80% (71 – 90%) of HIV-positive pregnant or breastfeeding women receiving ART for PMTCT, and 93% of those on treatment receiving lifelong HAART[130]. This has resulted in a 43% reduction in AIDS-related maternal deaths between 2009 and 2015, and a significant decline in the rate of vertical transmission (22.4% in 2009 to 8.9% in 2015)[130].

Source: Chimbwandira et al 2013[214]

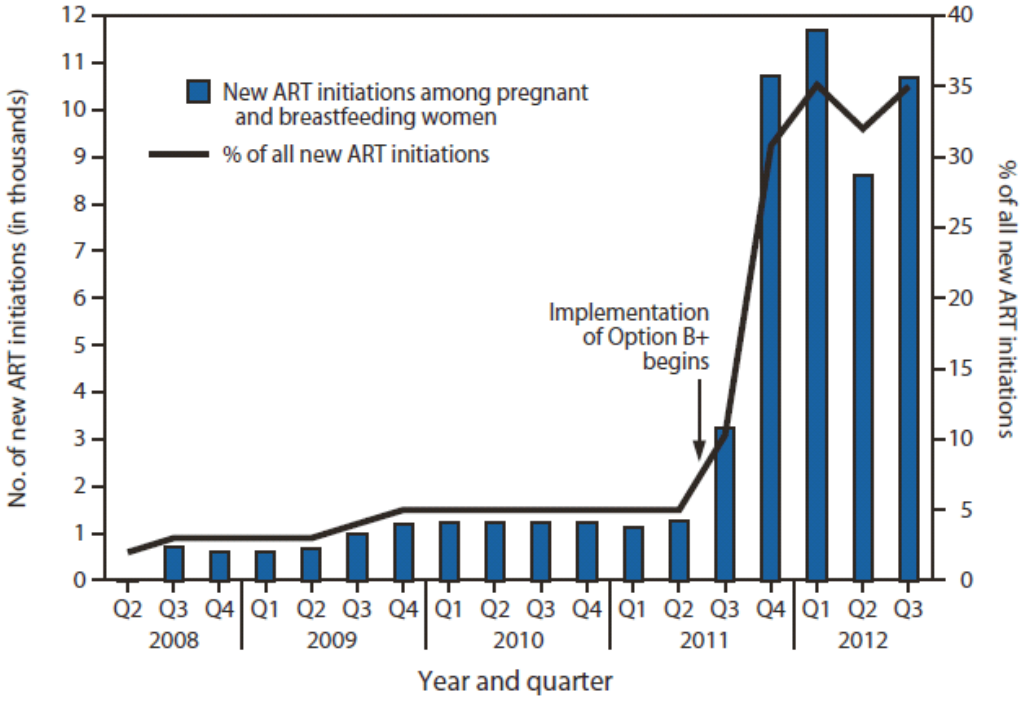


Figure 2.29: Number of new women enrolled in option B+ PMTCT program, and percentage of new adults initiating HAART in Malawian population.

Source: CDC, et al. 2013[214]

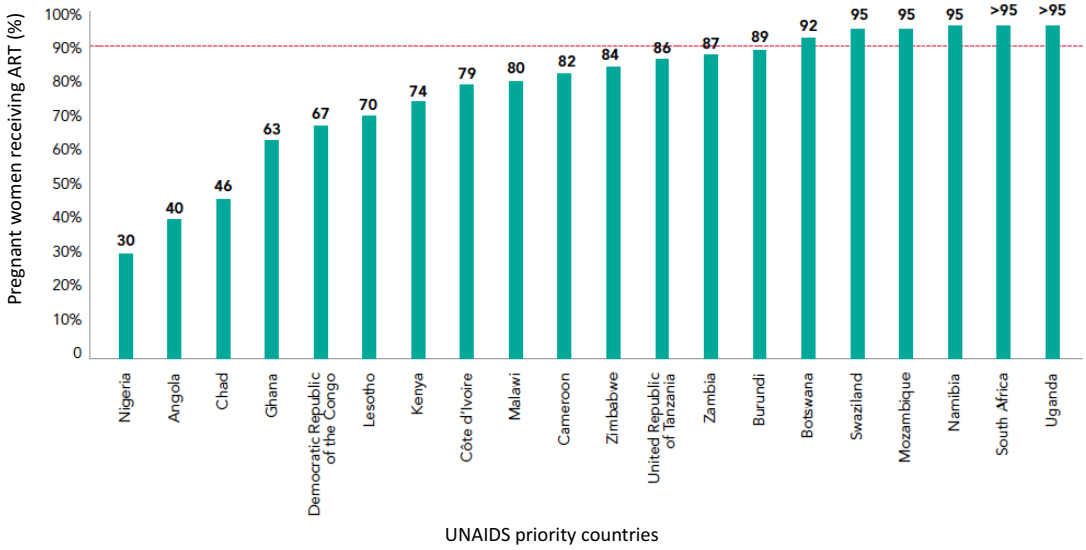


Figure 2.30: Percentage of pregnant HIV-positive women receiving ART for PMTCT, by country in 2015. Source: UNAIDS 2016[130]

2.4.2.2.2 Treatment as prevention (TasP)

Since the start of the epidemic, combination prevention strategies have failed to curb the incidence of HIV in adults, with unprotected sexual intercourse remaining the most frequent route of HIV transmission (80%)[92]. In 2011, the HIV Prevention Trial Network (HPTN) 052 RCT showed that virally suppressed HIV-positive women on HAART have a minimal risk of sexually transmitting the virus to their HIV-negative partner, particularly when HAART is initiated early ($CD4^+$ T-lymphocyte count: 350-550 cells/μL) (Figure 2.30), thereby extending the benefits of HAART from an individual to the population level[215]. Population-based studies have since confirmed these findings, showing a 37% lower risk of HIV-acquisition in communities with high ART coverage (30-40%)[148]. This has provided the impetus for developing countries, e.g. South Africa and Rwanda, to adopt WHO's universal test-and-treat strategy in an effort to reduce the incidence of HIV at a population level[216].

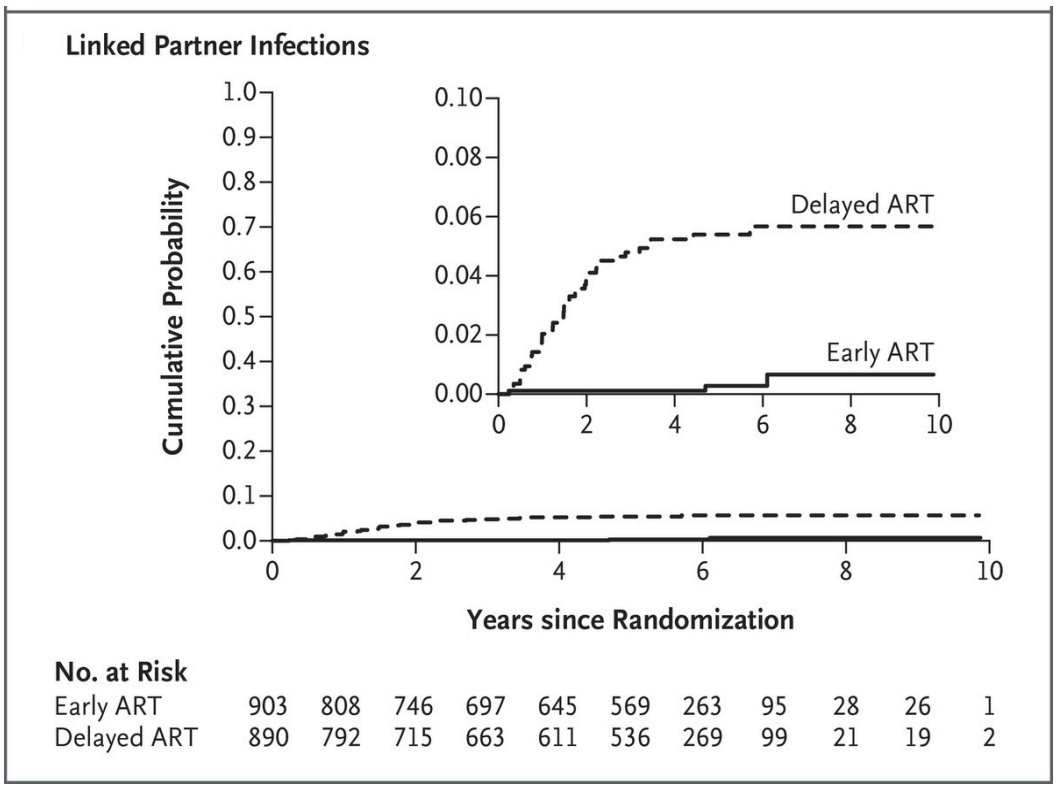


Figure 2.31: Kaplan-Meier estimates of the risk of HIV-1 infection among partners of index participants according to when HAART was initiated.

Source: Cohen et al 2011[215]

2.4.2.2.3 Pre-exposure prophylaxis

In addition to HIV-positive women who initiate lifelong ART through PMTCT, test-and-treat or TasP programs, HIV negative women at substantial risk of acquiring HIV through unprotected sexual intercourse are now also initiating ART as part of a combination prevention strategy known as pre-exposure prophylaxis (PrEP). RCT evidence has demonstrated the effectiveness of ART in preventing the acquisition of HIV-infection in women who adhere strictly to PrEP administered as an oral treatment[214] or a vaginal ring[13]. Tenofovir Disproxil Fumarate (TDF) used alone or in combination with emtricitabine (i.e. Travada) has a relatively high efficacy (TDF = 71%, TDF-FTC = 66%) when used consistently, and is the only antiviral licenced for use as PrEP[12,217]. WHO recommends PrEP for women in serodiscordant relationships and, in 2016, South Africa became the first African country to provide PrEP to sex-workers[12,218].

Take home points:

1. HIV-infection continues to affect millions of women of reproductive age, particularly in sub-Saharan Africa, with increased rates of mortality and morbidity in both the mother and infant.
2. ART has transformed HIV into a manageable chronic disease, with HIV-positive individuals living a near normal life expectancy.
3. The growing burden of HIV in women of reproductive age and the use of ART both for the treatment of those who are HIV-positive and for the prevention of HIV-infection in high-risk HIV-negative women means that, in the near future, a large proportion of HIV-positive and HIV-negative women in developing countries will conceive while on ART. Furthermore, assuming that all countries adopt the most recent WHO recommendations, almost all HIV-positive women will receive potent HAART throughout their entire pregnancy, exposing their children to the effects of ART at conception and while the fetus is developing *in utero*.

2.5 DPhil research questions: HIV, ART and adverse perinatal outcomes.

Studies from developed and developing countries have reported conflicting evidence on the association between maternal HIV-infection and adverse perinatal outcomes including PTB, LBW, SGA, stillbirth and NND[219-221]. Further inconsistent reports have suggested an association between ART and adverse perinatal outcomes with some proposing that the effect of ART on perinatal outcomes varies depending on the type of antiretroviral drug or regimen used, or the timing of treatment initiation[8-10]. Despite the fact that sub-Saharan Africa carries the greatest burden of HIV-infection and adverse perinatal outcomes globally, very little has been done in this region to address these questions, or to identify what proportion of the present adverse-outcome burden is attributable to antenatal exposure to HIV and ART. An understanding of these issues is now imperative considering the increasing burden of HIV in women of reproductive age and following new WHO regulations requiring all HIV-positive women to initiate life-long HAART during pregnancy, and an increasing number of HIV-negative women initiating ART in order to prevent of HIV-infection[12].

This thesis explores the association between maternal HIV infection, ART and adverse perinatal outcomes and aims to answers the following questions:

1. Chapter 3: Is ART-naïve maternal HIV-infection associated with adverse perinatal outcomes?
2. Chapter 4: Is ART associated with adverse perinatal outcomes? Furthermore, which ART is associated with the lowest risk of adverse perinatal outcome, and the highest efficacy for PMTCT?
3. Chapter 5: Do HIV-positive South African women with accurately dated pregnancies have a higher incidence of adverse perinatal outcomes compared to HIV-negative women?
4. Chapter 6: Does the association between maternal HIV-infection, HAART and perinatal outcomes persist after the correction for known confounders?

Section 2

Chapter 3: Perinatal outcomes associated with ART-naïve maternal HIV-infection: a systematic review and meta-analysis.

3.1 Introduction

It is well established that in the absence of ART, maternal HIV-infection is associated with significant maternal morbidity and mortality[133,137], and the majority of perinatally infected infants die before their fifth birthday[222]. What remains unclear is whether maternal HIV-infection also increases the risk of adverse perinatal outcomes, independent of MTCT. The majority of PTBs, stillbirths and NNDs, and a large proportion of all LBW and SGA infants occur in sub-Saharan Africa, where almost all HIV-positive pregnant women reside (91%)[3,6,38,63]. Given that sub-Saharan Africa carries the highest burden of maternal HIV-infection[3], as well as very high rates of adverse perinatal outcomes, it is crucial to determine whether maternal HIV-infection is associated with specific adverse perinatal outcomes.

While numerous studies have investigated the effect of ART-naïve maternal HIV on perinatal outcomes, the results have been inconsistent and contradictory[219-221]. Furthermore, as HIV-positive women are increasingly initiating lifelong HAART, it is unlikely that new evidence will provide a definite answer to this question[3,130]. Therefore, this chapter explored the association between ART-naïve maternal HIV-infection and adverse perinatal outcomes, using a systematic review and pairwise meta-analysis.

The results presented in this chapter have been published in a peer-reviewed academic Journal: Wedi et al. 2016. Lancet HIV 2016;3(1): e33-48[3].

3.1.1 Research question

1. Is ART-naïve maternal HIV-infection associated with 11 adverse perinatal outcomes, including; PTB, VPTB, LBW, VLBW, term-LBW, preterm-LBW, SGA, VSGA, miscarriage, stillbirth or neonatal death.

3.2 Methods

3.2.1 Search strategy

The Cochrane review guidelines were used to develop a robust systematic review protocol that informed each step of the systematic review process (Appendix 3.1)[223]. The protocol was registered online prior to commencing the review (PROSPERO registration: CRD42013005637)[224]. A specialist librarian searched five general electronic literature databases [PUBMED, CINAHL (Ebscohost), Global Health (OVID), EMBASE (OVID) and the Cochrane Central database] and one reviewer (OOW) searched four trial databases (WHO clinical trials database, Pan African Trials database, ClinicalTrials.gov database and ISRCTN register) for studies published between 1 January 1980 to 7 December 2014[3]. The search terms used included "HIV", "antiretroviral therapy", "pregnancy outcome", "preterm birth", "intrauterine growth restriction", "low birthweight", and "stillbirth". A combination of free-text terms (e.g. pregnancy outcome), word variants (e.g. new-born and newborn), synonyms (fetal death and fetal demise) and controlled vocabulary terms (MeSH and Emtree) were used in the search strategy in conjunction with the Boolean "OR" and "AND" operators to identify studies reporting relevant outcomes of interest[223]. The search was not restricted by methodology, language or geographic location, and full text articles and conference abstracts were eligible[3].

To ensure that no studies were missed, the search was conducted in two parts[3]. The first was a "specific search", targeting studies that reported on "[preterm birth or intrauterine growth restriction (IUGR) or stillbirth] and (HIV or ARV) "(Appendix 3.2). The second was a "general search" targeting studies that reported on "(pregnancy outcome) and (HIV or ARVs)" (Appendix 3.3). The final search strategy was adapted for all literature and trial electronic databases.

The references of all included studies and the preceding review[225], were assessed for additional citations, and authors were contacted in instances when the full text manuscript contained insufficient information[221]. All citations were imported into EndNote X6 electronic database.

3.2.2 Study selection and data-extraction

Two independent authors (OOW and RC) reviewed the citations retrieved and identified those that were relevant. OOW obtained and screened the full text articles for fulfilment of the following eligibility criteria[3]:

- **Study design**: RCTs, cohorts (prospective and retrospective), case-control and cross-sectional studies.
- **Population**: Pregnant women with known perinatal outcomes.
- **Exposure**: HIV-infection, contracted prior to or during pregnancy, irrespective of the route of transmission. HIV-positive women should not have been exposed to ART, with the exception of a single dose of ART at delivery. As the objective was to assess the association between untreated maternal HIV-infection (i.e. ART-naïve maternal HIV-infection during the antenatal period) and perinatal outcomes, and not vertical transmission, women who received a single-dose of ART at delivery were still considered as "ART-naïve".
- **Comparison**: HIV-negative pregnant women from the same community as the HIV-positive participants.
- **Outcomes of interest**: 11 outcomes were stratified into four categories according to whether untreated maternal HIV-infection influenced;
    1. GA at delivery: PTB (<37weeks 0 days' gestation)[49]; VPTB (<32 weeks 0 days' gestation[49]),
    2. Birthweight (BW): LBW (<2500g), VLBW (<1500g)[38],
    3. Birthweight and GA at delivery: Term-LBW (≥37 weeks' 0 days gestation and BW<2500g)[38]; preterm-LBW (<37 weeks' 0 days gestation and BW <2500g); SGA (BW for GA<10th centile)[226]; VSGA (BW for GA <3rd centile)[226].
    4. Fetal and neonatal mortality: miscarriage (spontaneous expulsion of fetus <24 weeks' gestation)[227]; stillbirth (any 3rd trimester delivery of stillborn infant with BW≥1000g[15] or ≥24 completed weeks[228] or ≥ 35cm body length[15]); NND (death of an infant within the first 28 days of life)[39].

Outcomes of interest were not extracted if they were not defined or the study definition differed to that stipulated in the protocol[3].

Any ambiguity in the eligibility of studies was resolved by discussion amongst three reviewers (OOW, JH, SK). A list of excluded studies is available from the authors upon request. In order to prevent duplicate publication bias, two independent reviewers (OOW and JH) assessed each study for uniqueness of the study population. When multiple studies reporting the same population/cohort were identified, the manuscript containing the most recent and complete information was retained. Furthermore, manuscripts reporting different outcomes of interest on the same cohort were both included[229,230]. One reviewer (OOW) extracted specific study data relating to the study design, study location, date when the study was performed, participant characteristics, method of determination of HIV-status, study definition of outcomes of interest, method used to assess gestational age, number of HIV-positive and HIV-negative participants in the cohort, number of events occurring in the HIV-positive and HIV-negative participants, and confounding factors assessed. Reported unadjusted and adjusted relative risk (RR) and odds ratio (OR), and 95% confidence intervals (CI), were used to assess the effect of adjusting for confounders. This was checked by a second reviewer (JH), and disagreements were resolved by discussion.

3.2.3 Methodological quality

In an effort to determine whether appropriate study design, conduct and statistical analyses were employed to minimize bias in the estimation of the association[231], a methodological quality assessment of each study was conducted by one reviewer (OOW) and checked by a second reviewer (JH). The Newcastle-Ottawa Scale was used to assess the methodological quality of cohort (prospective and retrospective) studies (Appendix 3.4) and case-control (Appendix 3.5) studies following modification to ensure relevance to this systematic review[232]. Cohort studies were assessed according to nine criteria that were categorised into three groups including; (1) selection of HIV-positive and negative participants (maximum 4 points), (2) comparability of exposed and unexposed

participants, and whether an effort was made to assess and correct for specific confounding factors (maximum 2 points), and (3) method used to assess the outcome of interest (maximum 3 points) (Table 3.1, Appendix 3.4).

Studies were classified as being of "good" quality if all nine criteria were met; as "average" if they fulfilled three of the "Selection" and "Outcome" criteria, or two of the "Selection" and "Outcome" as well as one of the "Comparability criteria (Table 3.2). Studies were deemed to be of poor quality if they did not qualify as a "good" or "average" quality study (Table 3.2)[231]. Case-control studies were graded in a similar way, with minor alterations to accommodate the study design (Table 3.2)[3].

Table 3.1: Adapted Newcastle Ottawa quality assessment scale

Adapted from Wells, et al. 2014[232].

Cohort (prospective and retrospective) and cross-sectional studies

1. **Selection of participants included in the study (maximum of 4 points):**
   - Representativeness of the exposed (HIV-positive) cohort?
   - Selection of the non-exposed (HIV-negative) cohort?
   - Ascertainment of exposure (e.g. HIV test)?
   - Demonstration that outcome of interest was not present at the start of the study?

2. **Comparability of exposed and unexposed participants (maximum of 2 points)**
   - Did the study control for any of the following factors: BMI, smoking, parity and maternal age?
   - Did the study control for any of the following additional factors, e.g. prior history adverse perinatal outcome, maternal hypertension, anaemia, illicit drug or alcohol use during pregnancy?

3. **Outcome (maximum of 3 points)**
   - Is the assessment of the outcome valid?
   - Was gestational age accurately assessed?
   - Adequacy of follow-up of cohorts?

Case-control studies

1. **Selection of cases and controls, and determination of gestational age (maximum of 5 points)**
   - Definition of cases (same as systematic review protocol)?
   - Representativeness of cases?
   - Selection of controls?
   - Definition of controls (same as systematic review protocol)?
   - Was gestational age accurately assessed in cases and controls?

2. **Comparability of cases and controls (maximum of 2 points)**
   - Did the study control for any of the following factors e.g. BMI, smoking, parity and maternal age?
   - Did the study control for additional factors e.g. prior history of adverse perinatal outcome, maternal hypertension, anaemia, illicit drug or alcohol use during pregnancy?

3. **Assessment of the exposure (maximum of 3 points)**
   - Is the assessment of exposure valid?
   - Same method used of ascertainment for cases and controls?
   - Non-response rate?

Table 3.2: Classification of primary studies according to the adapted Newcastle Ottawa quality assessment scale. Source: Wedi, et al. 2016[3].	
Prospective and retrospective cohort studies	
Good quality	9 points – all requirements met.
Average quality	3 points in "selection" and 3 points in "outcome" sections.
	≥2 points in the "selection" and "outcome" sections, as well as ≥1 points in the "comparability" section.
Poor quality	< 2 points in the "selection" and/or "outcome" sections.
	2 points in the "selection" and "outcome" sections, but no points in the "comparability" section.
Case-control studies	
Good quality	10 points – all requirements met.
Average quality	4 points in the "selection" and 3 points in the "exposure" sections.
	≥3 points in the "selection" and ≥2 points in the "exposure" sections, as well as ≥1 point in the "comparability" section.
Poor quality	< 3 points in the "selection" and/or <2 points in the "exposure" sections.
	3 points in the "selection" and 2 points in the "exposure" sections, but no points in the "comparability" section.

3.2.4 Data synthesis

Dichotomous perinatal outcome data were extracted from individual studies and recorded in 2x2 tables. This data was used to generate a RR (cohort studies) or OR (case-control studies), and 95% CI, irrespective of the quality of the studies. A meta-analysis was conducted when two or more studies, of the same study design, reported on the same perinatal outcome.

A random-effects model – which accounts for heterogeneity – was used to calculate a weighted summary effect estimate and 95%CI for the association between maternal HIV-infection and individual outcomes of interest[223,233]. The I^2 statistic was used to determine the proportion of heterogeneity in the effect estimate that could be attributed to clinical or methodological variability, as opposed to sampling error[223,234]. The degree of heterogeneity in individual meta-analyses was interpreted as none (<25%), low (25-49%), moderate (50-74%), and high (≥75%)[223]. All summary effect estimates and 95%CI for individual outcomes, according to study design, were represented on forest plots.

In order to assess the robustness of the systematic review results, sensitivity analyses were conducted to explore whether the methodological quality of included studies or underlying confounding influenced the effect estimate observed[235]. Pre-specified subgroup analyses explored the influence of covariates on the association between maternal HIV-infection and each perinatal outcome[223]. These included the geographic location where studies were conducted (sub-Saharan Africa, Asia, Europe, and the Americas & Caribbean) and the reported method used to determine GA [late ultrasound, LNMP, neonatal assessment (Ballard / Finnström / Dubowitz score), mixed–methods or unspecified].

The Peters' test for small study effect assessed each meta-analysis containing a minimum of 10 studies for the presence of publication bias[236]. The systematic review is reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines[237].

All statistical analyses were conducted using STATA version 13 (College Station, TX, USA).

3.3 Results

3.3.1 Results of search

The search yielded 60,750 citations; 1648 full text articles and abstracts were retrieved and assessed for the fulfilment of the eligibility criteria, and 35 studies were included in the final analysis[3]. The 35 studies reported data on 53,623 pregnant women, and included 20 prospective cohort studies, 12 retrospective cohort studies, and 3 case-control studies (Figure 3.1)[3]. No RCTs were found.

3.3.2 Description of included studies

The majority of studies were conducted in sub-Saharan Africa (n=25; 71%), and the remainder were conducted in the Americas and Caribbean (n=6; 17%), Europe (n=2; 6%) and Asia (n=2; 6%) (Table 3.3)[3]. The following perinatal outcomes were reported; LBW (n=26), PTB (n=24), SGA (n=7), term-LBW (n=7), NND (n=4), preterm-LBW (n=3), VSGA (n=1), VPTB (n=1) and VLBW (n=1) (Figure 3.1, Table 3.3)[3].

Two-thirds (n=38,076; 71%) of the 53,623 women assessed, were from rural and urban parts of sub-Saharan Africa. The majority of participants did not receive antenatal ART for PMTCT because the studies were conducted before 1994 (n=21; 60%) – i.e. when ART was shown as an effective intervention for PMTCT[205] (Table 3.3). The remaining participants did not receive antenatal ART because national regulations only recommended that ART be initiated during the intrapartum period (2 studies)[230,238]; HIV-positive status was only confirmed after delivery (2 studies)[239,240], antenatal ART was recommended in the national guidelines, but was not available (2 studies)[229,241], or ART was not recommended in the national guidelines (1 study)[242] (Table 3.3). The remaining 7 studies did not specify why antenatal ART was not provided (Table 3.3).

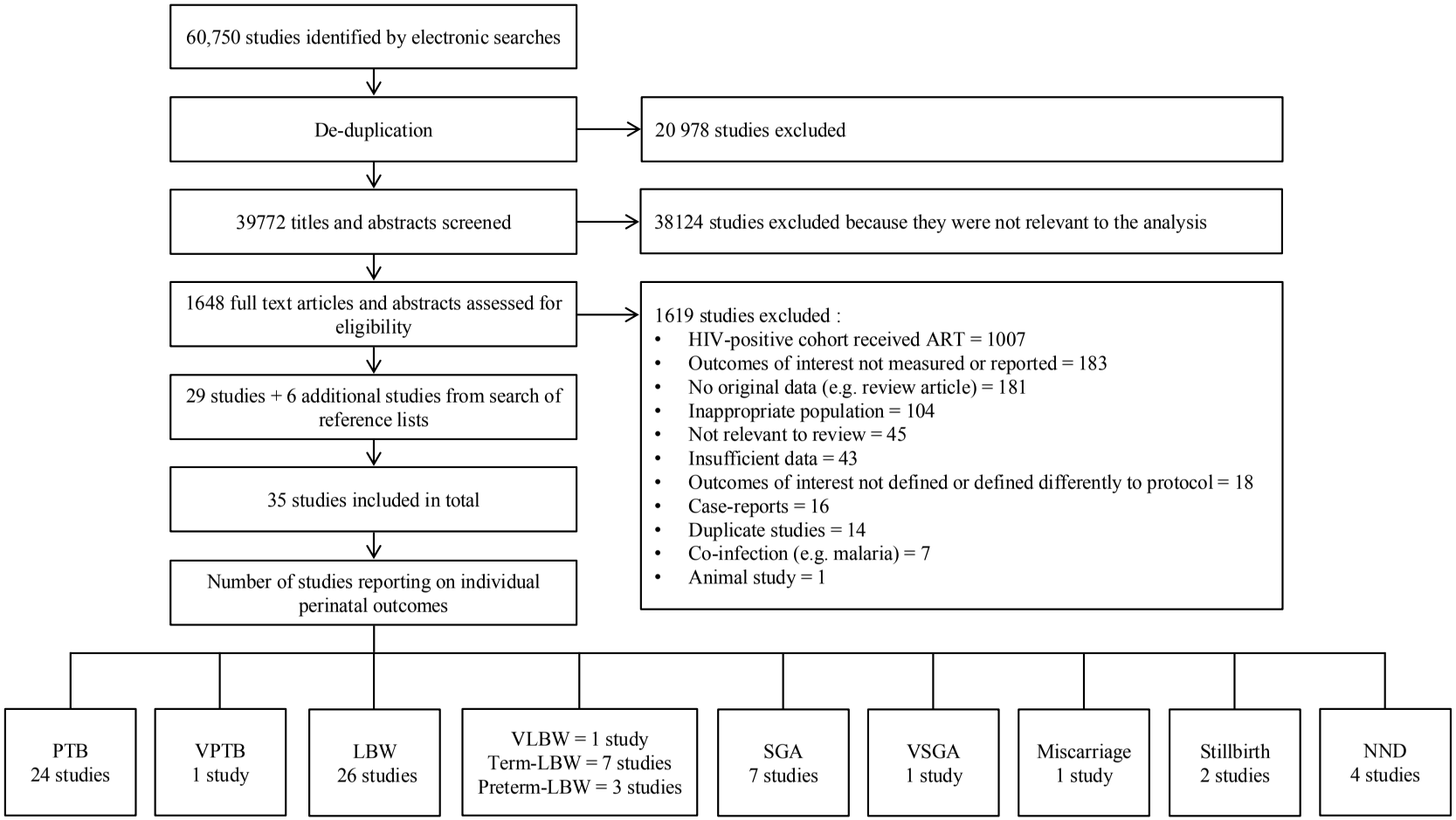


Figure 3.1: Schematic representation of study selection process.

ART = antiretroviral treatment, LBW = low birthweight, SGA = small for gestational age, NND = neonatal death, PTB = preterm birth, VLBW = very low birthweight, VPTB = very preterm birth, VSGA = very small for gestational age.

Image adapted from Wedi et al. 2016[3]

Table 3.3: Characteristics of studies included in the systematic review.

Table adapted from: Wedi et al 2016[3]

Study	Country	Study design	Recruitment time period	Number of pregnant women included in analysis (n)		Perinatal outcomes	Population characteristics	HIV disease stage and reason why ART not provided	Methods used to correct for confounding factors	Gestational age determination method	Quality Assessment
				HIV-positive	HIV-negative						
Sub-Saharan Africa											
Adjorlolo et al. 1991[243]	Ivory Coast	Retrospective cohort	Not reported	290	2768	LBW	Low SES, largest urban area in the country.	4-15% of women met criteria for AIDS. At time of study recruitment ART not yet recommended in pregnancy.	None	Unspecified	Poor
Ayisi et al. 2003[244]	Kenya	Prospective cohort	June 1996-March 1999	420	1503	PTB LBW SGA	Uncomplicated singleton pregnancies without comorbidities. Urban setting. All women delivered at a hospital.	No description on HIV disease stage or why ART not provided.	Regression analysis	Neonatal assessment (Dubowitz)	Average
Bergstrom et al. 1995[245]	Mozambique	Case-control	Not reported	51 cases	51 controls	LBW	No description.	No description on HIV disease stage. At time of study recruitment ART not yet recommended in pregnancy.	Matching	Unspecified	Average
Braddick et al. 1990[246] *	Kenya	Prospective cohort	January 1986-March 1989	177	326	PTB, LBW	Lower SES, urban setting, no history of intravenous drug use. All women delivered at a hospital.	55% of women were asymptomatic, 45% were symptomatic, one woman had AIDS. At time of study recruitment ART not yet recommended in pregnancy.	Risk factor analysis	Neonatal assessment (Ballard) and LNMP	Average
Bulterys et al. 1994[247]	Rwanda	Prospective cohort	October 1989-February 1992	308	299	PTB, LBW, SGA, SB, NND	Semirural setting where 75% of deliveries occur at home.	Majority of HIV-positive women were asymptomatic and none met the criteria for AIDS. At time of study	Risk factor analysis, regression analysis	Neonatal assessment (Ballard) and late ultrasound	Average

								recruitment ART not yet recommended in pregnancy.			
Coley et al. 2001[248]	Tanzania	Prospective cohort	April 1995-July 1997	522	485	PTB, LBW, Term-LBW, Preterm-LBW	Cohort representative of the general population. 93.5% of deliveries occurred at medical facility, 3.6% occurred at home and 2.9% occurred at non-medical facility.	83% of women were asymptomatic (WHO stage 1), 17% were symptomatic (WHO ≥2). No description on why ART not provided.	Risk factor analysis, regression analysis	Unspecified	Average
Ezeaka et al. 2009[249]	Nigeria	Prospective cohort	August 2002-August 2005	220	218	PTB, LBW	Participants recruited from a tertiary hospital in urban setting. None of the women reported the use of intravenous drugs, alcohol or tobacco. All women delivered at a hospital.	85.5% of women were asymptomatic and 14.5% met WHO criteria for AIDS. No description on why ART not provided.	Matching, risk factor analysis	LNMP and late ultrasound	Average
Friis et al. 2004[241]	Zimbabwe	Prospective cohort	1996-1997	360	725	PTB, LBW, Term-LBW	Low SES, urban setting.	No description on HIV disease stage or why ART not provided.	Regression analysis	LNMP and SFH	Poor
Habib et al. 2008[250]	Tanzania	Retrospective cohort	1999-2006	137	5436	PTB, SGA	Urban and rural settings in 6 districts in Tanzania. Study conducted at a referral hospital and data not representative of the population. All women delivered at a hospital.	No description on HIV disease stage or why ART not provided.	Regression analysis	LNMP	Poor
Ladner et al. 1998[251]	Rwanda	Prospective cohort	July 1992-August 1993	275	286	SB	Urban setting. All women delivered at a hospital.	No description on HIV disease stage. At time of study recruitment ART not yet recommended in pregnancy.	None	Late ultrasound	Poor
Lallemant et al. 1989[252]	Republic of the Congo	Prospective cohort	May 1987-March 1988	61	130	LBW	Urban setting. All women delivered at a hospital.	One woman met the WHO criteria for AIDS. At time of study recruitment ART not yet recommended in pregnancy.	Matching, risk factor analysis	Unspecified	Average

Lepage et al. 1991[253]	Rwanda	Retrospective cohort	November 1988-June 1989	215	216	LBW, NND	Urban setting. All women delivered at a hospital.	81% were asymptomatic and none met the WHO criteria for AIDS. At time of study recruitment ART not yet recommended in pregnancy.	Matching, risk factor analysis, regression analysis	Neonatal assessment (Finnström)	Average
Leroy et al. 1998[254]	Rwanda	Prospective cohort	July 1992-August 1993	364	365	PTB, LBW, Term-LBW Preterm-LBW	Urban setting. Study sample representative of the population. 12.3% of deliveries occurred at home and 87.7% occurred at a hospital.	92% of women were asymptomatic and one fulfilled the WHO criteria for AIDS. 7.5% of women had a CD4 count < 200 cells/µl. At time of study recruitment ART not yet recommended in pregnancy.	Matching, risk factor analysis, regression analysis, Mantel-Haenzel estimate	Neonatal assessment (Finnström) and late ultrasound	Average
Mmiro et al. 1993[251]	Uganda	Prospective cohort	December 1988-November 1990	557	697	PTB	Urban setting, no evidence of illicit drug use. 50% of deliveries occurred at home and 50% occurred at a hospital.	46.8% of women were symptomatic. At time of study recruitment ART not yet recommended in pregnancy.	Risk factor analysis	Unspecified	Poor
Musana et al. 2009[255]	Kenya	Prospective cohort	September 2004-April 2005	68	68	PTB, LBW	No description.	All women met the criteria for advanced stage of disease (WHO stage 3 and 4). No description on why ART not provided.	Matching, risk factor analysis	LNMP	Average
Mwanyumba et al. 2001[257]	Kenya	Retrospective cohort	May 1996-April 1999	1169	7188	LBW	Singleton pregnancy, no obstetric complications, illicit drug use or smoking. All women delivered at a hospital.	No description on HIV disease stage or why ART not provided.	Regression analysis	Unspecified	Poor
Ndirangu et al. 2012[229]	South Africa	Prospective cohort	August 2001-September 2004	1189	1179	PTB, VPTB, SGA, VSGA	Area with a high HIV-prevalence (39.5%). Rural setting, low SES. 11.8% of deliveries occurred at home or on the way to a healthcare facility and 88.2% of	5.5% of HIV-positive women had a CD4 count < 200 cells/µl. ART recommended nationally, but not available.	Risk factor analysis, regression analysis	Neonatal assessment (Ballard) and LNMP	Poor

							deliveries occurred at a hospital.				
Noble et al. 2005[239]	Zimbabwe	Case-control	July 1998-March 1999	53 cases	444 controls	PTB	Convenience sample of pregnant women who delivered term and preterm infants at a university affiliated hospital. Urban setting. All women delivered at hospital.	No description on HIV disease stage. HIV-diagnosis confirmed after delivery.	Risk factor analysis, regression analysis	Neonatal assessment (Dubowitz), LNMP and SFH	Average
Rollins et al. 2007[230]	South Africa	Prospective cohort	September 2001-September 2004	1449	1401	LBW	Low SES, rural, semirural and urban antenatal clinics in an area with a high HIV-prevalence (39.5%). 12.4% of deliveries occurred at home, 28% occurred at a clinic, 52.9% occurred at a hospital and 6.5% occurred in an unknown location.	11% of women had a CD4 count < 200 cells/µl. Participants received intrapartum ART only.	Regression analysis	Neonatal assessment (Ballard) and LNMP	Average
Ryder et al. 1989[258]	Democratic Republic of the Congo	Prospective cohort	30 April 1986-30 April 1987	466	606	LBW, NND	Urban setting, recruited from 2 hospitals, hospital A (women of low SES) and hospital B (women of high SES). No illicit drug use.	18% of women met criteria for AIDS. At time of study recruitment ART not yet recommended in pregnancy.	Matching	Neonatal assessment (Dubowitz)	Average
Sutton et al. 1999[259]*	Democratic Republic of the Congo	Retrospective cohort	October 1989-April 1990	215	206	LBW	Urban setting, singleton pregnancy. All women delivered at a hospital.	No description on HIV disease stage. At time of study recruitment ART not yet recommended in pregnancy.	Regression analysis	Neonatal assessment (Ballard) and LNMP	Average
Taha et al. 1995[260]	Malawi	Retrospective cohort	October 1989-October 1990	679	687	PTB, LBW, Term-LBW Preterm-LBW	Urban setting. All women delivered at a hospital.	No description on HIV disease stage. At time of study recruitment ART not yet recommended in pregnancy.	None	Neonatal assessment (Ballard) and LNMP	Poor
Temmerman et al. 1994[221]	Kenya	Prospective cohort	January 1989-December 1991	315	311	PTB, LBW, Term-LBW, NND	Low SES, urban setting, no illicit drug use. 20% of deliveries occurred at home or at another maternity unit.	52.2% of women were asymptomatic, 43.6% were symptomatic and 4.2% met the criteria for AIDS. At time of study	Matching, risk factor analysis	Late ultrasound	Poor

								recruitment ART not yet recommended in pregnancy.			
Ticconi et al. 2003[238]	Zimbabwe	Retrospective cohort	1 January-30 June 2000 and 1 January-30 June 2001	52	789	PTB, SGA	Referral hospital in malaria-endemic area. Recruitment only during peak malaria season.	No description on HIV disease stage. Participants received intrapartum ART only.	Regression analysis	LNMP	Poor
van den Broek et al. 2014[240]	Malawi	Case-control	February 2004-September 2005	351 cases	1729 controls	PTB	Rural setting, high HIV prevalence (26.2%), singleton pregnancy.	No description on HIV disease stage. HIV-diagnosis confirmed after delivery	Risk factor analysis, regression analysis	Late ultrasound	Average
Europe											
Bucceri et al. 1997[261]	Italy	Prospective cohort	1985-1993	151	164	PTB, LBW	Intravenous drug users, urban setting. All women delivered at a hospital.	93% of women were asymptomatic (CDC stage A) and 5% had a CD4 < 200 cell/µl. At time of study recruitment ART not yet recommended in pregnancy.	Regression analysis	Neonatal assessment (not specified), LNMP and late ultrasound	Average
Johnstone et al. 1996[262]	United Kingdom	Retrospective cohort	1983-1992	82	302	PTB	Intravenous drug user or a sexual partner of intravenous drug user, singleton pregnancy.	No description on HIV disease stage. At time of study recruitment ART not yet recommended in pregnancy.	Matching, regression analysis	Late ultrasound and LNMP	Poor
The Americas and Caribbean											
Alger et al. 1993[263]	USA	Prospective cohort	July 1987-July 1991	101	97	LBW	Low SES, inner city, current or former intravenous drug users or sexual partners of intravenous drug users.	One woman with an AIDS defining opportunistic infection and 15.8% of women had a CD4 count < 200 cells/µl. At time of study recruitment ART not yet recommended in pregnancy.	Risk factor analysis	Unspecified	Average
Ellis et al. 2002[242]	USA	Retrospective cohort	January 1988-December 1995	563	2252	PTB, LBW, SGA	Low SES, inner city, referral centre for high-risk obstetric	No description on HIV disease stage. At time of study recruitment ART	Regression analysis	Unspecified	Poor

							patients. All women delivered at a hospital.	not yet recommended in pregnancy.			
Halsey et al. 1990[264]	Haiti	Prospective cohort	August 1986-August 1988	199	1994	PTB, LBW	Low SES, with no history of intravenous drug use or cocaine. Sample was representative of the community.	All women were asymptomatic. At time of study recruitment ART not yet recommended in pregnancy.	None	Neonatal assessment (Dubowitz)	Average
Markson et al. 1996[265]	USA	Retrospective cohort	1989-1990	772	2377	PTB, LBW, Term-LBW	Urban setting, singleton pregnancy. Data drawn from New York city Medicaid claims database	No description on HIV disease stage. At time of study recruitment ART not yet recommended in pregnancy.	Matching, risk factor analysis, regression analysis	LNMP	Poor
Maynard et al. 1990[266]	USA	Retrospective cohort	March 1985-February 1988	17	41	PTB, LBW, VLBW	Intravenous drug users. All women delivered at a hospital.	82.4% of women were asymptomatic and 17.6% were symptomatic. At time of study recruitment ART not yet recommended in pregnancy.	None	Unspecified	Average
Selwyn et al. 1989[220]	USA	Prospective cohort	June 1985-1988	39	58	Term-LBW, Miscarriage	Inner city intravenous drug users. All women delivered at a hospital.	54% of women were asymptomatic (CDC II), 42% had persistent generalised lymphadenopathy (CDC III), 4% had advanced disease (CDC IV C.2) and none of the women met the criteria for AIDS. At time of study recruitment ART not yet recommended in pregnancy.	None	Unspecified	Poor
Asia											
Kumar et al. 1995[267]	India	Prospective cohort	January 1992-January 1993	150	152	PTB, LBW, SGA	Low SES, no illicit drug use, referral hospital. All women delivered at a hospital.	53% of women were asymptomatic (CDC 1/11), 38% were symptomatic (CDC 111) and 9% met the criteria for AIDS (CDC IV). At time of study recruitment ART not yet	Matching, risk factor analysis	Neonatal assessment (Dubowitz)	Average

								recommended in pregnancy.			
Mitgitti et al. 2008[268]	Thailand	Retrospective cohort	January 1997-December 2002	266	5872	PTB, LBW	Singleton pregnancy. All women delivered at a hospital.	No description on HIV disease stage. At time of study recruitment ART not yet recommended in pregnancy.	Risk factor analysis, regression	Unspecified	Poor

Abbreviations: AIDS = Acquired Immunodeficiency Syndrome, ART = Antiretroviral therapy, CDC = Centers for Disease Control and Prevention, HIV = Human Immunodeficiency Virus, LBW = low birthweight, LNMP = last normal menstrual period, MC = miscarriage, NND = neonatal death, PTB = preterm birth, SB = stillbirth, SES = socio-economic status, SFH = symphysio-fundal height, SGA = small-for-gestational-age, VLBW = very low birthweight, VPTB = very preterm birth, VSGA = very small-for-gestational-age, WHO = World Health Organisation.

* Studies designated as a case-control study by authors but found to be cohort studies and analysed as such for the purpose of this systematic review.

3.3.2.1 Methods used to estimate gestational age

A large proportion of the perinatal outcomes of interest were dependent on an accurate measure of GA (PTB, VPTB, term-LBW, preterm-LBW, SGA, VSGA, miscarriage and stillbirth)[3]. Two thirds (n=24; 69%) of included studies reported on the method used to estimate the GA (Table 3.3). Half of these studies (n=12; 34·3%) used a single method, including late ultrasound (≥14 weeks gestation) (n=3), LNMP (n=4) and neonatal assessment at birth (n=5). The remaining 12 (34.3%) studies used a combination of methods (mixed-method) as indicated in Table 3.4, and none of the studies used a first trimester ultrasound (<14 weeks gestation).

3.3.2.2 Assessment of methodological quality

Quality assessment of studies retrieved revealed 20 (57%) average and 15 (43%) poor quality studies (Table 3.3, Table 3.5)[3]. None of the studies were judged to be of good quality because they did not conduct a first trimester ultrasound (< 14 weeks' gestation) to determine GA (Table 3.5) and most studies failed to control for pre-specified confounding (Table 3.6).

Table 3.4: Combination of methods used to determine gestational age (mixed methods) in studies included in systematic review

Source: Wedi et al. 2016[3].

Method of gestational age determination	Number of studies
Late ultrasound and LNMP	2
Late ultrasound and neonatal assessment (Ballard score)	1
Late ultrasound and neonatal assessment (Finnström score)	1
LNMP and neonatal assessment (Ballard score)	5
LNMP and SFH	1
Late ultrasound, LNMP and neonatal assessment (type not specified)	1
LNMP, neonatal assessment (Dubowitz score) and SFH	1

LNMP = last normal menstrual period.

SFH = symphysio-fundal height.

Late ultrasound = ultrasound ≥14 weeks' gestation.

Table 3.5: Quality assessment scores of studies included in systematic review, according to the adapted Newcastle-Ottawa quality assessment checklist.

Table adapted from Wedi et al. 2016[3].

Study	Quality assessment			
	Selection (maximum 4 points) [Case-control study (cc): maximum 5 points]	**Comparability** (maximum 2 points)	**Outcome/exposure** (maximum 3 points)	**Overall quality assessment** (Good/Average/ Poor)
Sub-Saharan Africa				
Adjorlolo et al 1991[239]	★★★	-	-	Poor
Ayisi et al 2003[240]	★★★	★★	★★	Average
Bergstrom et al 1995 [241] (cc)	★★★	★	★★★	Average
Braddick et al 1990[242]	★★★★	★★	★★	Average
Bulterys et al 1994[243]	★★★★	★	★★	Average
Coley et al 2001[244]	★★★★	★★	★★	Average
Ezeaka et al 2009 [245]	★★★★	★★	★★	Average
Friis et al 2004[237]	★★★★	★	★	Poor
Habib et al 2008[246]	★★★	-	★	Poor
Ladner et al 1998[247]	★★★★	-	★★	Poor
Lallemant et al 1989[248]	★★★★	★★	★★	Average
Lepage et al 1991[249]	★★★	★	★★	Average
Leroy et al 1998[250]	★★★★	★★	★★	Average
Mmiro et al 1993[251]	★★★★	-	★★	Poor
Musana et al 2009[252]	★★★	★★	★★	Average
Mwanyumba et al 2001[253]	★★★	★★	★	Poor
Ndirangu et al 2012[225]	★★★★	★	★	Poor
Noble et al 2005 (cc)[235]	★★★★	★★	★★★	Average
Rollins et al 2007[226]	★★★★	★	★★	Average
Ryder et al 1989[254]	★★★★	★★	★★	Average
Sutton et al 1999[255]	★★★	★	★★	Average
Taha et al 1995[256]	★★★	-	★★	Poor
Temmerman et al 1994[216]	★★★★	★★	★	Poor
Ticconi et al 2003[234]	★★	★	★	Poor

Van den Broek et al 2014 (cc) [236]	★★★★	★★	★★	Average
Europe				
Bucceri et al 1997[257]	★★★	★★	★★	Average
Johnstone et al 1996[258]	★★	★★	★	Poor
The Americas and Caribbean				
Alger et al 1993[259]	★★★★	★★	★★	Average
Ellis et al 2002[238]	★★★	★★	★	Poor
Halsey et al 1990[260]	★★★★	★	★★	Average
Markson et al 1996[261]	★★★	★★	★	Poor
Maynard et al 1990[262]	★★	★	★★	Average
Selwyn et al 1989[218]	★★★	★	★	Poor
Asia				
Kumar et al 1995[263]	★★★★	★	★★	Average
Mitgitti et al 2008[264]	★★★	★	★	Poor

Table 3.6: Confounding factors adjusted for in individual studies.

Source: Wedi et al. 2016[3].

Study	Matching	Risk factor analysis	Regression analysis (Multivariable analysis)
Adjorlolo et al 1991[239]	-	-	-
Alger et al 1993[259]	-	Maternal age, parity, history of abortions, marital status, maternal weight	-
Ayisi et al 2003[240]	-	-	Maternal weight, maternal age, parity, education, ethnicity, season of delivery, hospitalization, sex of infant, hypertension, pre-eclampsia, polyhydramnios, abnormal presentation, POH (history of previous caesarean section, haemorrhage or repeated abortion)
Bergstrom et al 1995[241]	Maternal age and parity	-	-
Braddick et al 1990[242]	-	Maternal age, parity, smoking, STD	-
Bucceri et al 1997[257]	-	-	Maternal age, duration of IVDU, parity, smoking
Bulterys et al 1994[243]	-	BMI, mean body weight	Maternal age, parity, marital status, household income, education, smoking, STD
Coley et al 2001[244]	-	Education, marital status, height, weight, arm circumference, BMI	Maternal age, type of delivery, hypertension, smoking, maternal infection (malaria, tuberculosis), education, gravidity, height, occupation, marital status, arm circumference, STD
Ellis et al 2002[238]	-	-	Alcohol use, antenatal visits, hypertensive disorder, race, parity, maternal body weight, STD, diabetes, fetal distress
Ezeaka et al 2009[245]	Age and parity	Maternal height, social class, infant gender	-
Friis et al 2004[237]	-	-	Maternal age, gravidity, season of birth, twin pregnancies, infant gender, malaria, arm fat area

Habib et al 2008[246]	-	-	Year of baby's birth, maternal residence, maternal occupation and paternal tribe
Halsey et al 1990[260]	-	-	-
Johnstone et al 1996[258]	Maternal age, parity, ethnic group, ANC, delivery in the same year, deprivation score and smoking	-	Maternal age, smoking, parity, deprivation score, anaemia, maternal weight at 20 weeks' gestation
Kumar et al 1995[263]	Maternal age and parity	History of blood transfusion	-
Ladner et al 1998[247]	-	-	-
Lallemant et al 1989[248]	Maternal age, date of delivery, place of delivery	Occupation, marital status, POH, parity, gravidity, ANC visits, gestational age at first visit	-
Lepage et al 1991[249]	Maternal age and parity	Mothers occupation, father's occupation, place of origin, income	Immunological status, maternal age, parity, twins
Leroy et al 1998[250]	Maternal age and parity	Marital status, occupation, gestational age at enrolment, pregnancy complications	Maternal age, parity, STD, anaemia, malaria, education, prematurity
Markson et al 1996[261]	Location	Maternal age, education, ANC visits, year of delivery	Illicit drug use, race, maternal age, education, year of delivery, location, smoking, medicaid enrolment, antenatal visits
Maynard et al 1990[262]	-	-	-
Mitgitti et al 2008[264]	-	Parity, ethnicity, location of ANC, gestational age at delivery	Infant gender, maternal age, gestational age at delivery, antenatal visits
Mmiro et al 1993[251]	-	Marital status and transfusion	-
Musana et al 2009[252]	Maternal age and gestational age	Marital status, employment, gestational age at enrolment, hospitalization, mode of delivery, hypertension, chorioamnionitis, meconium staining in liquor	-
Mwanyumba et al 2001[253]	-	-	Maternal age, parity, sex of infant, religion, STD, anaemia, POH, hypertension, illicit drug use, smoking
Ndirangu et al 2012[225]	-	MUAC, place of delivery, mode of delivery, height, BMI	Infant gender, location, water-source, toilet type, delivery place, parity,

			maternal age, education, weight, height and MUAC
Noble et al 2005[235]	-	Parity, occupation, residence, history of STD, infant gender	Maternal age, gravidity, education, marital status, BMI, iron supplementation
Rollins et al 2007[226]	-	-	Maternal age, maternal residence, education, infant gender, MUAC
Ryder et al 1989[254]	Maternal age and parity	-	-
Selwyn et al 1989[218]	-	-	-
Sutton et al 1999[255]	-	-	Maternal age, parity, malaria, trichomoniasis, chorioamnionitis, SES
Taha et al 1995[256]	-	-	-
Temmerman et al 1994[216]	Maternal age and parity	Marital status, education, history of blood transfusion, history of oral contraception, POH, mode of delivery	-
Ticconi et al 2003[234]	-	-	Maternal age, parity, malaria, HIV treatment, malaria treatment
Van den Broek et al 2014[236]	-	Maternal age, parity, POH, BMI	Maternal age, BMI, weight gain, anaemia, malaria
ANC = antenatal care, BMI = body mass index, HIV = Human Immunodeficiency Virus, IVDU = Intravenous Drug Use, MUAC = mid-upper arm circumference, POH = past obstetric history, SES = socio-economic status, STD = sexually transmitted disease.			

3.3.3 Influence of maternal HIV-infection on gestation at delivery

3.3.3.1 PTB

14 prospective and eight retrospective cohort studies, and two case-control studies, including 34,337 women, reported on the association between maternal HIV-infection and PTB (Figure 3.2). Maternal HIV-infection significantly increased the risk of PTB in prospective (RR 1.50; 95%CI 1.24-1.82) and retrospective (RR 1.82; 95%CI 1.41-2.34) cohort studies. A high degree of heterogeneity was evident in the meta-analysis of retrospective studies (I^2 = 81.0%). The two case-control studies showed a non- significant association between maternal HIV-infection and PTB (Table 3.7).

This effect of HIV on PTB persisted across geographic regions, with a significant association observed in prospective and retrospective studies conducted in sub-Saharan Africa and the Americas and Caribbean (Figure 3.3, Figure 3.4). Among prospective studies, a significant risk of PTB was observed in HIV-positive women irrespective of the method used to determine GA, unless the method was not specified (Figure 3.5, Figure 3.6).

Sensitivity analyses according to the methodological quality showed a significant association between maternal HIV-infection and PTB in average (RR 1.64; 95%CI 1.23-2.20) and poor quality (RR 1.34; 95% CI 1.06-1.71) prospective studies (Figure 3.7), and poor quality retrospective studies (RR1.86; 95%CI 1.42-2.43) (Figure 3.8). The Peter test showed no evidence of publication bias (P =0.660).

3.3.3.2 VPTB

One prospective cohort study conducted in sub-Saharan Africa and including 2368 women reported a non-significant association between maternal HIV-infection and VPTB (RR 0.99; 95%CI 0.60-1.65) (Figure 3.9 – Figure 3.12).

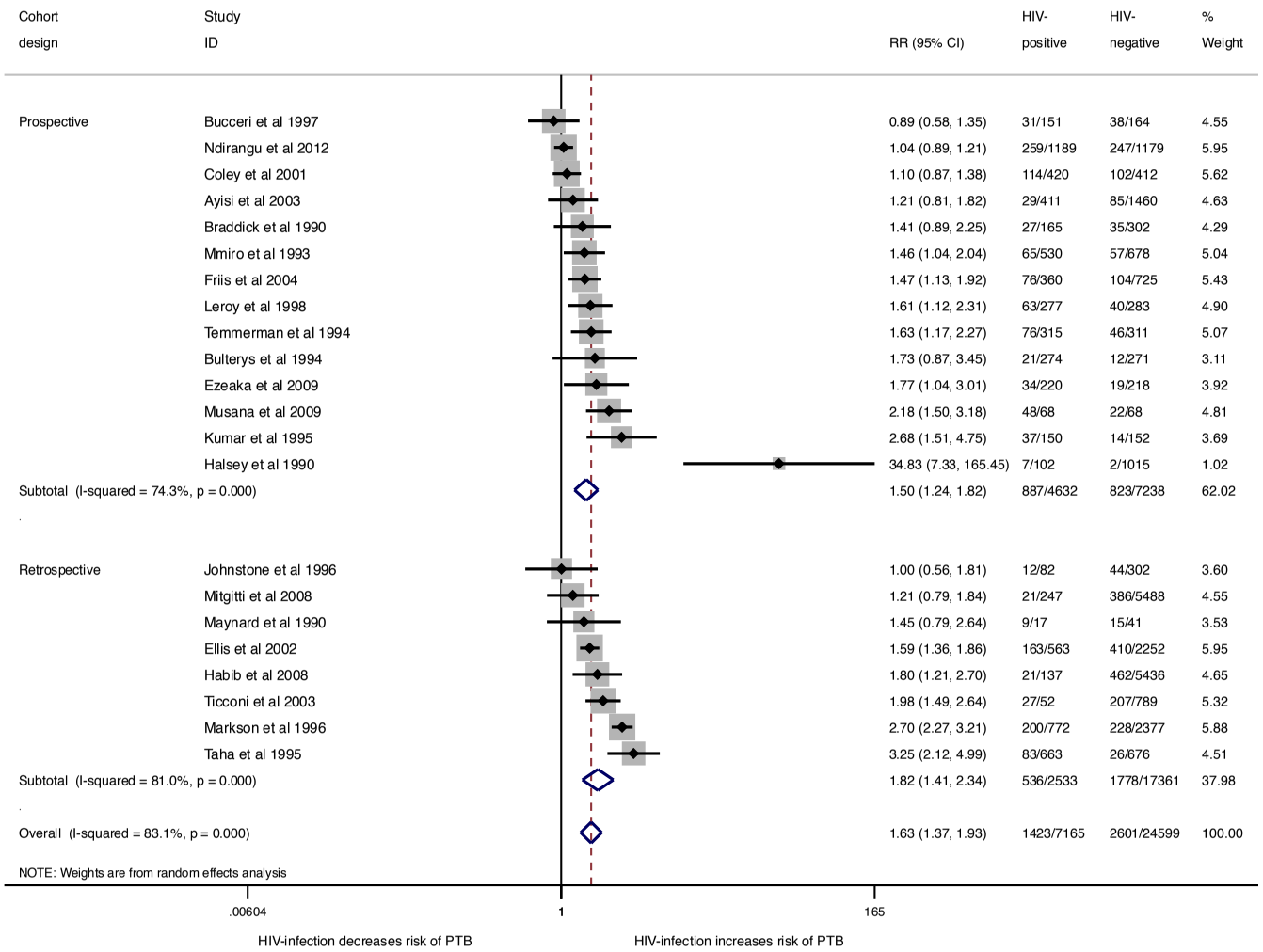


Figure 3.2: PTB associated with ART-naïve maternal HIV-infection, by study design.
Random-effect pairwise meta-analyses showed a significant increase in the risk of PTB in HIV-positive women, compared to HIV-negative women in prospective and retrospective studies. Abbreviations: HIV = Human Immunodeficiency Virus, PTB = preterm birth, RR = relative risk
Source: Wedi et al. 2016[3]

Table 3.7: Case-control studies assessing the association between ART-naïve maternal HIV-infection and perinatal outcomes.
Source: Wedi et al. 2016[3]

Study	Outcome	OR (95% CI)	HIV-positive women in cases	HIV-positive women in controls
Bergstrom et al 1995[241]	LBW	0.32 (0.01-8.38)	0/51	1/51
Noble et al 2005[235]	PTB	0.81 (0.43-1.52)	15/53	144/440
Van Den Broek et al 2014[236]	PTB	1.15 (0.89-1.49)	99/351	439/1729

Abbreviations: HIV = Human Immunodeficiency Virus, LBW = low birthweight, OR (95% CI) = odds ratio with 95% confidence interval, PTB = preterm birth.

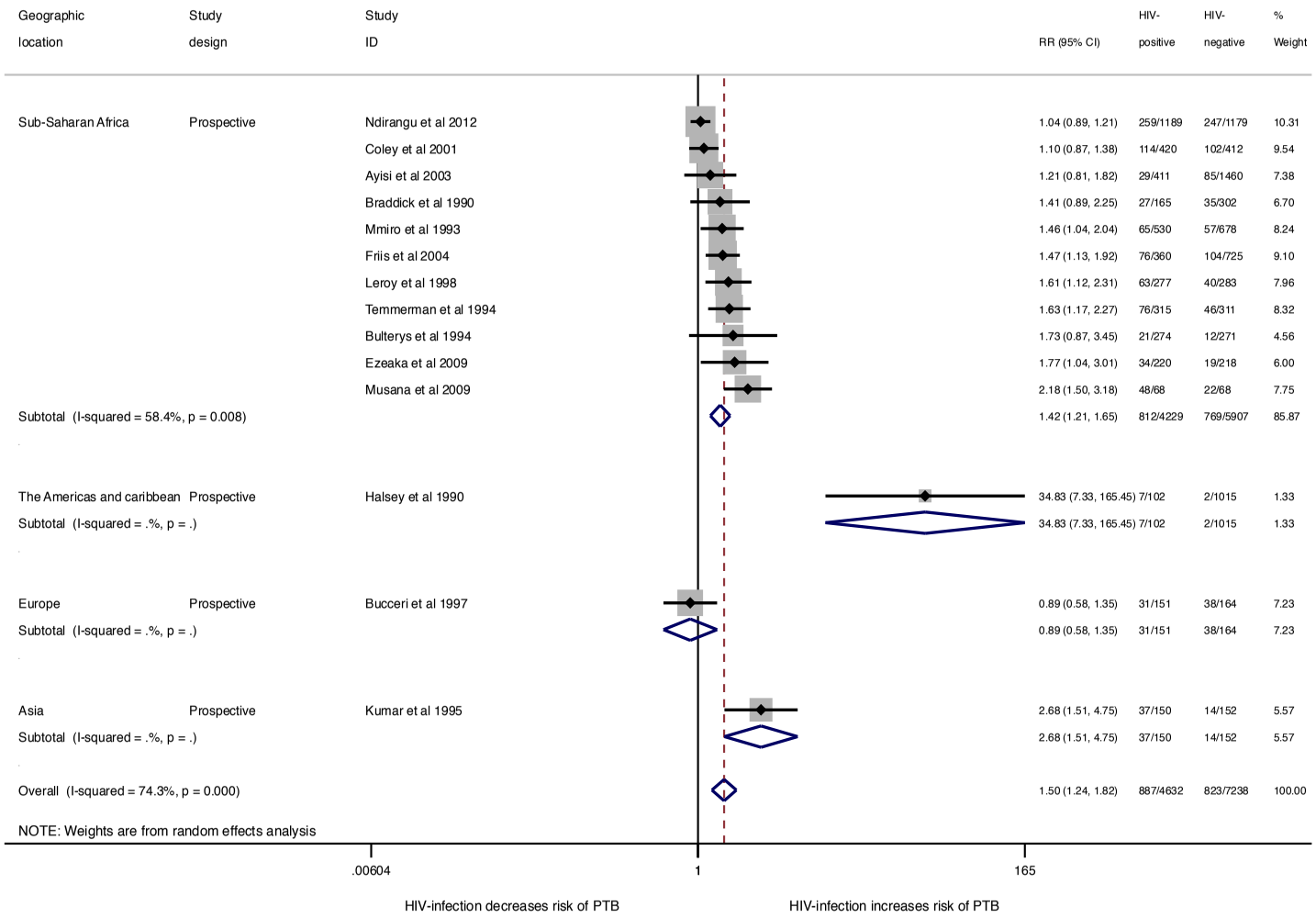


Figure 3.3: **PTB associated with ART-naïve maternal HIV-infection, by geographic region: prospective studies.**
Random-effect pairwise meta-analyses showed a significant increase in the risk of PTB in HIV-positive women residing in sub-Saharan Africa, Asia and the Americas and Caribbean. Abbreviations: HIV = Human Immunodeficiency Virus, PTB = preterm birth, RR = relative risk.
Source: Wedi et al. 2016[3]

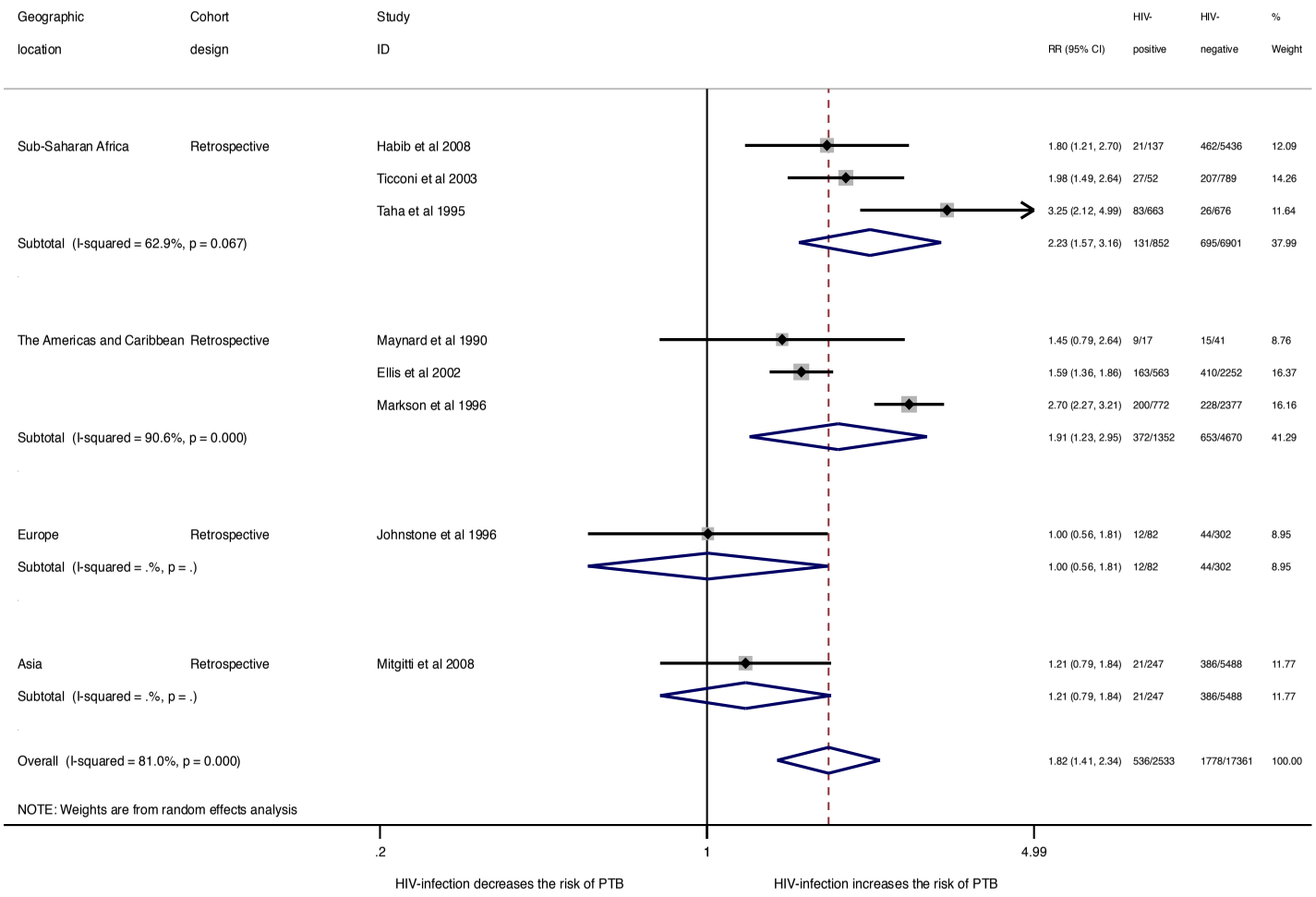


Figure 3.4: **PTB associated with ART-naïve maternal HIV-infection, by geographic region: retrospective studies.**
Random-effect pairwise meta-analyses showed a significant increase in the risk of PTB in HIV-positive women residing in sub-Saharan Africa and the Americas and Caribbean. Abbreviations: HIV = Human Immunodeficiency Virus, PTB = preterm birth, RR = relative risk.
Source: Wedi et al. 2016[3]

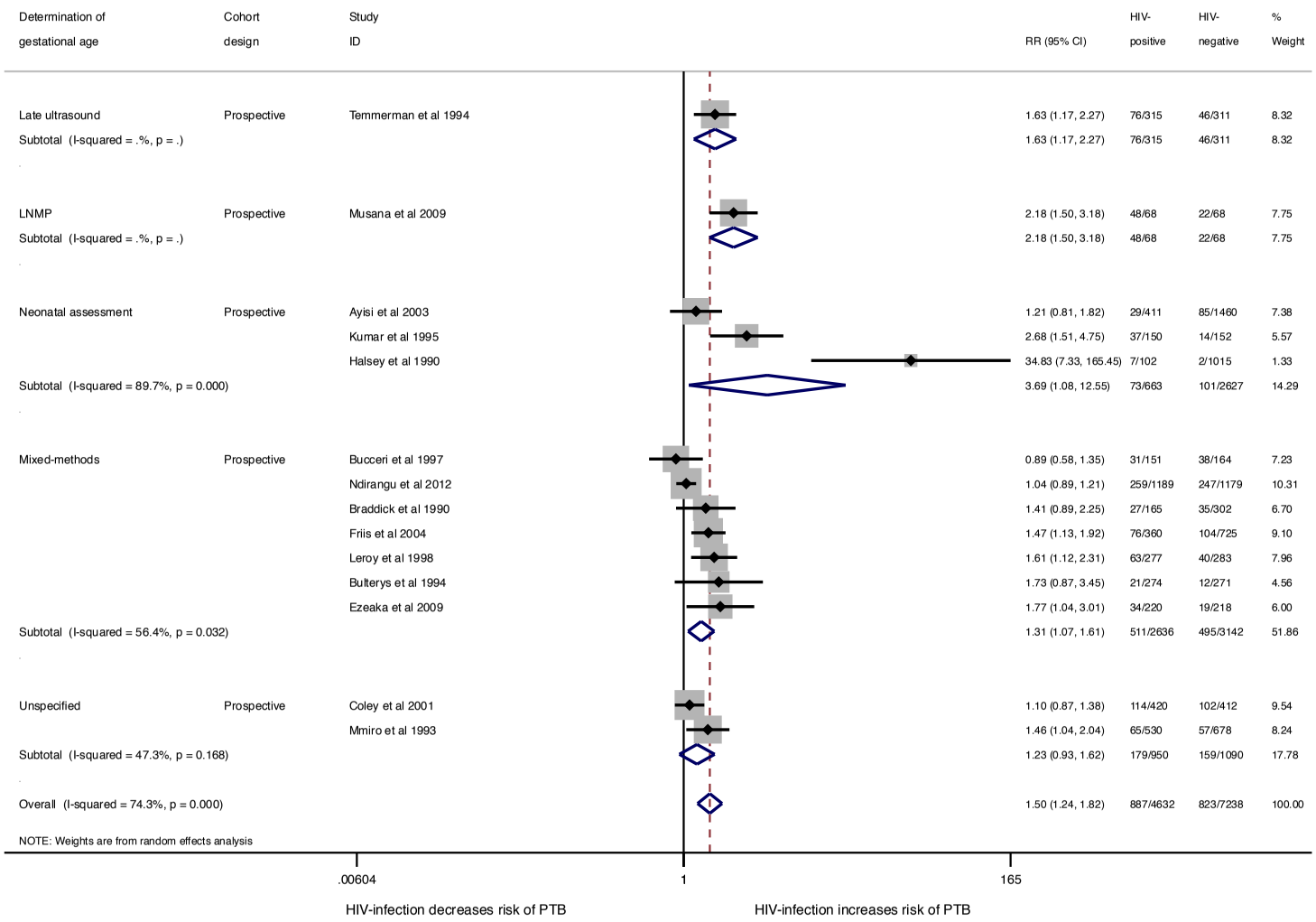


Figure 3·5: **PTB associated with ART-naïve maternal HIV-infection, by method used to determine gestational age: prospective studies.** Random-effect pairwise meta-analyses showed a significant increase in the risk of PTB in HIV-positive women in studies estimating GA with a late ultrasound, LNMP, neonatal assessment and a combination of methods (mixed-methods – see Table 3.4). Abbreviations: GA = gestational age, HIV = Human Immunodeficiency Virus, LNMP = last normal menstrual period, PTB = preterm birth, RR = relative risk. Source: Wedi et al. 2016[3]

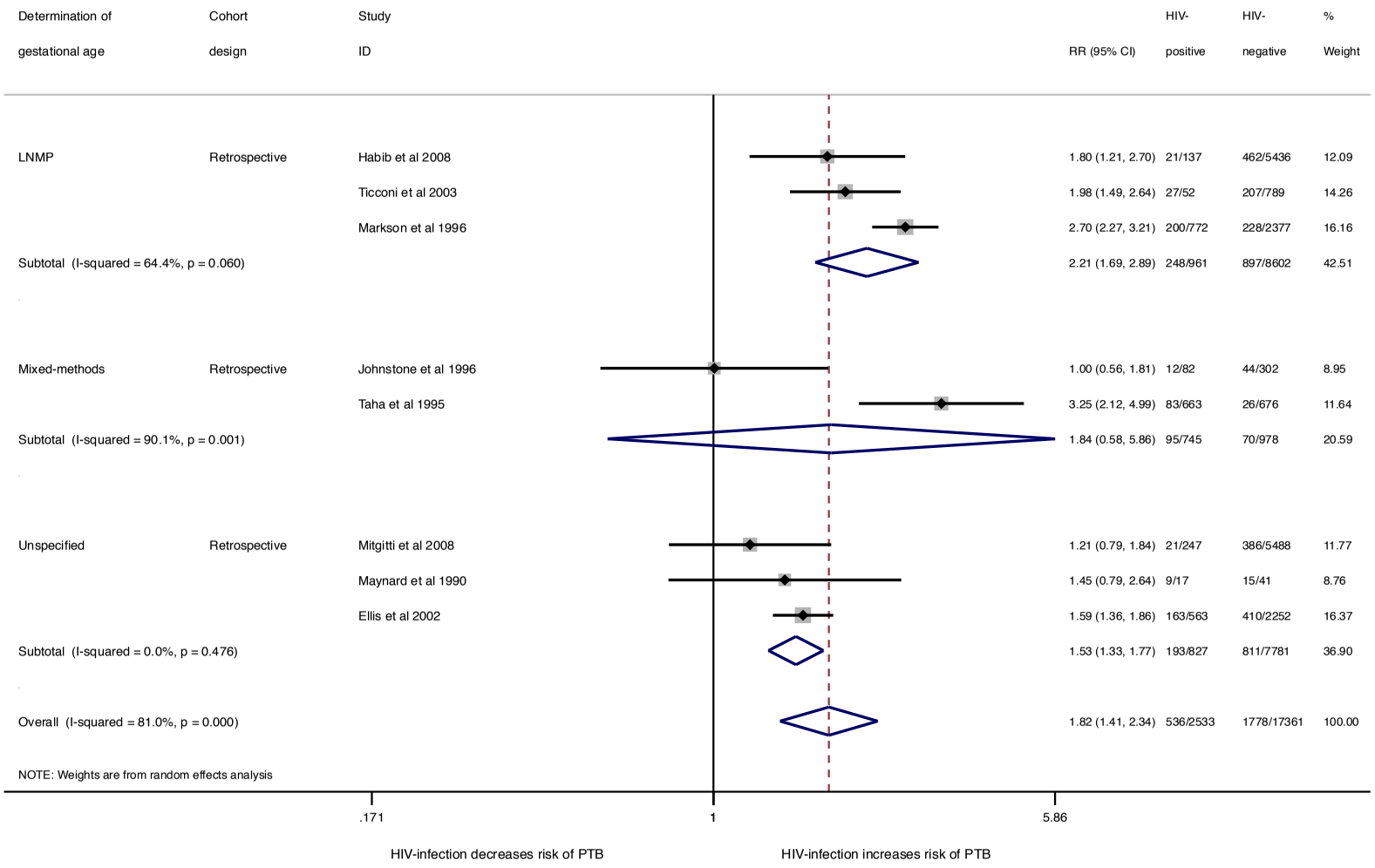


Figure 3.6: **PTB associated with ART-naïve maternal HIV-infection, by method used to determine gestational age: retrospective studies**. Random-effect pairwise meta-analyses showed a significant increase in the risk of PTB in HIV-positive women in studies estimating GA with a LNMP and in studies that did not report the method used. Abbreviations: GA = gestational age, HIV = Human Immunodeficiency Virus, LNMP = last normal menstrual period, PTB = preterm birth, RR = relative risk. Source: Wedi et al. 2016[3]

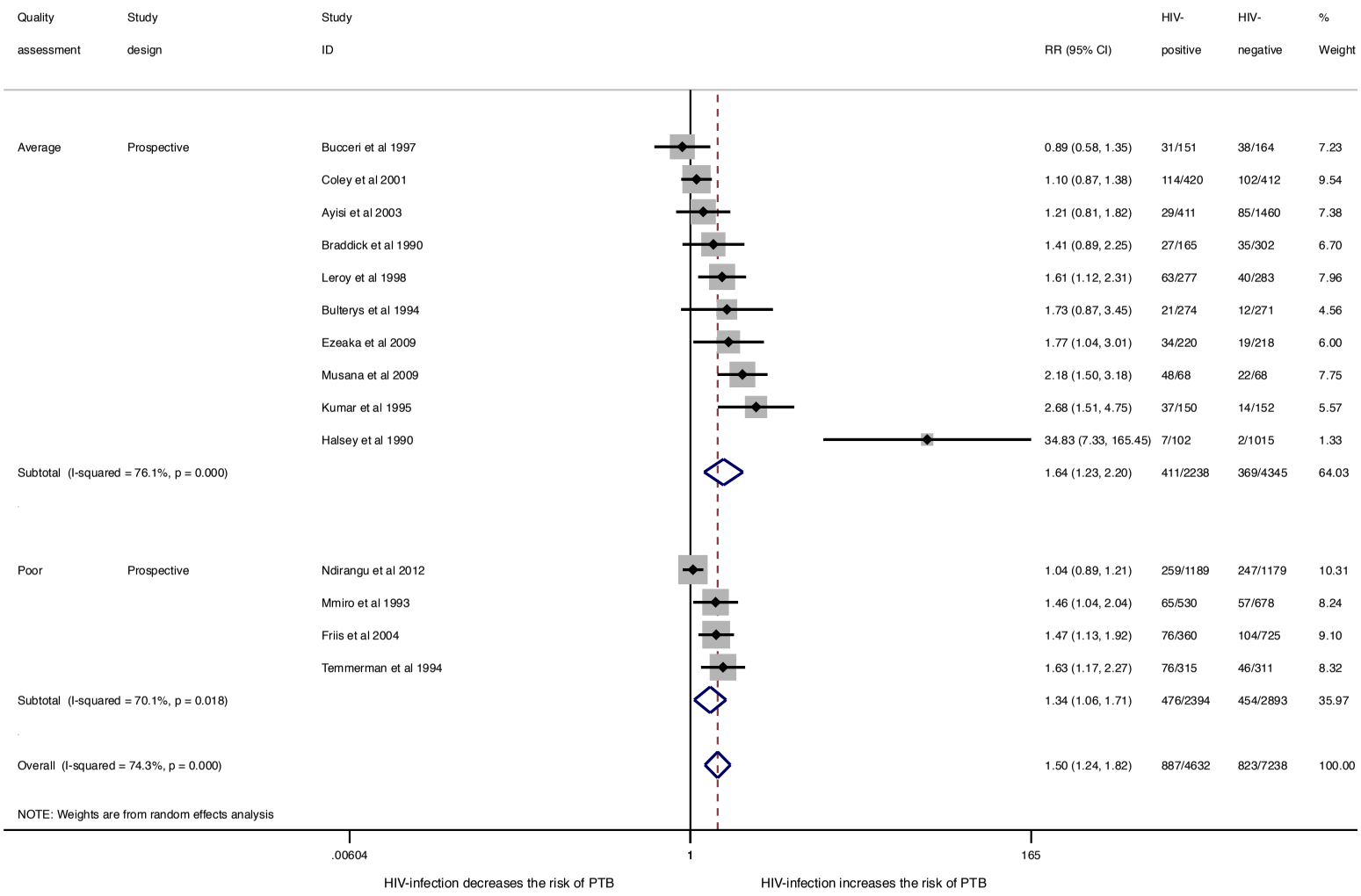


Figure 3.7: **PTB associated with ART-naïve maternal HIV-infection, by adapted Newcastle-Ottawa quality assessment of studies: prospective studies.** Random-effect pairwise meta-analyses showed a significant increase in the risk of PTB in HIV-positive women in average and poor quality studies. Abbreviations: HIV = Human Immunodeficiency Virus, PTB = preterm birth, RR = relative risk. Source: Wedi et al. 2016[3]

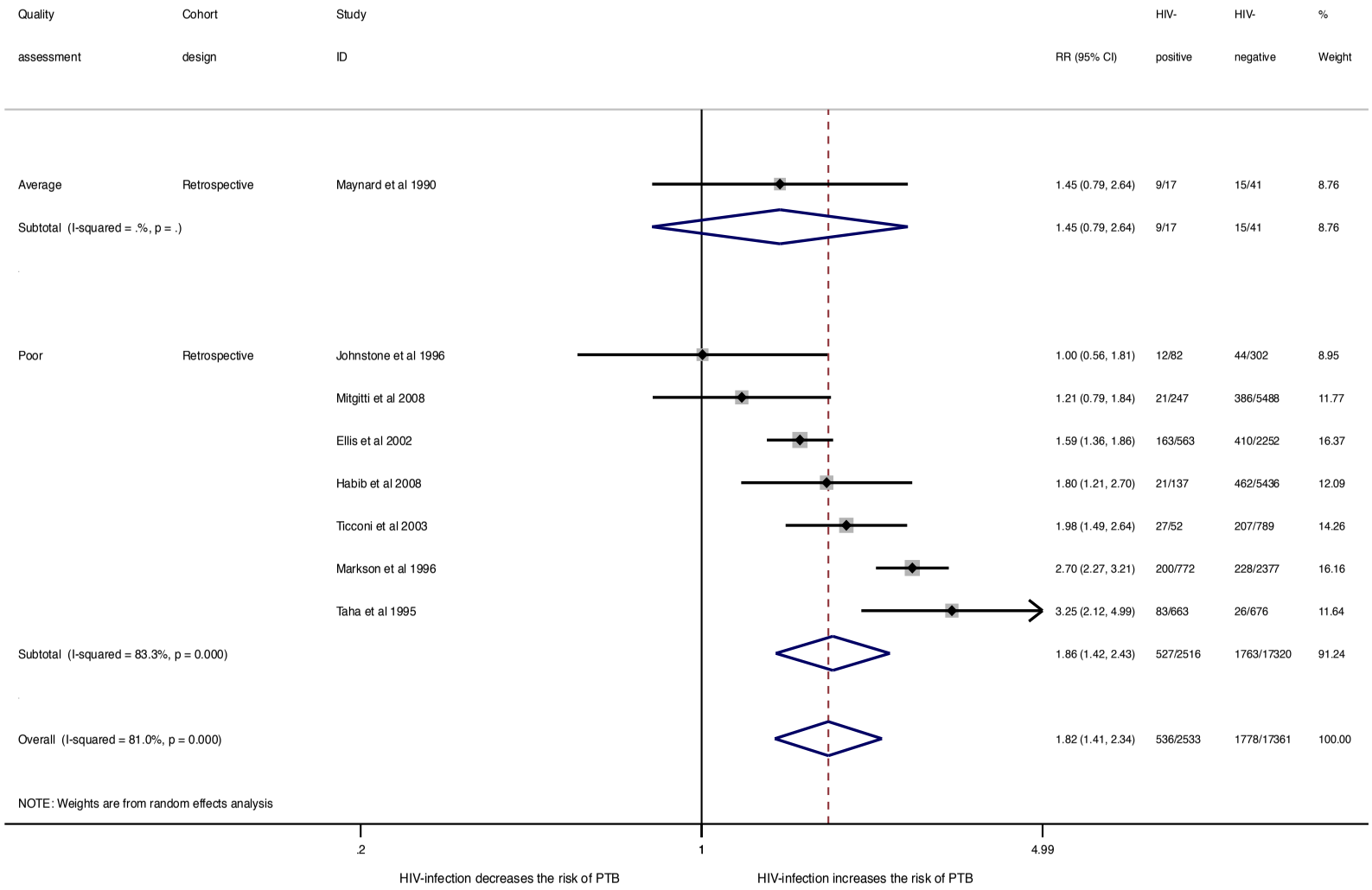


Figure 3.8: **PTB associated with ART-naïve maternal HIV-infection, by adapted Newcastle-Ottawa quality assessment of studies: retrospective studies.** Random-effect pairwise meta-analyses showed a significant increase in the risk of PTB in HIV-positive women in poor quality studies. Abbreviations: HIV = Human Immunodeficiency Virus, PTB = preterm birth, RR = relative risk. Source: Wedi et al. 2016[3]

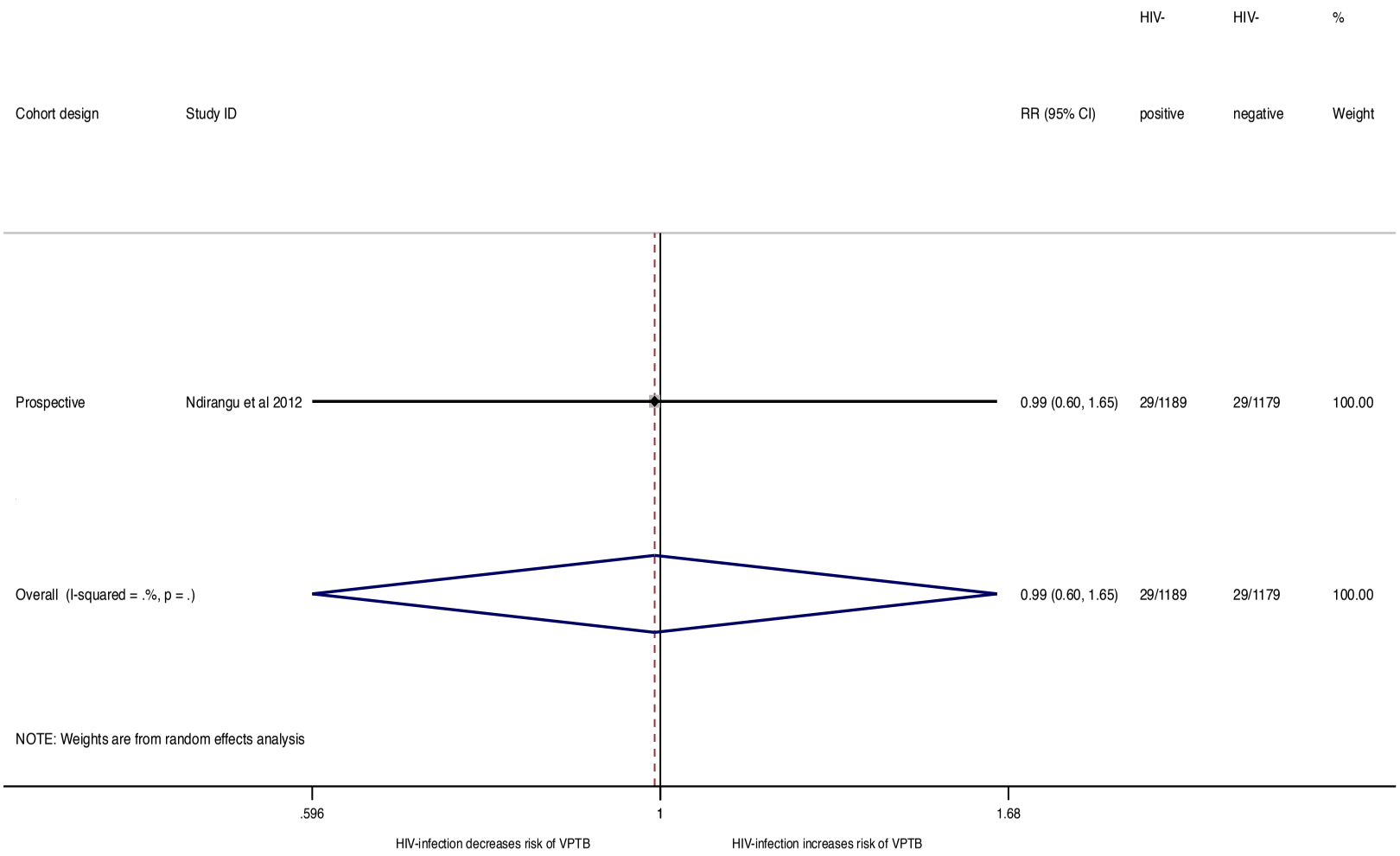


Figure 3.9: **VPTB associated with ART-naïve maternal HIV-infection, by study design.**
No significant association observed between maternal HIV-infection and VPTB. Abbreviations: HIV = Human Immunodeficiency Virus, RR = relative risk, VPTB = very preterm birth. Source: Wedi et al. 2016[3]

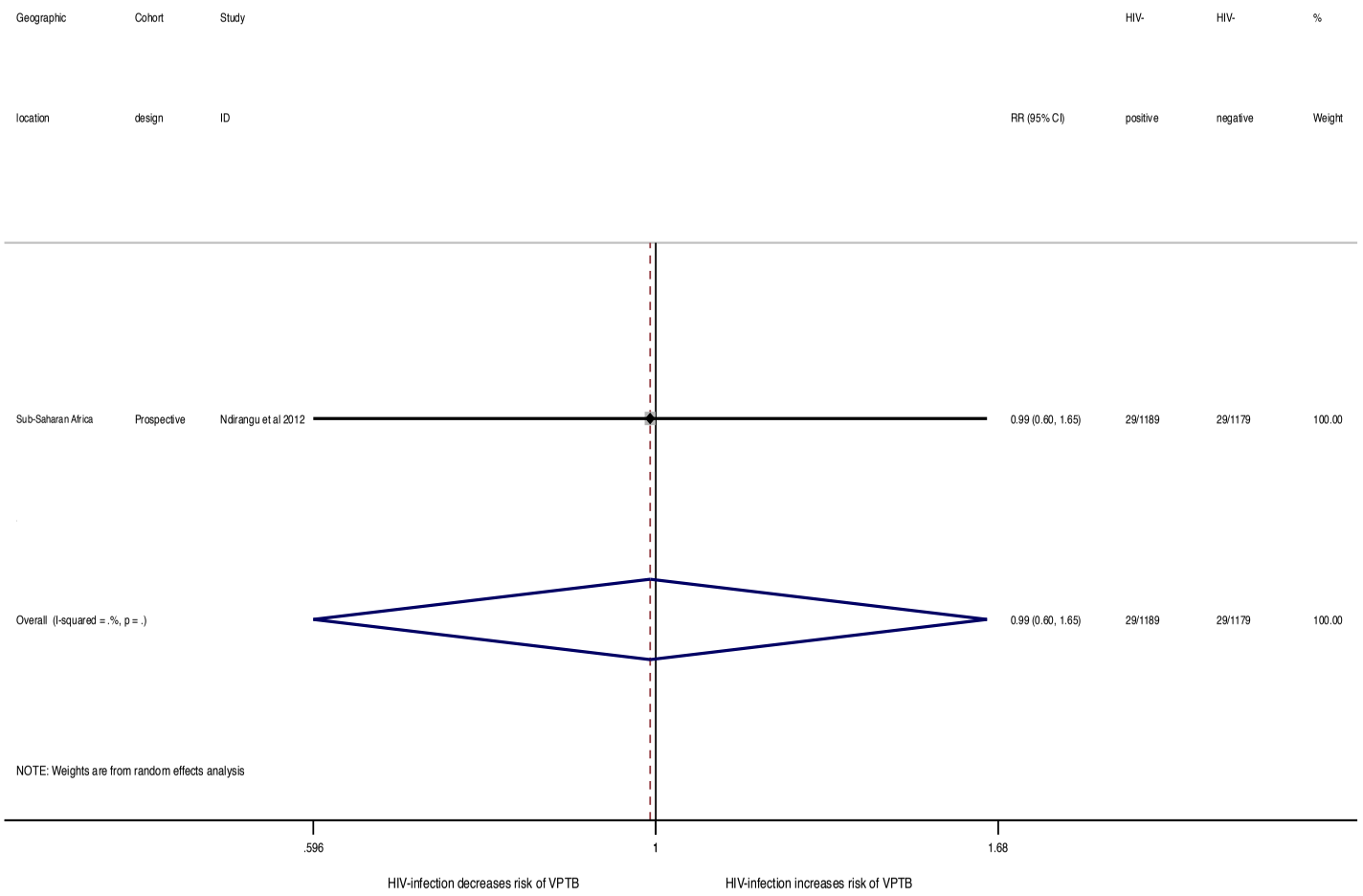
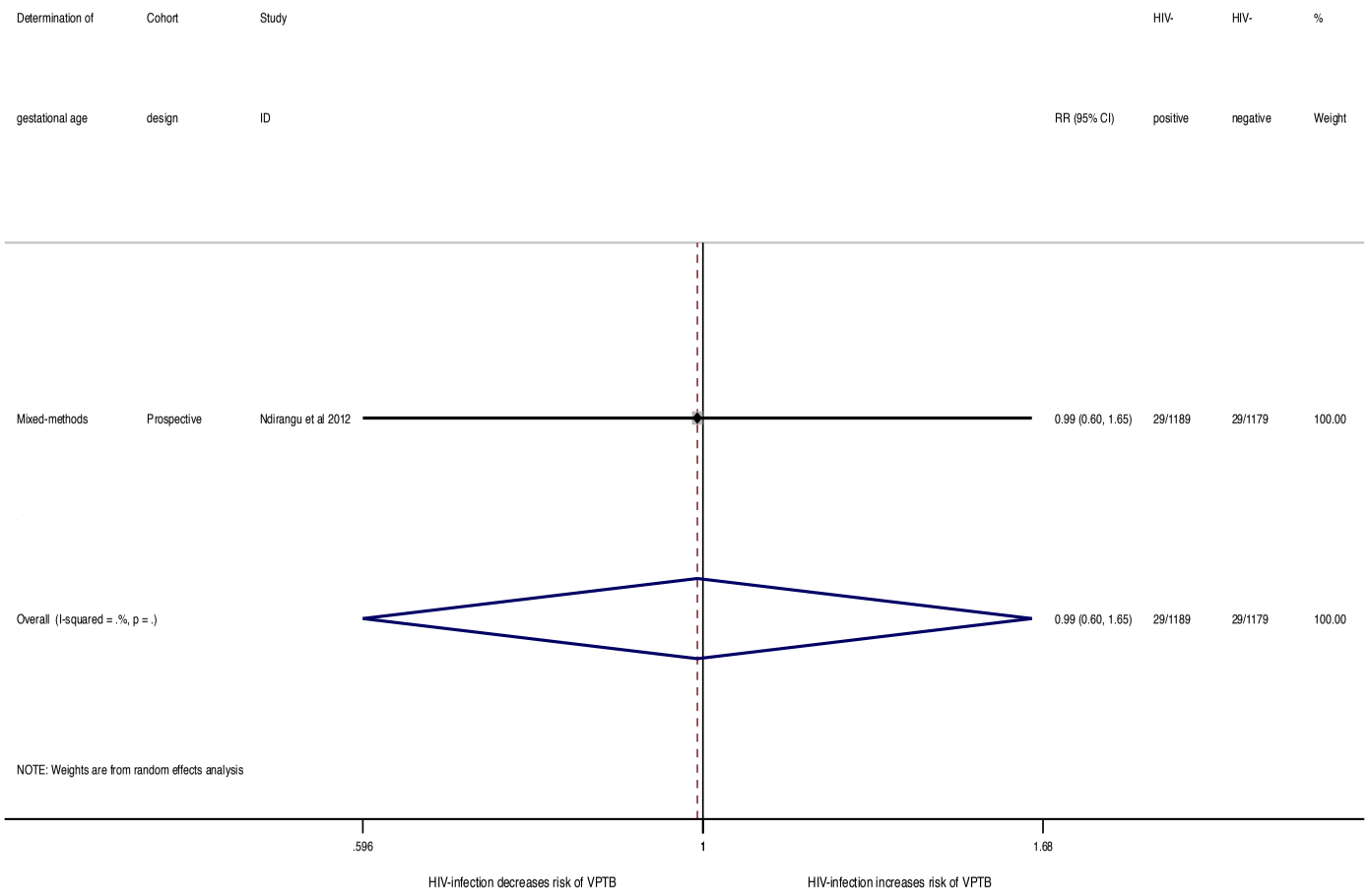


Figure 3. 10: VPTB associated with ART-naïve maternal HIV-infection, by geographic region: prospective study.
No significant association observed between maternal HIV-infection and VPTB. Abbreviations: HIV = Human Immunodeficiency Virus, RR = relative risk, VPTB = very preterm birth. Source: Wedi et al. 2016[3]



Figure·3.11: **VPTB associated with ART-naïve maternal HIV-infection, by method used to determine gestational age: prospective study.**
No significant association observed between maternal HIV-infection and VPTB. Abbreviations: HIV = Human Immunodeficiency Virus, RR = relative risk, VPTB = very preterm birth. Source: Wedi et al. 2016[3]

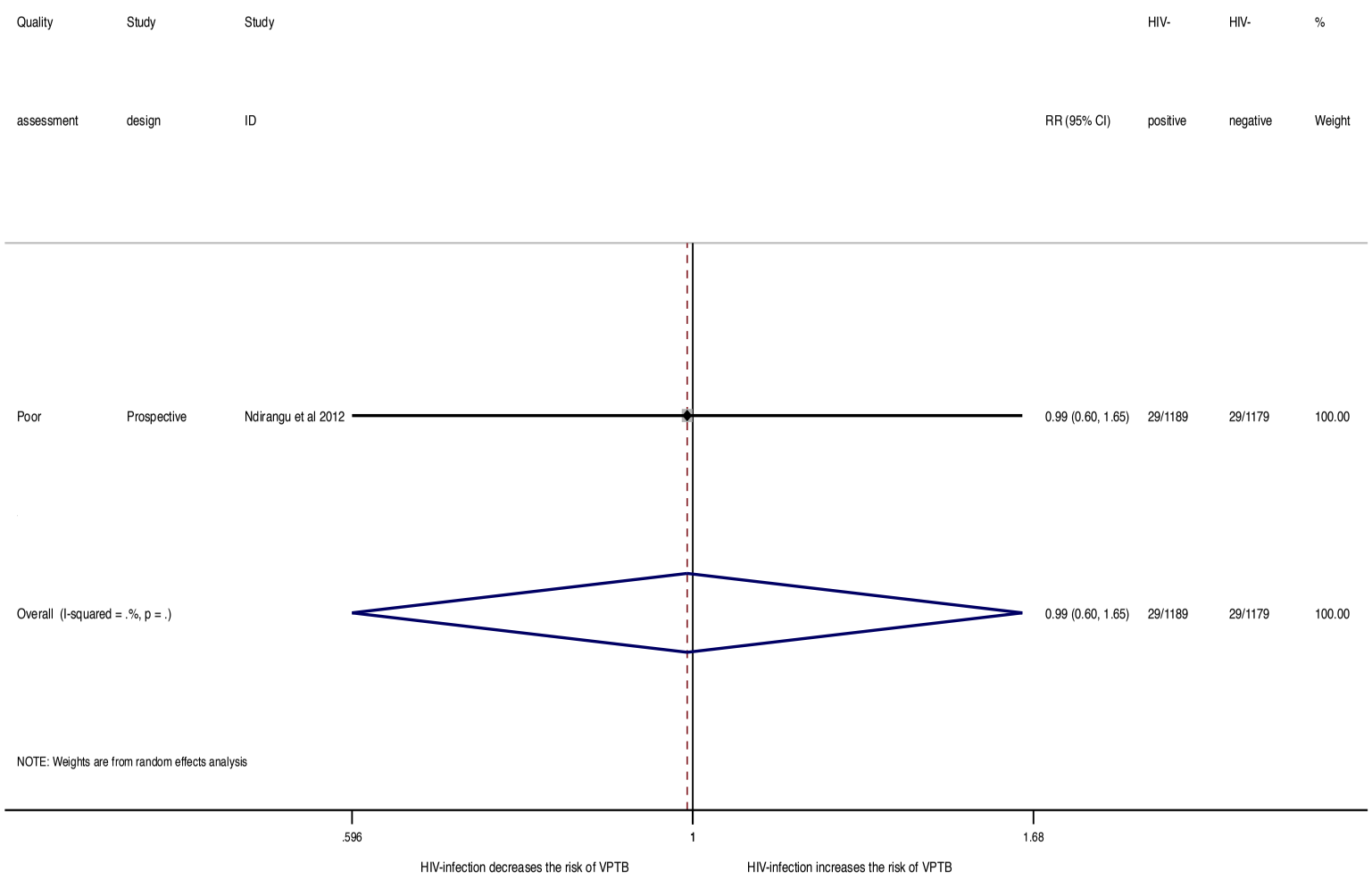


Figure 3·12: VPTB associated with ART-naïve maternal HIV-infection, by adapted Newcastle-Ottawa quality assessment of studies: prospective study. No significant association observed between maternal HIV-infection and VPTB. Abbreviations: HIV = Human Immunodeficiency Virus, RR = relative risk, VPTB = very preterm birth. Source: Wedi et al. 2016[3]

3.3.4 Influence of maternal HIV-infection on birthweight

3.3.4.1 Low birthweight

16 prospective and nine retrospective cohort studies, and one case-control study, including 36,312 women, reported on the association between maternal HIV-infection and LBW (Figure 3.13). Meta-analyses of prospective (RR 1.62; 95%CI 1.41-1.86) and retrospective (RR 1.93; 95%CI 1.48-2.52) cohort studies showed a significant risk of LBW in HIV-positive women compared to HIV-negative women (Figure 3.13). A high degree of heterogeneity was observed in the meta-analysis of retrospective studies (I^2 = 87.2%). There was no evidence of publication bias (P=0.630). The case-control study showed a negative but non-significant association between maternal HIV-infection and LBW (OR 0·32; 95%CI 0·01-8·38) (Table 3.7).

Prospective and retrospective studies showed that maternal HIV-infection significantly increased the risk of LBW in women residing in sub-Saharan Africa and Asia (Figure 3.14, Figure 3.15). Retrospective studies also showed a significant association between maternal HIV-infection and LBW in women residing in the Americas and Caribbean (RR 2.09; 95%CI 1.33-3.29).

Sensitivity analyses according to the quality assessment of studies showed a significant association between maternal HIV-infection and LBW, irrespective of the quality of prospective and retrospective studies (Figure 3.16, Figure 3.17).

3.3.4.2 Very low birth weight

One retrospective cohort study conducted in the Americas and Caribbean reported a non-significant association between maternal HIV-infection and VLBW (RR 4.82; 95%CI 0.47-49.72) (Figure 3.18 – Figure 3.20).

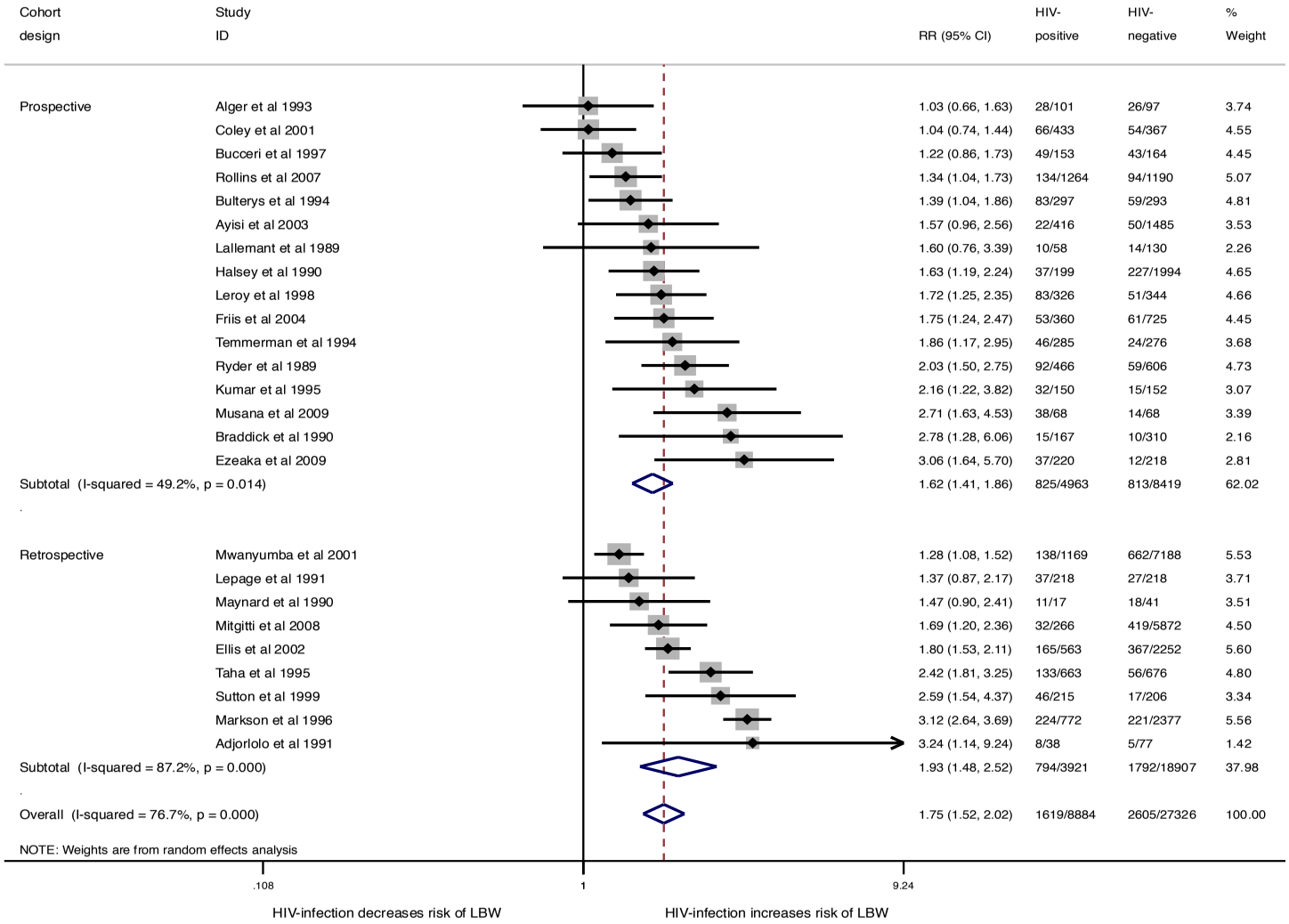


Figure 3.13: LBW associated with ART-naïve maternal HIV-infection, by study design.
Random-effect pairwise meta-analyses showed a significant increase in the risk of LBW in HIV-positive women, compared to HIV-negative women in prospective and retrospective studies. Abbreviations: HIV = Human Immunodeficiency Virus, LBW = low birthweight, RR = relative risk
Source: Wedi et al. 2016[3]

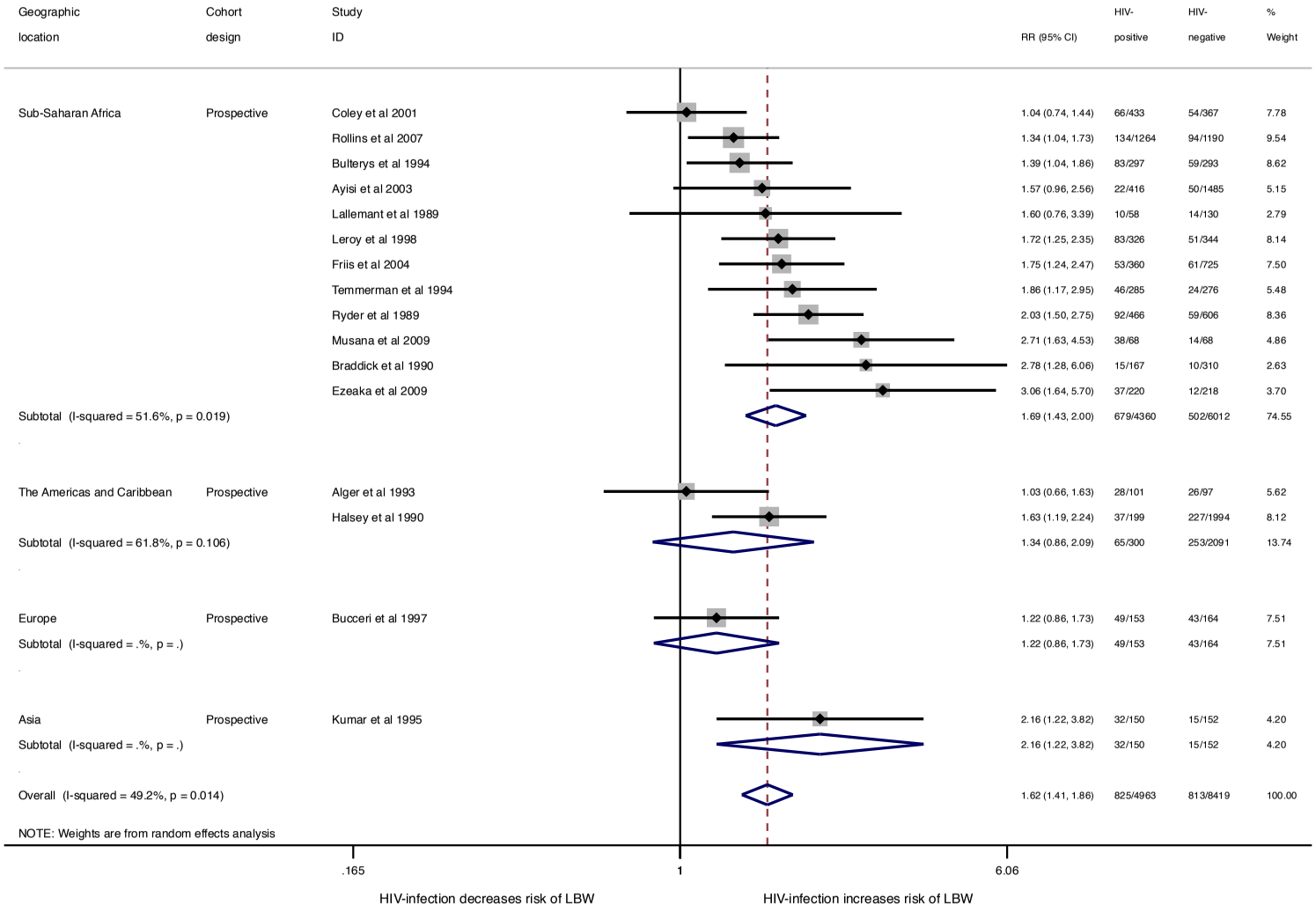


Figure 3.14: **LBW associated with ART-naïve maternal HIV-infection, by geographic region: prospective studies.**
Random-effect pairwise meta-analyses showed a significant increase in the risk of LBW in HIV-positive women residing in sub-Saharan Africa and Asia. Abbreviations: HIV = Human Immunodeficiency Virus, LBW = low birthweight, RR = relative risk.
Source: Wedi et al. 2016[3]

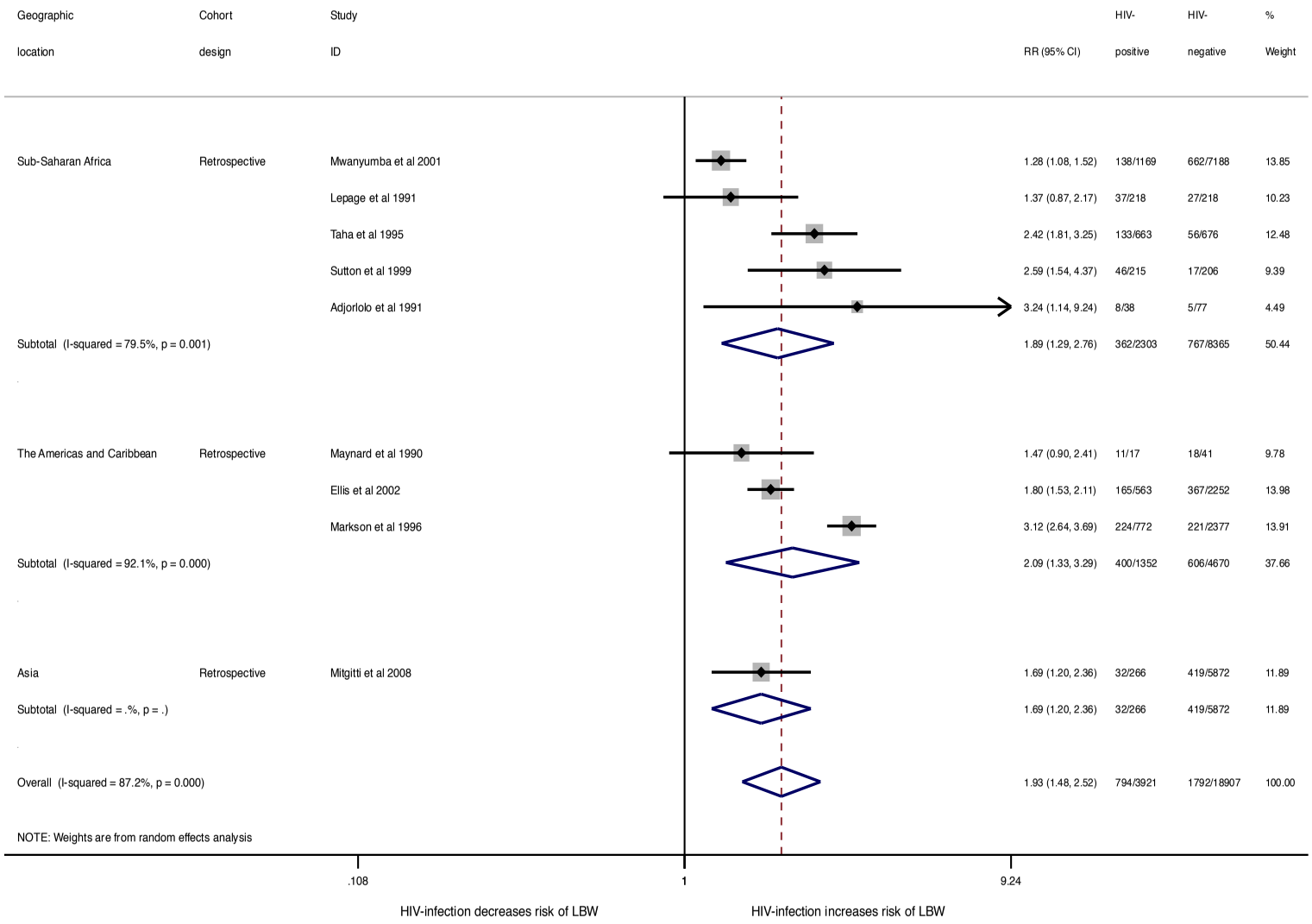


Figure 3.15: **LBW associated with ART-naïve maternal HIV-infection, by geographic region: retrospective studies.**
Random-effect pairwise meta-analyses showed a significant increase in the risk of LBW in HIV-positive women residing in sub-Saharan Africa, the Americas and Caribbean and Asia. Abbreviations: HIV = Human Immunodeficiency Virus, LBW = low birthweight, RR = relative risk.
Source: Wedi et al. 2016[3]

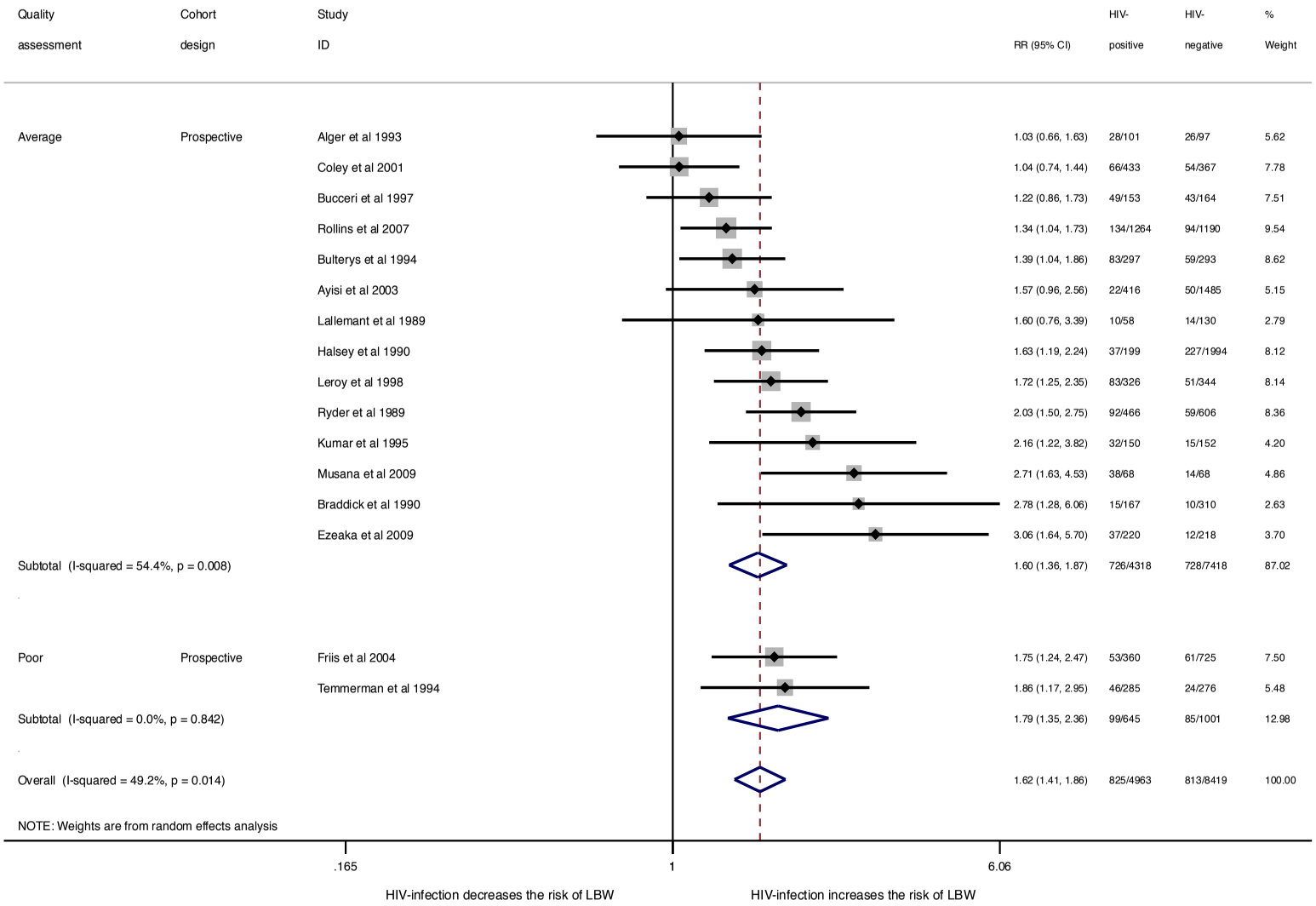


Figure 3.16: **LBW associated with ART-naïve maternal HIV-infection, by adapted Newcastle-Ottawa quality assessment of studies: prospective studies.** Random-effect pairwise meta-analyses showed a significant increase in the risk of LBW in HIV-positive women in average and poor quality studies. Abbreviations: HIV = Human Immunodeficiency Virus, LBW = low birthweight, RR = relative risk. Source: Wedi et al. 2016[3]

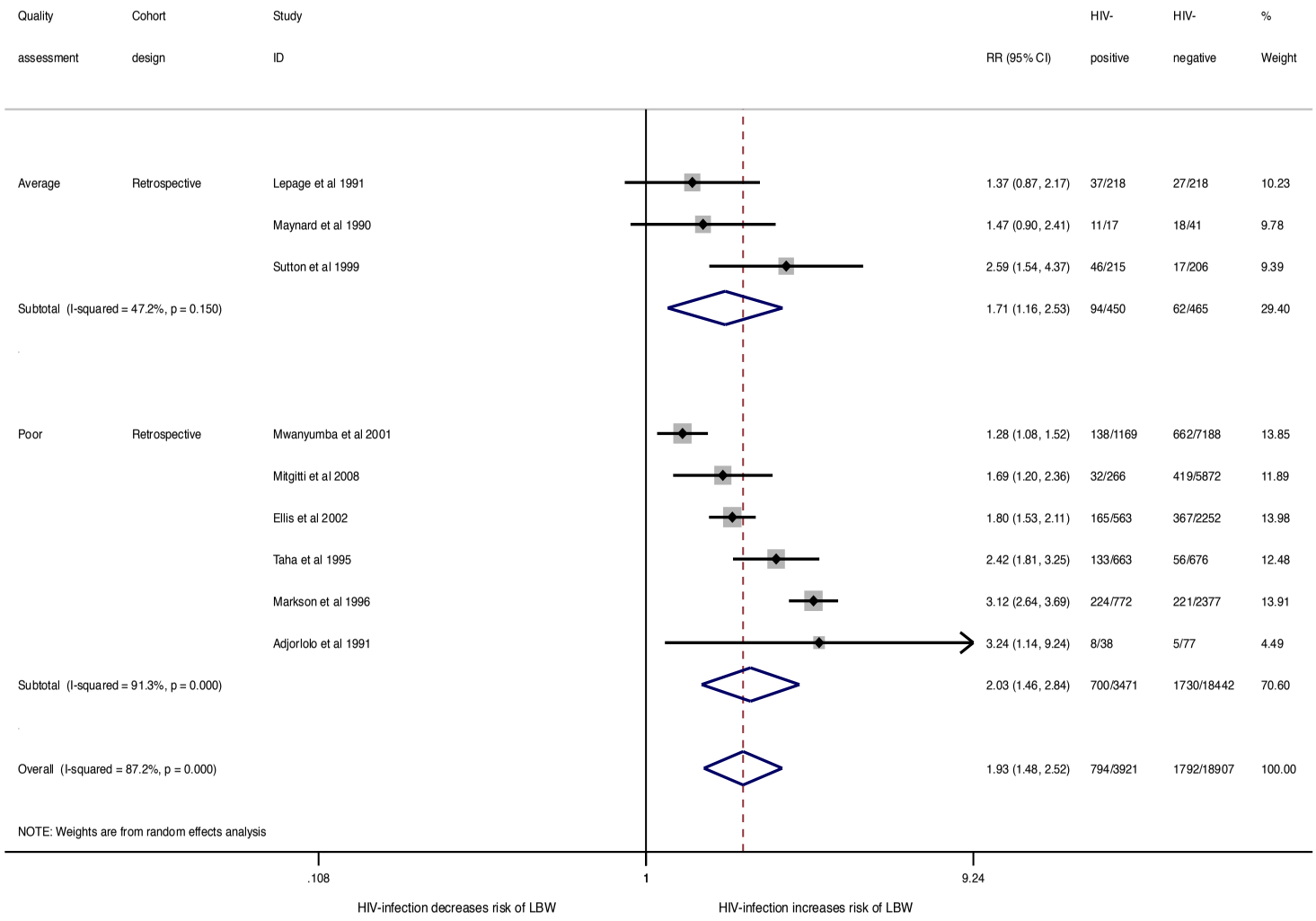


Figure 3.17: **LBW associated with ART-naïve maternal HIV-infection, by adapted Newcastle-Ottawa quality assessment of studies: retrospective studies.** Random-effect pairwise meta-analyses showed a significant increase in the risk of LBW in HIV-positive women in average and poor quality studies. Abbreviations: HIV = Human Immunodeficiency Virus, LBW = low birthweight, RR = relative risk. Source: Wedi et al. 2016[3]

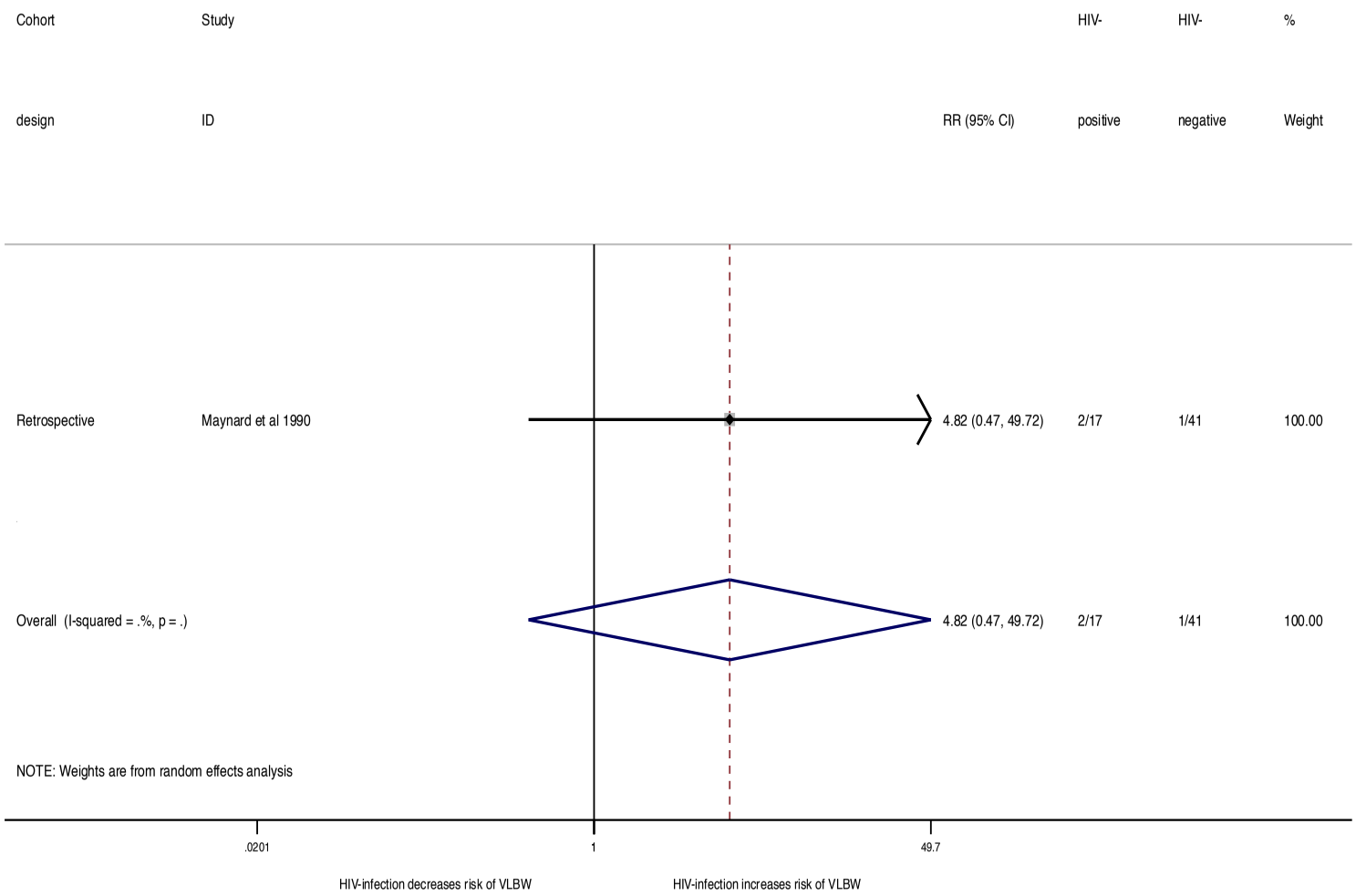


Figure 3.18: VLBW associated with ART-naïve maternal HIV-infection, by study design.
No significant association observed between maternal HIV-infection and VLBW. Abbreviations: HIV = Human Immunodeficiency Virus, RR = relative risk, VLBW = very low birthweight. Source: Wedi et al. 2016[3]

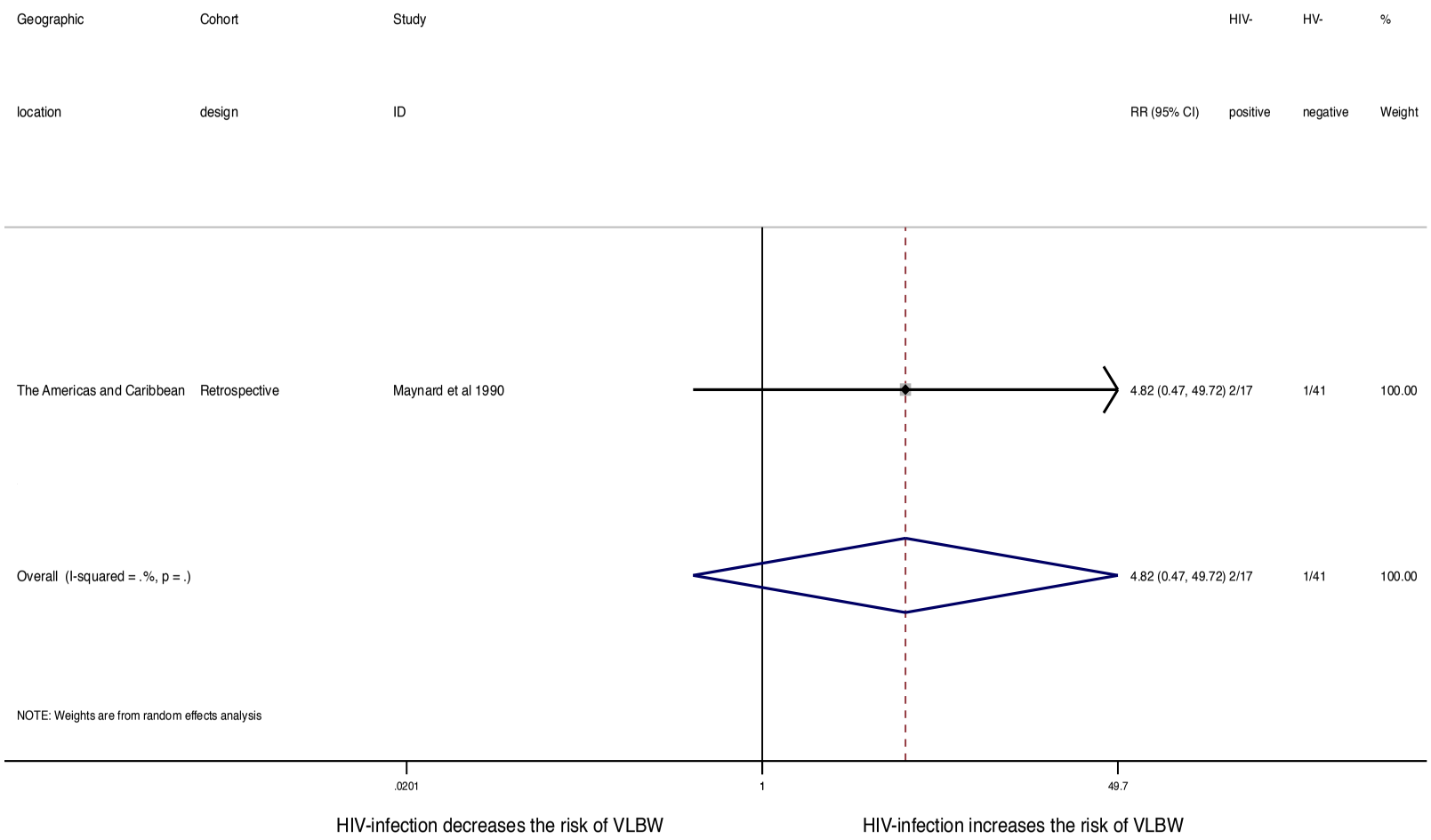


Figure 3.19: VLBW associated with ART-naïve maternal HIV-infection, by geographic region: retrospective study.
No significant association observed between maternal HIV-infection and VLBW. Abbreviations: HIV = Human Immunodeficiency Virus, RR = relative risk, VLBW = very low birthweight. Source: Wedi et al. 2016[3]

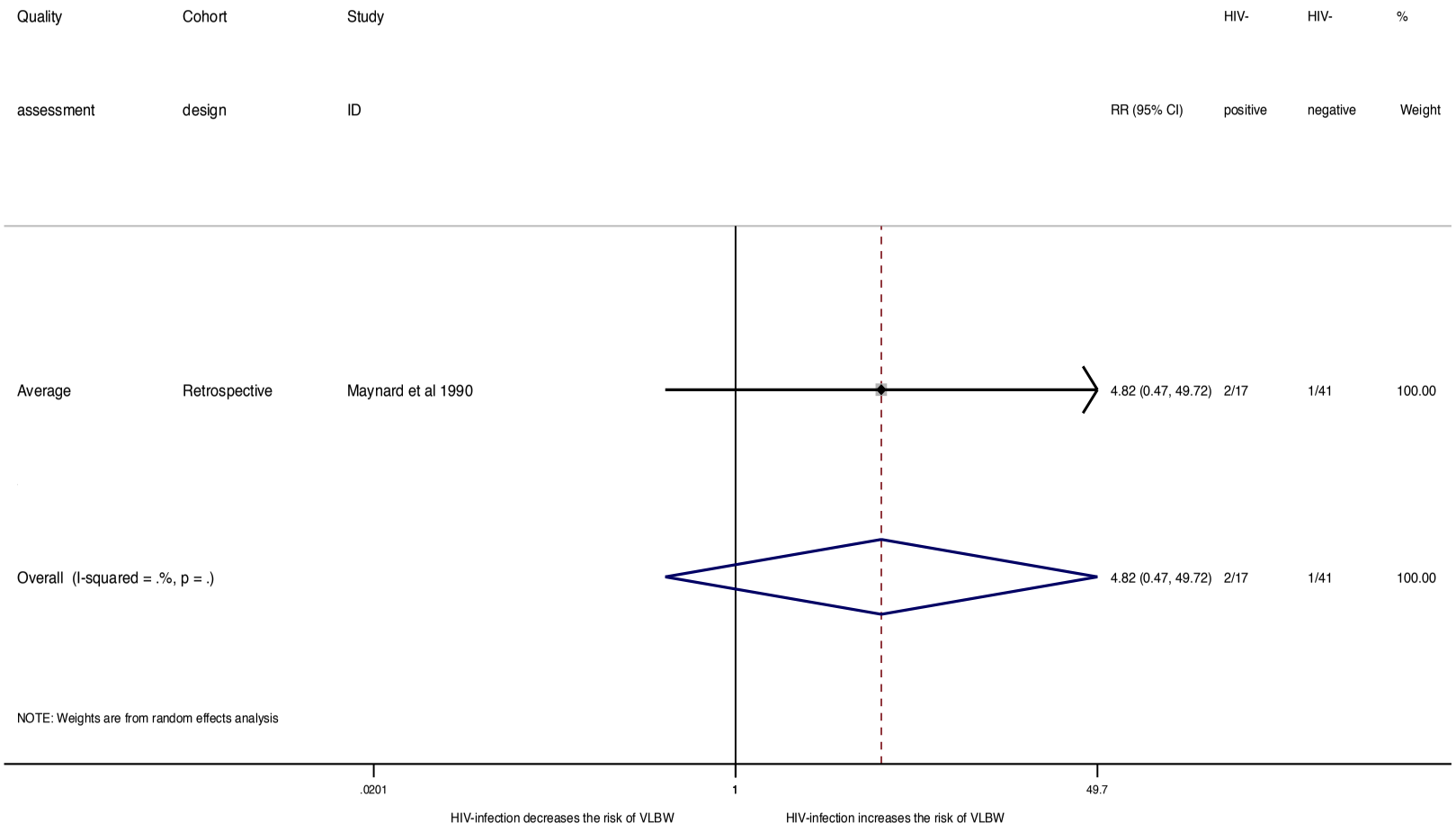


Figure 3.20: VLBW associated with ART-naïve maternal HIV-infection, by adapted Newcastle-Ottawa quality assessment of studies: retrospective study. No significant association observed between maternal HIV-infection and VLBW. Abbreviations: HIV = Human Immunodeficiency Virus, RR = relative risk, VLBW = very low birthweight. Source: Wedi et al. 2016[3]

3.3.5 Influence of maternal HIV-infection on birthweight and gestational age combined

3.3.5.1 Term-LBW

Seven cohort studies, including 7038 women, reported on the association between maternal HIV-infection and term-LBW (Figure 3.21). Retrospective studies showed that HIV-positive women were at significant risk of giving birth to a term-LBW infant (RR 2.62; 95%CI 1.15-5.93); however, a high degree of heterogeneity was observed (I^2 = 89.8%) (Figure 3.21). Prospective studies showed a non-significant association (Figure 3.21).

When studies were stratified according to the geographic region where the study was conducted (Figure 3.22, Figure 3.23), retrospective studies showed a significant association between maternal HIV-infection and term-LBW, in women residing in sub-Saharan Africa (RR1.70; 95%CI 1.09-2.64) and the Americas and Caribbean (RR3.89; 95%CI 2.93-5.18) (Figure 3.23) Prospective studies showed a non-significant association (Figure 3.22).

The stratification of studies according to the method used to estimate GA showed a significant association in studies that used a combination of methods to determine GA as well as those using the LNMP (RR3.89; 95%CI 2.93-5.18) (Figure 3.24, Figure 3.25).

Only poor quality retrospective cohort studies showed a significant association between HIV and term-LBW (RR2.62; 95%CI 1.15-5.93) (Figure 3.26, Figure 3.27); however, a high degree of heterogeneity was observed (I^2 = 89.8%).

3.3.5.2 Preterm-LBW

Two prospective and one retrospective cohort study including 2199 women, reported on preterm-LBW (Figure 3.28). The meta-analysis of prospective studies showed a non-significant increase in

the risk of preterm-LBW in HIV-positive women compared to HIV-negative (RR 1.18; 95%CI 0.91-1.54); however, one retrospective cohort study showed a significant association (RR 3.25; 95%CI 2.12-4.99) (Figure 3.28). Only the retrospective cohort study showed a significant association in the analysis of studies according to geographic location, method used to determine GA and quality assessment. It was conducted in sub-Saharan Africa, used mixed-methods to estimate GA, and was of poor quality (Figure 3.29 – Figure 3.34).

3.3.5.3 Small for gestational age

Four prospective and three retrospective cohort studies, including 14,315 women, reported on the association between maternal HIV-infection and SGA (Figure 3.35). Maternal HIV-infection was significantly associated with SGA in the meta-analyses of prospective (RR 1.31; 95%CI 1.14-1.51) and retrospective (RR 2.08; 95%CI 1.26-3.46) cohort studies (Figure 3.35). However, a high degree of heterogeneity was observed in the meta-analysis of retrospective studies (I^2 = 85.2%).

The association persisted when studies were stratified according to geographic location; a significant risk of giving birth to a SGA infant was observed in HIV-positive women from sub-Saharan Africa (prospective: RR 1.33; 95%CI 1.13-1.57, retrospective: RR 2.50; 95%CI 1.04-5.97) and the Americas and Caribbean (retrospective: RR2.08; 95%CI 1.26-3.46) (Figure 3.36, Figure 3.37). The association between maternal HIV-infection and SGA was maintained irrespective of the method used to estimate GA and the study quality, except for one poor quality prospective study (Figure 3.38 – Figure 3.41).

3.3.5.4 Very small for gestational age

One prospective study conducted in sub-Saharan Africa and including 2368 women, reported a positive but non-significant association between maternal HIV-infection and VSGA (RR 1.28; 95%CI 0.95-1.71) (Figure 3.42 – Figure 3.45).

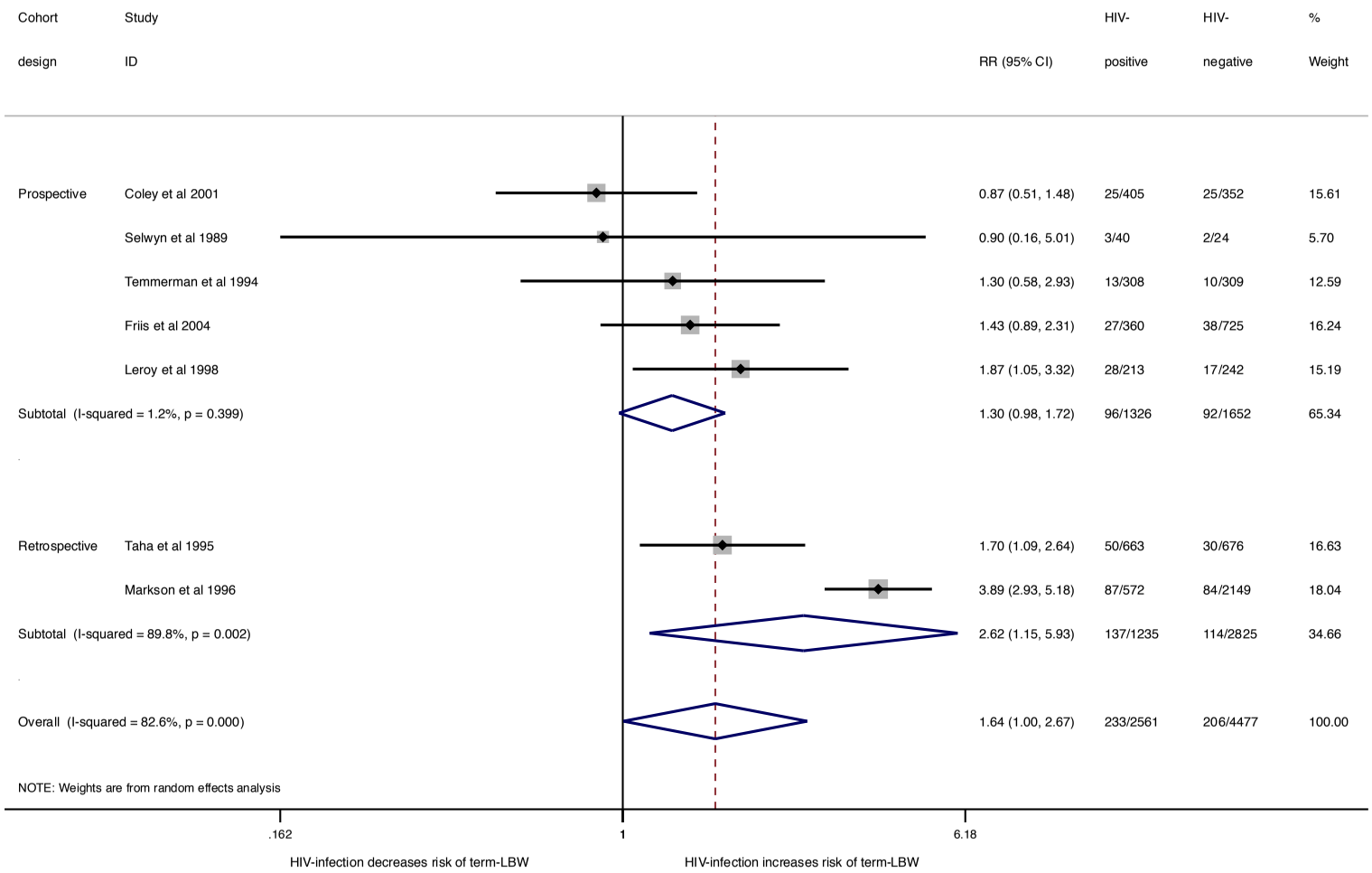


Figure 3.21: Term-LBW associated with ART-naïve maternal HIV-infection, by study design.
Random-effect pairwise meta-analyses showed a significant increase in the risk of term-LBW in HIV-positive women, compared to HIV-negative women in retrospective studies. Abbreviations: HIV = Human Immunodeficiency Virus, term-LBW = term low birthweight, RR = relative risk. Source: Wedi et al. 2016[3]

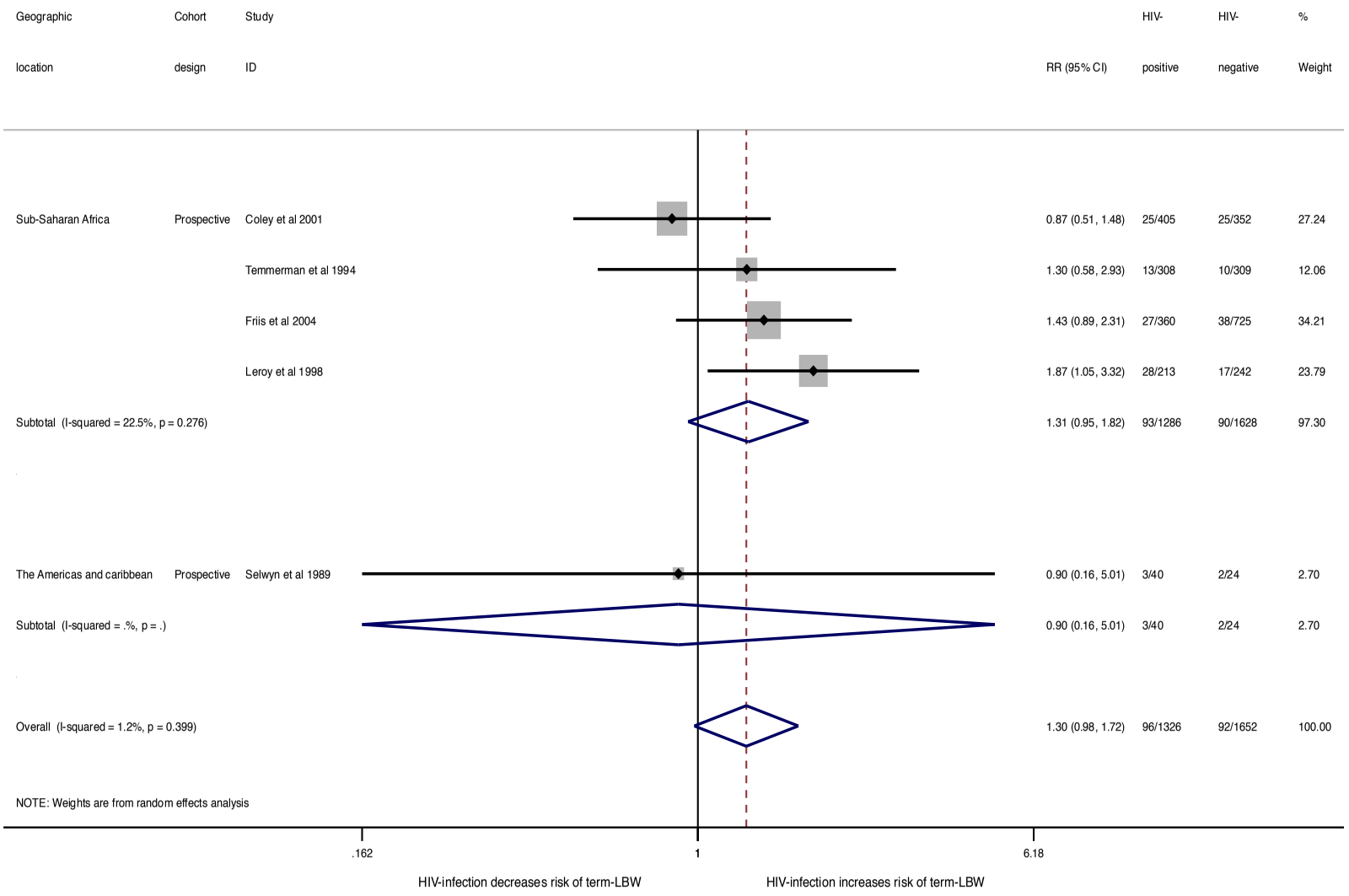


Figure 3.22: Term-LBW associated with ART-naïve maternal HIV-infection, by geographic region: prospective studies.
No significant association observed between maternal HIV-infection and term-LBW. Abbreviations: HIV = Human Immunodeficiency Virus, RR = relative risk, term-LBW = term low birthweight. Source: Wedi et al. 2016[3]

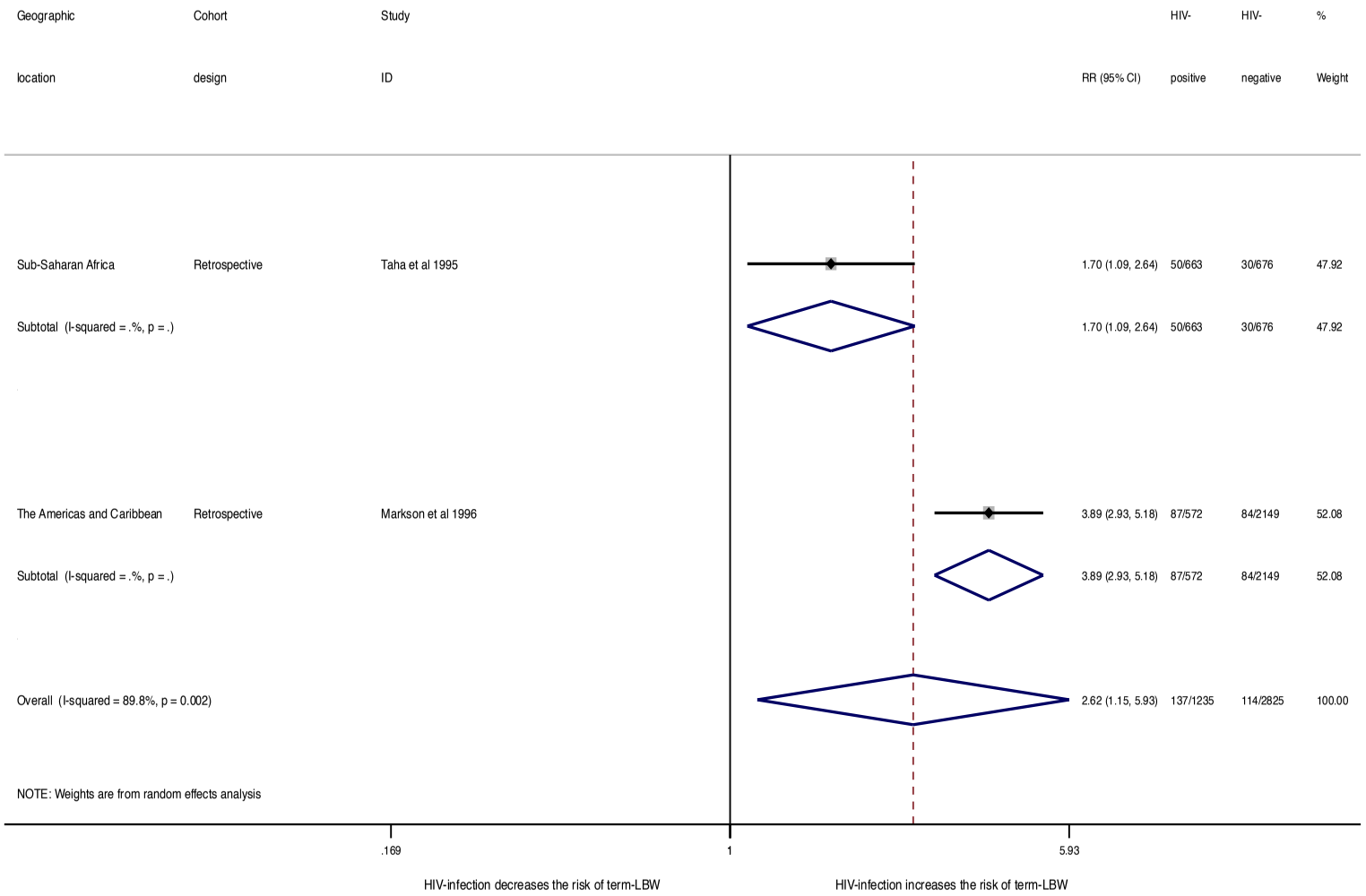


Figure 3.23: **Term-LBW associated with ART-naïve maternal HIV-infection, by geographic region: retrospective studies.**
A significant increase in the risk of term-LBW was observed in HIV-positive women residing in sub-Saharan Africa and the Americas and Caribbean. Abbreviations: HIV = Human Immunodeficiency Virus, RR = relative risk, term-LBW = term low birthweight. Source: Wedi et al. 2016[3]

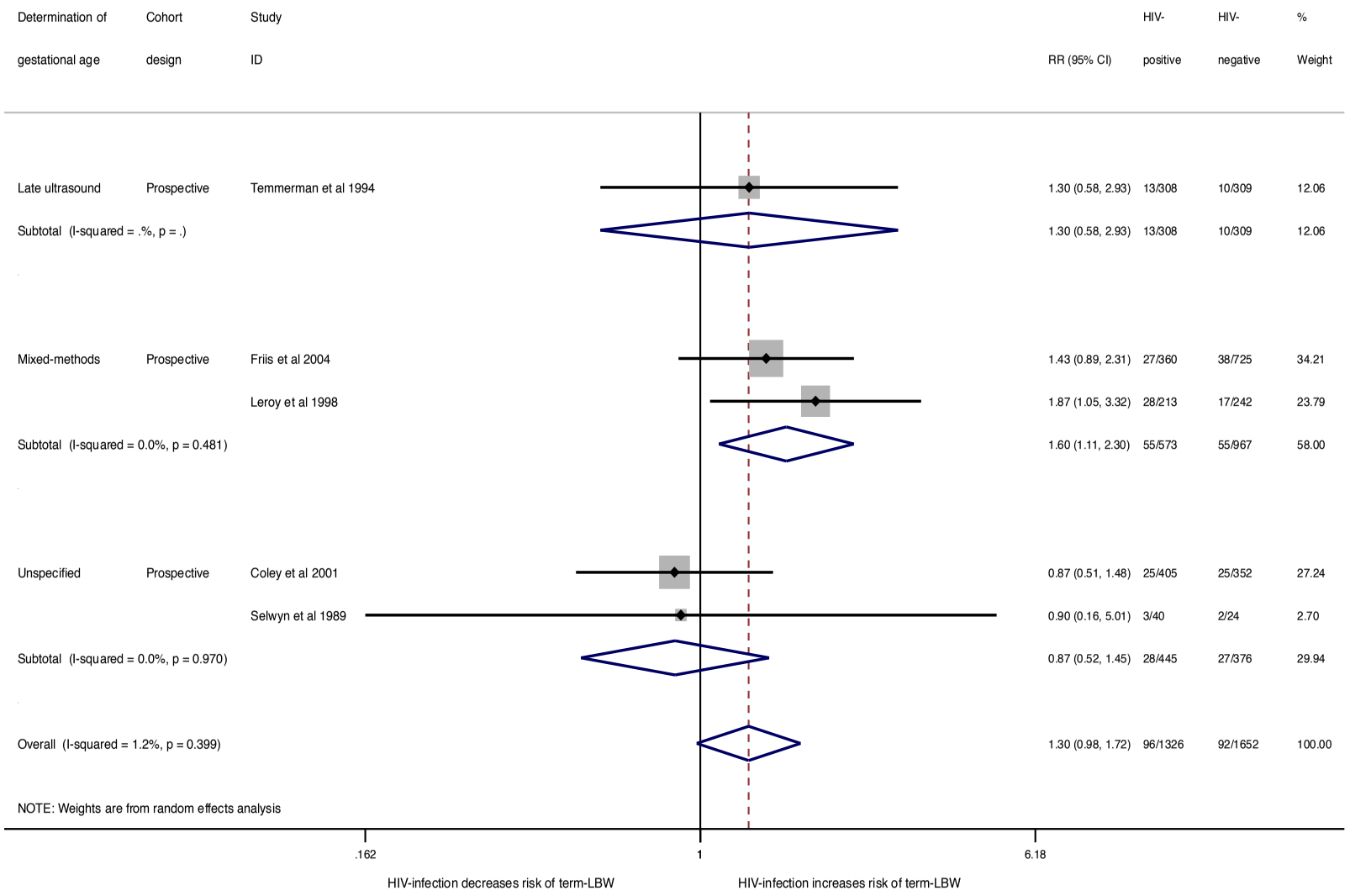


Figure 3.24: Term-LBW associated with ART-naïve maternal HIV-infection, by method used to determine gestational age: prospective studies. Random-effect pairwise meta-analyses showed a significant increase in the risk of term-LBW in HIV-positive women in studies using a combination of methods to estimate gestational age (mixed-methods – see Table 3.4). Abbreviations: HIV = Human Immunodeficiency Virus, RR = relative risk, term-LBW = term low birthweight. Source: Wedi et al. 2016[3] .

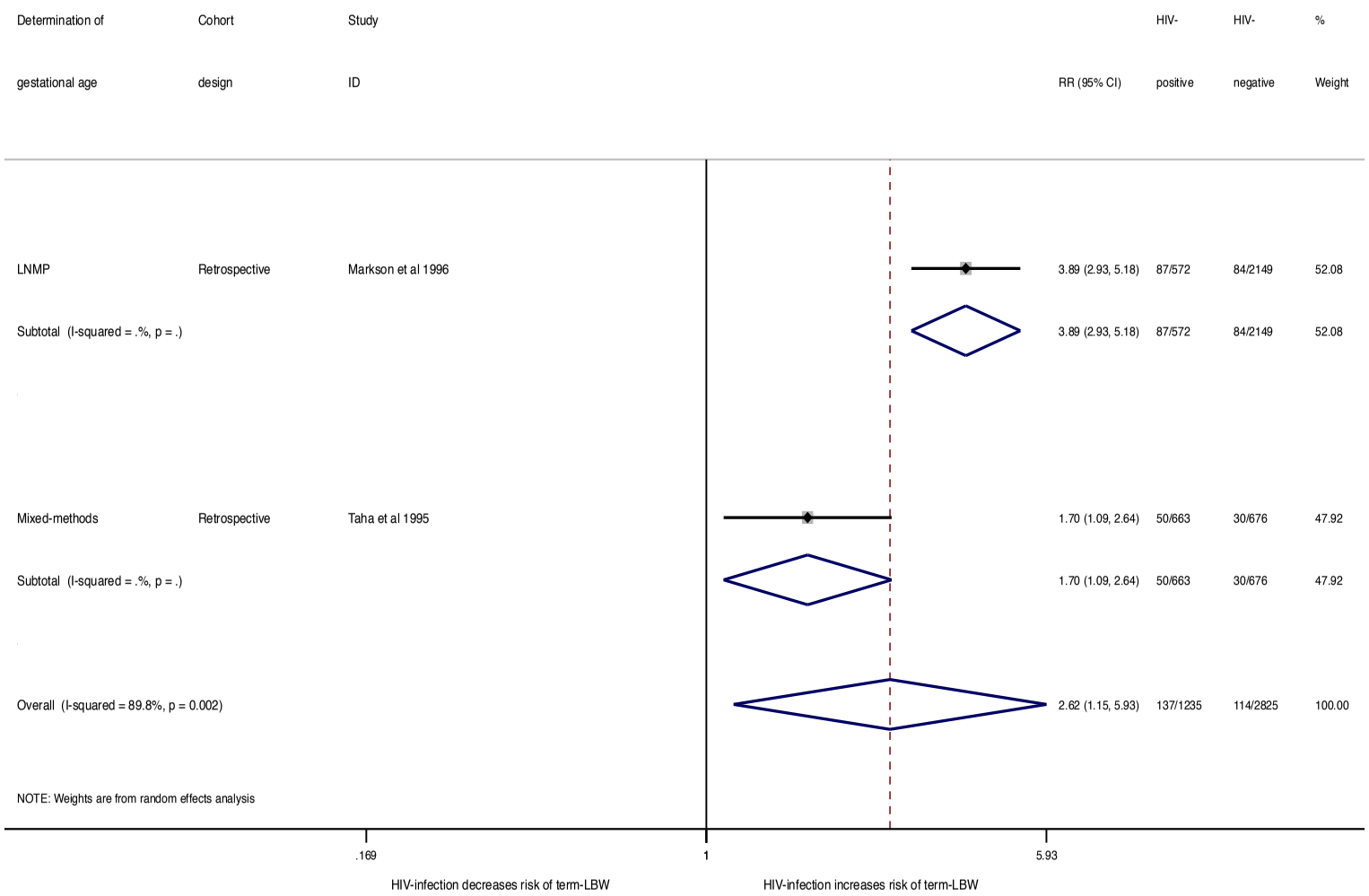


Figure 3.25: **Term-LBW associated with ART-naïve maternal HIV-infection, by method used to determine gestational age: retrospective studies.** A significant increase in the risk of term-LBW was observed in HIV-positive women in studies using the LNMP or a combination of methods to estimate GA (mixed-methods – see Table 3.4). Abbreviations: HIV = Human Immunodeficiency Virus, RR = relative risk, term-LBW = term low birthweight. Source: Wedi et al. 2016[3].

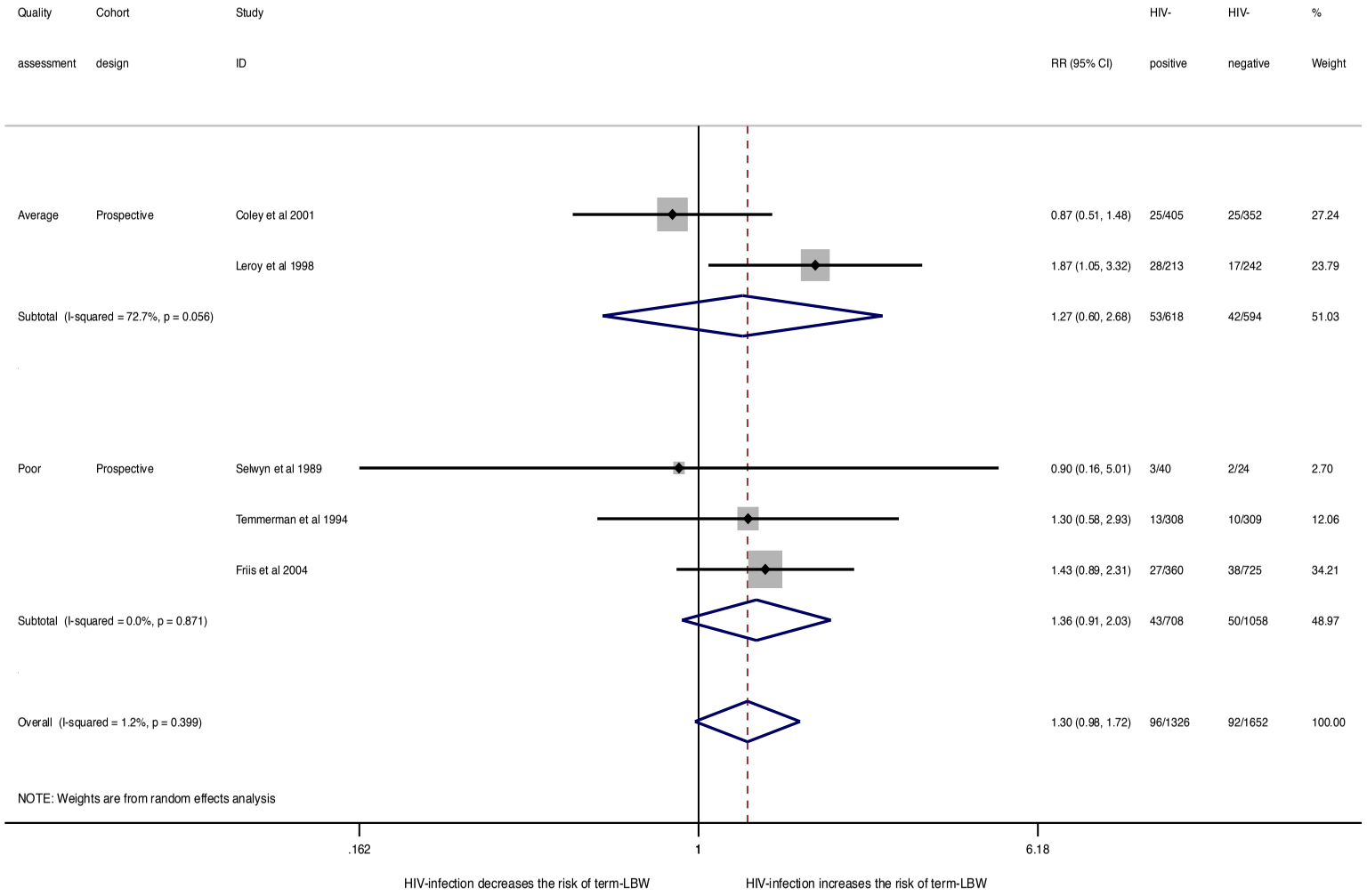


Figure 3.26: Term-LBW associated with ART-naïve maternal HIV-infection, by adapted Newcastle-Ottawa quality assessment of studies: prospective studies. No significant association observed between maternal HIV-infection and term-LBW. Abbreviations: HIV = Human Immunodeficiency Virus, RR = relative risk, term-LBW = term low birthweight. Source: Wedi et al. 2016[3].

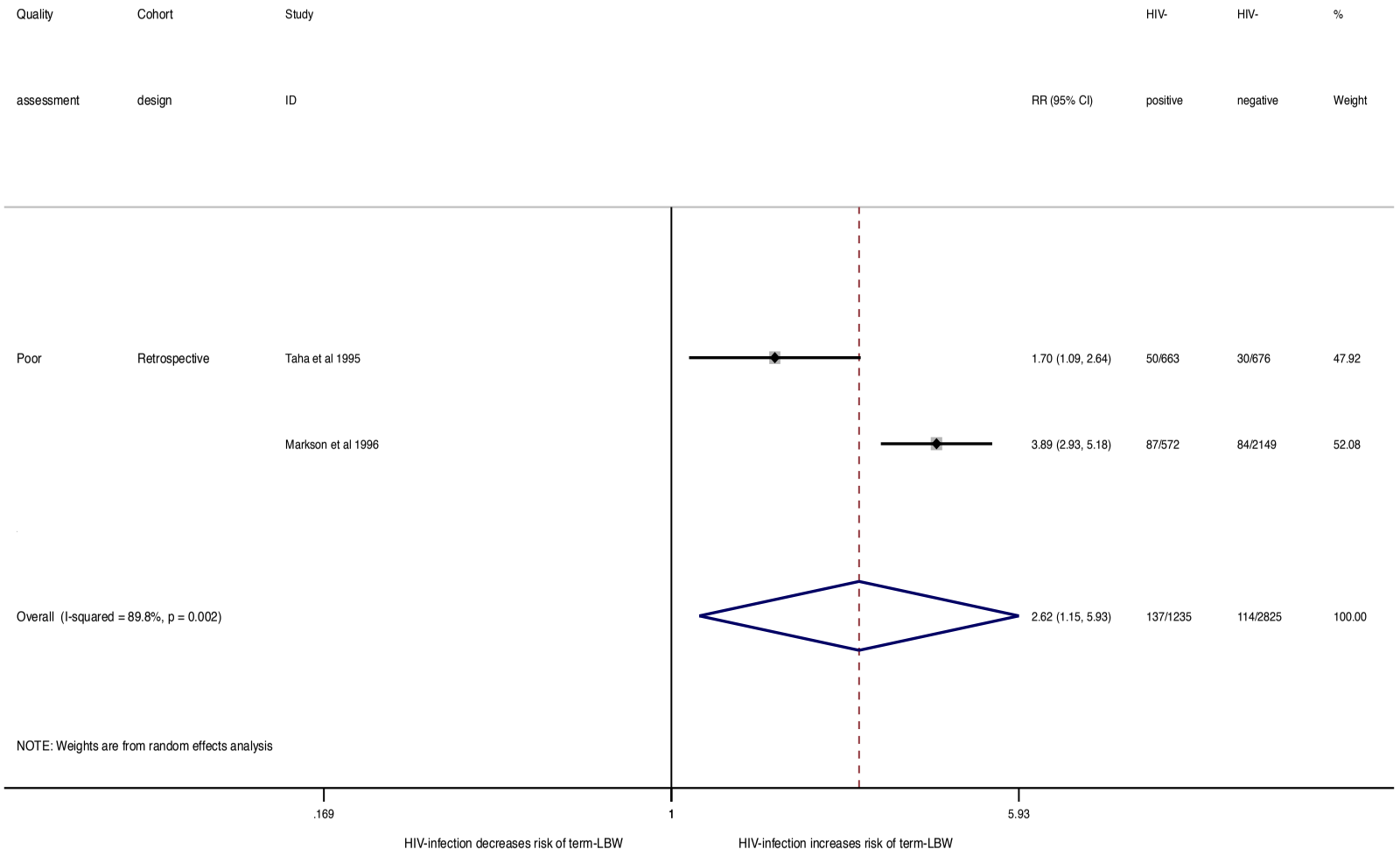


Figure 3.27: Term-LBW associated with ART-naïve maternal HIV-infection, by adapted Newcastle-Ottawa quality assessment of studies: retrospective studies. A random-effect pairwise meta-analysis of poor quality retrospective cohort studies showed a significant increase in the risk of giving birth to a term-LBW infant HIV-positive women. Abbreviations: HIV = Human Immunodeficiency Virus, RR = relative risk, term-LBW = term low birthweight. Source: Wedi et al. 2016[3].

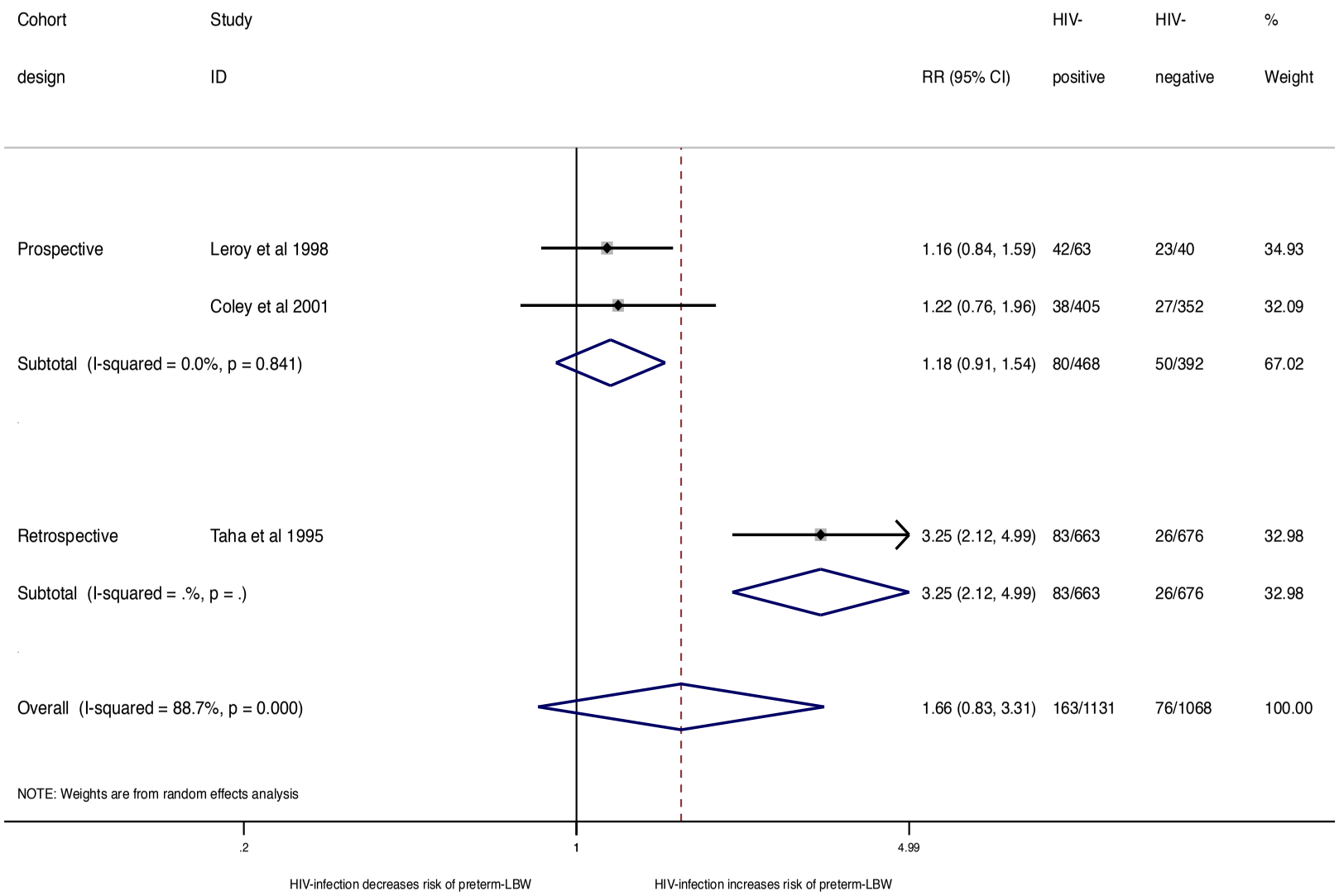


Figure 3.28: Preterm-LBW associated with ART-naïve maternal HIV-infection, by study design.
One retrospective study showed a significant increase in the risk of preterm-LBW in HIV-positive women, compared to HIV-negative women in retrospective studies. Abbreviations: HIV = Human Immunodeficiency Virus, preterm-LBW = preterm low birthweight, RR = relative risk. Source: Wedi et al. 2016[3].

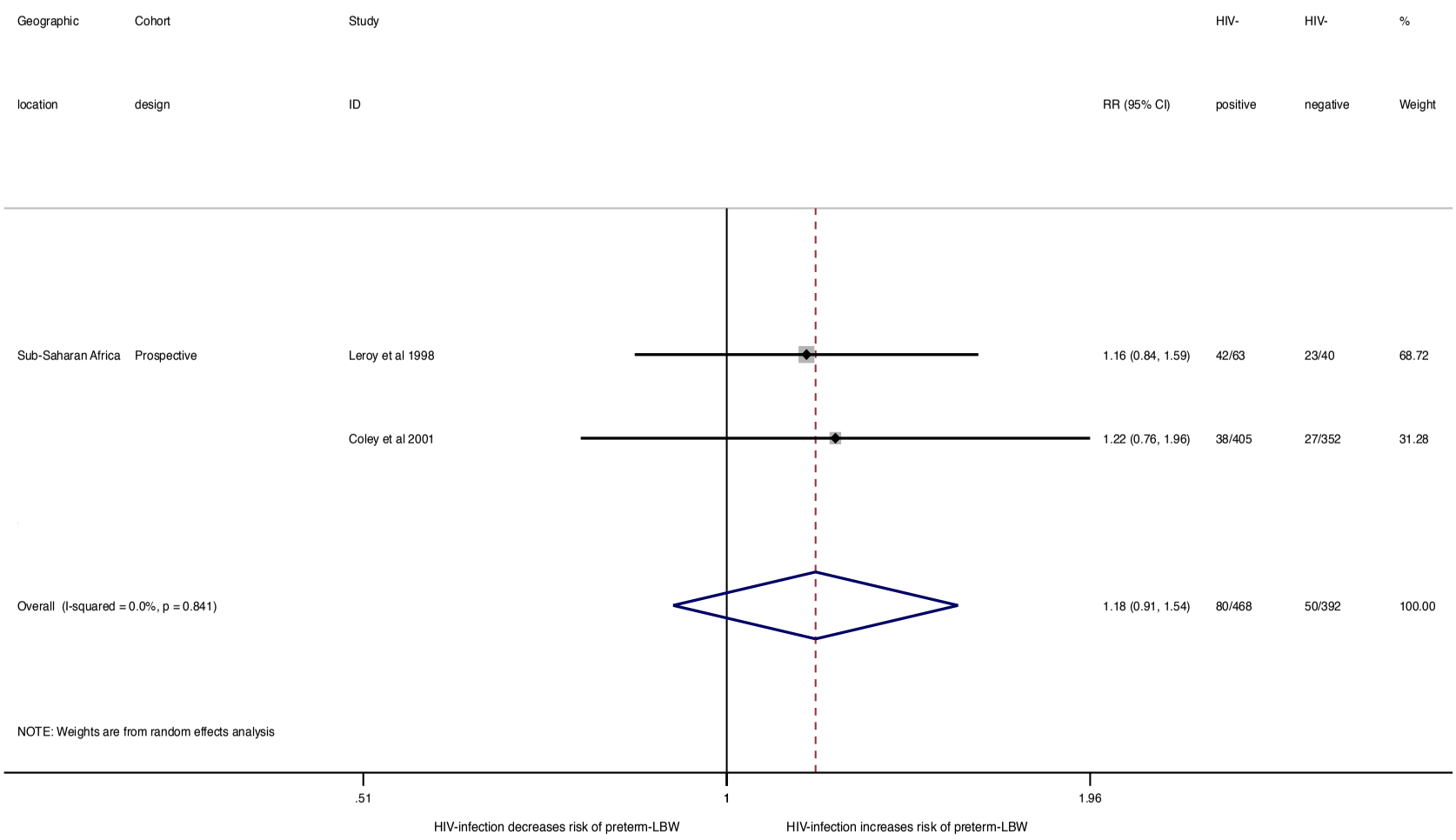


Figure 3.29: Preterm-LBW associated with ART-naïve maternal HIV-infection, by geographic region: prospective studies.
No significant association observed between maternal HIV-infection and preterm-LBW. Abbreviations: HIV = Human Immunodeficiency Virus, RR = relative risk, preterm-LBW = preterm low birthweight. Source: Wedi et al. 2016[3].

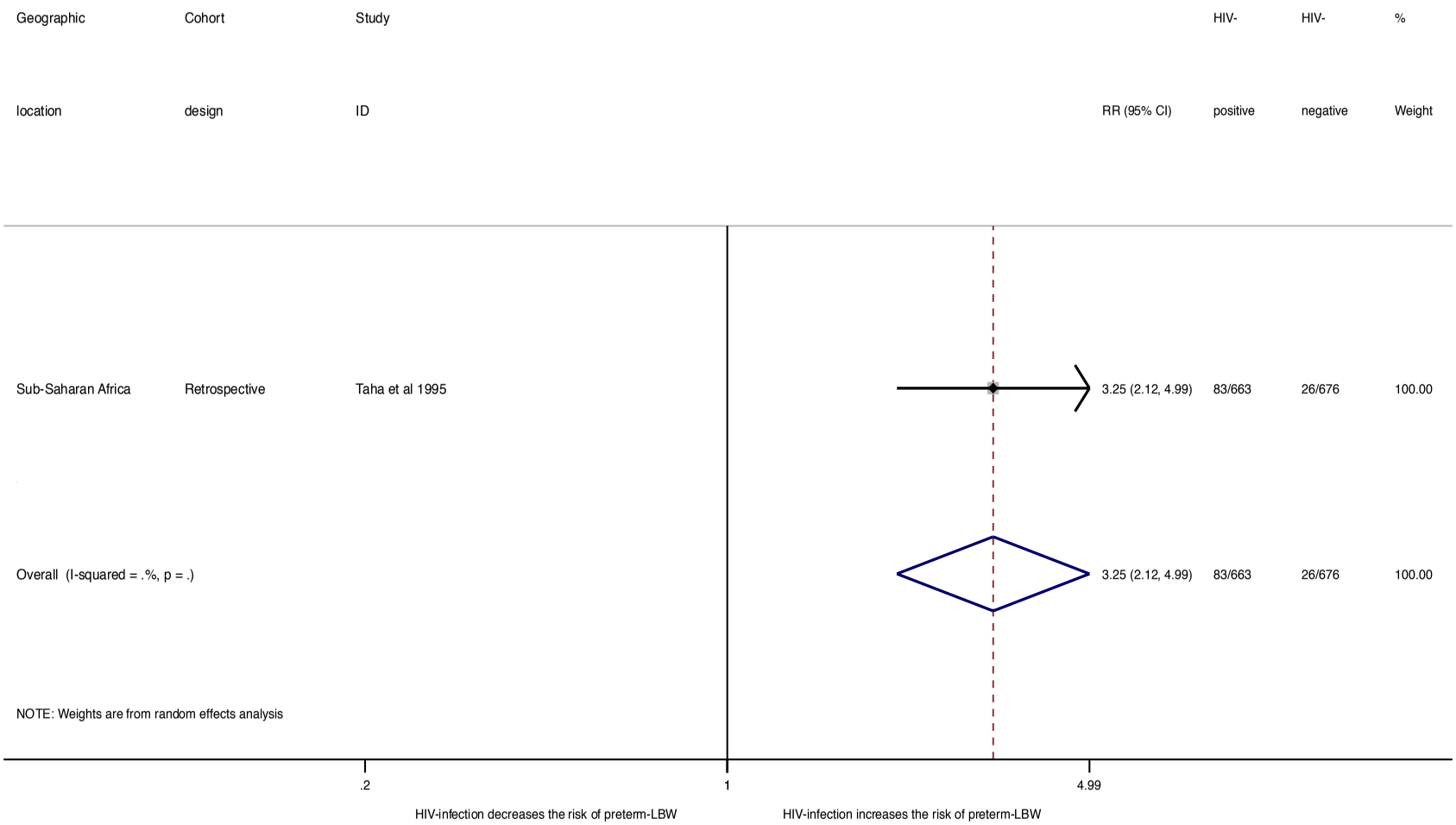


Figure 3.30: Preterm-LBW associated with ART-naïve maternal HIV-infection, by geographic region: retrospective study.
One retrospective cohort study showed a significant increase in the risk of preterm-LBW in HIV-positive women residing in sub-Saharan Africa. Abbreviations: HIV = Human Immunodeficiency Virus, RR = relative risk, preterm-LBW = preterm low birthweight. Source: Wedi et al. 2016[3].

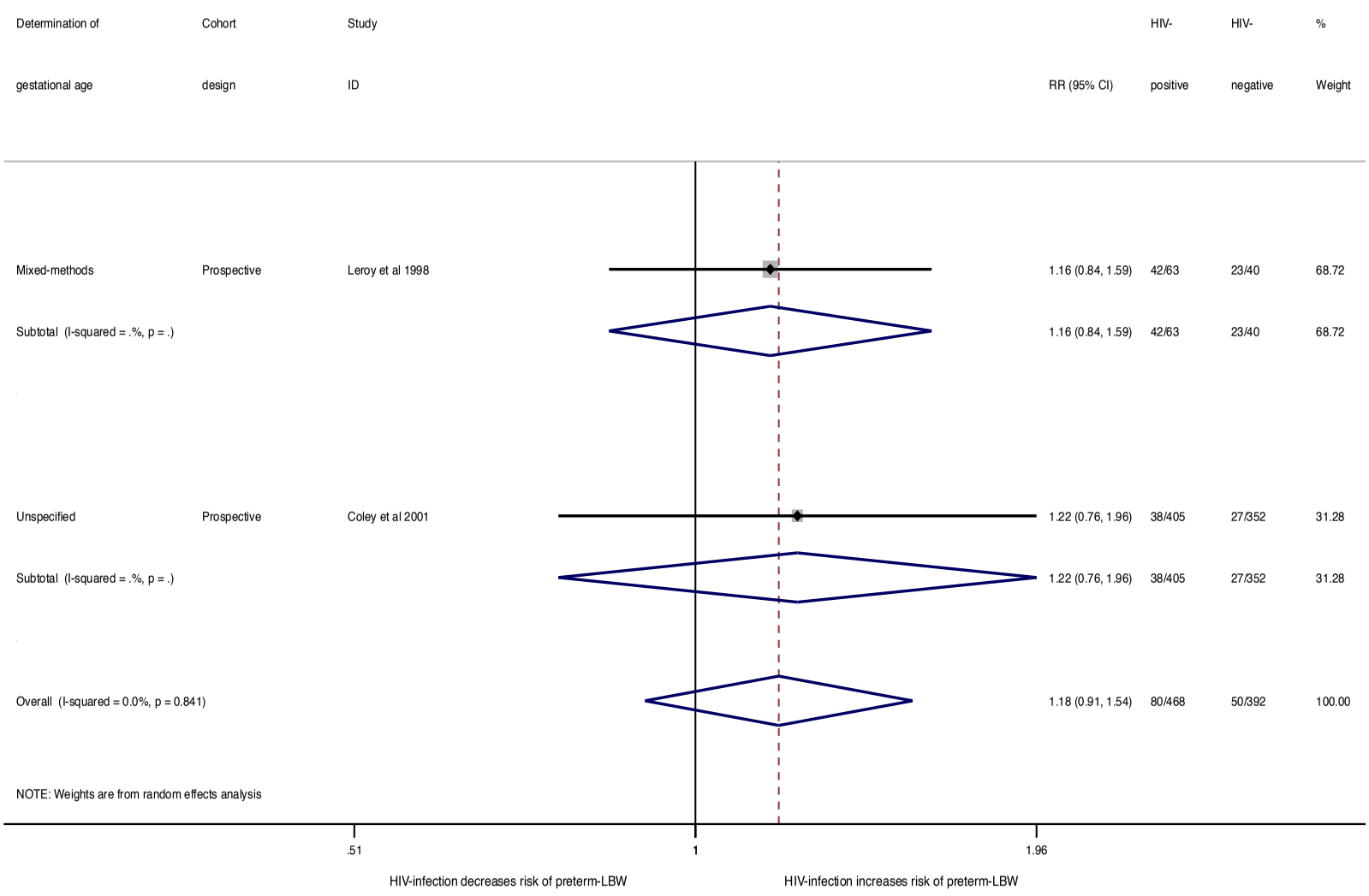


Figure 3.31: Preterm-LBW associated with ART-naïve maternal HIV-infection, by method used to determine gestational age: prospective studies. No significant association observed between maternal HIV-infection and preterm-LBW. Abbreviations: HIV = Human Immunodeficiency Virus, RR = relative risk, preterm-LBW = preterm low birthweight. Source: Wedi et al. 2016[3].

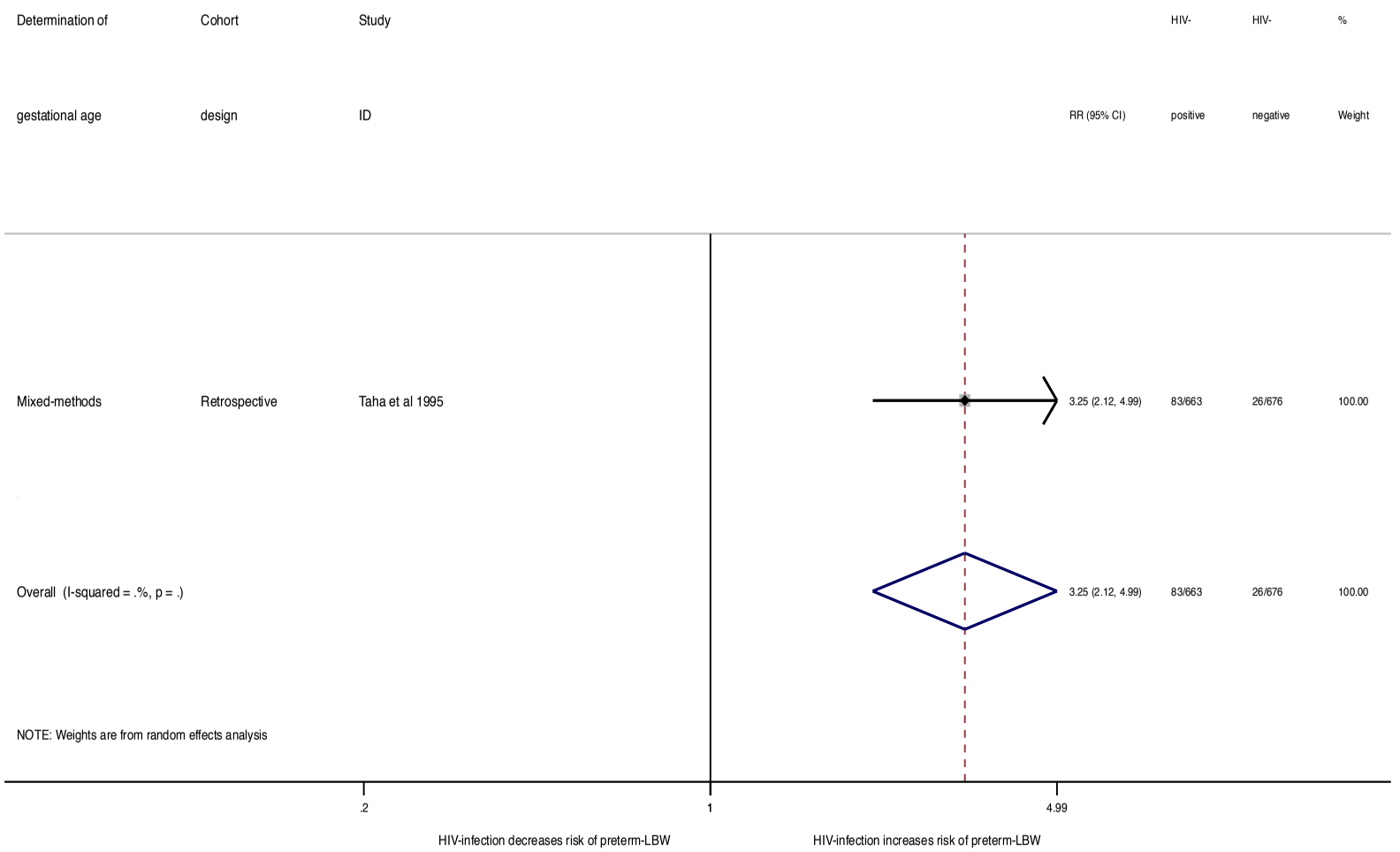


Figure 3.32: Preterm-LBW associated with ART-naïve maternal HIV-infection, by method used to determine gestational age: retrospective study. One study using a combination of methods to estimate GA (mixed-methods – see Table 3.4) showed a significant increase in the risk of preterm-LBW in HIV-positive women compared to HIV-negative women. Abbreviations: HIV = Human Immunodeficiency Virus, RR = relative risk, preterm-LBW = preterm low birthweight. Source: Wedi et al. 2016[3].

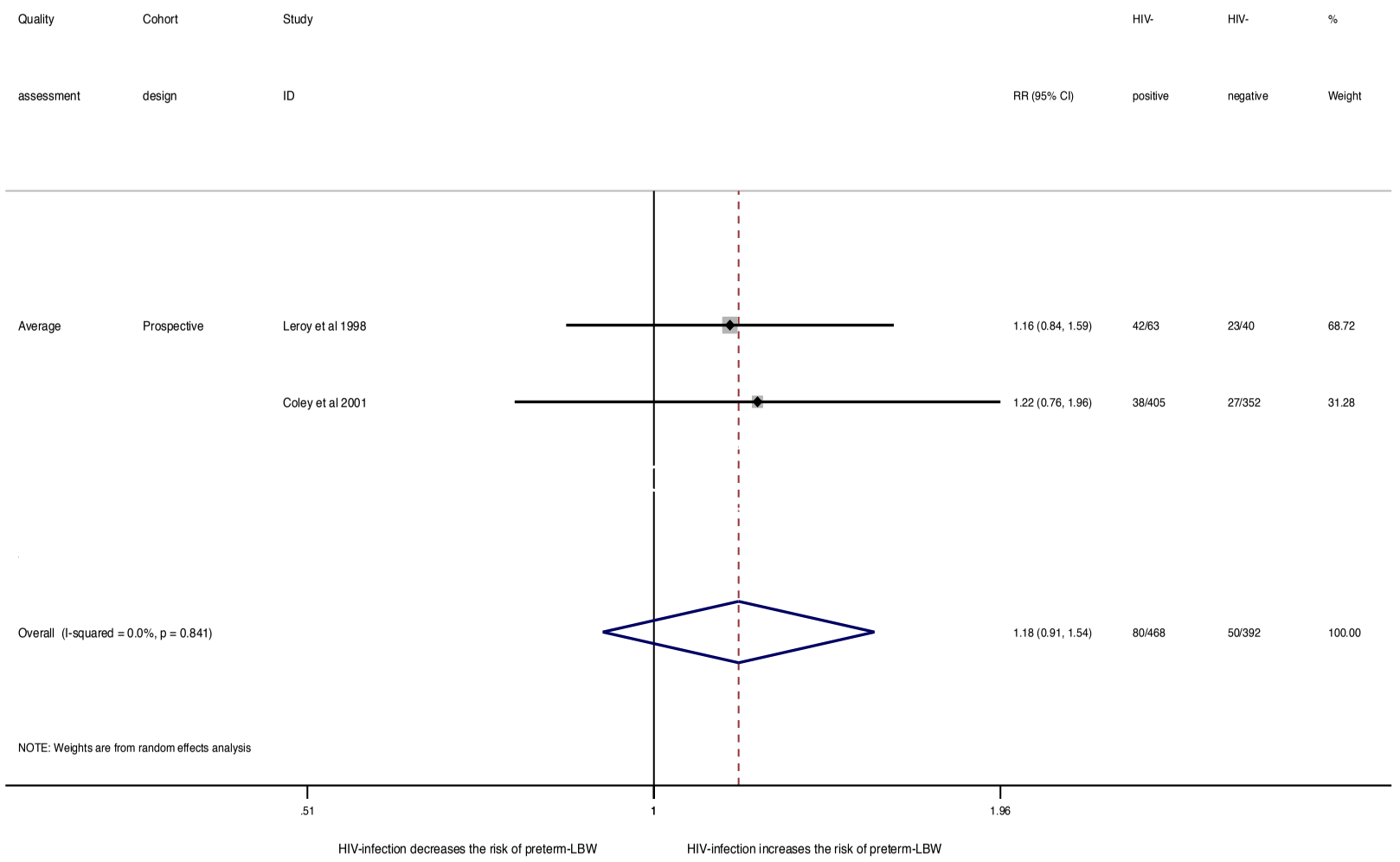


Figure 3.33: Preterm-LBW associated with ART-naïve maternal HIV-infection, by adapted Newcastle-Ottawa quality assessment of studies: prospective studies. No significant association observed between maternal HIV-infection and preterm-LBW. Abbreviations: HIV = Human Immunodeficiency Virus, RR = relative risk, preterm-LBW = preterm low birthweight. Source: Wedi et al. 2016[3]

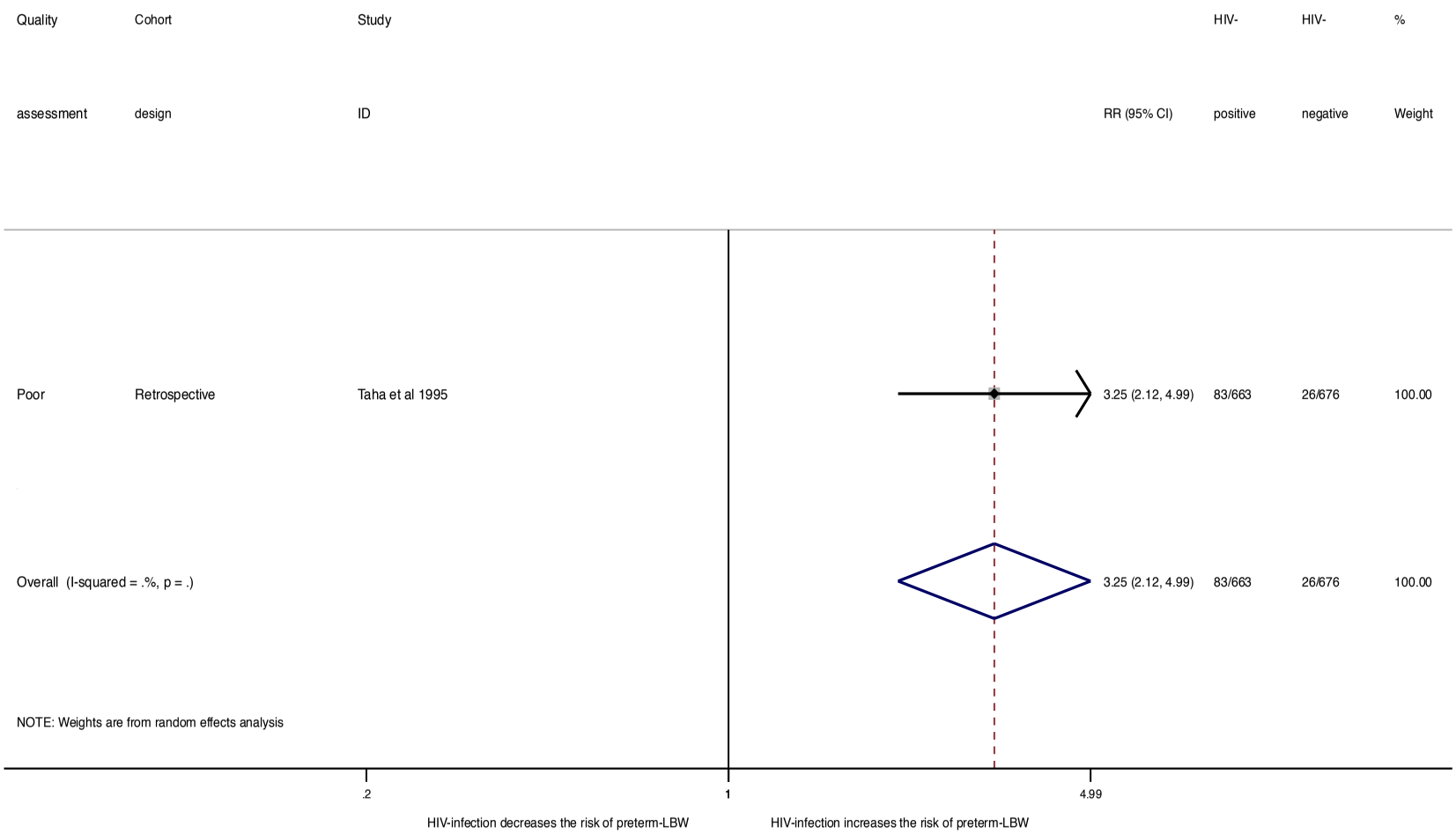


Figure 3.34: Preterm-LBW associated with ART-naïve maternal HIV-infection, by adapted Newcastle-Ottawa quality assessment of studies: retrospective study. One poor quality study showed a significant increase in the risk of preterm-LBW in HIV-positive women compared to HIV-negative women. Abbreviations: HIV = Human Immunodeficiency Virus, RR = relative risk, preterm-LBW = preterm low birthweight. Source: Wedi et al. 2016[3]

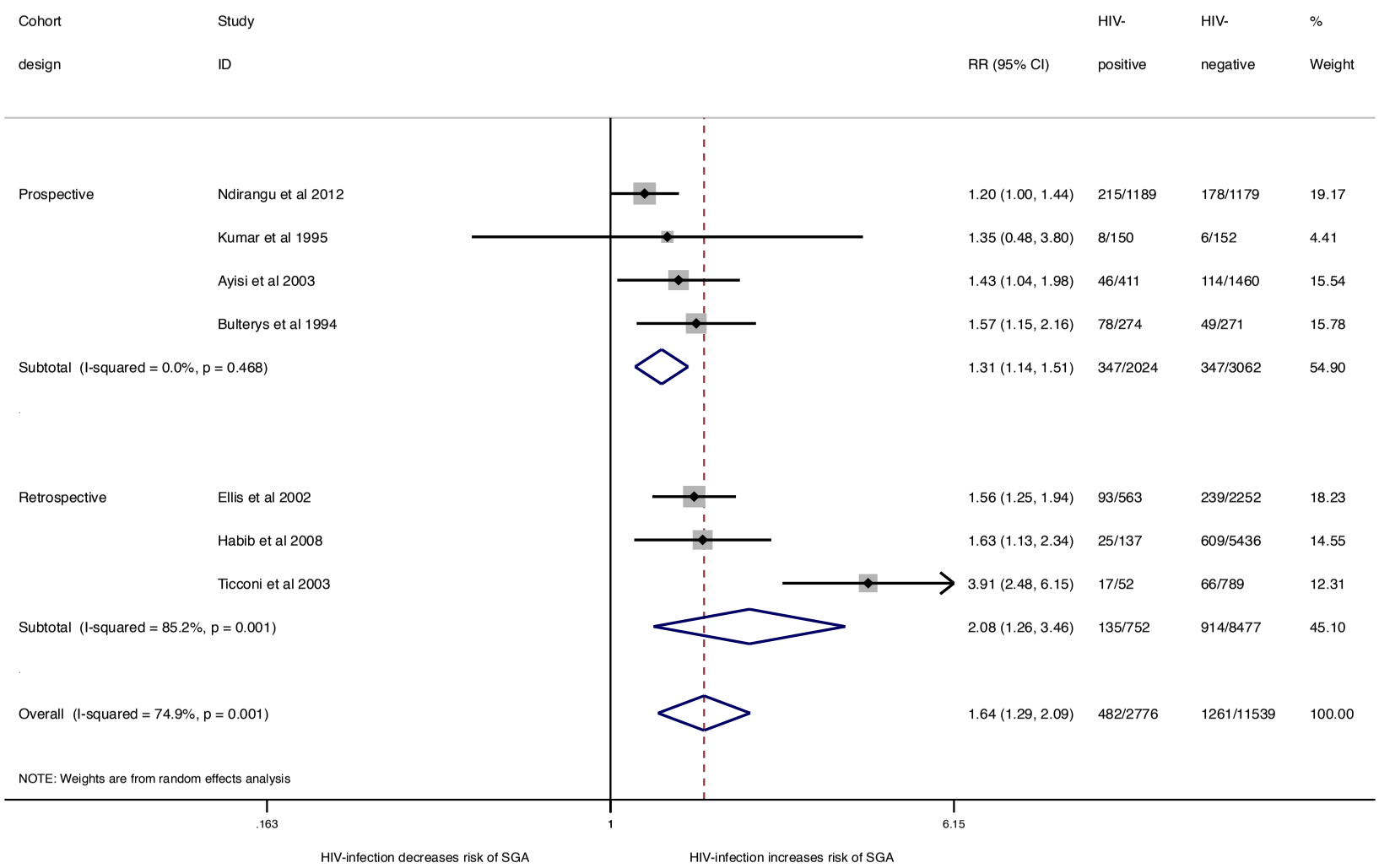


Figure 3.35: **SGA associated with ART-naïve maternal HIV-infection, by study design.**
Random-effect pairwise meta-analyses showed a significant increase in the risk of SGA in HIV-positive women, compared to HIV-negative women in prospective and retrospective studies. Abbreviations: HIV = Human Immunodeficiency Virus, RR = relative risk, SGA = small for gestational age. Source: Wedi et al. 2016[3].

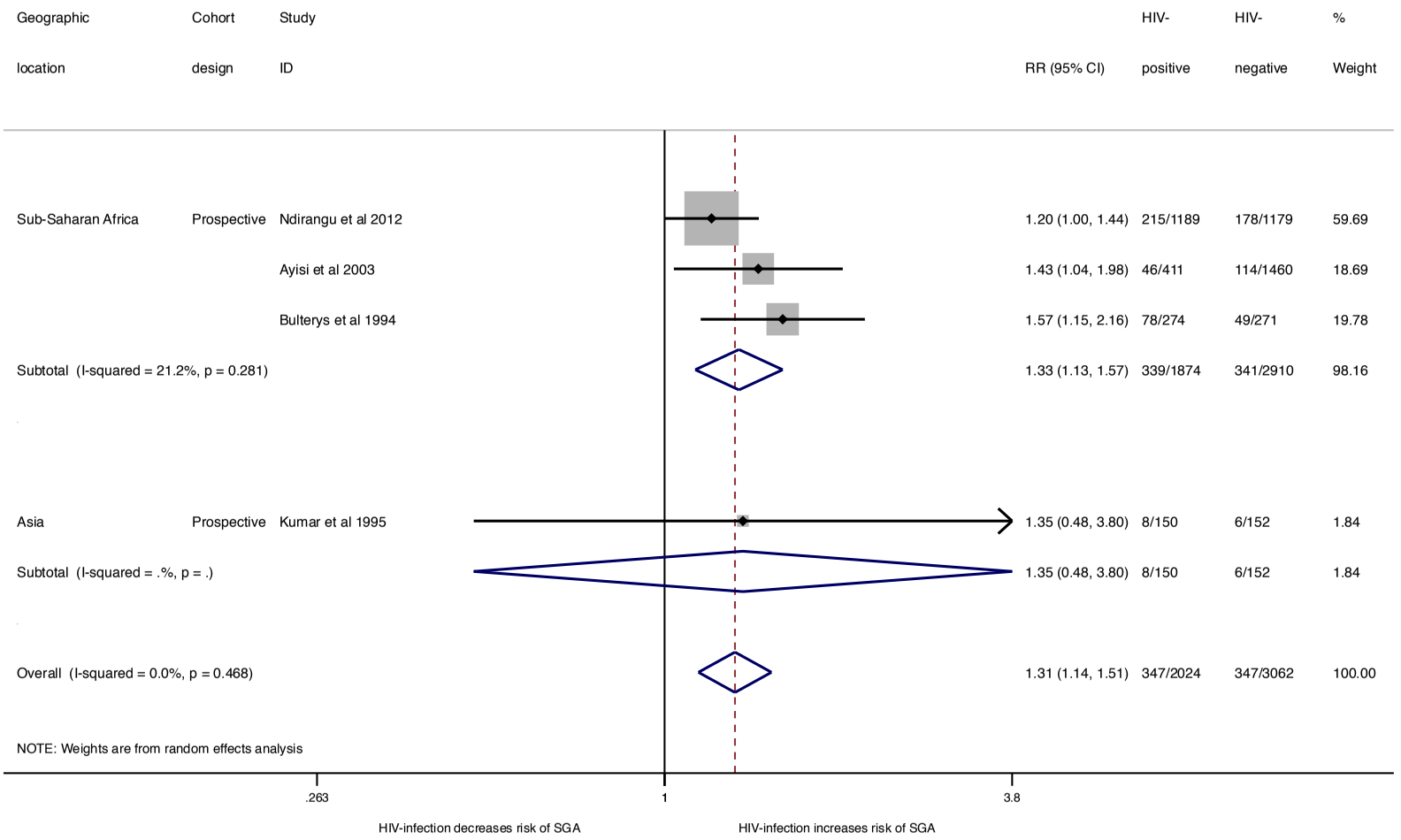


Figure 3.36: **SGA associated with ART-naïve maternal HIV-infection, by geographic region: prospective studies.**
Random-effect pairwise meta-analyses showed a significant increase in the risk of SGA in HIV-positive women residing in sub-Saharan Africa and Asia. Abbreviations: HIV = Human Immunodeficiency Virus, RR = relative risk, SGA = small for gestational age.
Source: Wedi et al. 2016[3].

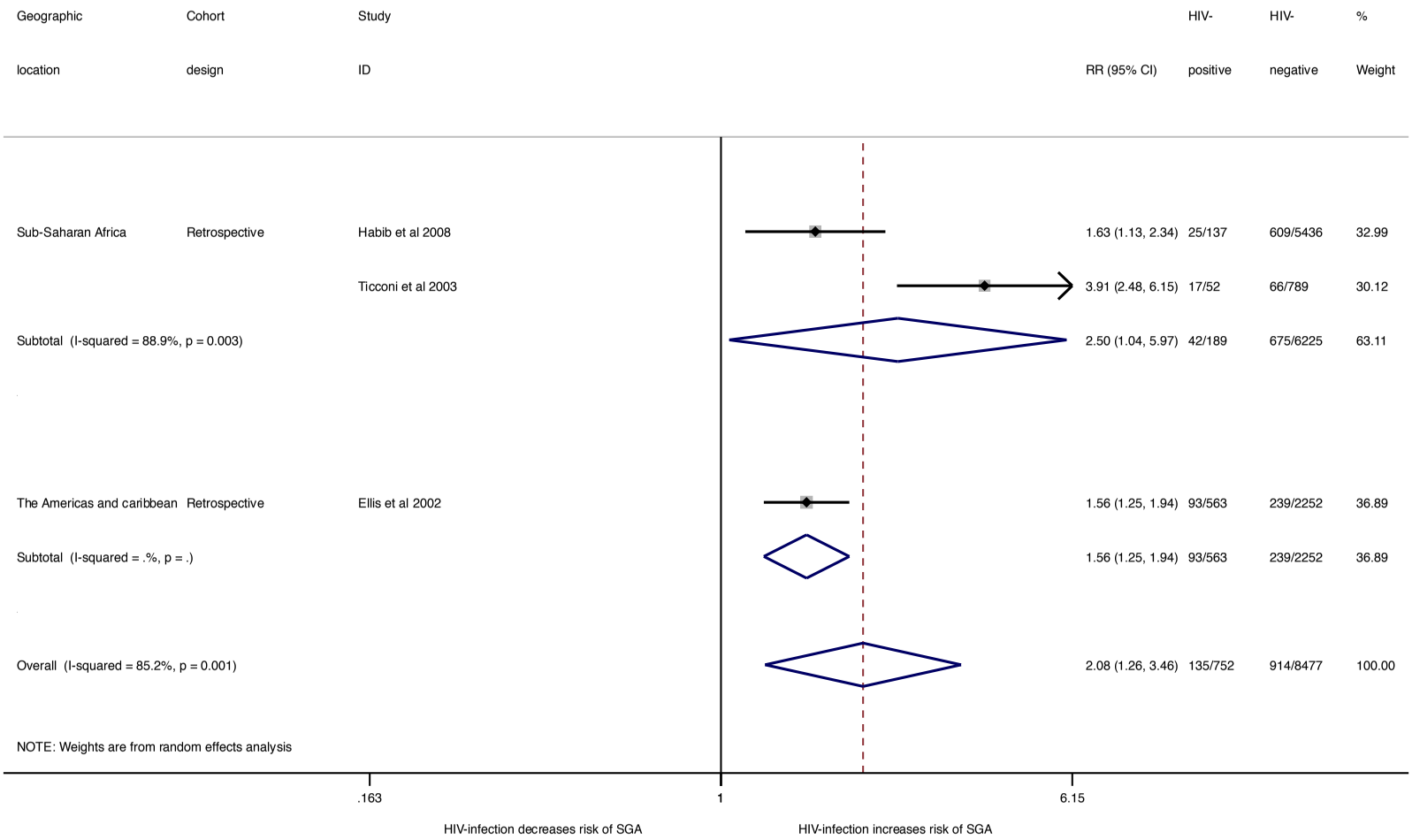


Figure 3.37: **SGA associated with ART-naïve maternal HIV-infection, by geographic region: retrospective studies.**
Random-effect pairwise meta-analyses showed a significant increase in the risk of SGA in HIV-positive women residing in sub-Saharan Africa and the Americas and Caribbean. Abbreviations: HIV = Human Immunodeficiency Virus, RR = relative risk, SGA = small for gestational age. Source: Wedi et al 2016[3].

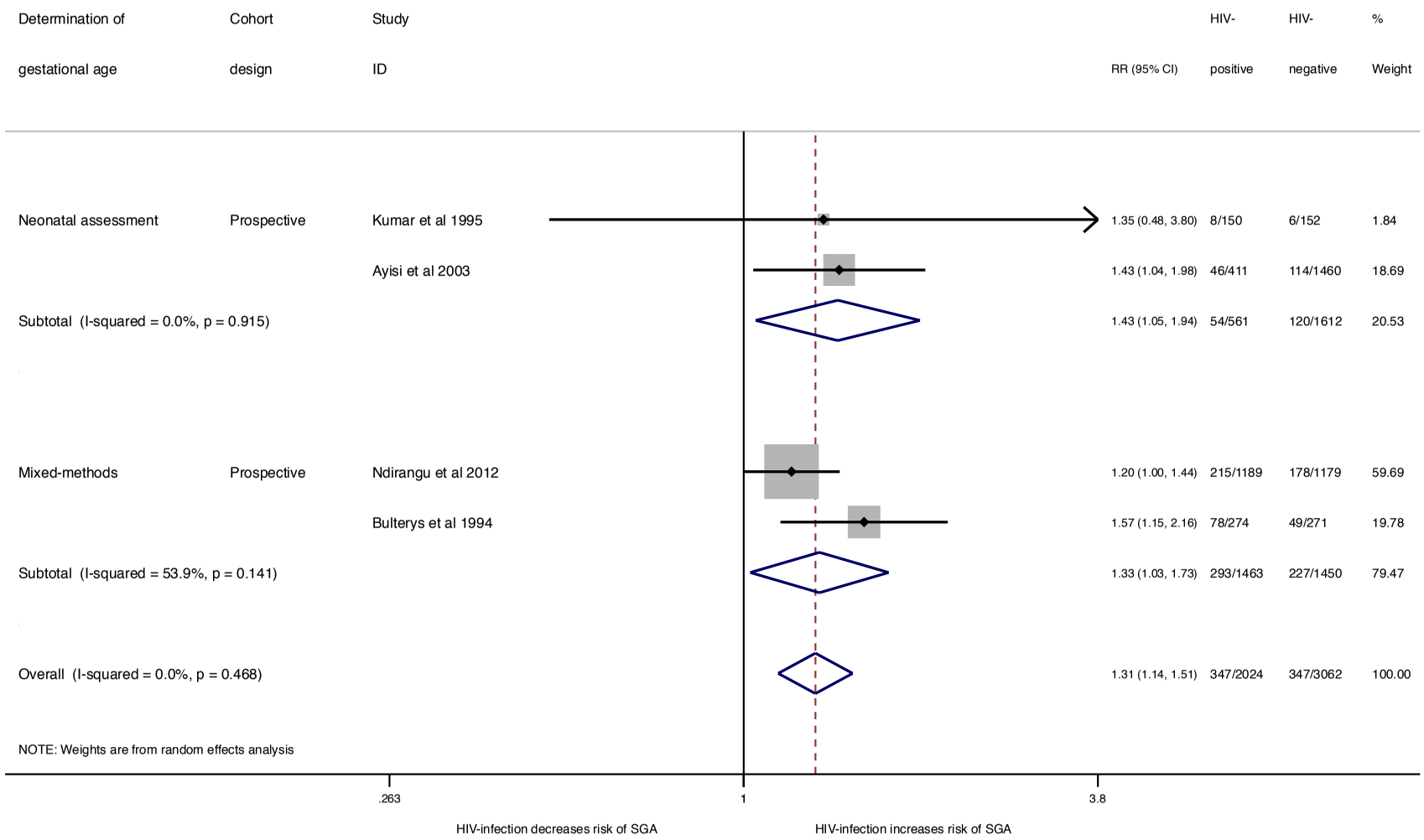


Figure 3.38: SGA associated with ART-naïve maternal HIV-infection, by method used to determine gestational age: prospective studies
Random-effect pairwise meta-analyses showed a significant increase in the risk of SGA in HIV-positive women in studies estimating GA with a neonatal assessment and a combination of methods (mixed-methods – see Table 3.4). Abbreviations: GA = gestational age, HIV = Human Immunodeficiency Virus, RR = relative risk, SGA = small for gestational age. Source: Wedi et al 2016[3].

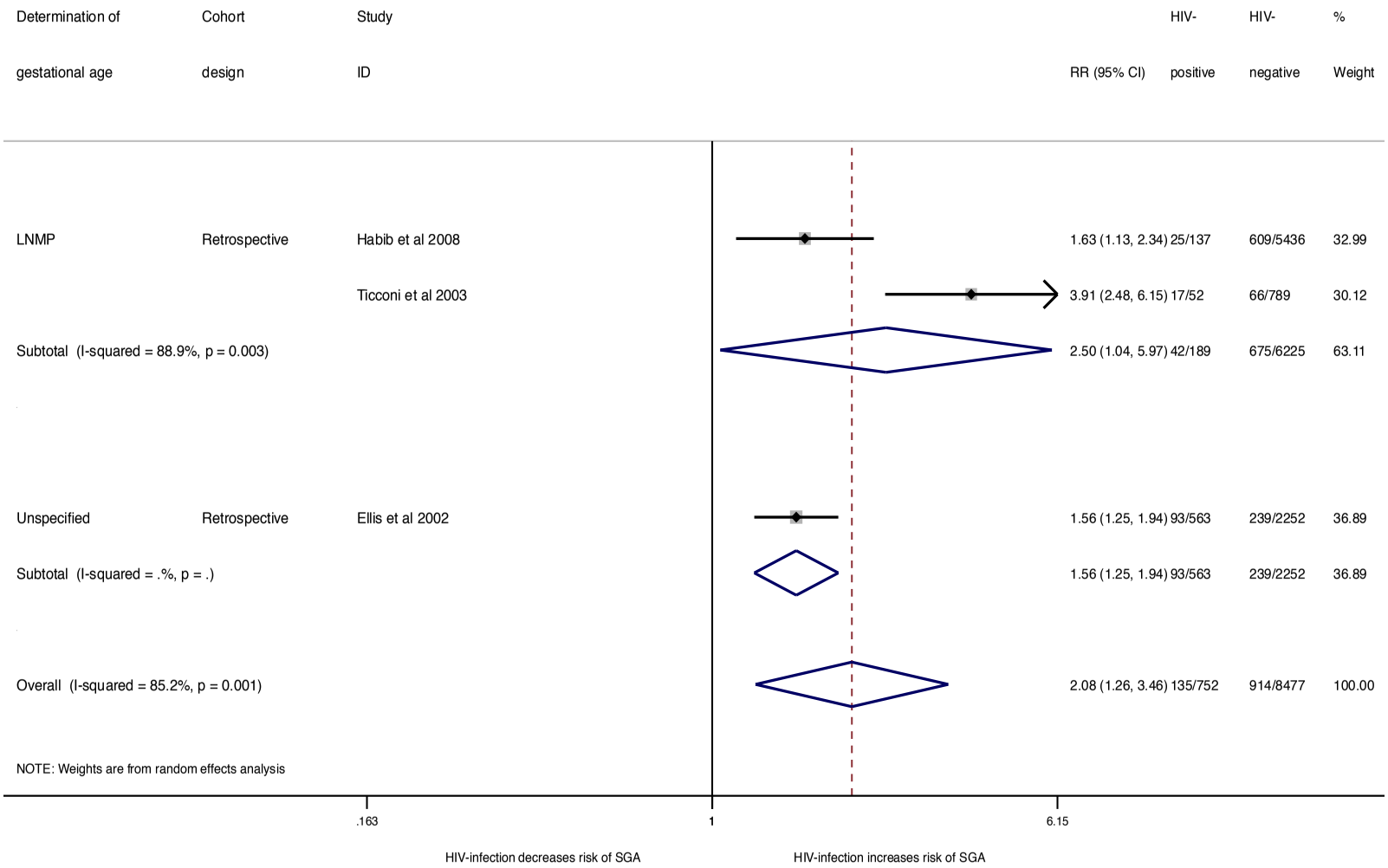


Figure 3.39: **SGA associated with ART-naïve maternal HIV-infection, by method used to determine gestational age: retrospective studies.** Random-effect pairwise meta-analyses showed a significant increase in the risk of SGA in HIV-positive women in studies estimating GA with the LNMP as well as one study that did not specify the method used to estimate GA. Abbreviations: GA = gestational age, HIV = Human Immunodeficiency Virus, LNMP = last normal menstrual period, RR = relative risk, SGA = small for gestational age. Source: Wedi et al. 2016[3].

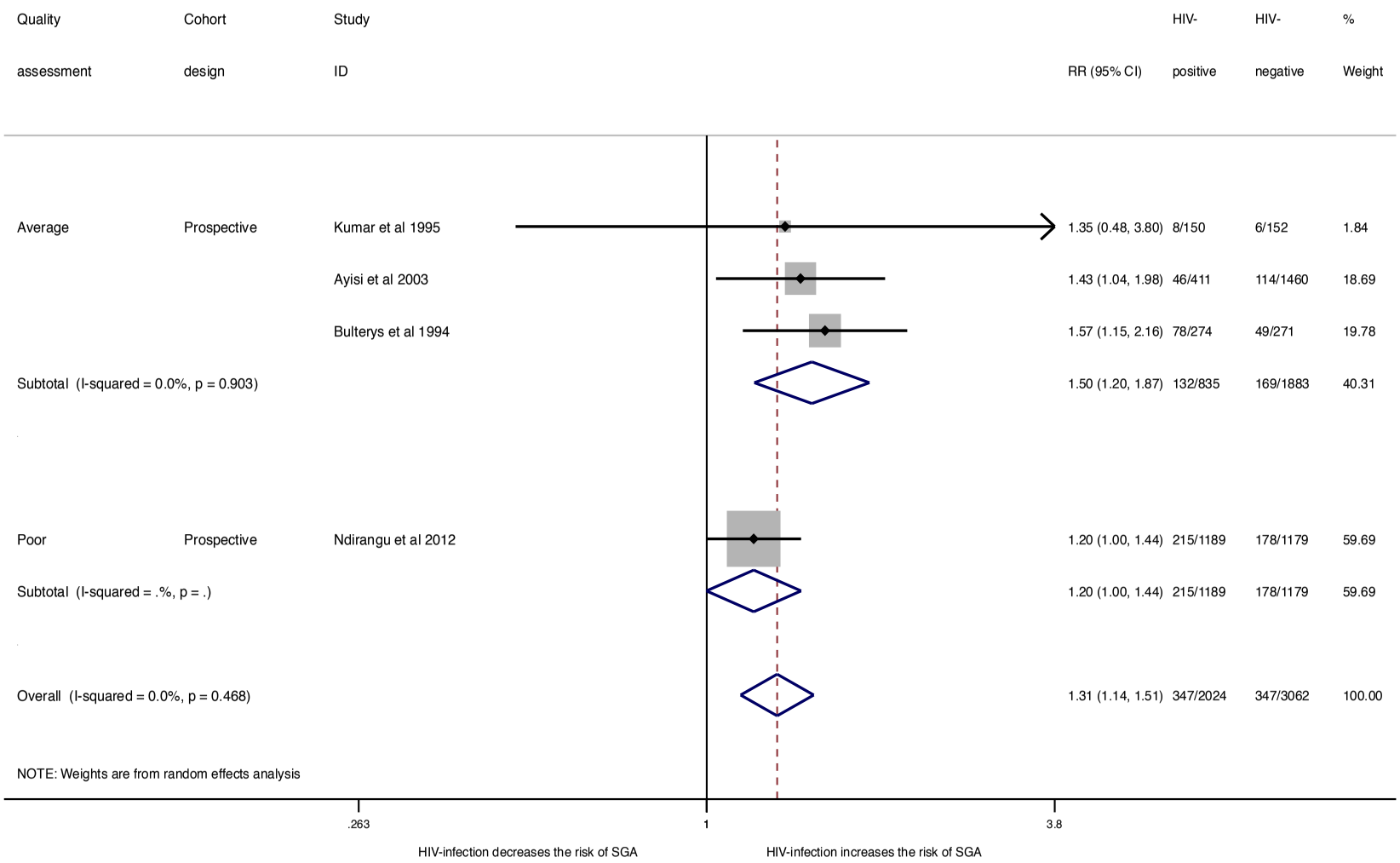


Figure 3.40: SGA associated with ART-naïve maternal HIV-infection, by adapted Newcastle-Ottawa quality assessment of studies: prospective studies. Random-effect pairwise meta-analyses showed a significant increase in the risk of SGA in HIV-positive women in average quality studies. Abbreviations: HIV = Human Immunodeficiency Virus, RR = relative risk, SGA = small for gestational age.
Source: Wedi et al. 2016[3].

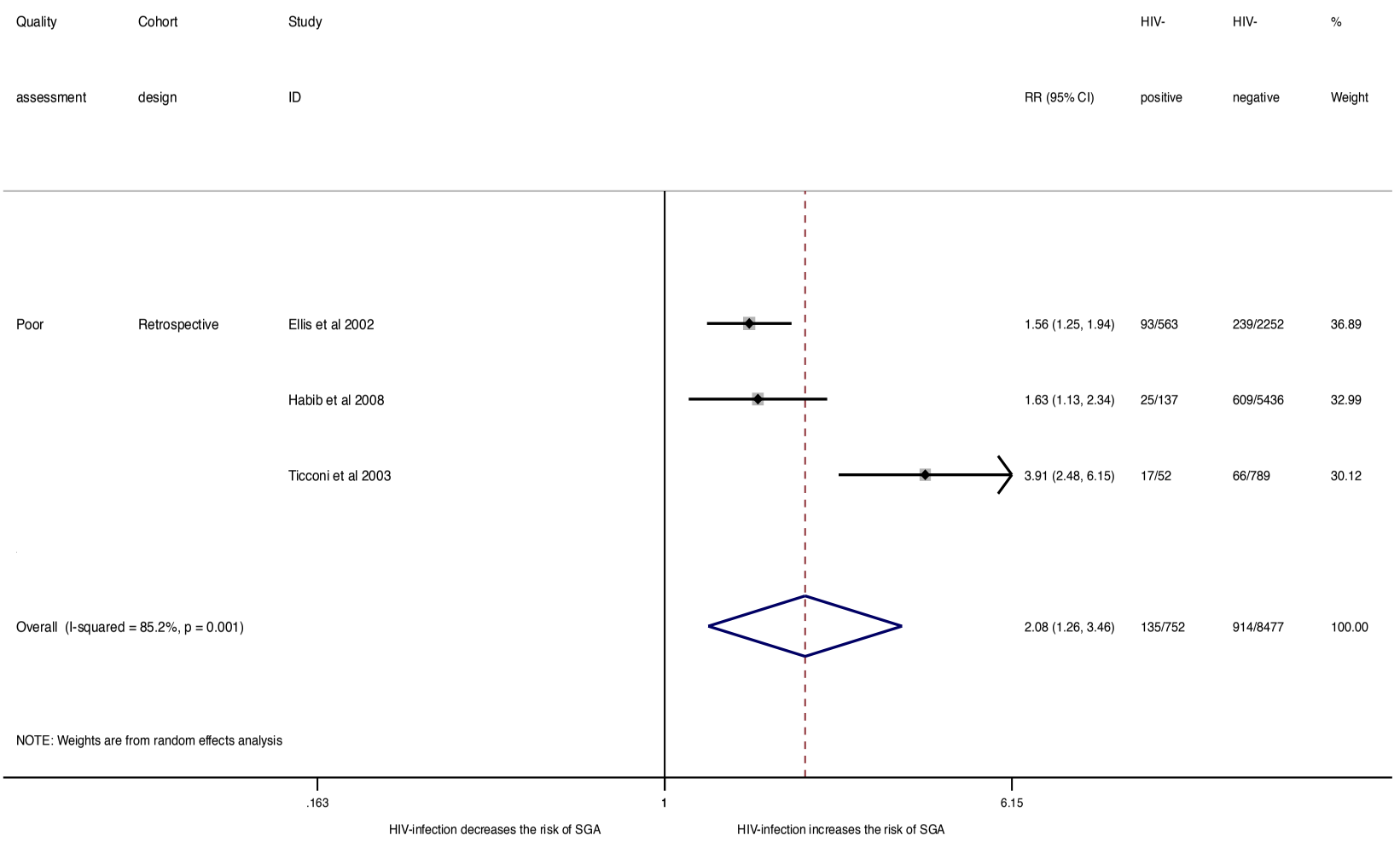


Figure 3.41: SGA associated with ART-naïve maternal HIV-infection, by adapted Newcastle-Ottawa quality assessment of studies: retrospective studies. Random-effect pairwise meta-analyses showed a significant increase in the risk of SGA in HIV-positive women in poor quality retrospective cohort studies. Abbreviations: HIV = Human Immunodeficiency Virus, RR = relative risk, SGA = small for gestational age. Source: Wedi et al. 2016[3].

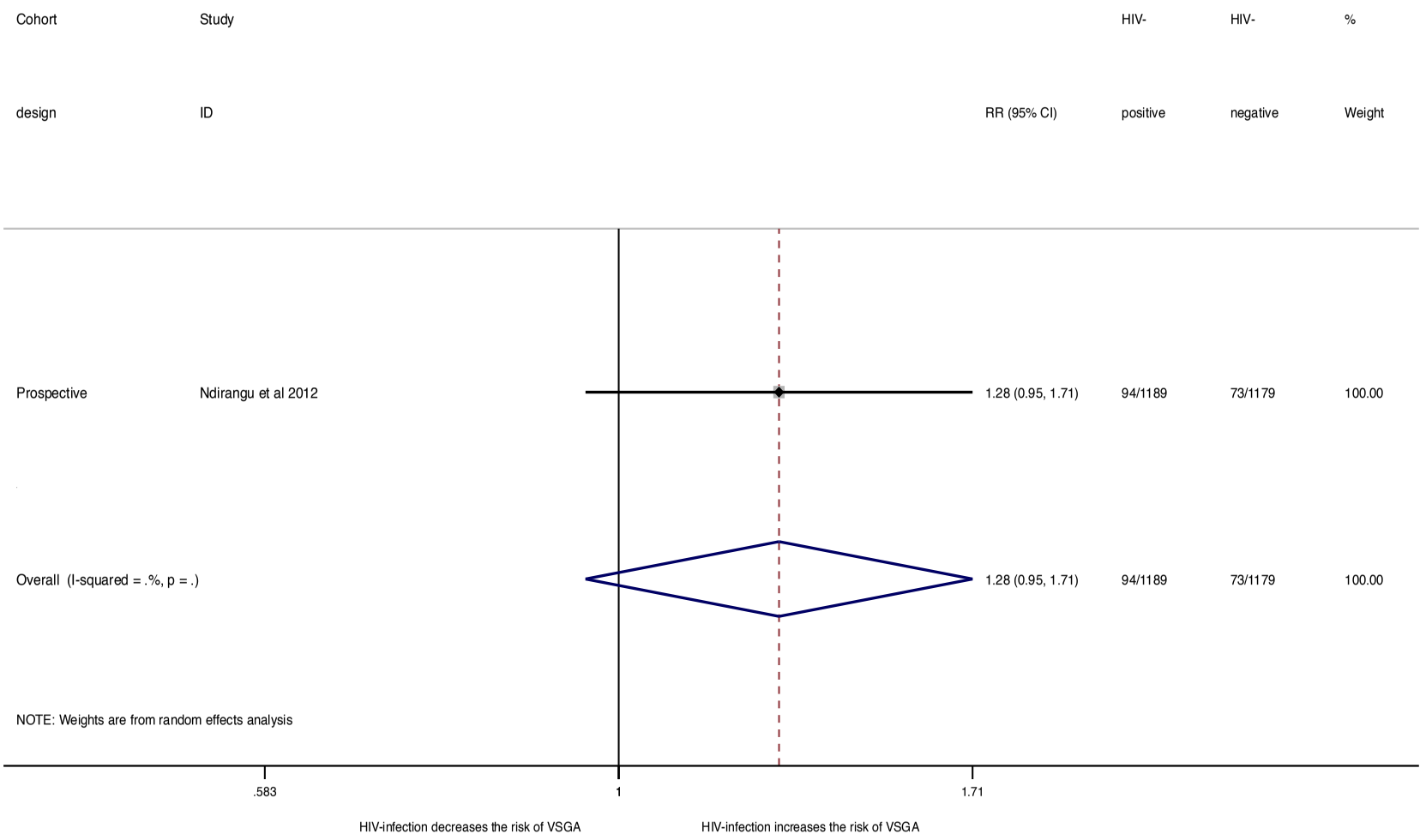


Figure 3.42: VSGA associated with ART-naïve maternal HIV-infection, by study design.
No significant association observed between maternal HIV-infection and VSGA. Abbreviations: HIV = Human Immunodeficiency Virus, RR = relative risk, VSGA = very small for gestational age. Source: Wedi et al. 2016[3].

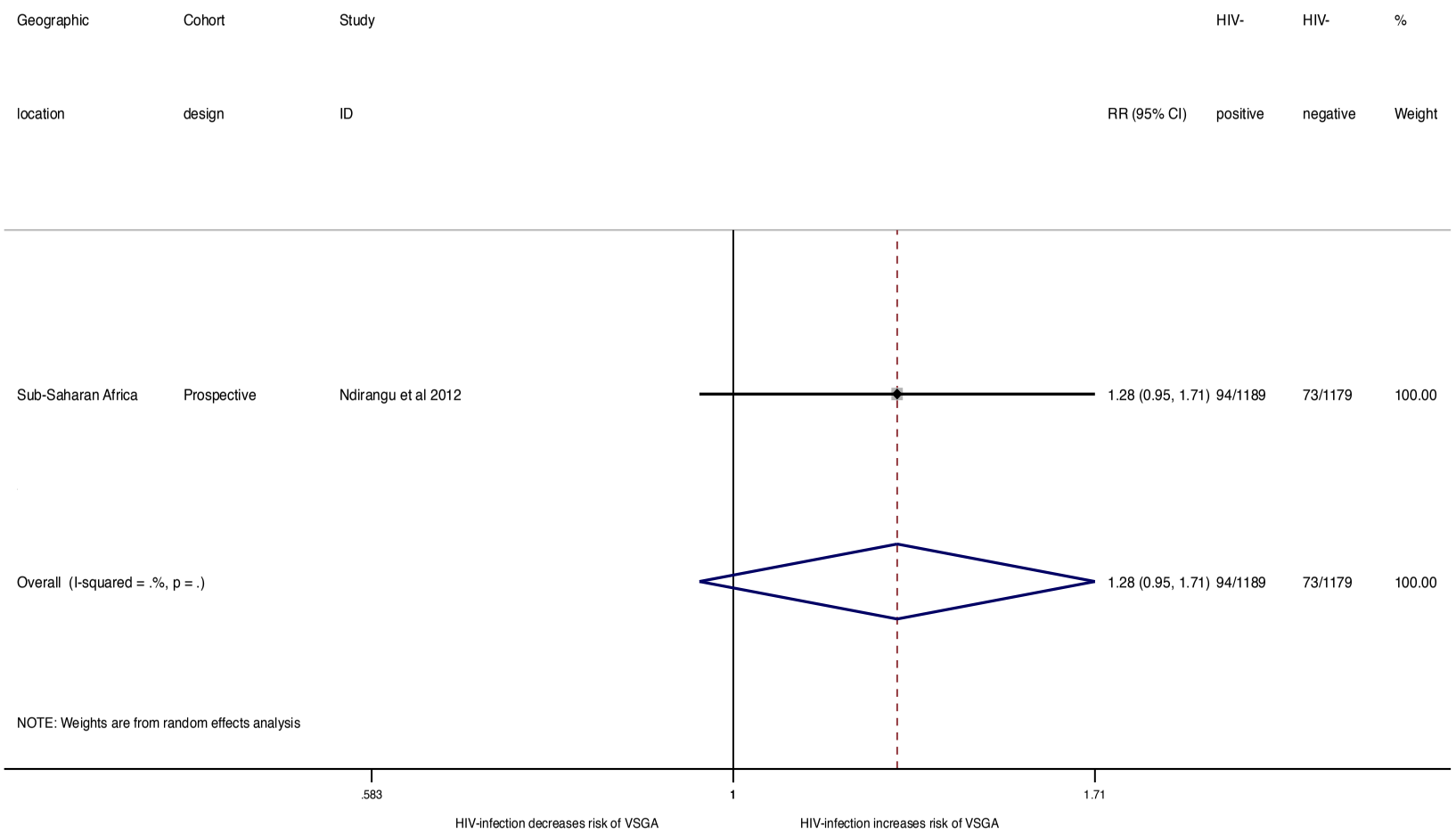


Figure 3.43: VSGA associated with ART-naïve maternal HIV-infection, by geographic region: prospective study.
No significant association observed between maternal HIV-infection and VSGA. Abbreviations: HIV = Human Immunodeficiency Virus, RR = relative risk, VSGA = very small for gestational age. Source: Wedi et al. 2016[3].

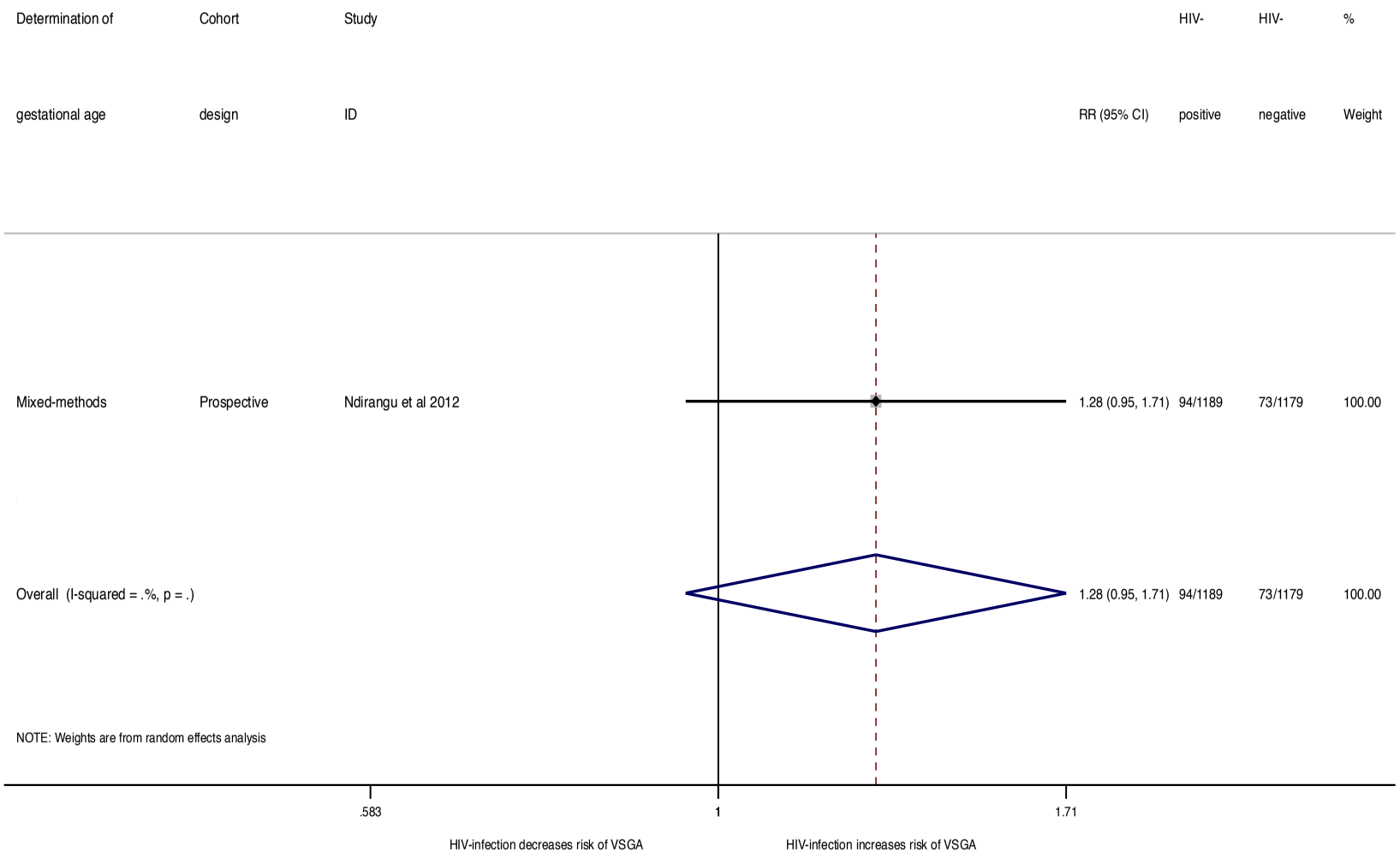


Figure 3.44: VSGA associated with ART-naïve maternal HIV-infection, by method used to determine gestational age: prospective study.
No significant association observed between maternal HIV-infection and VSGA. Abbreviations: HIV = Human Immunodeficiency Virus, RR = relative risk, VSGA = very small for gestational age. Source: Wedi et al. 2016[3].

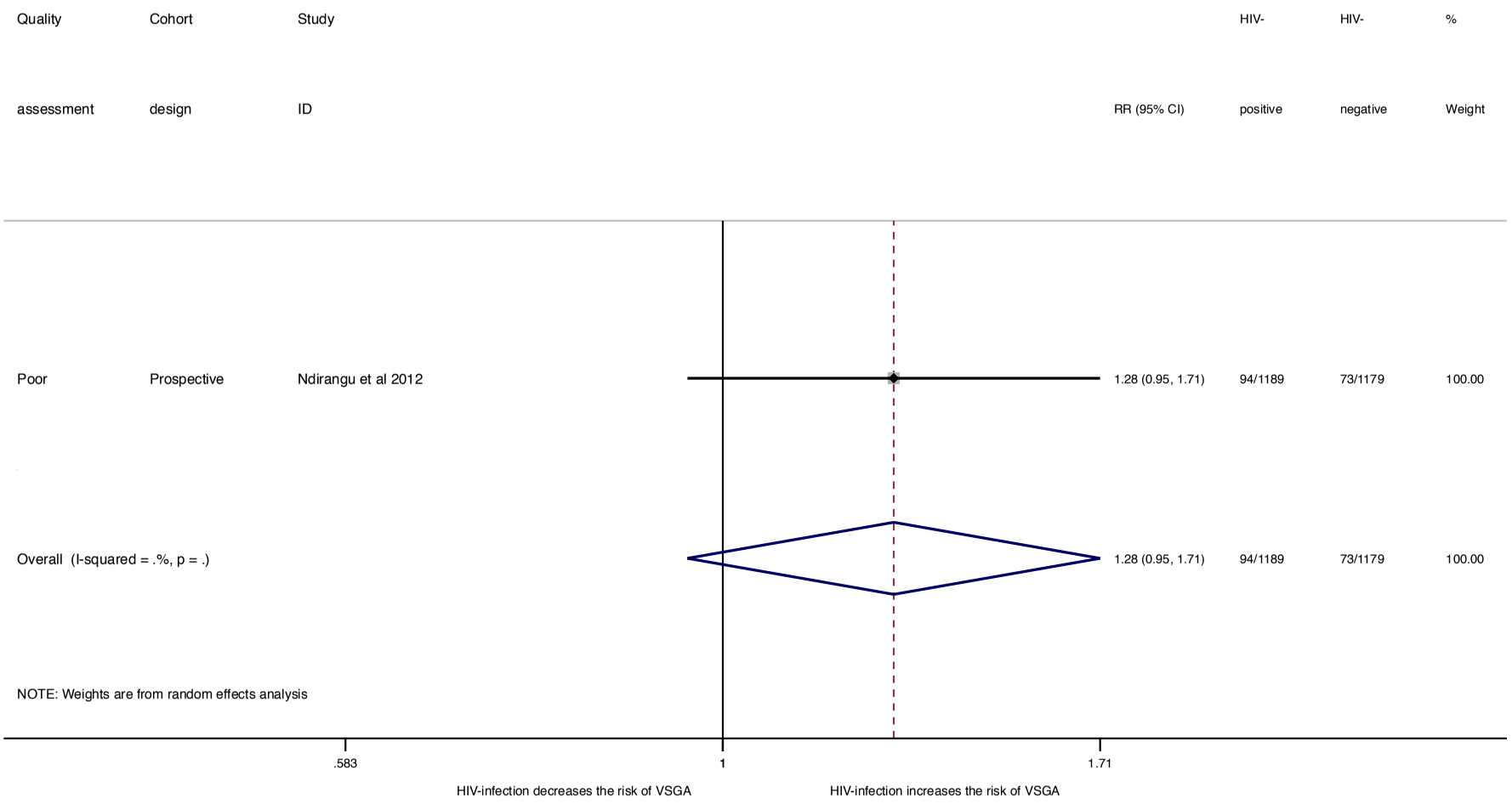


Figure 3.45: VSGA associated with ART-naïve maternal HIV-infection, by adapted Newcastle-Ottawa quality assessment of studies: prospective study. No significant association observed between maternal HIV-infection and VSGA. Abbreviations: HIV = Human Immunodeficiency Virus, RR = relative risk, VSGA = very small for gestational age. Source: Wedi et al. 2016[3].

3.3.6 Influence of maternal HIV-infection on fetal and neonatal mortality

3.3.6.1 Miscarriage

One poor quality prospective cohort study conducted in the Americas, and including 125 women, reported a positive but non-significant association between maternal HIV-infection and miscarriage (RR 1.40; 95%CI 0.37-5.36) (Figure 3.46 – to Figure 3.49).

3.3.6.2 Stillbirth

Two prospective cohort studies conducted in sub-Saharan Africa, and including 1168 women, reported a significant association between maternal HIV-infection and stillbirth (RR 1.67; 95%CI 1.05-2.66) (Figure 3.50, Figure 3.51). When studies were stratified according to quality and method used to estimate GA, the average quality study which used a mixed-method for GA determination, showed a significant association (Figure 3.52, Figure 3.53).

3.3.6.3 Neonatal death

Four studies conducted in sub-Saharan Africa, and including 2667 women, reported on the association between maternal HIV-infection and NND. Prospective (RR 1.68; 95%CI 0.45-6.29) and retrospective (RR 2.00; 95%CI 0.61-6.54) cohort studies showed a positive but non-significant associations between maternal HIV-infection and NND (Figure 3.54 – Figure 3.58).

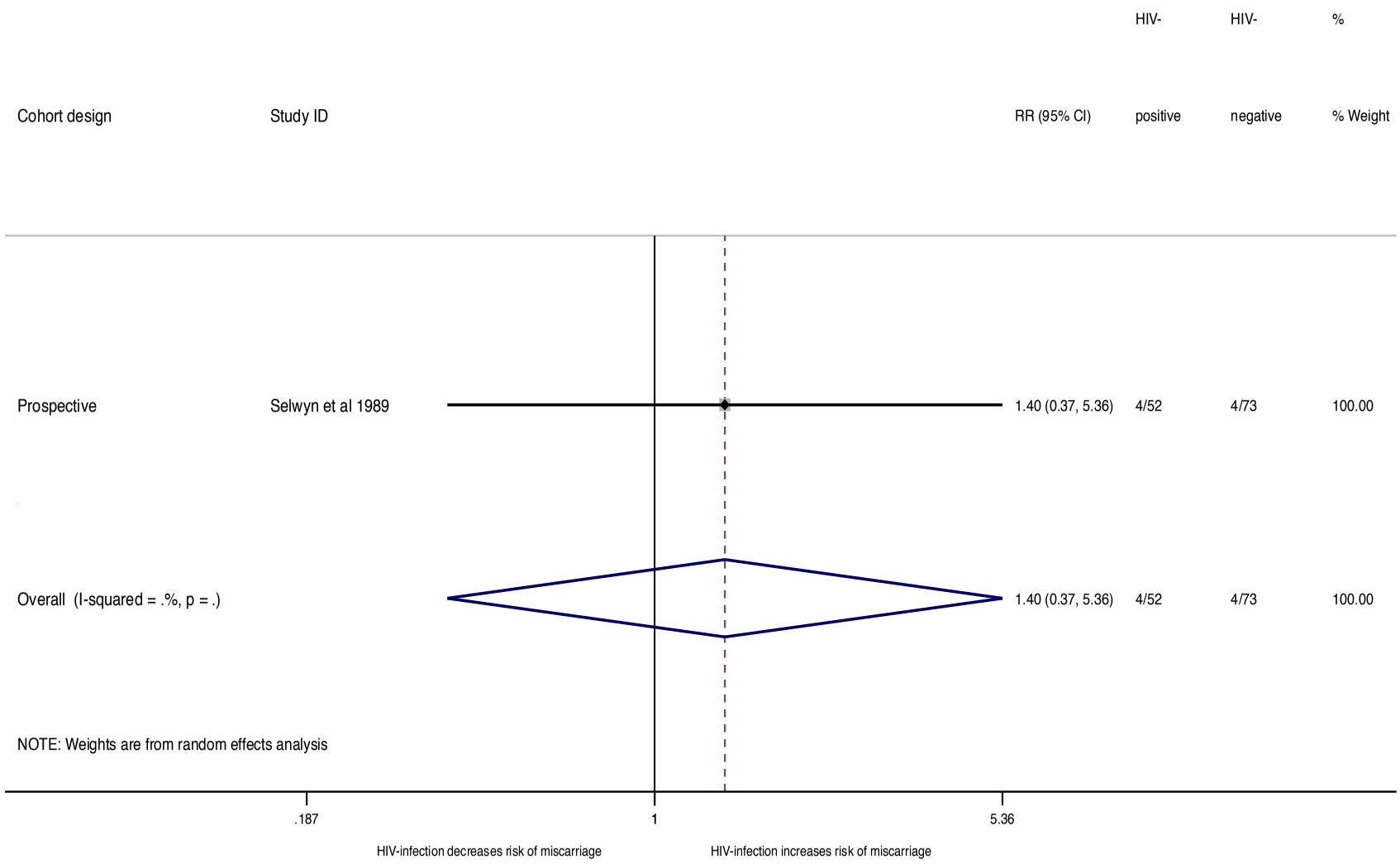


Figure 3.46: Miscarriage associated with ART-naïve maternal HIV-infection, by study design.
No significant association observed between maternal HIV-infection and miscarriage. Abbreviations: HIV = Human Immunodeficiency Virus, RR = relative risk. Source: Wedi et al. 2016[3].

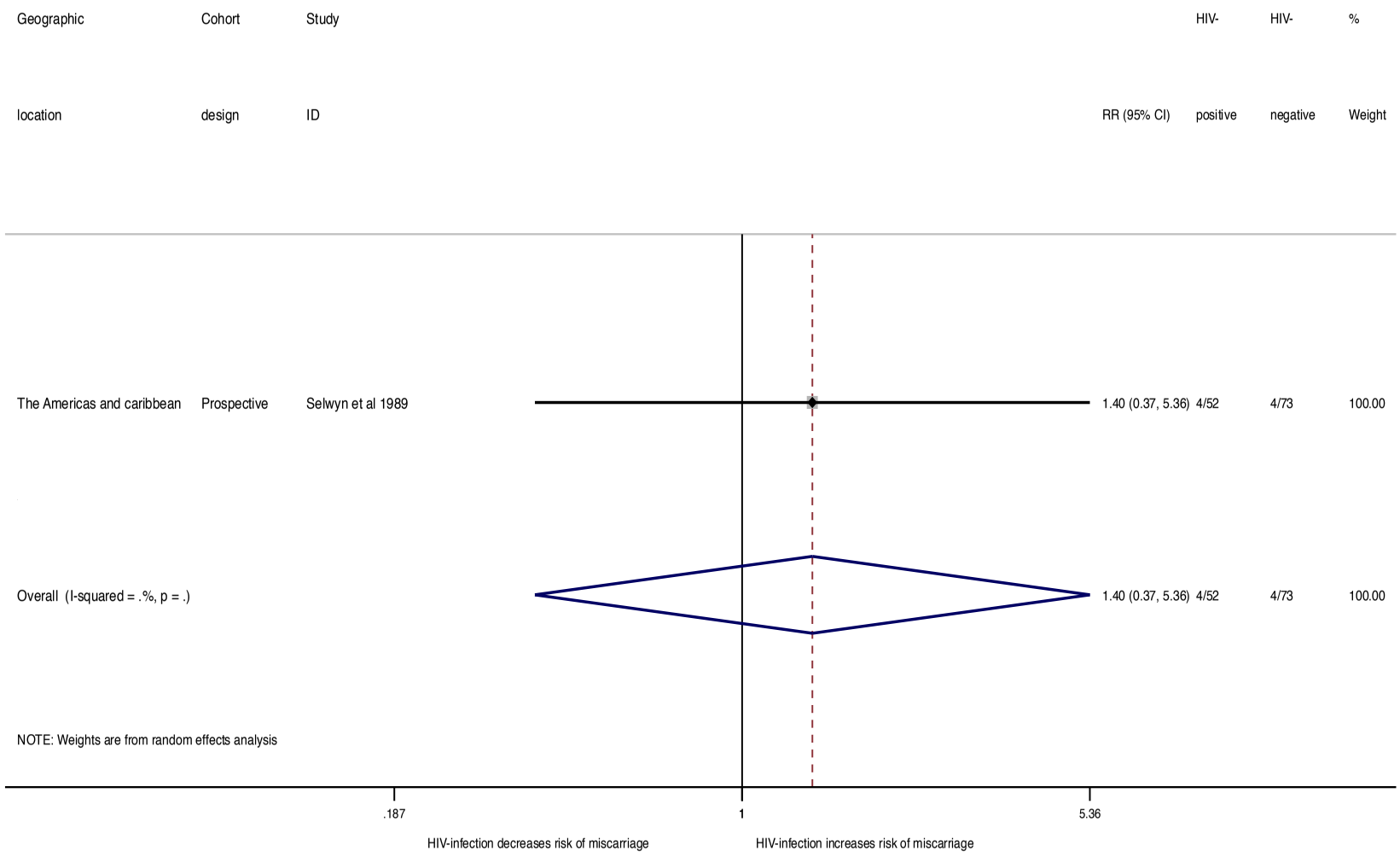


Figure 3.47: Miscarriage associated with ART-naïve maternal HIV-infection, by geographic region: prospective study.
No significant association observed between maternal HIV-infection and miscarriage. Abbreviations: HIV = Human Immunodeficiency Virus, RR = relative risk. Source: Wedi et al. 2016[3].

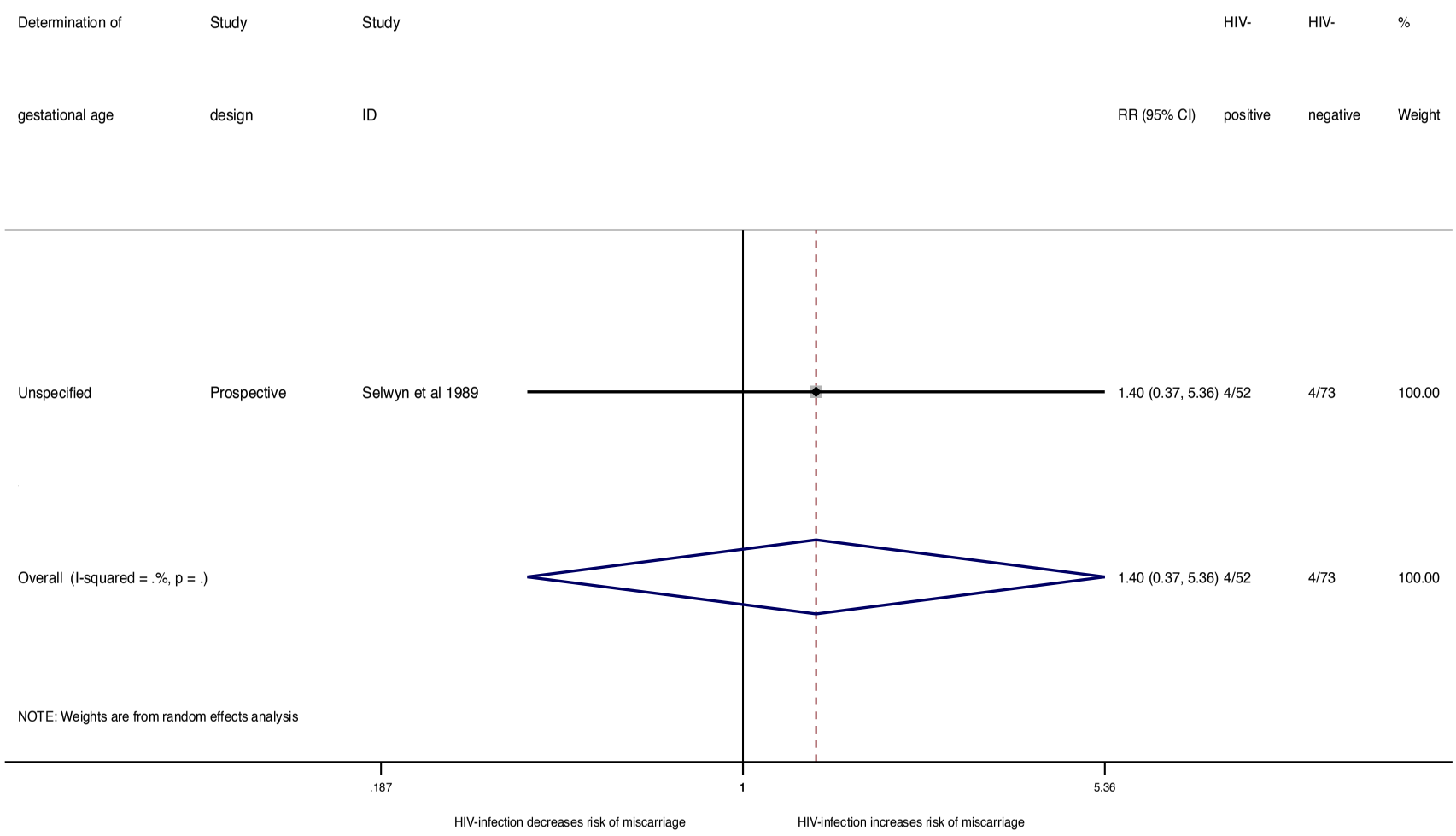


Figure 3.48: Miscarriage associated with ART-naïve maternal HIV-infection, by method used to determine gestational age: prospective study. No significant association observed between maternal HIV-infection and miscarriage. Abbreviations: HIV = Human Immunodeficiency Virus, RR = relative risk. Source: Wedi et al. 2016[3].

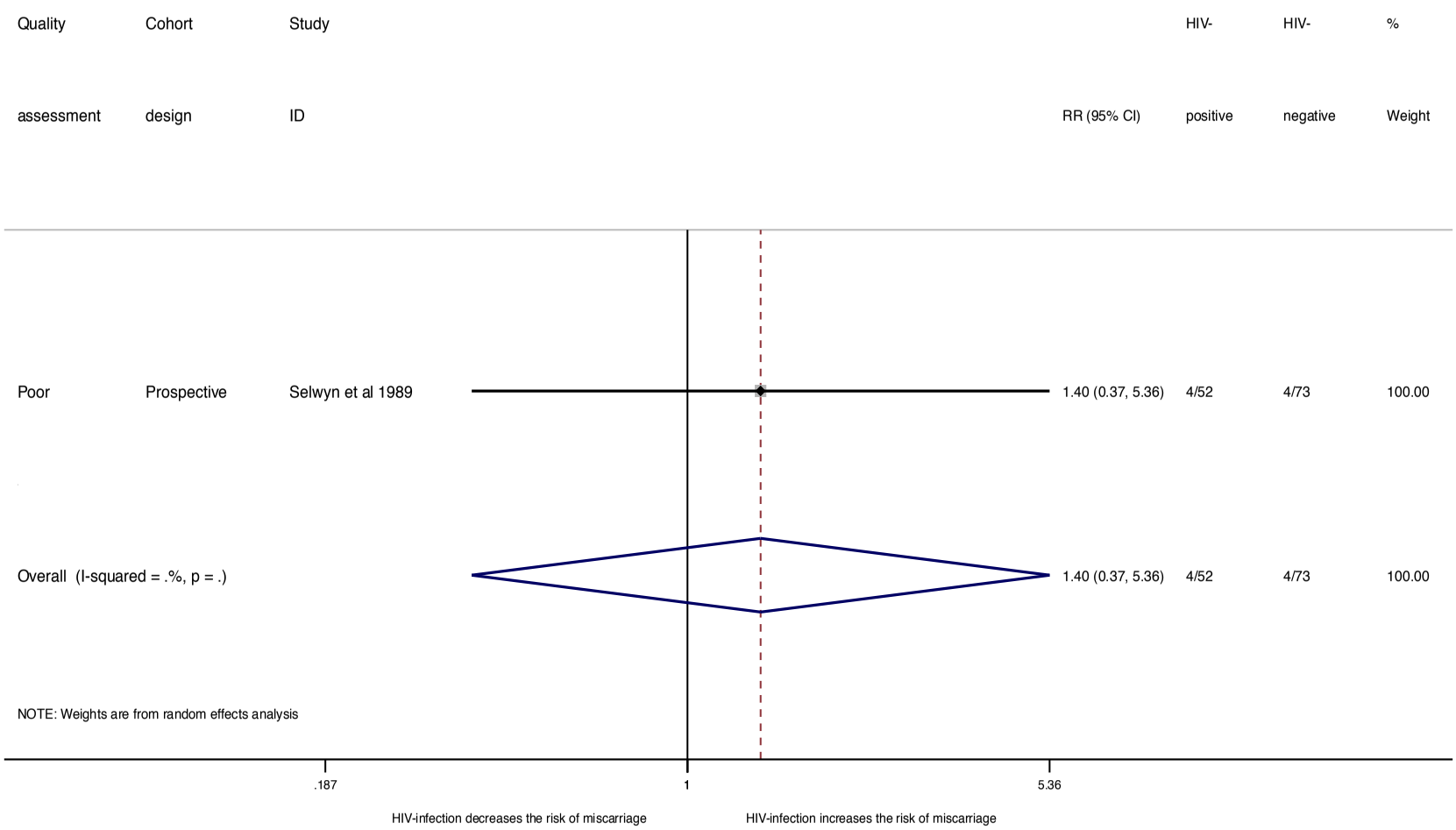


Figure 3.49: Miscarriage associated with ART-naïve maternal HIV-infection, by adapted Newcastle-Ottawa quality assessment of studies: prospective study. No significant association observed between maternal HIV-infection and miscarriage. Abbreviations: HIV = Human Immunodeficiency Virus, RR = relative risk. Source: Wedi et al. 2016[3].

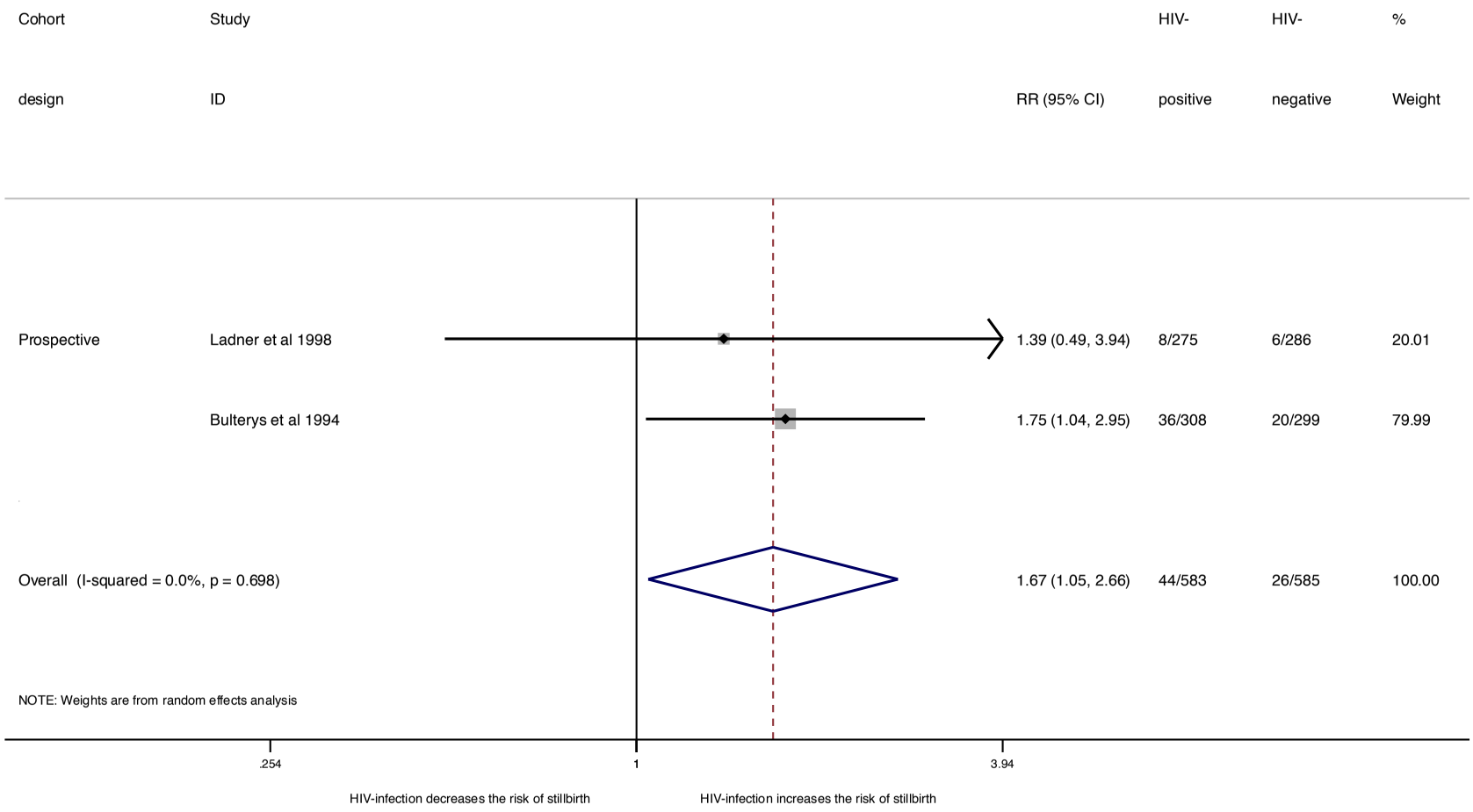


Figure 3.50: Stillbirth associated with ART-naïve maternal HIV-infection, by study design.
A random-effect pairwise meta-analysis of prospective studies showed a significantly increased risk of giving birth to a stillborn infant in HIV-positive women compared to HIV-negative women. Abbreviations: HIV = Human Immunodeficiency Virus, RR = relative risk. Source: Wedi et al. 2016[3].

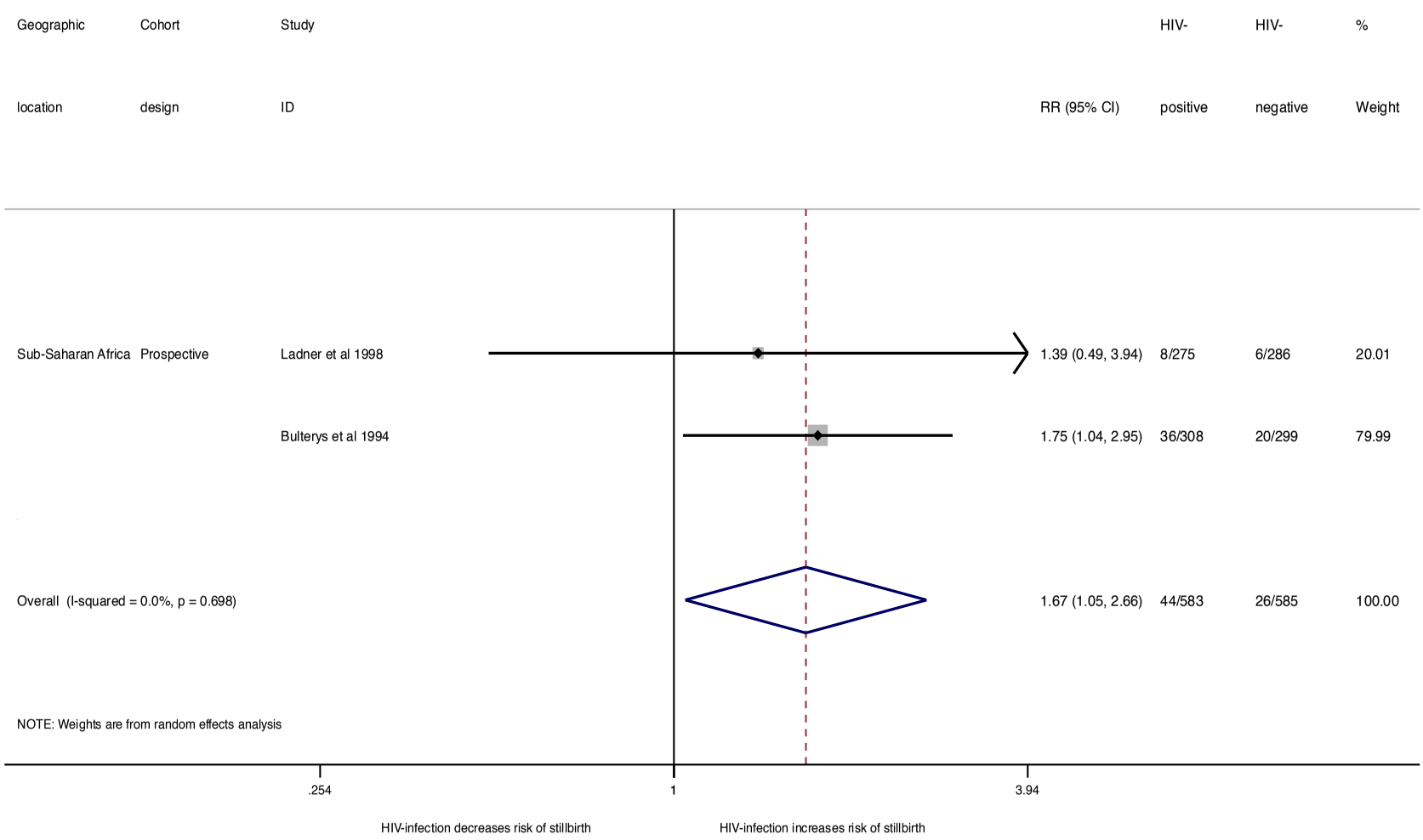


Figure 3.51: Stillbirth associated with ART-naïve maternal HIV-infection, by geographic region: prospective studies.
A random-effect pairwise meta-analysis of prospective studies showed a significantly increased risk of giving birth to a stillborn infant in HIV-positive women compared to HIV-negative women in studies conducted in sub-Saharan Africa. Abbreviations: HIV = Human Immunodeficiency Virus, RR = relative risk. Source: Wedi et al. 2016[3].

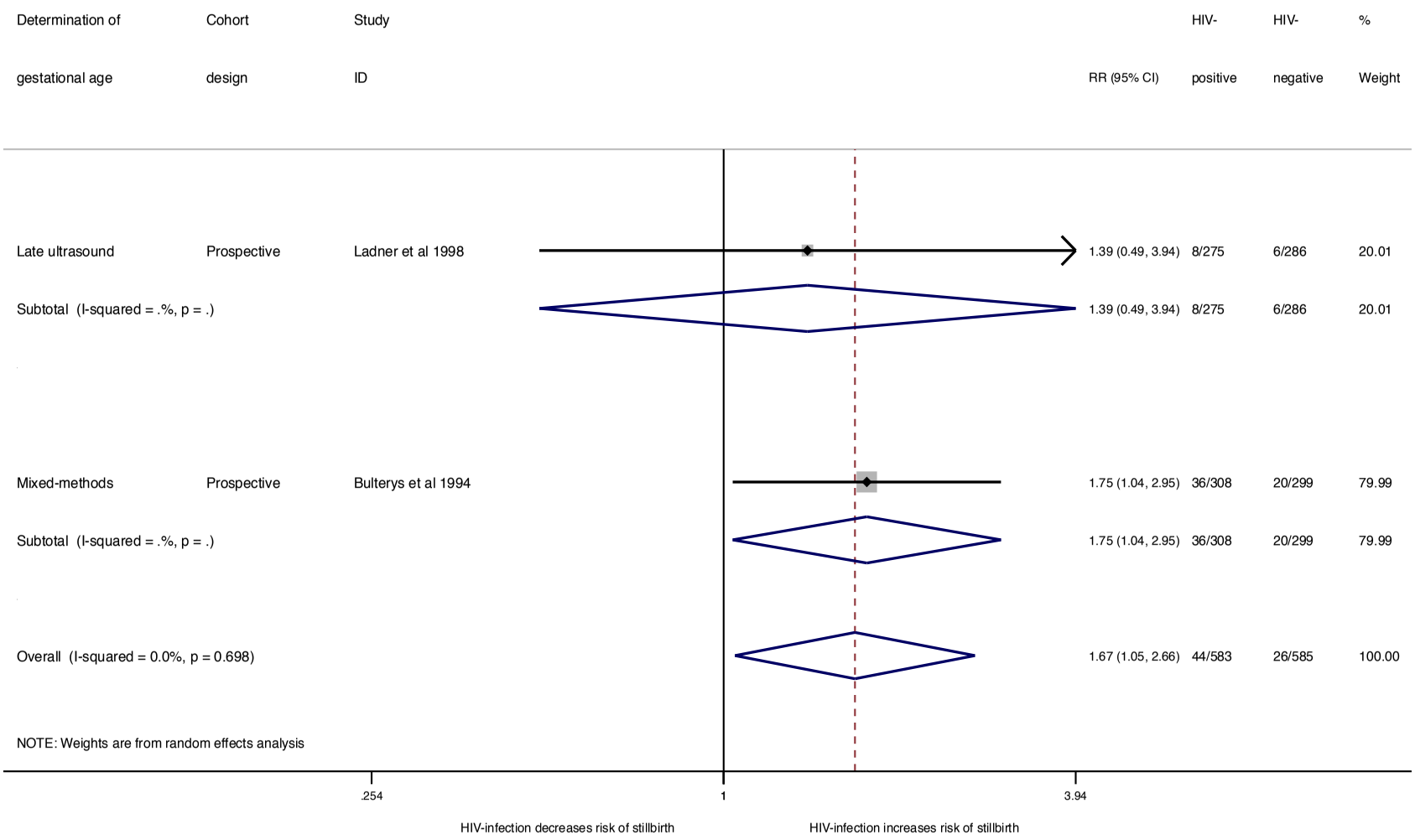


Figure 3.52: Stillbirth associated with ART-naïve maternal HIV-infection, by method used to determine gestational age: prospective studies. One study using a combination of methods to estimate GA (mixed-methods – see Table 3.4) showed a significantly increased risk of giving birth to a stillborn infant in HIV-positive women compared to HIV-negative women. Abbreviations: HIV = Human Immunodeficiency Virus, RR = relative risk. Source: Wedi et al. 2016[3].

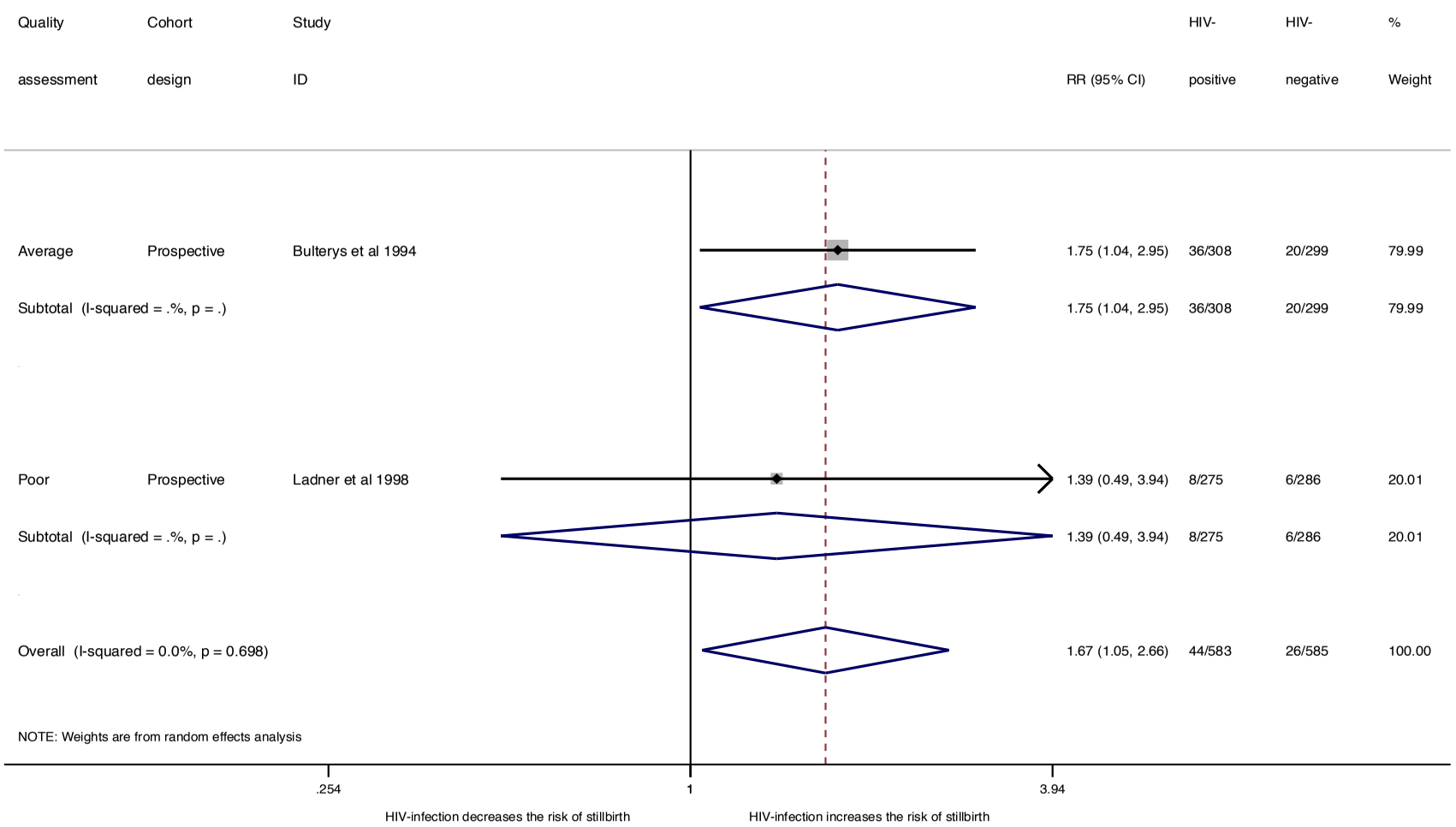


Figure 3.53: Stillbirth associated with ART-naïve maternal HIV-infection, by adapted Newcastle-Ottawa quality assessment of studies: prospective studies. One average quality study showed a significantly increased risk of giving birth to a stillborn infant in HIV-positive women compared to HIV-negative women. Abbreviations: HIV = Human Immunodeficiency Virus, RR = relative risk. Source: Wedi et al. 2016[3].

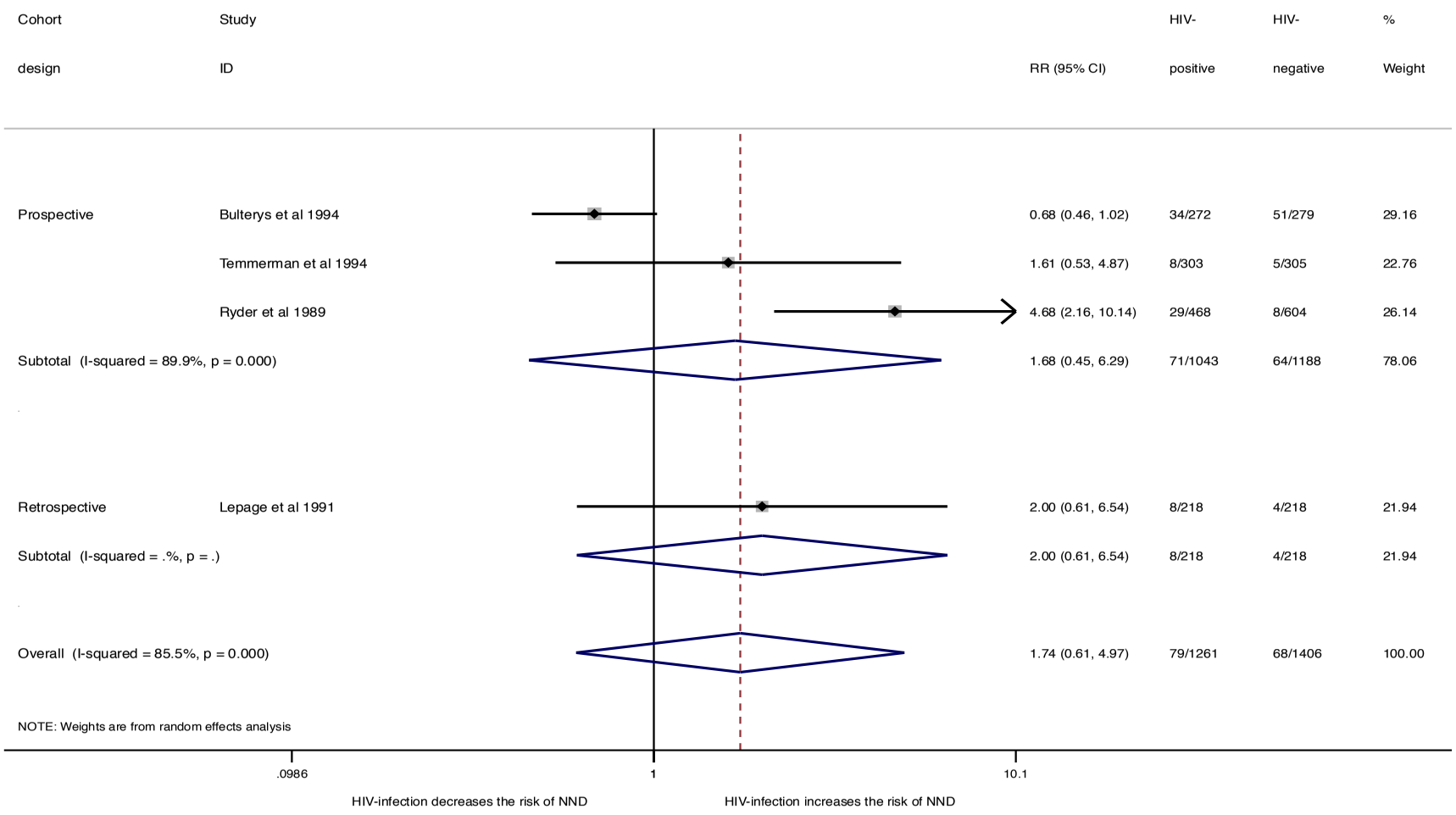


Figure 3.54: NND associated with ART-naïve maternal HIV-infection, by study design.
No significant association observed between maternal HIV-infection and NND. Abbreviations: HIV = Human Immunodeficiency Virus, NND = neonatal death, RR = relative risk. Source: Wedi et al. 2016[3].

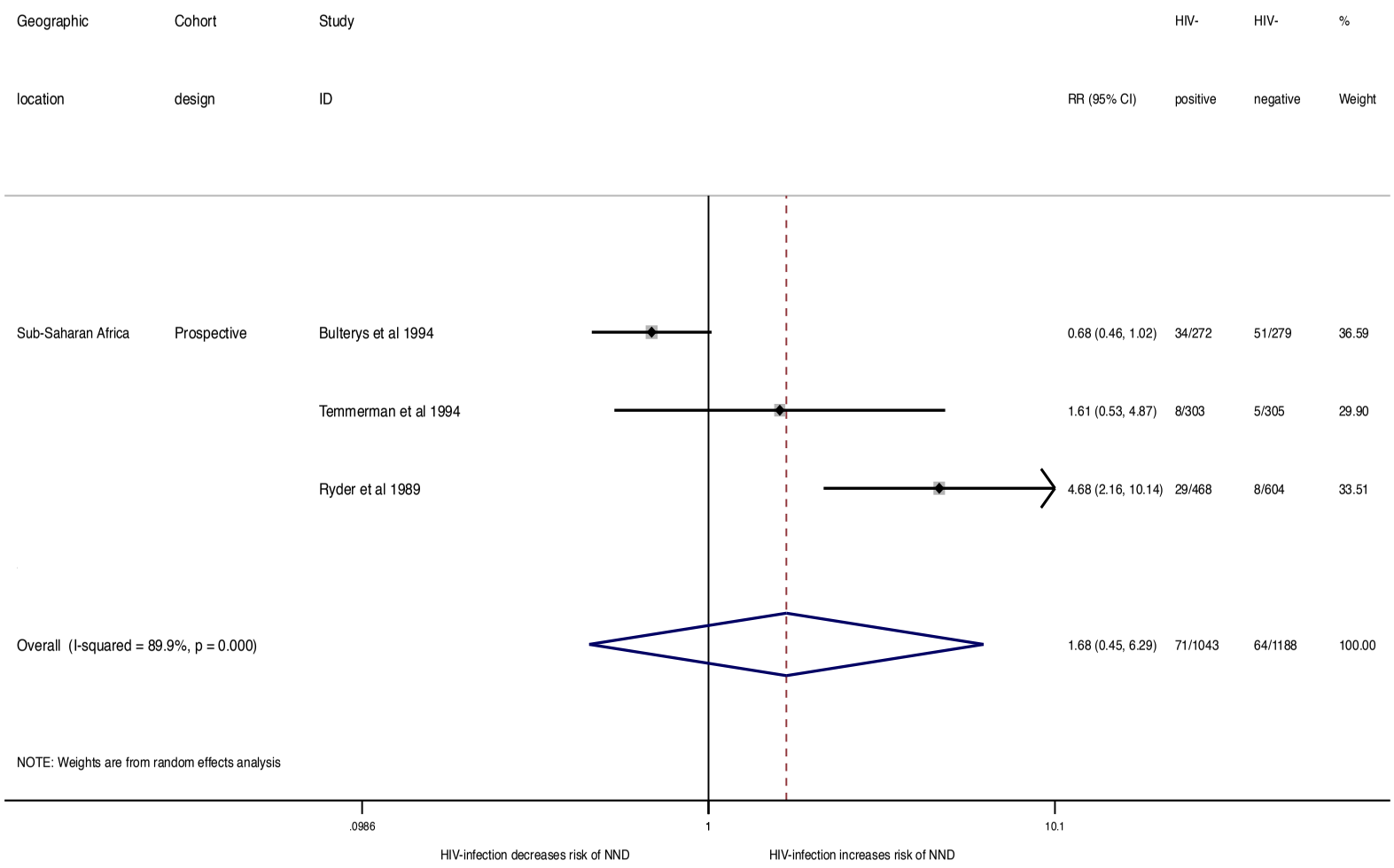


Figure 3.55: NND associated with ART-naïve maternal HIV-infection, by geographic region: prospective studies.
No significant association observed between maternal HIV-infection and NND. Abbreviations: HIV = Human Immunodeficiency Virus, NND = neonatal death, RR = relative risk. Source: Wedi et al. 2016[3].

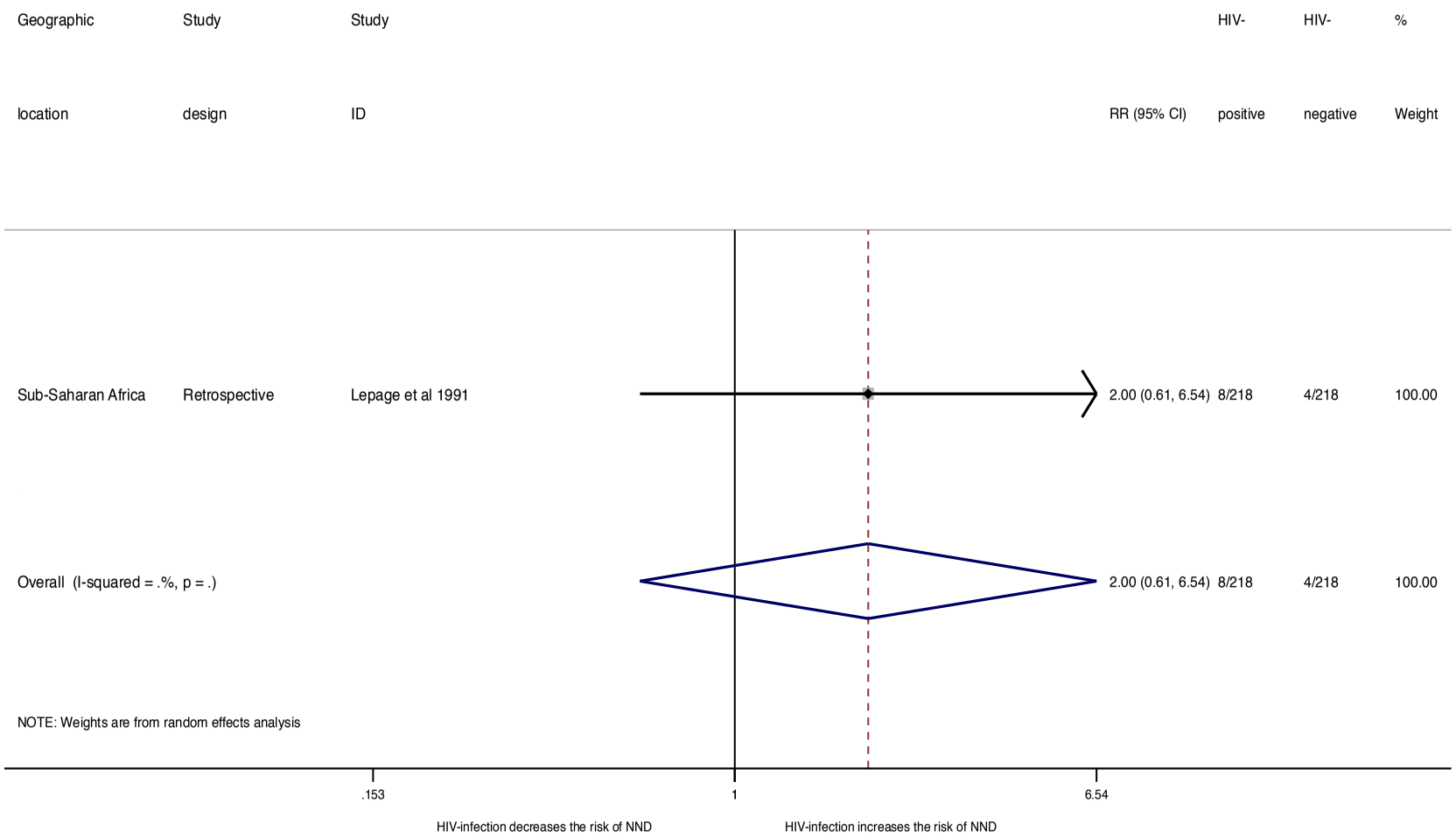


Figure 3.56: **NND associated with ART-naïve maternal HIV-infection, by geographic region: retrospective study.**
No significant association observed between maternal HIV-infection and NND. Abbreviations: HIV = Human Immunodeficiency Virus, NND = neonatal death, RR = relative risk. Source: Wedi et al. 2016[3].

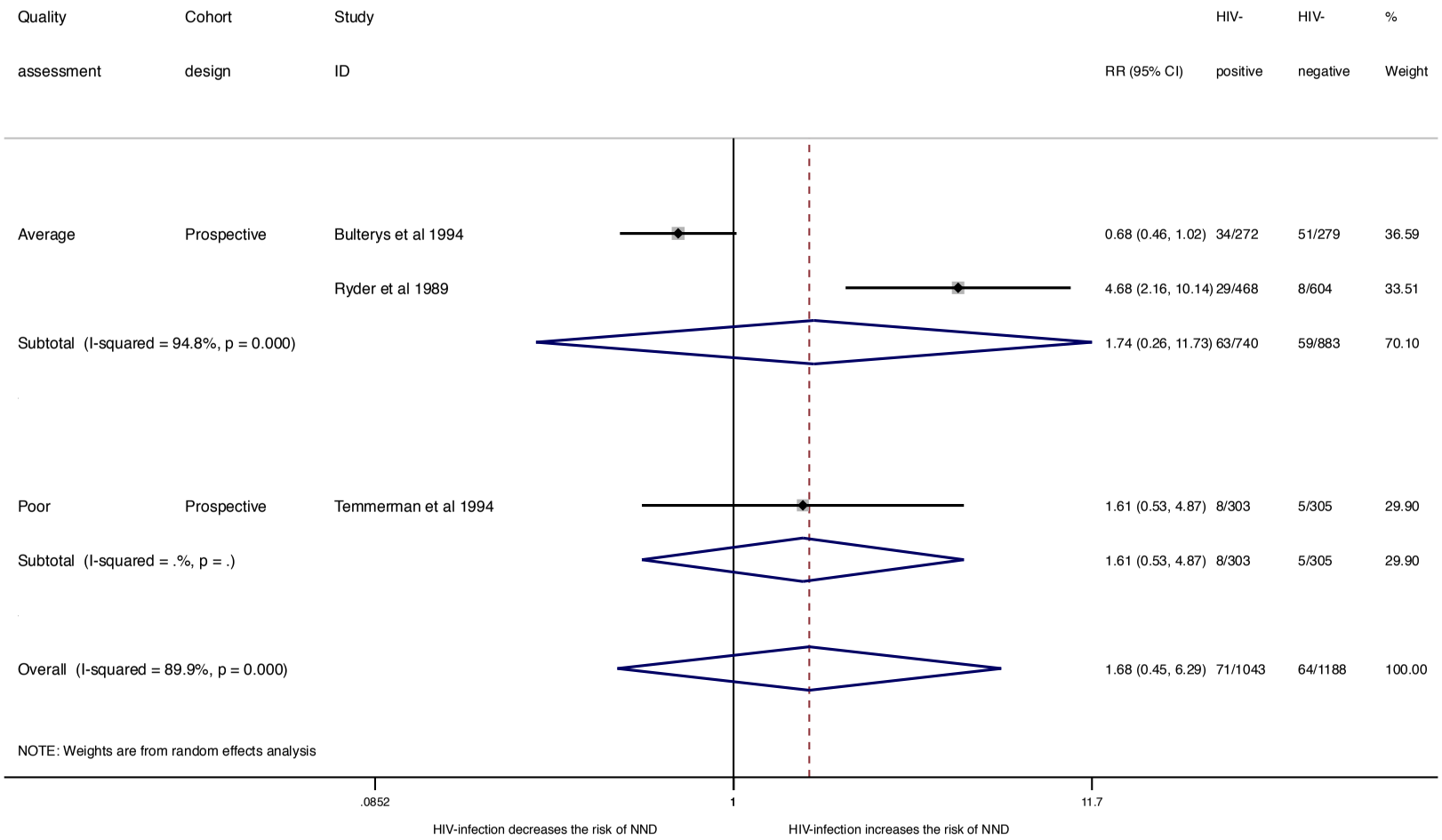


Figure 3.57: NND associated with ART-naïve maternal HIV-infection, by adapted Newcastle-Ottawa quality assessment of studies: prospective studies. No significant association observed between maternal HIV-infection and NND. Abbreviations: HIV = Human Immunodeficiency Virus, NND = neonatal death, RR = relative risk. Source: Wedi et al. 2016[3].

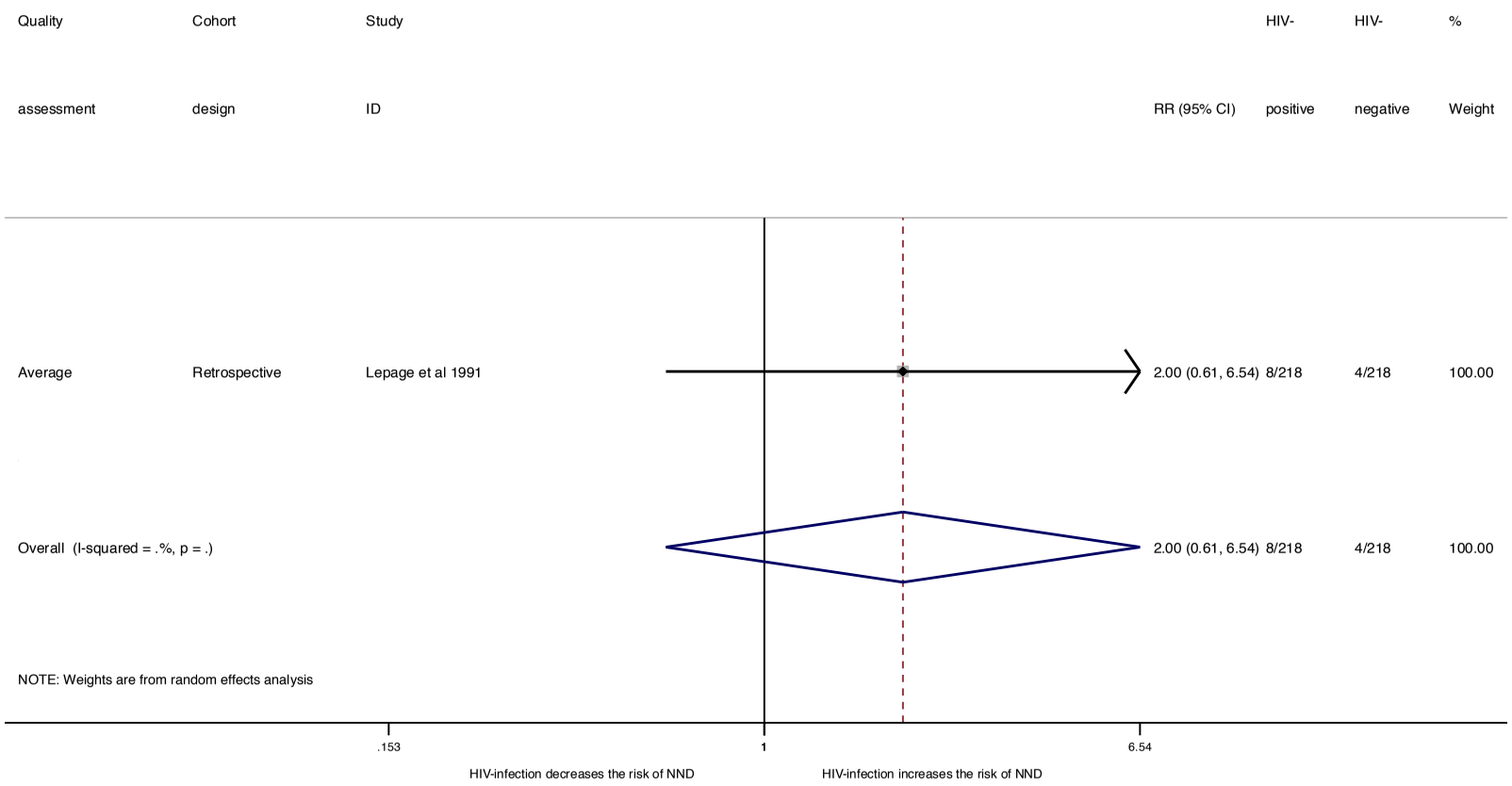


Figure 3.58: NND associated with ART-naïve maternal HIV-infection, by adapted Newcastle-Ottawa quality assessment of studies: retrospective study. No significant association observed between maternal HIV-infection and NND. Abbreviations: HIV = Human Immunodeficiency Virus, NND = neonatal death, RR = relative risk. Source: Wedi et al. 2016[3].

3.3.7 Summary points of perinatal outcome results.

A summary of the meta-analysis results for the association between maternal HIV-infection and perinatal outcomes according to study design, geographic location, method used to estimate GA, and the quality assessment of studies is shown in Figures 3.59 – Figure 3.65. Maternal HIV-infection significantly increased the risk of PTB, LBW, SGA and stillbirth in meta-analyses of prospective cohort studies, and term-LBW and preterm LBW in meta-analyses of retrospective cohort studies[3]. Meta-analyses of the association between maternal HIV-infection and VPTB, VLBW, VSGA, miscarriage and NND were not significant.

3.3.7.1 Significant associations observed in pairwise meta-analysis

3.3.7.1.1 PTB

- PTB was significantly associated with maternal HIV-infection in the meta-analysis of prospective and retrospective studies (Figure 3.59).
- This association persisted in prospective and retrospective studies conducted in sub-Saharan Africa and the America's and Caribbean (Figure 3.60, Figure3. 61).
- PTB remained significantly associated with maternal HIV-infection, irrespective of the method used to determine GA in prospective studies (Figure 3.62), and in retrospective studies that estimated GA with the LNMP and those that did not provide a specific method (Figure 3.63).
- Meta-analyses of average and poor quality prospective studies and poor quality retrospective studies showed a significant association between maternal HIV-infection and PTB (Figure 3.64, Figure 3.65).

3.3.7.1.2 LBW

- Meta-analyses of prospective and retrospective studies showed a significant association between maternal HIV-infection and LBW (Figure 3.59).

- The association persisted across prospective and retrospective studies conducted in sub-Saharan Africa and Asia, and retrospective studies conducted in the America's and Caribbean (Figure 3.60, Figure 3.61).
- Average and poor quality prospective and retrospective studies showed a significant association between maternal HIV-infection and LBW (Figure 3.64, Figure 3.65).

3.3.7.1.3 Term-LBW

- Maternal HIV-infection was significantly associated with term-LBW in retrospective studies (Figure 3.59).
- The association persisted in retrospective studies conducted in sub-Saharan Africa and the Americas and Caribbean (Figure 3.61).
- Prospective and retrospective studies using a combination of methods to estimate the GA, and retrospective studies using LNMP showed a significant association between maternal HIV-infection and term-LBW (Figure 3.62, Figure 3.63).
- Only poor quality retrospective studies showed a significant association between maternal HIV-infection and term-LBW (Figure 3.65).

3.3.7.1.4 Preterm-LBW

- A meta-analysis of retrospective studies showed a significant association between maternal HIV-infection and preterm-LBW (Figure 3.59).
- The effect persisted in retrospective studies conducted in sub-Saharan Africa (Figure 3.61).
- Retrospective studies using a combination of methods to estimate the GA showed a significant association between maternal HIV-infection and preterm-LBW.
- Only poor quality retrospective studies showed a significant association between maternal HIV-infection and preterm-LBW (Figure 3.65).

3.3.7.1.5 SGA

- Meta-analyses of prospective and retrospective cohort studies showed a significant association between maternal HIV-infection and SGA (Figure 3.59).

- The effect persisted in prospective and retrospective studies conducted in sub-Saharan Africa, and retrospective studies from the Americas and Caribbean (Figure 3.60, Figure 3.61).
- SGA remained significantly associated with maternal HIV-infection irrespective of the method used to estimate GA (Figure 3.62, Figure 3.63).
- Meta-analysis of average quality prospective studies and poor quality retrospective studies showed a significant association between SGA and maternal HIV-infection (Figure 3.64, Figure 3.65).

3.3.7.1.6 Stillbirth

- A meta-analysis of prospective cohort studies showed a significant association between maternal HIV-infection and stillbirth (Figure 3.59).
- The effect persisted in prospective studies conducted in sub-Saharan Africa (Figure 3.60).
- Only studies using a combination of methods to estimate GA showed a significant association between maternal HIV and stillbirth.
- A meta-analysis of average quality prospective cohort studies showed a significant association between stillbirths and maternal HIV-infection.

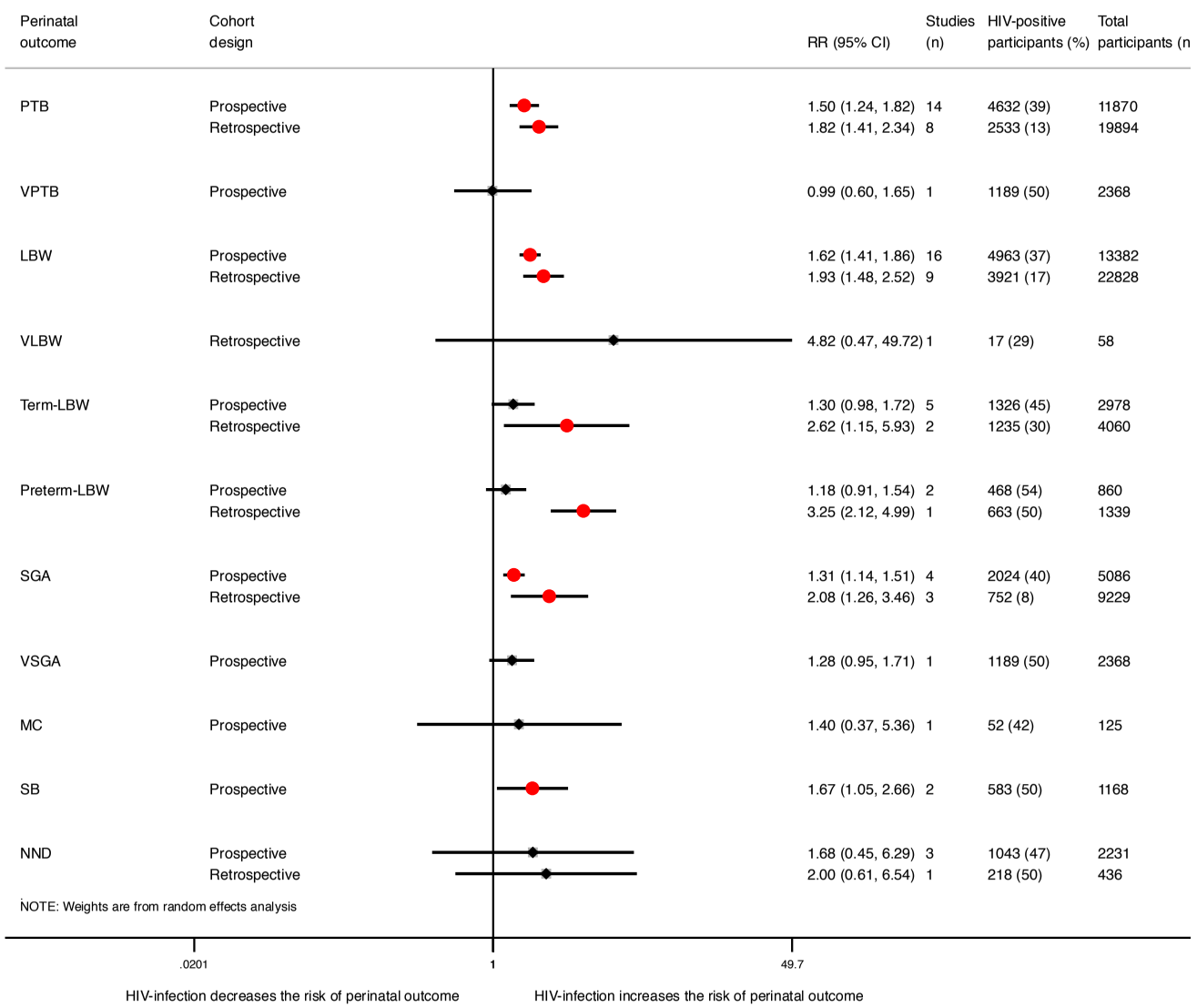


Figure 3.59: Perinatal outcomes associated with ART-naïve maternal HIV-infection, by study design.
Significant effects are presented in red. RR (95%CI) = relative risk with 95% confidence interval, n = number, % = number of HIV-positive participants as a proportion of the total number of participants in the included studies (for each outcome and study design). PTB = preterm birth, VPTB = very preterm birth, LBW = low birthweight, VLBW = very low birthweight, SGA = small for gestational age, VSGA = very small for gestational age, MC = miscarriage, SB = stillbirth, NND = neonatal death. Source: Wedi et al. 2016[3].

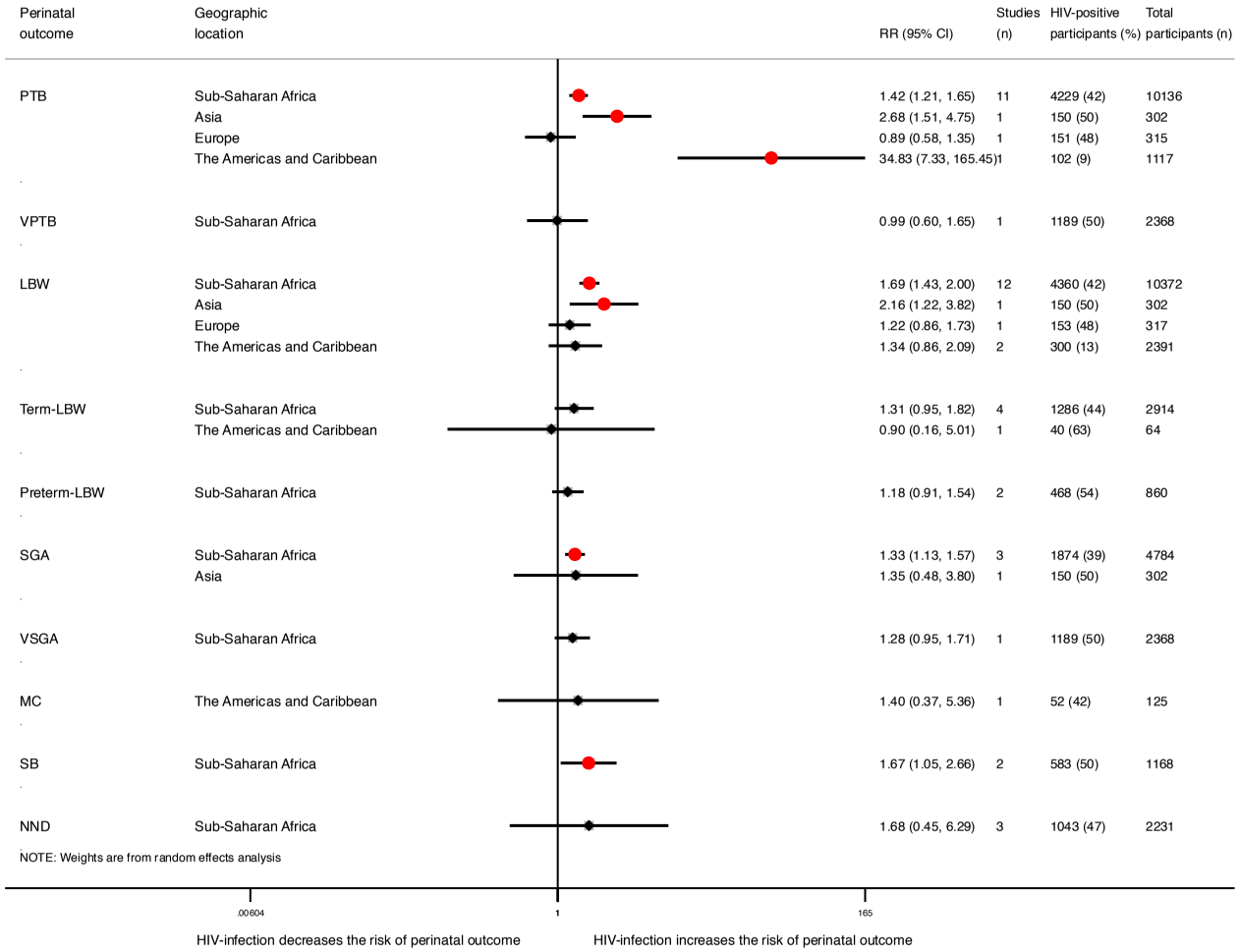


Figure 3.60 Perinatal outcomes associated with ART-naïve maternal HIV-infection, by geographic region where studies were conducted – Prospective cohort studies. Statistically significant effects are presented in red. RR (95%CI) = relative risk with 95% confidence interval, n = number, % = number of HIV-positive participants as a proportion of the total number of participants in the included studies (for each outcome and study design). PTB = preterm birth, VPTB = very preterm birth, LBW = low birthweight, SGA = small for gestational age, VSGA = very small-for-gestational-age, MC = miscarriage, SB = stillbirth, NND = neonatal death. Source: Wedi et al. 2016[3].

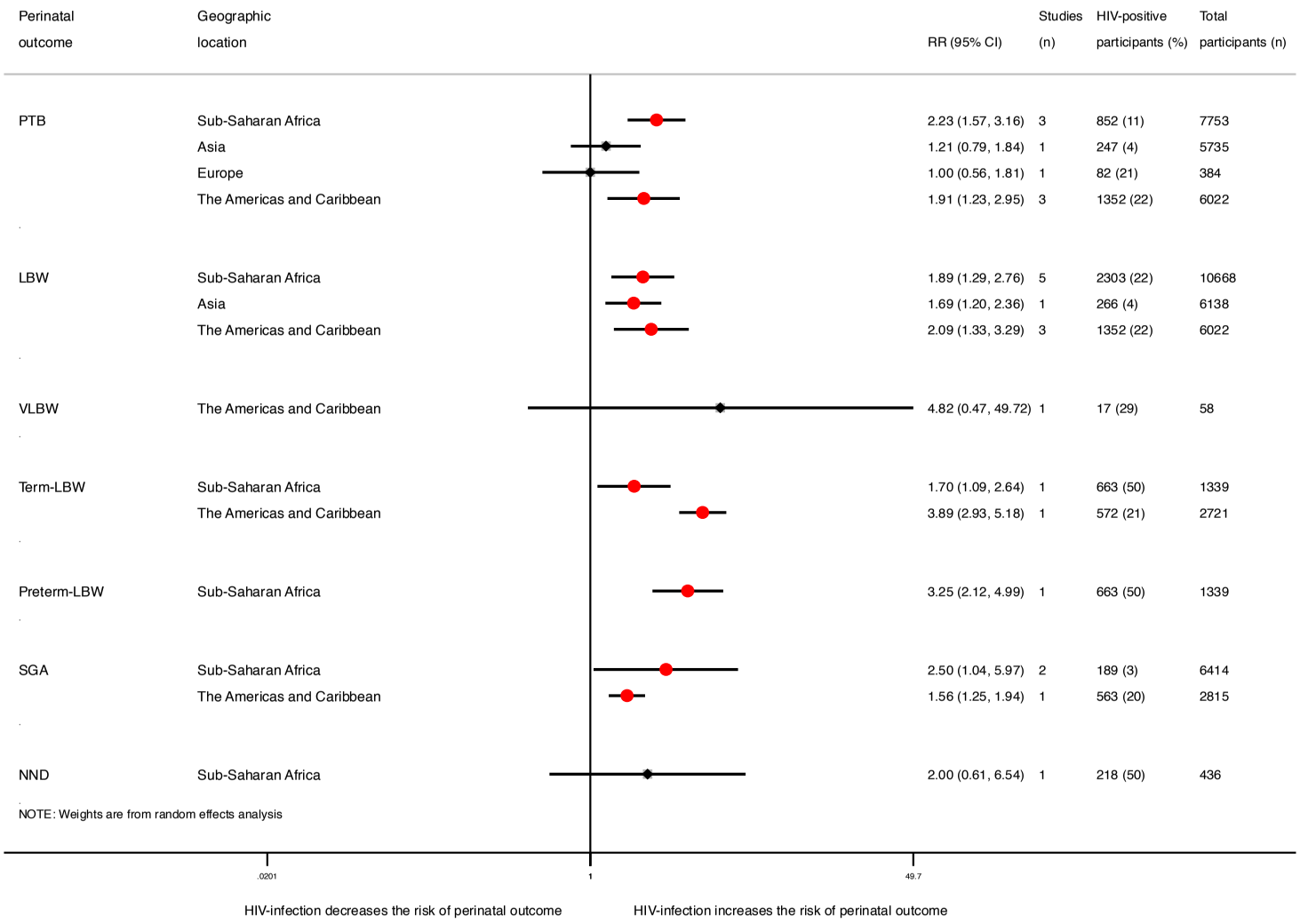


Figure 3.61: Perinatal outcomes associated with ART-naïve maternal HIV-infection, by geographic region where studies were conducted – Retrospective cohort studies. Statistically significant effects are presented in red. RR (95%CI) = relative risk with 95% confidence interval, n = number, % = number of HIV-positive participants as a proportion of the total number of participants in the included studies (for each outcome and study design). PTB = preterm birth, LBW = low birthweight, VLBW = very low birthweight, SGA = small for gestational age, NND = neonatal death. Source: Wedi et al. 2016[3].

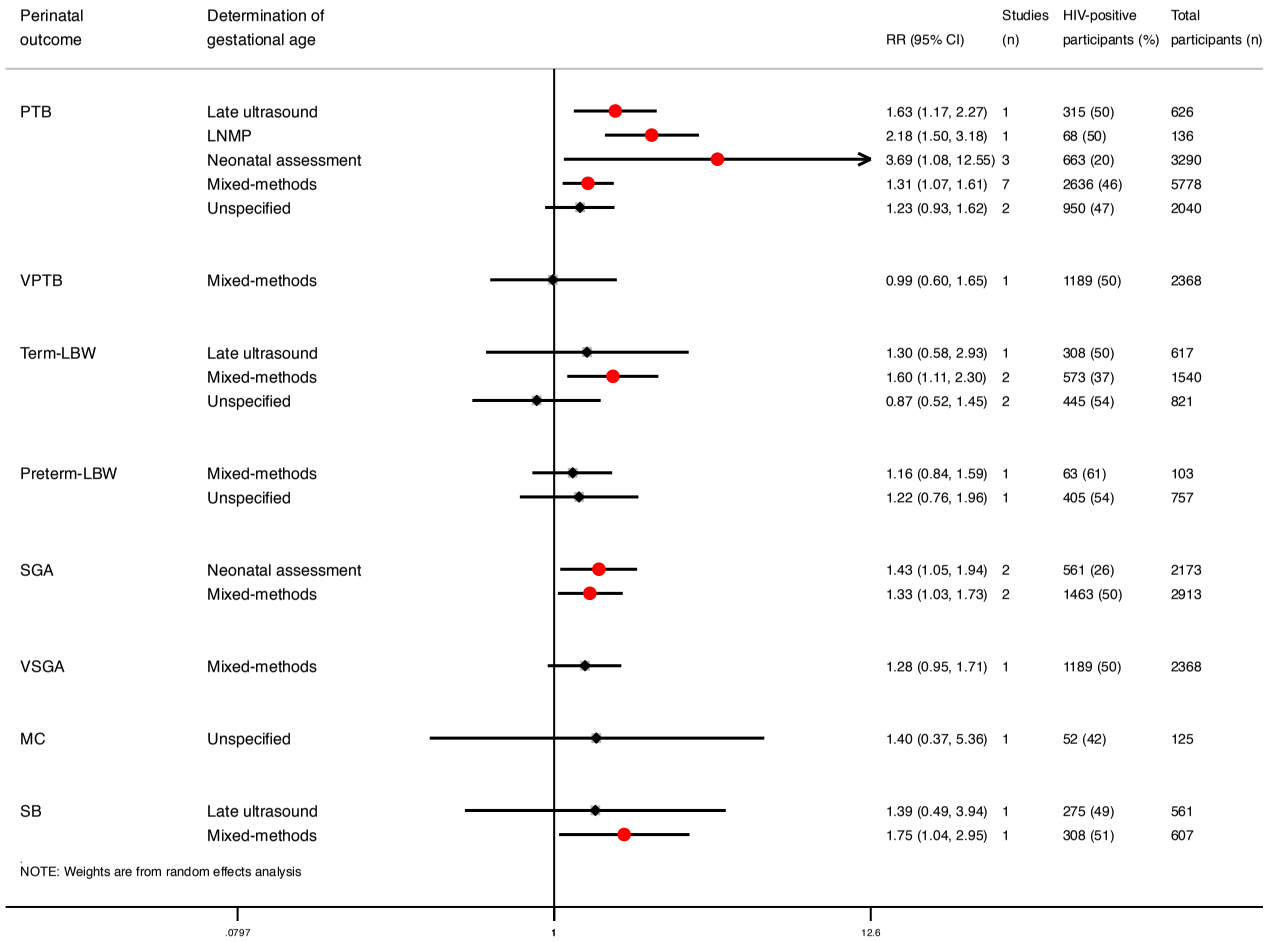


Figure 3.62: Perinatal outcomes associated with ART-naïve maternal HIV-infection, by method used to determine gestational age – prospective cohort studies. Statistically significant effects are presented in red. RR (95%CI) = relative risk with 95% confidence interval, n = number, % = number of HIV-positive participants as a proportion of the total number of participants in the included studies (for each outcome and study design). PTB = preterm birth, VPTB = very preterm birth, LBW = low birthweight, SGA = small for gestational age, VSGA = very small for gestational age, MC = miscarriage, SB = stillbirth, LNMP = last normal menstrual period. Details of mixed-methods is provided in Table 3.4. Source: Wedi et al. 2016[3].

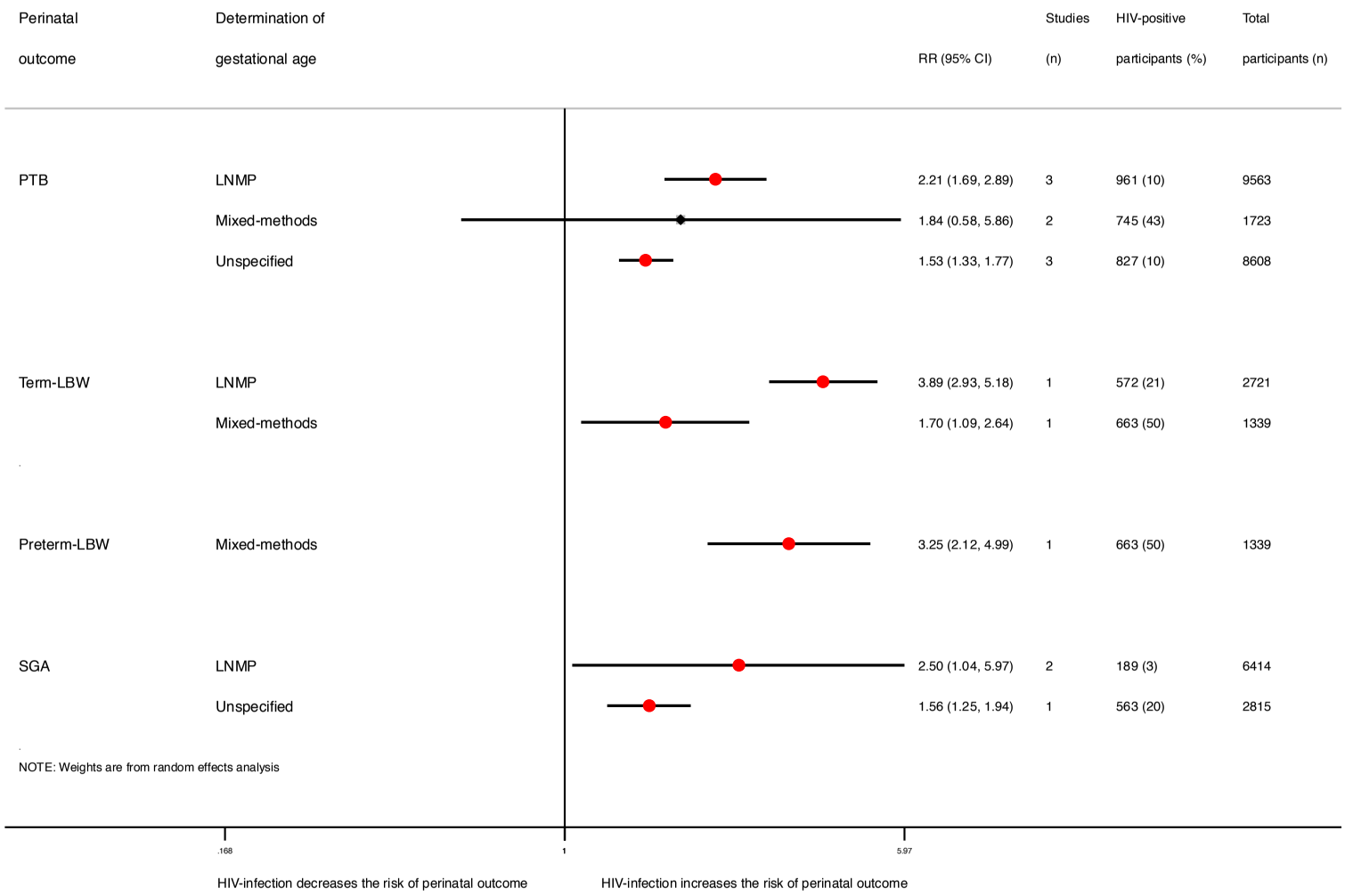


Figure 3.63: Perinatal outcomes associated with ART-naïve maternal HIV-infection, by method used to determine gestational age – retrospective cohort studies. Statistically significant effects are presented in red. RR (95%CI) = relative risk with 95% confidence interval, n = number, % = number of HIV-positive participants as a proportion of the total number of participants in the included studies (for each outcome and study design). PTB = preterm birth, LBW = low birthweight, SGA = small for gestational age, LNMP = last normal menstrual period. Details of mixed-methods is provided in Table 3.4. Source: Wedi et al. 2016[3].

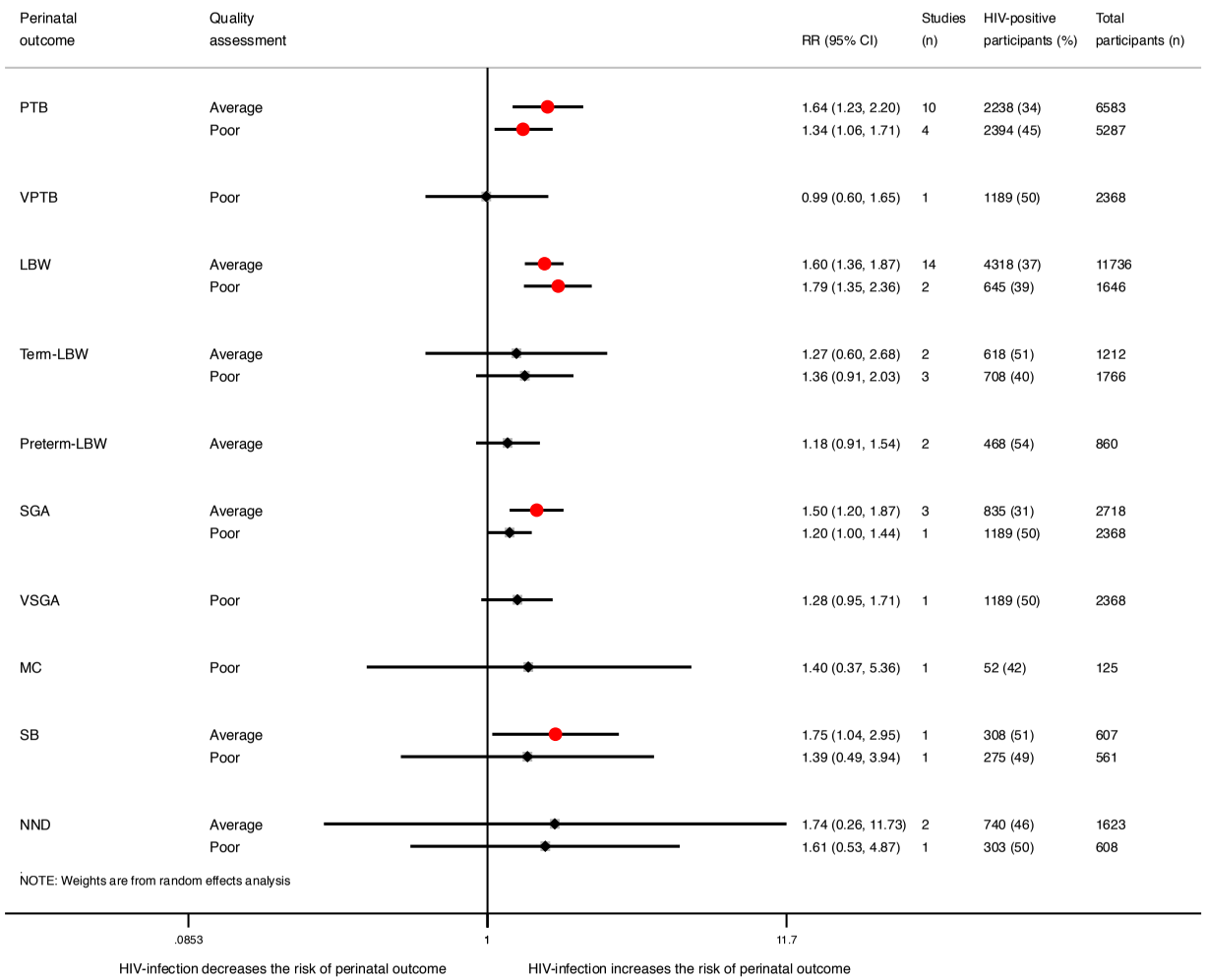


Figure 3.64: Perinatal outcomes associated with ART-naïve maternal HIV-infection, by the adapted Newcastle-Ottawa quality assessment of studies – prospective cohort studies. Statistically significant effects are presented in red. RR (95%CI) = relative risk with 95% confidence interval, n = number, % = number of HIV-positive participants as a proportion of the total number of participants in the included studies (for each outcome and study design). PTB = preterm birth, VPTB = very preterm birth, LBW = low birthweight, SGA = small for gestational age, VSGA = very small for gestational age, MC = miscarriage, SB = stillbirth, NND = neonatal death. Source: Wedi et al. 2016[3].

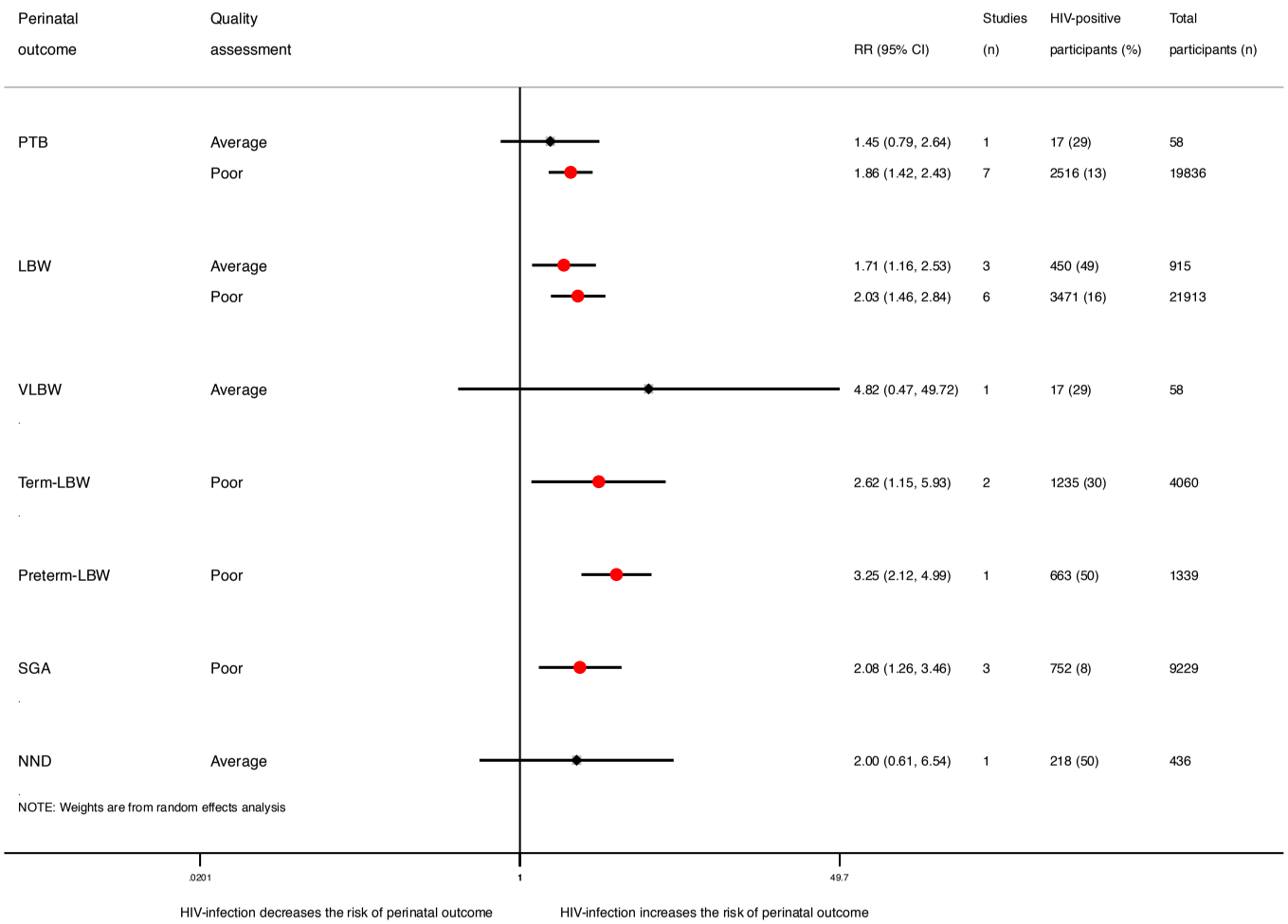


Figure 3.65: Perinatal outcomes associated with ART-naïve maternal HIV-infection, by the adapted Newcastle-Ottawa quality assessment of studies – retrospective cohort studies. Statistically significant effects are presented in red. RR (95%CI) = relative risk with 95% confidence interval, n = number, % = number of HIV-positive participants as a proportion of the total number of participants in the included studies (for each outcome and study design). PTB = preterm birth, LBW = low birthweight, VLBW = very low birthweight, SGA = small for gestational age, NND = neonatal death. Source: Wedi et al. 2016[3].

3.3.8 Sensitivity analysis: impact of HIV-disease stage and the adjustment for covariate factors on the association between maternal HIV-infection and perinatal outcomes

3.3.8.1 PTB

Seven studies reported both crude and adjusted effect estimates for the association between maternal HIV-infection and PTB. The studies adjusted for a variety of factors, and no two studies adjusted for the same variables (Table 3.6). The summary estimates were reported as RR in three studies (Figure 3.66), and OR in four studies (Figure 3.67). The adjustment for confounding did not change the direction of the association, and only induced minor changes in the magnitude of the effect estimate.

Two studies reported on the influence of the clinical stage of disease on the risk of giving birth preterm (Figure 3.68). Coley et al. (2001)[244] showed an increased risk of giving birth preterm in women with AIDS compared to asymptomatic HIV-positive or HIV-negative pregnant women (Figure 3.68). Similarly, Temmerman et al. (1994)[216] showed higher rates of of PTBs in women with AIDS (28.6%), compared to those who presented with AIDS-related signs and symptoms (23.1%), were asymptomatic (19.3%) or women who were HIV-negative (9.9%). Temmerman et al 1994 is not represented on the forest plot due to the limited data provided[216].

3.3.8.2 LBW

Nine studies reported both crude and adjusted effect estimates, including two that reported RR (Figure 3.66) and seven that reported OR (Figure 3.67). While the adjustment for confounders did influence the magnitude of effect estimate, it did not change the direction of the effect.

Two prospective cohort studies showed a significant increase in the risk of LBW in women presenting with AIDS compared to asymptomatic HIV-positive or HIV-negative women (Figure 3.68).

3.3.8.3 Term-LBW

Three studies reported crude and adjusted effect estimates (Figure 3.66, Figure 3.67). The adjustment for confounding in one prospective cohort study reporting RR (Figure 3.66) changed the direction of the association between term-LBW and maternal HIV-infection, from a non-significant negative association to a significant and positive association. The adjustment for confounders in the remaining studies did not change the direction of the association.

There was no significant association between the clinical stage of disease and term-LBW (Figure 3.68).

3.3.8.4 Preterm-LBW

The adjustment for confounding in two prospective cohort studies did not change the direction of the association between maternal HIV-infection and preterm-LBW (Figure 3.66, Figure 3.67).

One prospective cohort study showed a significant increase in the risk of preterm-LBW in women presenting with AIDS compared to asymptomatic HIV-positive or HIV-negative women (Figure 3.68).

3.3.8.5 SGA

The adjustment for confounders in five studies reporting crude and adjusted effect estimates did not change the direction of the association (Figure 3.66, Figure 3.67).

3.3.8.6 VSGA

The adjustment for confounders in one prospective cohort study did not change the direction of the association (Figure 3.66).

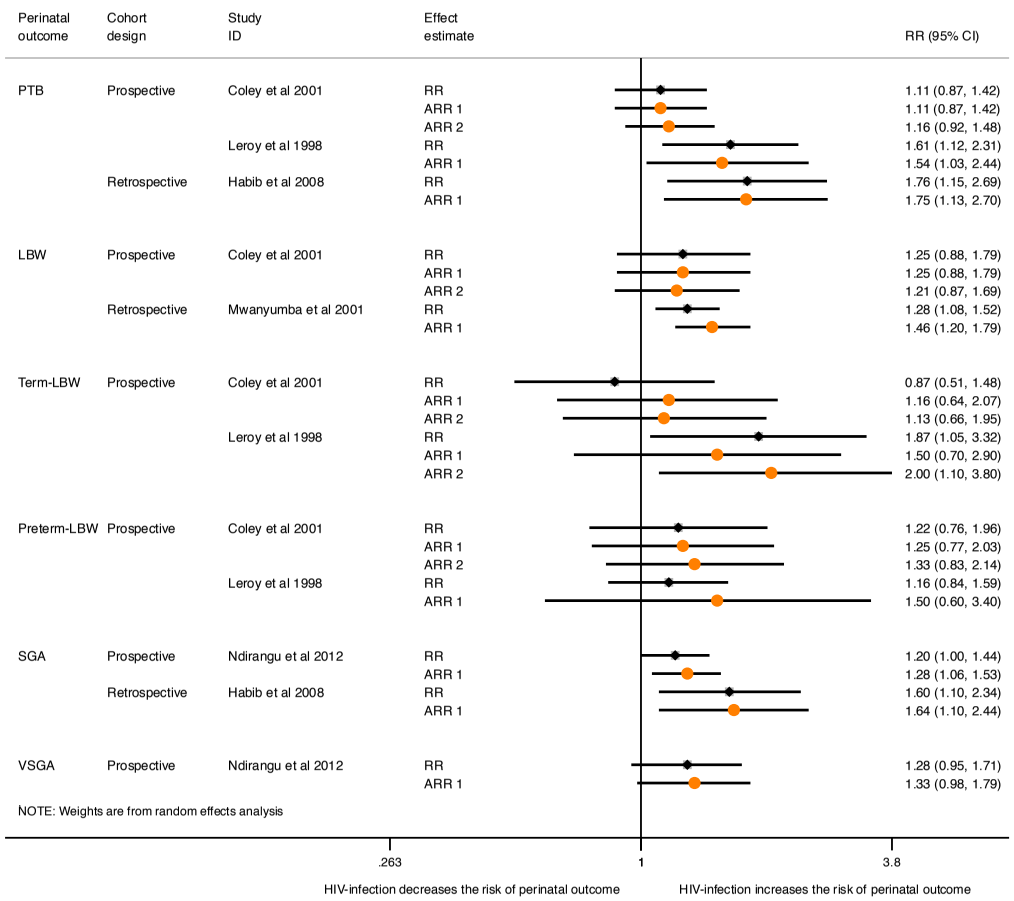


Figure 3.66: Sensitivity analysis of the adjustment for confounding factors in studies that assessed the association between ART-naïve maternal HIV-infection and perinatal outcomes using relative risk (RR) estimates. Sensitivity analysis displaying the reported unadjusted relative risk (RR) and adjusted relative risk (ARR) for the association between ART-naïve maternal HIV-infection and perinatal outcomes. Adjusted effect estimates are presented in orange. Studies are listed in alphabetical order of first author within each category. Covariate factors adjusted for are detailed in Table 3.6. For all analyses ARR 1 = multivariate analysis. For Coley et al. (2001)[244] ARR 2 = Age-adjusted RR. For Leroy et al. (1998)[250] ARR 2 = Mantel-Haenzel estimate adjusting for prematurity. RR (95%CI) = relative risk with 95% confidence interval. PTB = preterm birth, LBW = low birthweight, SGA = small for gestational age, VSGA = very small for gestational age. Source: Wedi et al. 2016[3].

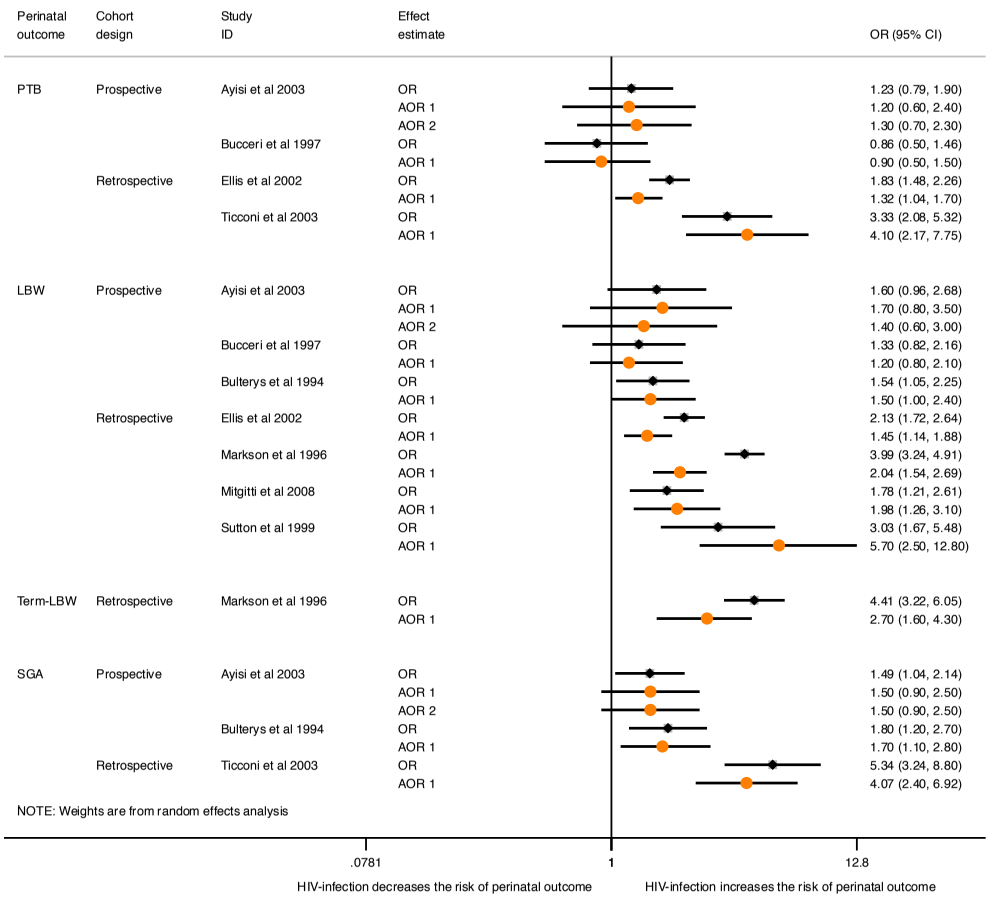


Figure 3.67: Sensitivity analysis of the adjustment for confounding factors in studies that assessed the association between ART-naïve maternal HIV-infection and perinatal outcomes using odds ratio (OR) estimates. Sensitivity analysis displaying the reported unadjusted odds ratio (OR) and adjusted odds ratio (AOR) for the association between ART-naïve maternal HIV-infection and perinatal outcomes. Adjusted effect estimates are presented in orange. Studies are listed in alphabetical order of first author within each category. Covariate factors adjusted for are detailed in Table 3.6. For all analyses ARR 1 = multivariate analysis. For all analyses AOR 1 = multivariate analysis, except for Ayisi et al. (2003)[240] where AOR 1 = multivariate analysis in primigravidae, and AOR 2 = multivariate analysis in multigravidae. OR (95%CI) = odds ratio with 95% confidence interval. PTB = preterm birth, LBW = low birthweight, SGA = small for gestational age, VSGA = very small for gestational age. Source: Wedi et al. 2016[3].

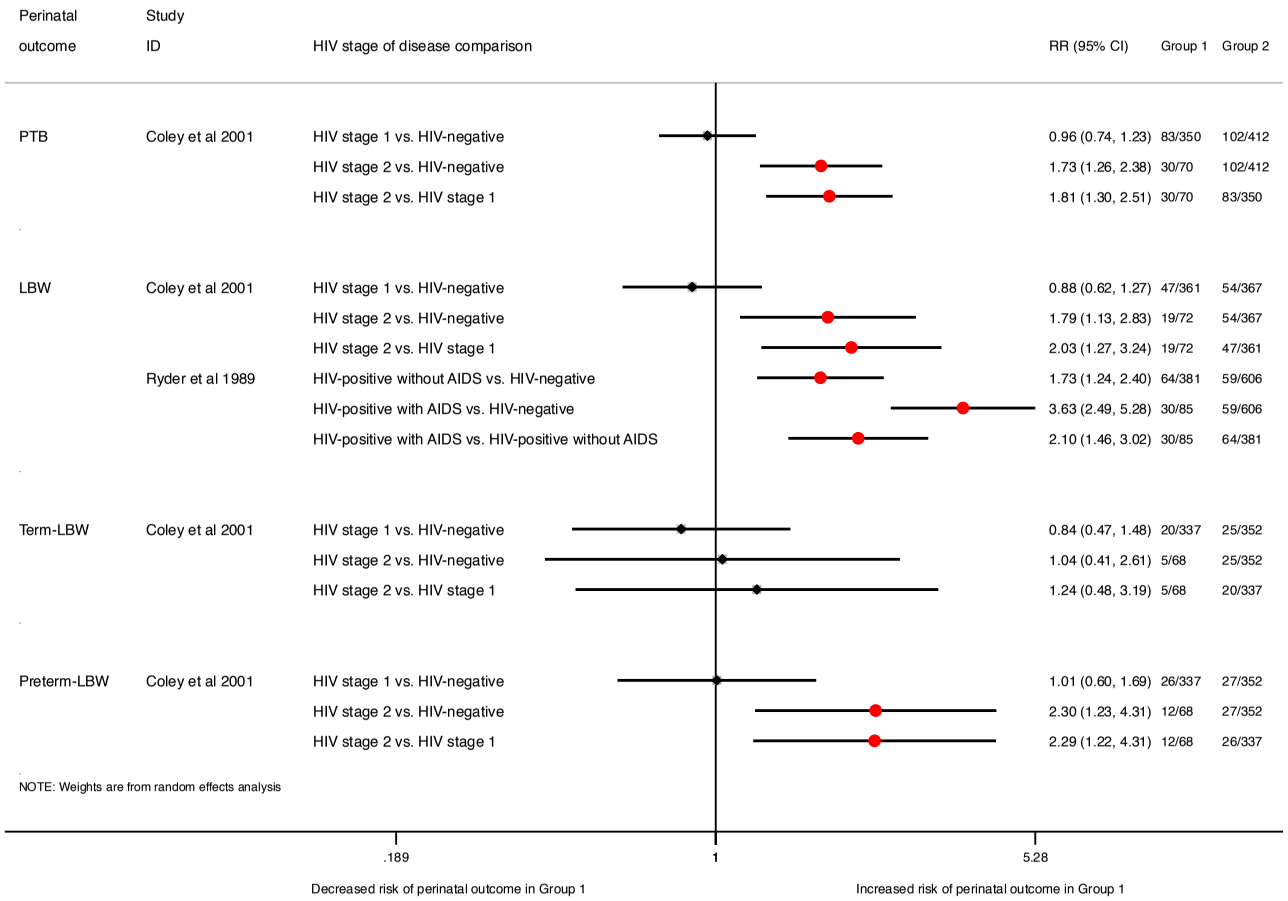


Figure 3.68. Perinatal outcomes associated with ART-naïve maternal HIV infection, by HIV stage of disease.
Summary forest plot displaying the association between ART-naïve maternal HIV-infection and perinatal outcomes, stratified according to the HIV stage of disease. Relative risks displayed as Group 1/Group 2. Group 2 = group adjacent to line of no effect (e.g. Coley et al. (2001)[244] - HIV stage 1 vs. HIV-negative: HIV-stage 1 = Group 1, HIV-negative = Group 2). Statistically significant effects presented in red. RR (95%CI) = relative risk with 95% confidence interval. PTB = preterm birth, LBW = low birthweight. Source: Wedi et al. 2016[3].

3.4 Discussion

The aim of this systematic review and meta-analysis was to assess whether ART-naïve maternal HIV-infection increased the risk of adverse perinatal outcomes.

3.4.1 Summary of findings

The evidence presented shows that maternal HIV-infection significantly increases the risk of PTB, LBW, SGA and stillbirth, with weak evidence to support an association with term-LBW and preterm-LBW. There was no evidence to support an association between maternal HIV and VPTB, VSGA, VLBW, miscarriage or NND.

The HIV epidemic is characterised by strong geographic polarisation, with more than two-thirds of the disease burden occurring in 15 countries, 10 of which are in sub-Saharan Africa[124]. Consequently, the majority of included studies (n=25; 71%) were conducted in sub-Saharan Africa, and the effect of HIV on perinatal outcomes was most prominent and consistent in these studies[3]. Subgroup and sensitivity analyses – which addressed underlying causes of heterogeneity – showed a persisting association between maternal HIV-infection and perinatal outcomes irrespective of the quality of studies, the method used to estimate GA and the adjustment for confounding factors. Furthermore, the effect of HIV-infection on perinatal outcomes increased exponentially with more advanced clinical stage of disease.

3.4.2 Comparison of results to previous systematic review

Brocklehurst et al. (1998) conducted the only other review to assess the association between ART-naive maternal HIV-infection and perinatal outcomes[269]. This review was conducted during the early stages of the epidemic and only included a small number of prospective studies – most of which were abstracts – published between 1983–96 (17 full text articles and 14 abstracts). The majority of perinatal outcomes assessed in the previous review were poorly or not defined; the methods used to

estimate GA were not assessed and heterogeneity was not explored, thus increasing the risk bias. A fixed-effect model was used to pool the outcome data, despite the presence of substantial between-study heterogeneity. Despite these limitations, Brocklehurst et al. (2008) showed a significant increase in the risk of PTB (OR 1.83; 95%CI 1.63-2.06), stillbirth (OR3.91; 95%CI 2.65-5.77) and LBW (OR 2.09, 95%CI 1.86-2.35), which supports the findings of the present review[221].

3.4.3 Strengths

This systematic review has multiple strengths. In order to minimise any bias in the conduct and reporting of the review, a rigorous, protocol driven approach was used as recommended by the Cochrane and PRISMA guidelines[223,237]. The scientific literature search was extensive and rigorous, and was not restricted by language, study design or geographic location. The outcomes of interest were clearly defined *a priori*, and two independent reviewers assessed the eligibility and methodological quality of studies in a strict and transparent manner. A random-effects model was used to account for heterogeneity, and unexplained heterogeneity, bias and confounding were explored using subgroup and sensitivity analyses[3]. There was no evidence of publication bias[237].

3.4.4 Implications of findings

The evidence generated from this review suggests that a large proportion of adverse perinatal outcomes that occur in sub-Saharan Africa can be attributed to the large burden of maternal HIV-infections in the region. Three of these outcomes – PTB, LBW and SGA – are well established causes of neonatal and under-five mortality[6,35]. The increase in these outcomes, coupled with high rates of MTCT explains why the majority of the 21 priority countries failed to meet the child-related Millennium Development Goals (MDG) – i.e. reduce child mortality – and many saw an increase in the rate of neonatal and under-5 deaths between 1990-2015[130,270].

While the relationship between ART and perinatal outcomes is controversial, it is well established that ART improves maternal health and reduces MTCT[194,203]. This review has shown an association between the clinical stage of disease and adverse perinatal outcomes, which suggests that improved

maternal health following the initiation of ART will reduce the risk of adverse perinatal outcomes in HIV-positive women. The global commitment to diagnose, treat and suppress 90% of all HIV-positive individuals, and reduce MTCT to <5% has led to a rapid increase in PMTCT coverage in sub-Saharan Africa; however, progress has been uneven across countries and significant challenges remain[130]. ART coverage in the region remains heterogeneous and incomplete, and in 2015, more than 70% of HIV-positive pregnant women in Nigeria, and 20% of HIV-positive pregnant women globally did not receive ART for PMTCT[130], and were at increased risk of PTB, LBW, SGA and stillbirth.

3.4.4.1 Theories regarding the underlying mechanism of action

The mechanisms underlying adverse perinatal outcome in HIV-infection remains poorly understood; however, a causal association is biologically plausible. Pregnancy is a complex process, dependent on a symbiotic interaction between the maternal endometrium and a semi-allogeneic fetus[271]. The conceptus' survival and optimal growth is dependent on placental function following adequate trophoblast invasion of the endometrium and maternal spiral arteries[271,272]. Maternal HIV-infection induces a chronic state of inflammation and immune activation, leading to generalised immune dysregulation[273]. This heightened inflammation and immune activation far exceeds the controlled and mild inflammatory activity observed in normal pregnancy, which could increase the risk of adverse perinatal outcomes[274]. Some have postulated that HIV-infection may affect placental growth and function[275]; others suggest that the effect may be due to intrauterine HIV-infection of the infant[276,277]. The majority of vertical HIV transmissions occur after 36 weeks, and in utero HIV infection is rarely associated with stillbirth[278,279], which makes a role of in utero HIV-infection in the pathogenesis of adverse perinatal outcome less likely[3]. Some have attributed these outcomes to the systemic effects of HIV-infection on maternal health[248], and while ART improves maternal health[124], increased rates of adverse perinatal outcomes continue to occur in HIV-positive women[280,281]. Urgent research is required on the biological mechanisms underlying these associations, as well as the role of ART in the development or prevention of these outcomes[3].

3.4.5 Limitations

This systematic review has a few limitations. The strict eligibility criteria meant that only a few studies were included, and some perinatal outcomes were not assessed. While this reduced the power of the review, the strict eligibility criteria reduced bias and ensured maximal validity for the perinatal outcomes reported. Very few studies adjusted for confounding factors, which precluded the exploration of confounders that are known to increase the risk of adverse perinatal outcomes (BMI, maternal VL, $CD4^+$ T-lymphocyte count and clinical stage of disease). Furthermore, the variety of confounders adjusted for in different studies precluded the pooling of adjusted estimates as is recommended[225]. The method used to estimate GA was not reported by a third of studies, and none estimated the GA with the most accurate method (i.e. early ultrasound <14 weeks)[282]. This limitation might have contributed to some of the heterogeneity observed in the meta-analyses of perinatal outcomes dependent on an accurate measure of GA (PTB, SGA, term-LBW, preterm-LBW, miscarriage, and stillbirth).

3.4.6 Conclusion

Given the contribution of PTB, SGA, LBW and SB to the global burden of perinatal, neonatal and child mortality and morbidity, the increase of these outcomes seen in maternal HIV infection, and possible further exacerbation caused by maternal ART, it is clear that maternal HIV infection should be a top priority in the global research, public health and development agendas. Moreover, because HIV infection, and maternal and child health are interlinked it will be very difficult to make progress in one area without addressing the other areas, as is highlighted by the slow progress towards MDG 4, 5 and 6[270]. Integrated approaches are needed which combine antenatal maternal HIV testing and treatment, skilful assistance at delivery, neonatal care, HIV testing and treatment of the newborn, and management of the short and long-term consequences of adverse outcomes in infants who survive the neonatal period.

Chapter 4: Perinatal outcomes associated with ART: a systematic review, pairwise and network-meta-analysis of RCTs.

4.1 Introduction:

In 2015, more than 80% of the estimated 1.2 million HIV-positive pregnant women in the world received antenatal ART for PMTCT[130]. Consequently only 8.9% of these women transmitted the virus to their infants; a significant decline from the pre-ART era where an estimated 40% of all exposed infants were infected[113]. The risk of MTCT is directly correlated with the maternal viral load, and the majority of infections occur during the antenatal (25-40%) and intrapartum periods (50%)[5]. The use of ART during pregnancy reduces the risk of MTCT transmission to <1%,[9] by decreasing the maternal viral load to undetectable levels, while simultaneously providing the fetus with both pre- and post-exposure prophylaxis[202].

In 1994, the ground-breaking PACTG 076 placebo-controlled trial showed that AZT monotherapy successfully reduced the risk of MTCT by 67.5% (95%CI 40.7 – 82.1)[205]. Since then, newer, more potent antiretrovirals have been developed and introduced during pregnancy as monotherapy, dual therapy or HAART, with the latter proving to be the most effective ART for PMTCT[203]. The advantages of HAART extend beyond PMTCT; it significantly improves maternal health, reduces the development of non-AIDS related non-communicable diseases, and prevents the transmission of HIV to seronegative partners[139,140,147,194]. These individual and population level benefits gave impetus to WHO to recommend that all HIV-positive individuals – including HIV-positive pregnant women – be initiated on life-long HAART at diagnosis irrespective of $CD4^+$ T-lymphocyte count or clinical stage of disease[12].

While the benefits of ART in pregnancy cannot be disputed, accumulating evidence from Europe[8,283-285] and sub-Saharan Africa[9,10,280] suggest a possible association between ART and adverse perinatal outcomes, including PTB, LBW, SGA and stillbirth. This has been disputed by US based researchers, who found no association between ART and perinatal outcomes, and attributed the observed effects to the underlying HIV infection[11,286,287]. These conflicting results have generated substantial debate on the safety of ART in pregnancy, with many questioning whether, in the urgency to reduce MTCT, ART policies were hastily adopted with insufficient consideration given to the safety profile of these drugs within the context of pregnancy[288-292].

Currently, almost all HIV-positive pregnant women receive ART for PMTCT, and in the near future all HIV-positive women will be receiving HAART at conception and throughout pregnancy. Therefore, it is imperative to clarify whether an unintended negative consequence of the most effective treatment for HIV and PMTCT is an increase in the burden of adverse perinatal outcomes.

This chapter explores the association between antenatal ART and perinatal outcomes, using a systematic review of RCTs, pairwise and network meta-analyses. Furthermore, this is the first network meta-analysis to determine which antenatal ART is safe (i.e. associated with the lowest risk of adverse perinatal outcomes) and still maintains a high efficacy for PMTCT (i.e. associated with the lowest risk of MTCT).

4.2 Research questions

This chapter aims to answer the following research questions:

1. Is ART associated with the following adverse perinatal outcomes: PTB, VPTB, spontaneous PTB (sPTB), spontaneous VPTB (sVPTB), LBW, VLBW, preterm-LBW, term-LBW, miscarriage, stillbirth and NND?
2. Which ART is associated with the lowest risk of adverse perinatal outcomes and the highest efficacy for PMTCT (i.e. lowest risk of MTCT)?

4.3 Methodology specific to this chapter

4.3.1 Search strategy

A systematic review was developed *a priori* using the Cochrane guidelines[223], and registered online (PROSPERO registration: CRD42013005637) (Appendix 4.1)[293]. A detailed description of the search strategy is found in Chapter 3; in short, five electronic literature databases [PubMed, CINAHL (Ebscohost), Global Health (Ovid), EMBASE (Ovid), and the Cochrane Central Register of Controlled Trials) and four trial databases (WHO International Clinical Trial Registry Platform, the Pan African Clinical Trials Registry, ClinicalTrial.gov database, and the ISRCTN Registry) were searched in order to identify studies that were published between 1 January 1980 to 19 November 2016. The references of all included studies and the preceding or relevant reviews[203,281,294-305] were checked for additional citations. All citations were imported into EndNote X6 electronic database.

4.3.2 Study selection and data extraction

One reviewer (OOW) independently scrutinized all the citations and abstracts retrieved from the search, and identified studies that were relevant to the systematic review. Three "second reviewers" (RC, CN and SH), also reviewed the search results and agreed with the titles and abstracts selected for inclusion. The relevant titles and abstracts were recorded in a predesigned, standardized Access database.

4.3.2.1 Eligibility criteria

One reviewer (OOW) obtained and screened the full text manuscripts for fulfilment of the following eligibility criteria:

- **Study design**: RCTs reporting sufficient data for the generation of summary statistics [relative risk (RR) and odds ratio (OR)].
- **Population**: Pregnant women with known perinatal outcomes.

- **Intervention**: ART received for a minimum of 30 days during the antenatal period. The ART received had to be described in sufficient detail so that one could ascertain the regimen (monotherapy, dual therapy or HAART), class (NRTI, NNRTI, PI) and drug (e.g. zidovudine). ART regimens were ranked as follows: (1) monotherapy - one drug, dual therapy - two drugs, HAART - three or more drugs. The ART class of the regimen was defined by the most complex antiretroviral drug in the regimen: (1) boosted PI, (2) un-boosted PI, (3) NNRTI, and (4) NRTI or NtRTI. HAART was defined according to the third unique drug included in the regimen (i.e. NNRTI), and the two other drugs were regarded as the backbone (e.g. NRTI)[178].
- **Comparison**: (1) Pregnant HIV-negative women from the same community, (2) pregnant HIV-positive women who did not receive any ART or placebo during the antenatal period, or (3) pregnant HIV-positive women who received a different ART than the index population.
- **Outcomes:** 12 perinatal outcomes were assessed including: (1) PTB (<37 weeks 0 days' gestation), (2) VPTB (<32 weeks 0 days' gestation), (3) sPTB (PTB after spontaneous initiation of labour), (4) sVPTB (VPTB after spontaneous initiation of labour) (5) LBW (BW <2500g), VLBW (BW <1500g), (6) term-LBW (≥37 weeks 0 days' gestation and BW <2500g), (7) preterm-LBW (<37 weeks 0 days' gestation and BW <2500g), (8) SGA (BW for GA <10th centile) or (9) VSGA (BW for GA <3rd centile) according to reference charts used by study sites, (10) miscarriage (spontaneous expulsion of a fetus <24 weeks 0 days' gestation), (11) stillbirth (any third trimester delivery of a stillborn infant with BW ≥1000g[15] or ≥24 weeks 0 days' completed weeks gestation[228] or ≥ 35cm body length[15]), (12) NND (death within the first 28 days of life[39]), (12) MTCT.

Studies were excluded if they reported ambiguous eligibility criteria, used an inappropriate control group, the duration of ART received during the antenatal period for the index population was <30 days, or participants in one group received an additional intervention (e.g. anti-tuberculosis treatment). Furthermore, studies were excluded if the perinatal outcomes of interest were not defined or the study definition differed to that specified in the study protocol (Appendix 4.1).

Two reviewers (OOW & JH) resolved any ambiguity about study eligibility and the uniqueness of the study population. Multiple manuscripts on the same cohort were included if they reported different perinatal outcomes, or ART comparisons. One reviewer (OOW) extracted the study, population and treatment level data on pre-designed standardised Excel worksheets, which was checked by second reviewers (CN and SH). A third reviewer (JH) served as an arbiter for any disagreements between the first (OOW) and second reviewers. In instances where additional information was required, the original author of the manuscript was contacted.

4.3.3 Risk of bias assessment for RCT

The Cochrane risk of bias tool was used to assess the methodological quality of RCTs (Appendix 4.2). Each trial was investigated for 6 sources of bias (Table 4.1) using the following domains: allocation sequence, allocation concealment, blinding (staff, participants or outcome assessors), incomplete outcome data, selective outcome reporting, other sources of bias. The risk of bias for each trial was graded as high or low depending on whether bias was detected or not (Appendix 4.3). In instances where there was insufficient information provided to determine whether a form of bias was present – or absent – the trial was regarded as having an "unclear" risk of bias (Appendix 4.3).

Table 4.1: Bias assessed in randomised controlled trials

Source: adapted from Higgins et al 2008[223]

Type of bias	Description	Domain in risk of bias tool
Selection bias	Systematic differences in the baseline characteristics of the groups being compared	Sequence generation Allocation concealment
Performance bias	Systematic differences between the groups in the exposure to factors or the care provided other than the intervention of interest	Blinding Other sources of bias
Attrition bias	Systematic differences between groups in the withdrawal from the trial	Incomplete outcome data Blinding
Detection and misclassification bias	Systematic differences between groups in how outcomes were determined	Blinding Other sources of bias
Reporting bias	Systematic differences between reported findings and unreported findings [a].	Selective outcome reporting

[a] This was assessed by comparing the reported outcomes in the manuscript to outcomes pre-specified in the trial protocol.

4.3.4 Statistical analysis

4.3.4.1 Data extraction on ART and perinatal outcomes

One reviewer (OOW) extracted dichotomous perinatal outcome data for participants who did and did not experience the outcome of interest, according to the ART they received during the antenatal period. ART data were stratified according to the regimen (e.g. monotherapy), class (e.g. NRTI), drug (e.g. lamivudine), and when treatment was initiated (i.e. preconception, antenatal, mixed or unspecified). The perinatal outcome data – stratified by treatment groups – were recorded in 2x2 tables, and used to compute a relative risk (RR) and 95%CI as part of the pairwise and network meta-analyses.

4.3.4.2 Pairwise meta-analysis

In order to assess the association between ART and perinatal outcomes, pairwise meta-analyses were conducted when two or more trials reported evidence on the same ART and perinatal outcome. A random-effect model was used to calculate the weighted summary RR and 95%CI, and a confidence interval including 1 (e.g. 0.2 – 1.5) was considered as not significant. Three pairwise meta-analyses were conducted for each outcome – provided there were sufficient data – the first assessed the association between the specific ART (e.g. AZT/3TC/EFV) reported and adverse perinatal outcome. The second and third pairwise meta-analyses assessed the association between the antiretroviral regimen (i.e. HAART), class of antiretroviral (e.g. NRTI) and perinatal outcome. The I^2 statistic was used to measure the proportion of between-study heterogeneity not attributable to random error, and was interpreted as no heterogeneity (<25%), low heterogeneity (25-49%), moderate heterogeneity (50-75%) and high heterogeneity (>75%)[230]. All results are represented on forest plots.

4.3.4.3 Network meta-analysis

There are currently more than 25 competing antiretroviral drugs in clinical use for the management of HIV-infection; however, pairwise meta-analysis only allows one to compare two treatments at any given time, (e.g. A versus B) (Figure 4.1A). If a third drug is to be compared with treatment A or B, a second pairwise meta-analysis must be conducted (e.g. A versus C), assuming sufficient

direct evidence (i.e. data derived from studies directly comparing the two treatments) is reported (Figure 4.1 A)[306]. A second limitation of pairwise meta-analysis is its reliance on direct evidence for all comparisons assessed[306]. In order to obtain approval for drug licensing, most ART are compared to the standard of care or a placebo in a head-to-head RCT[306]. The nature of these comparisons mean that direct evidence is only available for new ARTs versus placebo or standard of care; therefore, one cannot compare the relative efficacy of two new or competing antiretroviral drugs using a pairwise meta-analysis[306].

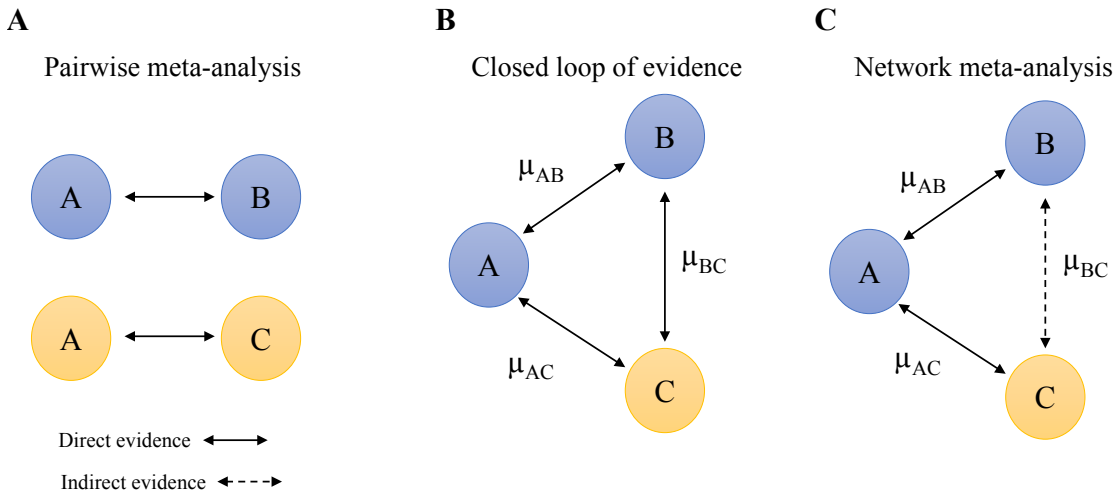


Figure 4.1. Pairwise meta-analysis versus network meta-analysis.
Image adapted from Salanti et al 2012[306], Cameron et al 2015[307]

A network meta-analysis (i.e. multi-drug meta-analysis) allows one to statistically compare and rank all treatment options available for a specific medical condition according to its relative efficacy or side-effect profile, thus allowing one to choose the best treatment option available[308]. Furthermore, it improves the precision of the estimated effect size and allows one to estimate effects for comparisons where head-to-head trials have not been conducted or reported[306].

The assumption underlying network meta-analysis is that of transitivity[308]. In figure 4.1B, three effect estimates can be generated from the evidence network:

- μ_{AB} = comparison between treatment A and B

- μ_{AC} = comparison between treatment A and C
- μ_{BC} = comparison between treatment B and C

This is referred to as a "closed loop of evidence" as it provides both direct and indirect evidence for each comparison (Figure 4.1B)[308]. The transitivity assumption refers to the inter-relatedness of the three effect estimates, and assumes that the indirect estimate is a true reflection of the comparison that has not been measured in a head-head RCT (e.g. μ_{BC} in figure 4.1 C)[306]. This assumption is represented by the following consistency model[308]:

$$\mu_{BC} = \mu_{AC} - \mu_{AB} \quad (1)$$

The principle underlying the consistency model (1), and the assumption of consistency – an extension of the transitivity assumption across a closed loop of evidence – is that there is agreement between the indirect and direct estimates[302]. Therefore, one can determine the effect estimate for each of the three treatment comparisons (e.g. μ_{BC}), using either direct evidence (i.e. studies reporting on comparisons between drug B versus C), indirect evidence (i.e. studies reporting on comparisons between drug A versus C and A versus B) or both (direct and indirect evidence = mixed effect estimate)[302,304].

In an effort to rank the ART, antiretroviral regimens and classes of antiretrovirals according to their relative risk of adverse perinatal outcome, and contrast this with their relative efficacy for PMTCT, I conducted network meta-analyses for RCTs that reported on the same perinatal outcome, and the rate of MTCT within each treatment arm. For each adverse perinatal outcome and MTCT, I conducted the following six analyses:

4.3.4.3.1 Network plot

The Stata function "network plot" was used to generate a network plot. This plot graphically represents the direct and indirect comparisons between ARTs assessed in the network meta-analysis[309]. Within each network plot, each ART being compared is represented by a circle (Figure 4.2); the size of the circle represents the number of participants in individual comparisons who received the ART. The lines connecting individual circles represent the direct comparisons made in

each trial between ART (e.g. zidovudine versus placebo). The width of the lines represents the number of trials that report on each comparison and the colours of the lines indicate the risk of bias of the trials which conducted the comparison; green = low risk of bias, red = high risk of bias, and yellow = unclear risk of bias.

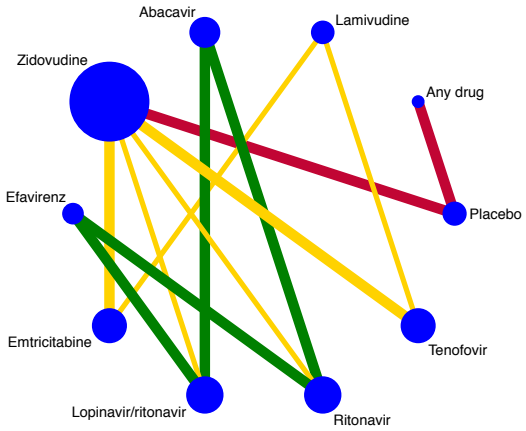


Figure 4.2. Network plot of antiretroviral drug comparisons

4.3.4.3.2 Network meta-analysis

A contrast-based network meta-analysis, using multivariate random-effects meta-analysis and meta-regression was used to assess the association between ART and individual perinatal outcomes[310,311]. This model assumes a common heterogeneity parameter for all comparisons[310, 311]. The STATA function "network set up was used to convert the raw data into the appropriate format for the analysis, and the Stata function "network meta c" was used to perform the network meta-analyses[308]. The effect estimates generated are reported as a RR with a 95%CI. All summary effects are represented on a forest plot, and league table.

4.3.4.3.3 Surface under the cumulative ranking curve (SUCRA)

The estimates generated from the network meta-analysis are then used to rank each ART according to its associated risk of adverse perinatal outcome (i.e. safety) and, secondly, its ability to prevent MTCT (i.e. efficacy). The Stata function "network rank min" was used to generate the ranking

probabilities for each ART to assume each possible rank among the available treatment options in the network[312]. These ranking probabilities are represented as a cumulative ranking curve on a rankogram[310]. The Stata function "sucraprob*" is then used to rank each ART according to its safety or efficacy using the surface under the cumulative ranking (SUCRA) probabilities[310]. SUCRA is a simple transformation of the mean rank of each ART, and compares each ART to an imaginary ART that is always best (i.e. associated with the lowest risk of adverse perinatal outcome or MTCT), with no uncertainty[313,314]. It is expressed as a percentage, with 100% indicating that the ART is the best treatment – i.e. associated with the lowest risk of adverse perinatal outcome or MTCT – and 0% indicates that the treatment is the worst regimen – i.e. associated with the highest risk of adverse perinatal outcome or MTCT[310]. In addition to the SUCRA percentage, the mean rank (MeanRank) of each ART and the probability for each ART being the best (PrBest) is also reported.

4.3.4.3.4 Cluster ranking plot of individual adverse perinatal outcomes and PMTCT

In order to directly compare each ARTs associated risk of adverse perinatal outcome (i.e. safety), with its efficacy for PMTCT, the Stata function "clusterank" was used to plot each ART's SUCRA value for safety against its SUCRA value for PMTCT[311].

4.3.4.3.5 Inconsistency

A test for inconsistency allows one to determine whether differences between the direct and indirect estimates - for the same treatment comparison - is due to bias or genuine diversity[308,310]. Each network was investigated for global inconsistency (global test of inconsistency) using a design-by-treatment interaction model, and a p-value >0.05 was considered as evidence of inconsistency[51]. Evidence of statistically significant inconsistency was explored by comparing individual direct and indirect estimates within the network by side-splitting[310].

4.3.4.4 Publication bias

The Peters' test was used to identify any presence of publication bias in pairwise meta-analyses including at least 10 studies[236]. Comparison-adjusted funnel plots were used to assess the presence of publication bias in network meta-analyses[309].

STATA version 13 (College Station, TX, USA) was used to conduct all analyses, and results are reported according to PRISMA guidelines for network meta-analyses.

4.4 Results

4.4.1 Results of search

The search strategy yielded 69,173 citations of which 1925 full text articles and abstracts were retrieved and screened for eligibility (Figure 4.3). Eighty (80) manuscripts met the eligibility criteria, and an additional 8 manuscripts were identified by screening the references of the included studies and previous reviews (Figure 4.3). The 88 manuscripts identified included 10 RCTs and 78 cohort studies. For the purposes of this analysis, I focus on the 10 RCTs[9,204-206,209,315-319].

4.4.2 Description of included trials

Table 4.2 provides detailed information on the characteristics of each trial. The 10 RCT manuscripts reported on 7 unique trials – ANRS 135 PRIMEVA[209]; Kesho Bora[315]; PACTG076[205,319]; PHPT-5[206]; PROMISE[9]; PROMOTE[316,317] and Mma Bana[204, 318] – including 6287 pregnant women randomly assigned to 8 different ART groups (Figure 4.4, Table 4.2). These 7 trials reported on the association between ART and the following perinatal outcomes; MTCT (7 trials), PTB (7 trials), sPTB (3 trials), VPTB (4 trials), LBW (6 trials), VLBW (4 trials), SGA (2 trial) and NND (1 trial) (Figure 4.3). None of the trials reported on the following outcomes – as defined by the systematic review protocol – sVPTB, preterm-LBW, term-LBW, miscarriage or stillbirth.

The trials were conducted between 1991 and 2014. Four trials (57.1%) were conducted in sub-Saharan Africa and contributed to 83.3% (5176/6287) of participants included in this analysis. The remaining 3 trials enrolled participants from Europe, the Americas and Asia (Table 4.2).

4.4.2.1 Risk of bias

All the included trials were found to have a high risk of bias (Table 4.2, Table 4.3) mostly because of a lack of blinding (6 trials; 85.7%) or due to a high probability of misclassification bias because they used inaccurate methods to estimate the GA (5 trials; 71.4%) (Table 4.3).

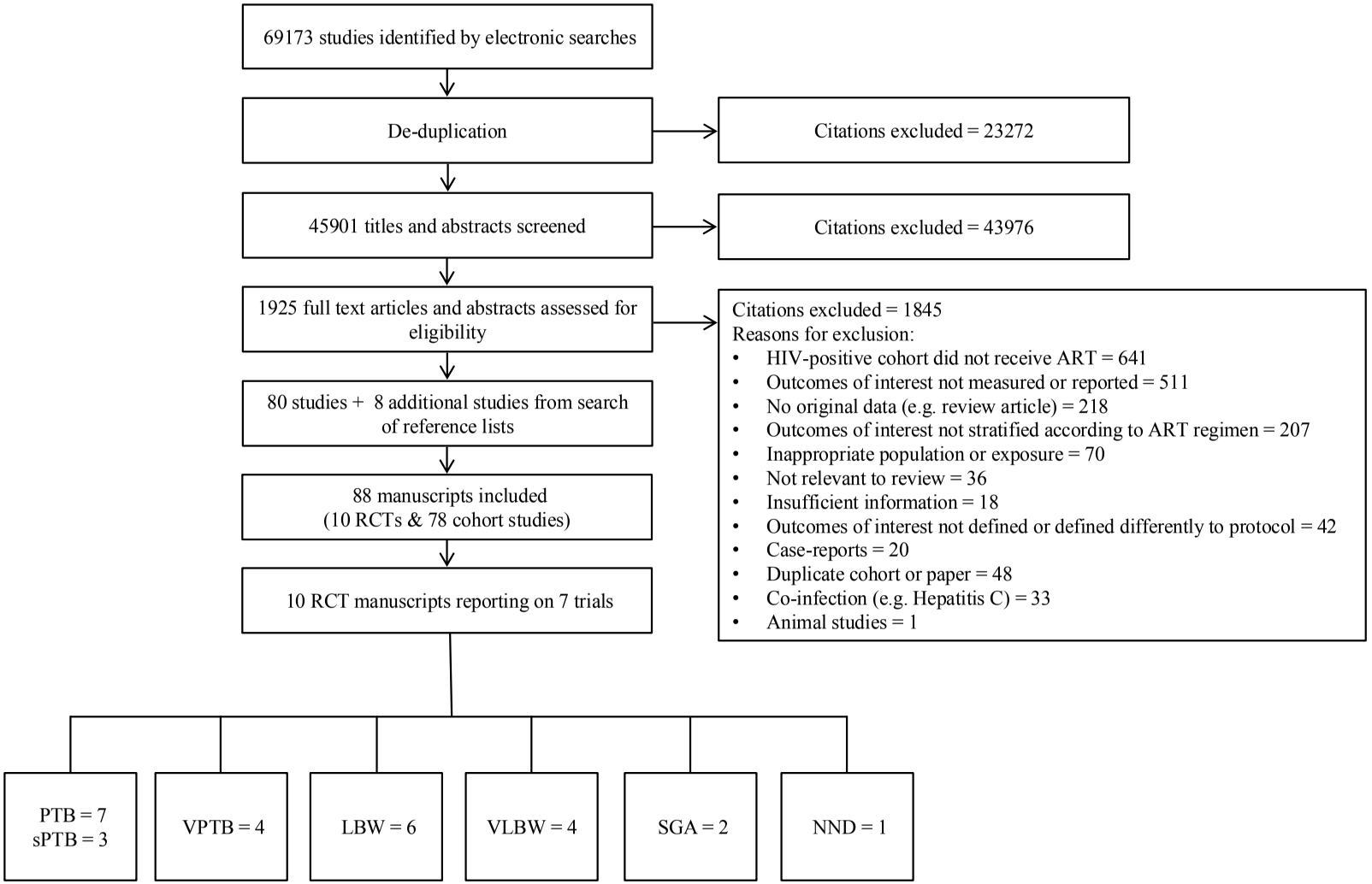


Figure 4.3: Schematic representation of study selection process.

Abbreviations: ART = antiretroviral treatment, HIV = Human Immunodeficiency Virus, LBW = low birthweight, NND = neonatal death, PTB = preterm birth, RCT = randomised controlled trial, SGA = small for gestational age, sPTB = spontaneous PTB, VLBW = very low birthweight, VPTB = very preterm birth.

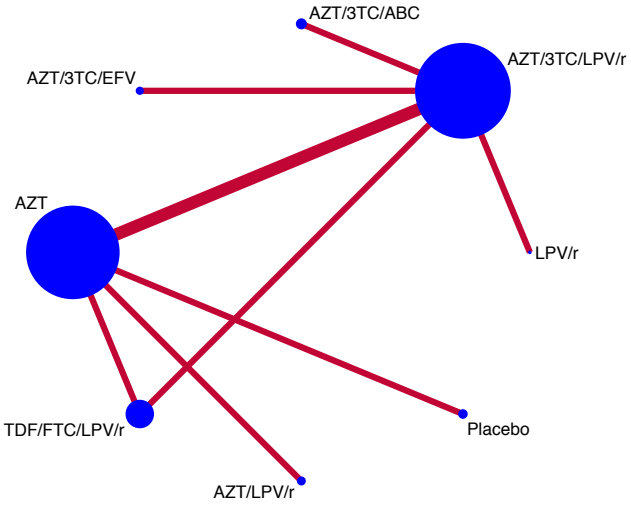


Figure 4.4: Network plot illustrating direct comparisons between ART reported by RCTs. Each circle represents a treatment group. The size of the circle represents the number of participants who received the ART. The lines connecting the circles represent direct comparisons of ART reported by the RCTs. The red colour of the connecting lines represents a high risk of bias in trials reporting the treatment comparison and the width of the lines represents the number of trials reporting on the treatment comparison.

4.4.2.2 Treatment arms

The majority of trials (5 trials; 71.4%) had two treatment arms, and the PHPT-5 trial and PROMISE trial had three treatment arms (Table 4.2). The results of the PROMISE trial pertaining to perinatal outcomes are reported over two periods (period 1 and 2, and period 2 only) in order to maintain randomisation. In the initial study period (period 1 and 2), participants were randomised to one of two antenatal treatment groups, namely NRTI-based monotherapy or NRTI-based PI HAART. During the second half of the study (period 2 only), a NtRTI-based PI HAART regimen was included as a third treatment arm; therefore, participants could now be assigned to one of three treatment arms following randomisation (Table 4.2). A detailed description of the treatment arms will be discussed in section 4.3.4, and detailed information on additional ART received during labour (intrapartum treatment) is available in Appendix 4.4.

Table 4.2: Trial characteristics

Study	Trial	Country	Recruitment time period	Number of sites	Twin pregnancies	Number of pregnant women randomised (n)	Perinatal outcomes	Antenatal treatment comparisons	Methods to correct for confounding factors	Gestational age estimation method	Risk of bias
Sub-Saharan Africa											
Fowler, et al. 2016 (period 1 and 2)[9]	Promoting Maternal and Infant Survival Everywhere - PROMISE	India, Malawi, South Africa, Tanzania, Uganda, Zambia, Zimbabwe,	April 2011 - 10 September 2014	14	Yes	3088	PTB, LBW, VLBW	1. NRTI-based monotherapy, 2. NRTI-based PI-HAART	Stratification, randomisation	Obstetric estimate (unspecified), neonatal assessment (Ballard)	High
Fowler, et al. 2016 (period 2 only)[9]	The Promoting Maternal and Infant Survival Everywhere - PROMISE	India, Malawi, South Africa, Tanzania, Uganda, Zambia Zimbabwe	October 2012 - 10 September 2014	14	Yes	1230	PTB, LBW, VLBW	1. NRTI-based monotherapy, 2. NRTI-based PI HAART, 3. NtRTI-based PI HAART	Stratification, randomisation	Obstetric estimate (unspecified), neonatal assessment (Ballard)	High
Koss, et al. 2014[316]	PROMOTE - Pregnant Women and Infants Study	Uganda	December 2009 - September 2012	1	No (All singleton live births)	356	PTB, sPTB, VPTB	1. Boosted PI-based HAART, 2. NNRTI-based HAART	Stratification, randomisation, regression	LNMP confirmed by ultrasound (early and late)	High
Natureeba, et al. 2014[317]	PROMOTE - Pregnant Women and Infants Study	Uganda	December 2009 - March 2013	1	Yes	389	LBW, NND	1. Boosted PI-based HAART, 2. NNRTI-based HAART	Stratification, randomisation	LNMP confirmed by ultrasound (early and late)	High
Powis, et al. 2011[318]	Mma Bana Study	Botswana	July 2006 - May 2008	4	No (All singleton live births)	530	sPTB	1. NRTI-based HAART, 2. Boosted PI-based HAART	Stratification, randomisation, regression	LNMP confirmed by ultrasound (early and late)	High

Shapiro, et al. 2010[204]	Mma Bana Study	Botswana	July 2006 - May 2008	4	Yes	560	PTB, VPTB, LBW, VLBW	1. NRTI-based HAART, 2. Boosted PI-based HAART	Stratification, randomisation	LNMP confirmed by ultrasound (early and late)	High
The Kesho Bora Study Group, et al. 2011[315]	Kesho Bora Study	Burkina Faso, Kenya, South Africa	June 2005 - August 2008	5	Yes	824	PTB, VPTB, LBW, VLBW	1. NRTI-based monotherapy, 2. Boosted PI-based HAART	Stratification, randomisation	LNMP ultrasound (unspecified), SF-height	High
Europe and the Americas											
Connor, et al. 1994[205]	Pediatric AIDS Clinical Trials Group Protocol 076 Study Group (PACTG 076)	USA and France	11 April 1991 - 20 December 1993	59	Yes	477	LBW, VLBW	1. Placebo, 2. NRTI-based monotherapy	Stratification, randomisation, blinding	LNMP, ultrasound (late)	High
Sperling, et al. 1998[319]	Pediatric AIDS Clinical Trials Group Protocol 076 Study Group (PACTG 076)	USA and France	11 April 1991 - 20 December 1993	55	No (All singleton births)	477	PTB, SGA	1. Placebo, 2. NRTI-based monotherapy	Stratification, randomisation, blinding	LNMP, ultrasound (late)	High
Tubiana, et al. 2013[209]	The ANRS 135 PRIMEVA Phase 2/3 Randomised Trial	France	14 June 2007 - 29 June 2010	19	No (All singleton births)	107	PTB, sPTB, VPTB	1. Boosted PI-based monotherapy, 2. Boosted PI-based HAART	Randomisation	LNMP	High
Asia											
Lallemant, et al. 2015[206]	Perinatal HIV Prevention Trial-5 (PHPT-5)	Thailand	9 January 2009 - 30 September 2010	43	Yes	435	PTB, LBW, SGA	1. NRTI-based monotherapy, 2. NRTI-based monotherapy, 3. Boosted PI-based dual therapy	Stratification, randomisation	Unspecified	High

Abbreviations: AIDS = Acquired Immunodeficiency Syndrome, HAART = Highly Active Antiretroviral Treatment, LBW = low birthweight, LNMP = last normal menstrual period, NND = neonatal death, NNRTI = Non-Nucleoside Reverse Transcriptase Inhibitors, NRTI = Nucleoside Reverse Transcriptase Inhibitors (NRTIs), NtRTI = Nucleotide Reverse Transcriptase Inhibitors, PI = Protease Inhibitor, PTB = preterm birth, SF-height = symphysio-fundal height, SGA = small for gestational age, sPTB = spontaneous PTB, USA = United States of America, VLBW = very low birthweight, VPTB = very preterm birth.

Table 4.3: Risk of bias assessment

Study	Trial	Sequence generation		Allocation concealment		Blinding		Incomplete outcome data		Selective outcome reporting		There are no other sources of bias		Overal risk of bias
			Specify		Specify		Specify		Specify		Specify:		Specify:	
Sub-Saharan Africa														
Fowler, et al. 2016-period 1 and 2[9]	PROMISE	Yes*	Restricted randomisation was done. In period 1: If participant was HBV-negative, she was randomised in a 1:1 ratio to one of two arms (AZT or AZT/3TC/LPV/r). Participants who were co-infected with HIV/HBV were randomised in a 1:1:1 ratio to one of three arms (AZT, AZT/3TC/LPV/r or TDF/FTC/LPV/r). In period 2: Women were randomised in a 1:1:1 ratio to one of three arms (AZT, AZT/3TC/LPV/r or TDF/FTC/LPV/r). Randomisation was stratified according HBV infection status (positive or negative) and by country. Randomisation done using the Data Management Centre (DMC) Subject enrolment system. HBV-positives individuals who received TDF/FTC/LPV/r in period 1 were excluded from the analysis.	Yes*	Central allocation was used as randomisation was provided by DMC Subject Enrolment System.	No	No blinding, it's an open-label trial.	Yes*	Missing outcome data balanced in numbers across intervention groups, with similar reasons for missing data across groups. Intent-To-Treat analysis used	Yes*	Interim results reported as specified in study protocol.	No	Method used to determine gestational age was either by neonatal assessment (Ballard) or other obstetric measures, thus outcomes prone to misclassification bias	High
Fowler, et al. 2016-period 2 only[9]	PROMISE	Yes*	Restricted randomisation was done. Women were randomised in a 1:1:1 ratio to one of three arms (AZT, AZT/3TC/LPV/r or TDF/FTC/LPV/r). Randomisation was stratified according to	Yes*	Central allocation was used as randomisation was provided by DMC Subject Enrolment System.	No	No blinding, it's an open-label trial.	Yes*	Missing outcome data balanced in numbers across intervention groups, with similar reasons for missing data across groups.	Yes*	Interim results reported as specified in study protocol.	No	Method used to determine gestational age was either by neonatal assessment (Ballard) or other obstetric	High

			HBV infection status (positive or negative) and by country. Randomisation done using the Data Management Centre (DMC) Subject enrolment system.						Intent-To-Treat analysis used				measures, thus outcomes prone to misclassification bias	
Koss, et al. 2014[316]	PROMOTE	Yes*	Restricted randomisation was done. They randomised women in a 1:1 ratio after stratification by gravidity (gravida 1 vs gravida ≥ 2) and gestational age at enrolment (<24 weeks vs ≥24 weeks). Randomisation was performed in permuted blocks of 2 or 4. Randomisation was performed by computer generation.	Yes*	Sequentially numbered, opaque, sealed envelopes	No	No blinding, it's an open-label trial.	Yes*	Missing outcome data balanced in numbers across intervention groups, with similar reasons for missing data across groups. Intent-To-Treat analysis used	Yes*	This was a planned secondary analysis of preterm birth	Yes*	Gestational age determined by LNMP confirmed by ultrasound	High
Natureeba, et al. 2014[317]	PROMOTE	Yes*	Restricted randomisation was done. They randomised women in a 1:1 ratio after stratification by gravidity (gravida 1 vs gravida ≥ 2) and gestational age at enrolment (<24 weeks vs ≥24 weeks). Randomisation was performed in permuted blocks of 2 or 4. Randomisation was performed by computer generation.	Yes*	Sequentially numbered, opaque, sealed envelopes	No	No blinding, it's an open-label trial.	Yes*	Missing outcome data balanced in numbers across intervention groups, with similar reasons for missing data across groups. Intent-To-Treat analysis used	Yes*	Outcomes reported specified in study protocol	Yes*	Gestational age determined by LNMP confirmed by ultrasound	High
Powis, et al. 2011[318]	Mma Bana Study	Yes*	Restricted randomisation was done. They used permuted blocks stratified according to clinical sites, and randomisation was performed by a computer-generated list	Yes*	Central allocation was used, where each study site phoned the data management centre in Gaborone for the randomisation assignment.	No	The healthcare workers, participants and outcome assessors had knowledge of the HAART regimen.	Yes*	Missing outcome data balanced in numbers across intervention groups, with similar reasons for missing data across groups	Yes*	Outcomes reported specified in study protocol and this was a planned secondary	Yes	Gestational age determined by LNMP confirmed by ultrasound	High

										analysis of the data.				
Shapiro, et al. 2010[204]	Mma Bana Study	Yes*	Restricted randomisation was done. They used permuted blocks stratified according to clinical sites, and randomisation was performed by a computer-generated list.	Yes*	Central allocation was used, where each study site phoned the data management centre in Gaborone for the randomisation assignment.	No	The healthcare workers, participants and outcome assessors had knowledge of the HAART regimen.	Yes*	Missing outcome data balanced in numbers across intervention groups, with similar reasons for missing data across groups. Intent-To-Treat analysis used.	Yes*	Outcomes reported specified in study protocol	Yes	Gestational age determined by LNMP confirmed by ultrasound	High
The Kesho Bora Study Group, et al. 2011[315]	Kesho Bora Study	Yes*	Restricted randomisation was done. Randomisation occurred at 34 weeks' gestation initially, and at 28 weeks from Dec 2006, and was stratified by centre and planned infant feeding mode, balanced in blocks of six or eight by a computer generated random sequence.	Yes*	Sealed envelopes containing group assignments were prepared at the study coordinating centre and were marked externally with the randomisation sequence number. The assigned envelope was only opened once all enrolment procedures had been completed	No	Patients and study investigators were not blinded to treatment allocation.	Yes*	Intent-To-Treat analysis used and all participants accounted.	Yes*	Outcomes reported specified in study protocol	No	Gestational age determined by LNMP, symphysio-fundal height, ultrasound when available, prone to misclassification bias:	High
Europe and the Americas														

Connor, et al. 1994[205]	PACTG 076	Unclear	Women were stratified by gestational age and then randomised, however no description given of randomisation process	Unclear	No description of allocation method	Unclear	Study described as double-blinded but no description on how this was enforced. They also state that open label zidovudine was prescribed for one woman in each group before delivery.	Yes*	Missing outcome data balanced in numbers across intervention groups, with similar reasons for missing data across groups. Furthermore, they conducted an intention-to-treat analysis.	Unclear	Unable to assess the full extent of selective reporting as the original study protocol is not available	No	Sperling et al 1998 states that gestational age estimation based on LNMP unless corrected by pre-entry ultrasound; therefore, possible misclassification bias as some women were enrolled in the third trimester. While the study was stopped early, which could introduce bias, they report that this was done at the first planned interim efficacy analysis.	High
Sperling, et al. 1998[319]	PACTG 076	Unclear	No description of randomisation process. Connor et al 1994 reported that stratification was used as well.	Unclear	No description of allocation method	Unclear	Study described as double-blinded but no description on how this was enforced	Yes*	Missing outcome data balanced in numbers across intervention groups, with similar reasons for missing data across groups, and they conducted an intention-to-treat analysis	Unclear	Unable to assess the full extent of selective reporting as the original study protocol is not available.	No	Gestational age calculation based on LNMP unless corrected by pre-entry ultrasound; therefore, possible misclassification bias as some women were enrolled in the third trimester	High
Tubiana, et al. 2013[209]	ANRS 135 PRIMEVA	Unclear	The only information provided is that centralized randomisation was performed. No specification given on how this was done	Yes*	Central allocation was used, where the treatment group assignment was communicated to the site investigator via fax.	No	Patients and physicians were not blinded to the treatment group assignment.	Yes*	Intent-To-Treat analysis used and all participants accounted for.	Unclear	Unable to assess the full extent of selective reporting as the original study protocol is not available.	No	Gestational age determined by LNMP, so possibility of misclassification bias.	High
Asia														

Lallemant, et al. 2015[206]	PHPT-5	Yes*	Restricted randomisation was used. Women were randomised in a 1:1:1 ratio, and randomly assigned in blocks of six, stratified by site, gestation (below or ≥32 weeks) and until 4 June 2010 by $CD4^+$ T-lymphocyte count (below or ≥350 cells/μl).	Yes*	Central allocation was performed by fax, and randomisation lists were encrypted in a database before study initiation.	No	No blinding conducted for the antenatal component of the treatment received, only the intrapartum component.	No	The trial was stopped early because the Thai Ministry of Public Health recommended that all pregnant women be initiated on AZT/3TC/LPV/r. All women who delivered after the trial was stopped and unblinded were not included in the analysis.	Unclear	Unable to assess the full extent of selective reporting as the original study protocol is not available	No	While they do not specify how gestational age was assessed, all women were enrolled in the third trimester; therefore, there is a possibility of misclassification bias.	High

Abbreviations: 3TC = lamivudine, ABC = abacavir, ART = antiretroviral treatment, AZT = zidovudine, DMC = data management centre, FTC = emtricitabine, HBV = hepatitis B, HIV = Human Immunodeficiency Virus, LNMP = last normal menstru period, LPV/r = lopinavir/ritonavir, PTB = preterm birth, RR = relative risk, TDF = tenofovir disoproxil fumarate.

4.4.3 Participant characteristics

Table 4.4 provides detailed information on participants recruited in each trial. The majority of women were Black African, from rural and urban settings in sub-Saharan Africa. The median maternal age at enrolment was 27 years; all women were enrolled during the second trimester (median = 26 weeks' gestation), and most births were vaginal deliveries.

Demographic factors were well distributed between study arms (Appendix 4.5), and non-infectious comorbidities were low and well distributed between treatment arms in each trial (Table 4.4). There were some differences between trials in the prevalence of co-infections: 33% of women in PROMOTE and 1% of women in Kesho Bora were diagnosed with malaria during pregnancy; almost half the participants in the PACTG076 trial were diagnosed with a bacterial (44%), viral (42%) or fungal co-infection (21%), and 11.9% of women in the Mma Bana trial had at least one STD during pregnancy as opposed to 21.5% of women included in PACTG076. Most trials reported a very low incidence of smoking and alcohol use within the cohorts, and only PACTG076 reported the use of intravenous drugs by 15.9% of included participants (Table 4.4). The rates of breastfeeding varied, between regions, with the highest rates observed in sub-Saharan Africa, and very low rates in other regions (Appendix 4.6).

The median $CD4^+$ T-lymphocyte count across the trials at study enrolment was 451.5 cells/µl, and the median VL was 11294.5 copies/ml (Table 4.4). The majority of trials (6 trials, 84.7%) only enrolled participants with a $CD4^+$ T-lymphocyte count above 200 cells/µl, and almost all participants were asymptomatic. PROMOTE was the only trial not to use a specific $CD4^+$ T-lymphocyte count threshold at study entry (Appendix 4.6). Additional information on the distribution of $CD4^+$ T-lymphocyte count and VL is available in Appendix 4.6.

Table 4.4: Participant characteristics

Study	Antenatal treatment group	Maternal age (years)	Race	Gestational age at recruitment (weeks) [Mean ± SD/ Median (IQR)]	Population characteristic	Caesarean section rate	$CD4^+$ T-lymphocyte count (cells/µL) [Median(IQR)] Enrolment	$CD4^+$ T-lymphocyte count (cells/µL) [Median(IQR)] Delivery	HIV stage of disease	Plasma HIV-1 RNA (VL) (copies/ml) [Median(IQR)] Enrolment	Plasma HIV-1 RNA (VL) (copies/ml) [Median(IQR)] Delivery
Sub-Saharan Africa											
Fowler, et al. 2016 (period 1 and 2)[9]	NRTI-based monotherapy	26 (22 - 30)	Black African = 96.9% Native Indian = 3.0% Other = 0.1%	26 (21 - 30)	Majority of cohort from Africa: 46.6% from East Africa (Uganda, Malawi, Tanzania), 33.1% from South Africa and 17.3% from Southern Africa (Zimbabwe, Zambia). Only 3.0% of cohort from India. 3.0% of participants had a positive hepatitis B status at enrolment. 1.0% of participants had an infection or infestation.	-	533 (433 - 678)	-	WHO 1 = 96.5% WHO 2 = 3.4% WHO 3 = 0.1% WHO 4 = 0.0%	6309 (1584 - 25118)	1258 (251 - 6309)
	NRTI-based PI-based HAART	26 (23 - 30)	Black African = 97.0% Native Indian = 3.0% Other = 0.1%	25 (21 - 30)		-	526 (439 - 650)	-	WHO 1 = 97.7% WHO 2 = 2.2% WHO 3 = 0.1% WHO 4 = 0.0%	7943 (1995 - 31622)	63 (39 - 251)
Fowler, et al. 2016 (period 2 only)[9]	NRTI-based monotherapy	25 (22 - 29)	Black African = 100% Native Indian = 0.0% Other = 0.0%	26 (21 - 31)	Majority of cohort from Africa: 56.3% from East Africa (Uganda, Malawi, Tanzania), 16.5% from South Africa and 27.0% from Southern Africa (Zimbabwe, Zambia). Only 0.2% of cohort from India. 3.0% of participants had a positive hepatitis B status at enrolment. 1.0% of participants had an infection or infestation.	-	530 (431 - 687)	-	WHO 1 = 96.6% WHO 2 = 3.4% WHO 3 = 0.0% WHO 4 = 0.0%	6309 (1584 - 25118)	1258 (251 - 6309)
	NRTI-based PI HAART	26 (23 - 30)	Black African = 100% Native Indian = 0.0% Other = 0.0%	26 (21 - 31)		-	540 (451 - 657)	-	WHO 1 = 97.3% WHO 2 = 2.7% WHO 3 = 0.0% WHO 4 = 0.0%	6309 (1584 - 25118)	39 (39 - 199)
	NtRTI-based PI HAART	26 (22 - 30)	Black African = 100% Native Indian = 0.0% Other = 0.0%	26 (22 - 31)		-	537 (432 - 691)	-	WHO 1 = 98.3% WHO 2 = 1.7% WHO 3 = 0.0% WHO 4 = 0.0%	7943 (1995 - 31622)	79 (39 - 398)
Koss, et al. 2014[316]	Boosted PI-based HAART	29 (25 - 33)	-	21 (17 - 25)	Women recruited from an antenatal clinic at a district hospital and other health centres situated in a rural area with high-intensity malaria transmission. 33.4% of participants were diagnosed with placental malaria. All women also received daily TMP-SMX prophylaxis against malaria in pregnancy, long lasting insecticide treated nets, multivitamins and condoms. 80.4% of deliveries occurred at a healthcare facility, and 19.6% occurred at home.	5.3%	368 (281 - 510)	-	WHO 1 = 96%	14125 (2398 - 58884)	-
	NNRTI-based HAART	30 (26 - 33)	-	21 (18 - 24)		5.3%	369 (271- 487)	-	WHO 1 = 96%	20417 (2884 - 72444)	-

Natureeba, et al. 2014[317]	Boosted PI-based HAART	29.0 ± 5.4	-	21.2 ± 4.3	Women recruited from an antenatal clinic at a district hospital and other health centres situated in a rural area with high-intensity malaria transmission. 33.3% of participants were diagnosed with placental malaria. All women also received daily TMP-SMX prophylaxis against malaria in pregnancy and long lasting insecticide treated nets as well as multivitamins and condoms. 80.4% of deliveries occurred at a healthcare facility, and 19.6% occurred at home.	5.3%	368 (282 - 506)	-	WHO 1 = 97.4% WHO 2 = 2.6% WHO 3 = 0.0% WHO 4 = 0.0%	12589 (1995 - 50119)	-
	NNRTI-based HAART	29.5 ± 5.4	-	21.1 ± 4.1		5.3%	374 (270 - 485)	-	WHO 1 = 92.1% WHO 2 = 6.7% WHO 3 = 0.5% WHO 4 = 0.0%	19952 (3162 - 63095)	-
Powis et al. 2011[318]	NRTI-based HAART	26.8 (23.0 - 31.6)	-	26 - 28 = 67.3% 29 - 31 = 16.7% 32 - 34 = 16.0%	Women recruited from government run antenatal clinics in urban and rural communities. All singleton live births. 11.9% of cohort had at least 1 sexually transmitted disease before 32 weeks' gestation. 3.8% of participants had a positive hepatitis B status at enrolment. The prevalence of maternal use of tobacco, alcohol or illicit substances is reported to be low.	0%	388 (305 - 516)	-	No AIDS defining disease	8709 (871 - 38018)	-
	Boosted PI-based HAART	26.0 (23.0 - 30.4)	-	26 - 28 = 67.4% 29 - 31 = 23.6% 32 - 34 = 9.0%		0%	405 (297 - 519)	-	No AIDS defining disease	8317 (1585 - 36308)	-
Shapiro et al. 2010[204]	NRTI-based HAART	26	-	27.1 (26.4 - 29.9)	Women recruited from government run antenatal clinics in urban and rural communities. 11.9% of cohort had at least 1 sexually transmitted disease before 32 weeks' gestation. 4.3% of participants were hepatitis B positive at enrolment. The prevalence of maternal use of tobacco, alcohol or illicit substances is reported to be low.	-	393 (305 - 514)	-	No AIDS defining disease	13,300 (2340 - 50.900)	-
	Boosted PI-based HAART	25	-	27.1 (26.4 - 29.9)		-	403 (297 - 514)	-	No AIDS defining disease	9,100 (2210 - 39,900)	-
The Kesho Bora Study Group et al. 2011[315]	NRTI-based monotherapy	27 (23 - 31)	-	<32 [a]	Participants recruited from antenatal clinics in 5 study sites. 8.3% of participants had an infectious disease including tuberculosis (2.3%), malaria (1.0%) and pneumonia (1.1%).	12.5%	339 (267 - 408)	416 (331 - 530)	WHO 1, 2 or 3	16218 (3802 - 54954)	-
	Boosted PI-based HAART	27 (24 - 31)	-	<32 [a]		10.9%	336 (282 - 408)	463 (383 - 603)	WHO 1, 2 or 3	16982 (4571 - 56234)	-

Europe and the Americas											
Connor et al. 1994[205]	Placebo	25	Caucasian = 16.6%, Black = 55.4%, Hispanic = 26.6%, Other = 1.3%	27 (14 – 34) [a]	Participants recruited from healthcare centres. 15.9% of participants had a history of injection drug use. 21.5% of participants had a sexually transmitted disease during pregnancy. 44% of participants had a bacterial infection (urinary tract infection, upper respiratory infections, bacterial vaginosis), 42% of had a viral infection (hepatitis B, hepatitis C, cytomegalovirus, herpes zoster, upper respiratory infections, herpes simplex virus, human papillomavirus, unclassifiable viral illness), 21% of participants had a fungal infection (fungal vulvovaginitis, tinea corporis) All hospital deliveries.	25.2%	538	-	CDC-B = 21% CDC-C = 2%	-	-
	NRTI-based monotherapy	25	Caucasian = 20.7% Black = 46.1% Hispanic = 30.6% Other = 2.6%	26 (14 – 34) [a]		29.4%	560	-	CDC-B = 21% CDC-C = 2%	-	-
Sperling et al. 1998[319]	Placebo	25	Caucasian = 16.6%, Black = 55.4%, Hispanic = 26.6%, Other = 1.3%	27 (14 – 34) [a]	Participants recruited from healthcare centres. 15.9% of participants had a history of injection drug use. 21.5% of participants had a sexually transmitted disease during pregnancy. 44% of participants had a bacterial infection (urinary tract infection, upper respiratory infections, bacterial vaginosis), 42% of participants had a viral infection (hepatitis B, hepatitis C, cytomegalovirus, herpes zoster, upper respiratory infections, herpes simplex virus, human papillomavirus, unclassifiable viral illness), 21% of participants had a fungal infection (fungal vulvovaginitis, tinea corporis) All hospital deliveries.	23.6%	538	-	CDC-B = 21% CDC-C = 2%,	-	-
	NRTI-based monotherapy	25	Caucasian = 20.7% Black = 46.1% Hispanic = 30.6% Other = 2.6%	26 (14 – 34) [a]		27.7%	560	-	CDC-B = 21% CDC-C = 2%	-	-

Tubiana et al. 2013[209]	Boosted PI-based monotherapy	30 (18 - 44)	-	26 (24 - 27)	72.5% of participants in the monotherapy group and 77.8% of participants in the HAART group were originally from sub-Saharan Africa. 2.9% of cohort smoked ≥10 cigarettes/day.	50.7%	498 (222 - 1020)	610 (278 - 1150)	-	2687 (70 - 28258)	-
	Boosted PI-based HAART	29 (18 - 42)	-	26 (25 - 27)		47.2%	504 (243 - 881)	659 (391 - 1266)	-	1887 (48 - 46100)	-
Asia											
Lallemant et al. 2015[206]	NRTI-based monotherapy	28 (23.0 - 33.0)	-	28.6 (28.1 - 30.4)	Women participating in the Thai national PMTCT program. 4.9% of participants infected with hepatitis B and 3.2% infected with hepatitis C virus. All hospital deliveries.	31.3%	452 (360 - 568)	515 (401 - 681)	WHO 1 = 98.4% WHO 2 = 1.6%	7943 (2512 - 19953)	3162 (1259 - 10000)
	NRTI-based monotherapy	27 (23.0 - 32.0)	-	28.7 (28.3 - 31.0)		28.9%	456 (366 - 577)	533 (400 - 728)	WHO 1 = 97.1% WHO 2 = 2.9%	10000 (3162 - 39811)	5012 (1000 - 15849)
	Boosted PI-based dual therapy	27 (23.0 - 32.0)	-	28.4 (28.1 - 30.4)		31.7%	451 (363 - 583)	558 (434 - 679)	WHO 1 = 97.9% WHO 2 = 2.1%	12589 (2512 - 31623)	40 (20 - 100)

[a] This estimate reflects the gestational age at which women were recruited. Maternal age reported as mean ± SD or median or median (IQR). Gestational age at recruitment reported as a mean ± SD or median or median (IQR). $CD4^+$ T-lymphocyte cou and plasma HIV-1 RNA reported as a median or median (IQR). Abbreviations: AIDS = Acquired Immune Deficiency Syndrome, CDC = The Centre for Disease Control and prevention, HAART = Highly Active Antiretroviral Treatment, HIV = Huma Immunodeficiency Virus, IQR = inter quartile range, NNRTI = Non-Nucleoside Reverse Transcriptase Inhibitors, NRTI = Nucleoside Reverse Transcriptase Inhibitors, NtRTI = Nucleotide Reverse Transcriptase Inhibitors, PI = Protease Inhibitor, PMTC = prevention of mother to child transmission, RNA = ribonucleic acid, TMX-SMX = Trimethoprim/sulfamethoxazole, USA = United States of America, WHO = World Health organisation.

4.4.4 ART arms

Table 4.5 provides detailed information on the ART arms reported in each trial. The vast majority (99.9%; 6285/6287) of participants were assigned to an ART arm after randomisation, barring 2 participants in the ANRS 135 PRIMEVA trial[209]. ART was initiated after the first trimester (median = 26.6 weeks) specifically for PMTCT. None of the participants were initiated on treatment for maternal health reasons, thus eliminating indication bias. The majority (53.2%) of participants were initiated on HAART (n=3347/6287), consisting of a PI (85.7%; n=2867/3347), NRTI (3.5%; n=285/3347) or NNRTI (5.8%; n=195/3347) antiretroviral drug, and a NRTI backbone (Figure 4.5). The remaining participants were initiated on monotherapy (40.5%; n=2547/6287), dual therapy (2.4%; n=152/6287) or placebo (3.8%; n=239/6287) (Figure 4.5). The majority of trials compared a HAART regimen to another HAART regimen or monotherapy (Figure 4.5.) Only one trial compared the index treatment to placebo or dual therapy (AZT/LPV/r) (Figure 4.5). The dosage and route of administration of the assigned antiretroviral drugs were similar across all trials, barring PACTG 076, which administered zidovudine at a dose of 500mg po daily, as opposed to 300mg po daily used by the other trials. Lopinavir/ritonavir was the only protease inhibitor used across all studies. Participants received the assigned ART for 11 weeks (median of the medians reported) before delivery, and the majority of women adhered to the assigned treatment regimen.

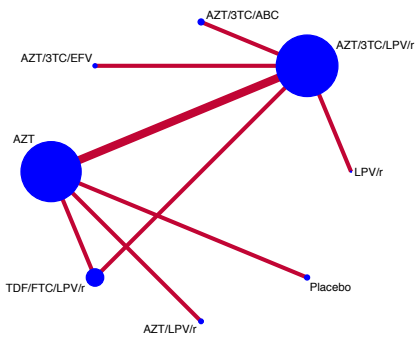


Figure 4.5: Network plot illustrating ART reported by RCTs.
Each circle represents a treatment group. The size of the circle represents the number of participants who received the ART. The lines connecting the circles represent direct comparisons of ART reported by the RCTs. The red colour of the connecting lines represents a high risk of bias in trials reporting the treatment comparison and the width of the lines represents the number of trials reporting on the treatment comparison.

Table 4.5: Characteristics of ART arms.

Study	Treatment group	Treatment initiation [median (range)/ Mean ± SD weeks]	Antiretroviral treatment received during antenatal period			Indication	Duration of treatment [Median (range) weeks]	Assessment of adherence	Adherence %	Mother to child transmission rate %(95% CI) (age at diagnosis)
			Regimen	Class	Drug (dose)					
Sub-Saharan Africa										
Fowler, et al. 2016 (period 1 and 2)[9]	NRTI-based monotherapy	Antenatal [26 (21 – 30). Distribution: <28 = 62.7% 28 - <34 = 26.7% 34 - <37 = 7.6% ≥37 = 2.9%]	Monotherapy	NRTI	Zidovudine (300mg po twice daily)	PMTCT	Until delivery (Unspecified)	Adherence questionnaire	Entire cohort: 4% of cohort discontinued the assigned antiretroviral regimen prematurely. 0.7% discontinuation possibly related to trial drug.	1.8% (In utero transmission)
	Boosted PI-based HAART	Antenatal [25 (21 – 30) Distribution: <28 = 65.8% 28 - <34 = 23.9% 34 - <37 = 7.2% ≥37= 3.1%]	HAART	Boosted PI & 2 NRTI	Lopinavir/ritonavir [(400mg/100mg po twice daily) from 14 to <28 weeks' gestation and (600mg/150mg po twice daily) ≥ 28 weeks' gestation] & zidovudine (300mg po twice daily) & lamivudine (150mg po twice daily)	PMTCT	Until delivery (Unspecified)	Adherence questionnaire	Entire cohort: 4% of cohort discontinued the assigned antiretroviral regimen prematurely. 0.7% discontinuation possibly related to trial drug.	0.5% (In utero transmission)
Fowler, et al. 2016 (period 2 only)[9]	NRTI-based monotherapy	Antenatal [26 (21 – 31) Distribution: <28 = 62.1% 28 - <34 = 24.6% 34 - <37 = 10.6% ≥37 = 2.7%	Monotherapy	NRTI	Zidovudine (300mg po twice daily)	PMTCT	Until delivery (Unspecified)	Adherence questionnaire	Entire cohort: 4% of cohort discontinued the assigned antiretroviral regimen prematurely. 0.7% discontinuation possibly related to trial drug	1.8% (In utero transmission)
	NRTI-based PI HAART	Antenatal [26 (21 – 31) Distribution: <28 = 61.0% 28 - <34 = 25.7% 34 - <37 = 9.6% ≥37 = 3.7%	HAART	Boosted PI & 2 NRTI	Lopinavir/ritonavir [(400mg/100mg po twice daily) from 14 to <28 weeks' gestation and (600mg/150mg po twice daily) ≥ 28 weeks' gestation] & zidovudine (300mg po twice daily) & lamivudine (150mg po twice daily)	PMTCT	Until delivery (Unspecified)	Adherence questionnaire	Entire cohort: 4% of cohort discontinued the assigned antiretroviral regimen prematurely. 0.7% discontinuation possibly related to trial drug	0.5% (In utero transmission)

	NtRTI-based PI HAART	Antenatal [26 (22 – 31) Distribution: <28 = 59.9% 28 - <34 = 26.3% 34 - <37 = 10.0% ≥37 = 3.8%	HAART	Boosted PI & 1 NRTI & 1 NtRTI	Lopinavir/ritonavir [(400mg/100mg po twice daily) from 14 to <28 weeks' gestation and (600mg/150mg po twice daily) ≥ 28 weeks' gestation] & emtricitabine-tenofovir disoproxil fumarate (200mg/300mg po once daily)	PMTCT	Until delivery (Unspecified)	Adherence questionnaire	Entire cohort: 4% of cohort discontinued the assigned antiretroviral regimen prematurely. 0.7% discontinuation possibly related to trial drug	0.6% (In utero transmission)
Koss, et al. 2014[316]	Boosted PI-based HAART	Antenatal (21 (17 - 25)]	HAART	Boosted PI & 2 NRTI	Lopinavir/ritonavir (400mg/100mg twice daily) until 30 weeks' gestation, increased to (600mg/150mg po twice daily thereafter) & zidovudine (300mg twice daily) & lamivudine (150mg twice daily). Tenofovir in case of zidovudine intolerance	PMTCT and prevention of malaria.	Until delivery (Unspecified)	Self-reported recall	99%	0.5% (In utero transmission)
	NNRTI-based HAART	Antenatal [21 (18 - 24)]	HAART	NNRTI & 2 NRTI	Efavirenz (600mg once daily), zidovudine (300mg twice daily) lamivudine (150mg twice daily)	PMTCT and prevention of malaria.	Until delivery (Unspecified)	Self-reported recall	97%	0% (In utero transmission)
Natureeba, et al. 2014[317]	Boosted PI-based HAART	Antenatal [21.2 ± 4.3]	HAART	Boosted PI & 2 NRTI	Lopinavir/ritonavir (400mg/100mg twice daily) until 30 weeks' gestation, increased to (600mg/150mg po twice daily thereafter) & zidovudine (300mg twice daily) & lamivudine (150mg twice daily). Tenofovir in case of zidovudine intolerance.	PMTCT and prevention of malaria.	Until delivery (Unspecified)	Self-reported recall	99%	0.5% (In utero transmission)
	NNRTI-based HAART	Antenatal [21.1 ± 4.1]	HAART	NNRTI & 2 NRTI	Efavirenz (600mg once daily), zidovudine (300mg twice daily) & lamivudine (150mg twice daily). Tenofovir in case of zidovudine intolerance.	PMTCT and prevention of malaria.	Until delivery (Unspecified)	Self-reported recall	97%	0% (In utero transmission)

Powis, et al. 2011[318]	NRTI-based HAART	Antenatal (26 - 28 = 67.3%; 29 - 31= 16.7%; 32 - 34 = 16.0%)	HAART	3 NRTI	Abacavir (300mg twice daily), zidovudine (300mg twice daily), lamivudine (150mg twice daily)	PMTCT	11.6 (8.3 – 13.3)	Self-report, pill counts	Still receiving originally assigned HAART regimen at delivery = 99.6%	1.4% (In utero transmission)
	Boosted PI-based HAART	Antenatal (26 - 28 = 67.4% 29 - 31 = 23.6% 32 - 34 = 9.0%)	HAART	Boosted PI & 2 NRTI	Lopinavir/ritonavir (400mg/100mg twice daily) zidovudine (300mg twice daily), lamivudine (150mg twice daily)	PMTCT	11.0 (8.1 – 12.7)	Self-report, pill counts	Still receiving originally assigned HAART regimen at delivery = 99.6%	0.4% (In utero transmission)
Shapiro, et al. 2010[204]	NRTI-based HAART	Antenatal [27.1 (26.4 - 29.9)]	HAART	3 NRTI	Abacavir (300mg twice daily), zidovudine (300mg twice daily), lamivudine (150 mg twice daily)	PMTCT	11	Self-report and pill counts	Missed 1 day or more of HAART during pregnancy or breastfeeding = 22%	1.4% (In utero transmission)
	Boosted PI-based HAART	Antenatal [27.1 (26.4 - 29.9)]	HAART	Boosted PI & 2 NRTI	Lopinavir/ritonavir (400mg/100mg twice daily), zidovudine (300mg twice daily), lamivudine (150mg twice daily)	PMTCT	11	Self-report and pill counts	Missed 1 day or more of HAART during pregnancy or breastfeeding = 22%	0.4% (In utero transmission)
The Kesho Bora Study Group, et al. 2011[315]	NRTI-based monotherapy	Antenatal (28 - 36)	Monotherapy	1 NRTI	Zidovudine (300mg twice daily)	PMTCT	6.4 (4.3 - 8.8)	Self-reported recall	No missed doses before pregnancy = 73%	2.5% (95%CI 1.3 - 4.6) (In utero transmission)
	Boosted PI-based HAART	Antenatal (28 - 36)	HAART	Boosted PI & 2 NRTI	Lopinavir/ritonavir (400mg/100mg twice daily), zidovudine (300mg twice daily) lamivudine (150mg twice daily)	PMTCT	6.0 (4.3 - 8.8)	Self-reported recall	No missed doses before pregnancy = 73%	1.8% (95%CI 0.9 - 3.7) (In utero transmission
Europe and the Americas										
Connor, et al. 1994[205]	Placebo	Antenatal (14 - 34)	Placebo	None	Placebo	PMTCT	11 (0 – 26)	Unspecified	Incomplete = 6.3%	24.4% (>32 weeks)
	NRTI based monotherapy	Antenatal (14 - 34)	Monotherapy	NRTI	Zidovudine (100mg po 5 times daily)	PMTCT	11 (0 – 26)	Unspecified	Incomplete = 3.8%	7.4% (>32 weeks)

Sperling, et al. 1998[319]	Placebo	Antenatal (14 - 34)	Placebo	None	Placebo	PMTCT	11 (0 – 26)	Unspecified	Unspecified	24.4% (>32 weeks)
	NRTI based monotherapy	Antenatal (14 - 34)	Monotherapy	NRTI	Zidovudine	PMTCT	11 (0 – 26)	Unspecified	Unspecified	7.4% (>32 weeks)
Tubiana, et al. 2013[209]	Boosted PI-based monotherapy	Antenatal [26.0 (24 - 27)]	Monotherapy	Boosted PI	Lopinavir/ritonavir (400mg/100mg twice daily)	PMTCT	90 (50 – 111) days	Pill count, Self-administered questionnaire at week 8	At 8 weeks of treatment 75.4% did not miss a pill in the last 4 days or in the last 4 weeks	0% (95% upper limit = 5.2%) (In utero transmission)
	Boosted PI-based HAART	Antenatal [26.0 (25 - 27)]	HAART	Boosted PI & 2 NRTI	Lopinavir/ritonavir (400mg/100mg twice daily), zidovudine (300mg twice daily), lamivudine (150mg twice daily)	PMTCT	90 (57 – 111) days	Pill count, self-administered questionnaire at week 8	At 8 weeks of treatment 75.4% did not miss a pill in the last 4 days or in the last 4 weeks	2.8% (95%CI 0.07 - 14.5%) (In utero transmission)
Asia										
Lallemant, et al. 2015[206]	NRTI-based monotherapy	28 (23.0 - 33.0)	Monotherapy	NRTI	Zidovudine (300mg po twice daily)	PMTCT	10.3 (9.1 – 11.4)	Pill count at each visit, and the intake of intrapartum treatment (single-dose nevirapine or placebo) was directly observed.	Adherence more than 90% for zidovudine = 96.8%	Intent-to-treat analysis: 1.6% (0.2% - 5.6%) As treated analysis: 3.2% (0.9% - 8.0%) (In utero transmissions)
	NRTI-based monotherapy	27 (23.0 - 32.0)	Monotherapy	NRTI	Zidovudine (300mg po twice daily)	PMTCT	10.1 (8.1 – 11.1)	Pill count at each visit, and the intake of intrapartum treatment (single-dose nevirapine or placebo) was directly observed.	Adherence more than 90% for zidovudine = 96.8%	Intent-to-treat analysis: 3.8% (1.2% - 8.6%) As treated analysis: 2.2% (0.5% - 6.3%)
	Boosted PI-based dual therapy	27 (23.0 - 32.0)	HAART	Boosted PI & 1 NRTI	Lopinavir/ritonavir (400mg/100mg po twice daily) & zidovudine (300mg po twice daily)	PMTCT	10.0 (8.6 – 11.1)	Pill count at each visit, and the intake of intrapartum treatment (single-dose nevirapine or placebo) was directly observed.	Adherence more than 90% for zidovudine = 96.8% Adherence more than 90% for lopinavir/ritonavir = 95.2%	Intent-to-treat analysis: 1.4% (0.4% - 5.1%) As treated analysis: 1.5% (0.2% - 5.2%) (In utero transmissions

Abbreviations: HAART = Highly Active Antiretroviral Treatment, NNRTI = Non-Nucleoside Reverse Transcriptase Inhibitors, NRTI = Nucleoside Reverse Transcriptase Inhibitors, NtRTI = Nucleotide Reverse Transcriptase Inhibitors, PI = Protease Inhibitor, PMTCT = prevention of mother to child transmission.

4.4.5 Mother to child transmission rate

The rate of MTCT reported for individual treatment arms reflected the ability of the ART to reduce or eliminate antenatal HIV transmissions (in-utero transmissions) (Table 4.5). The highest rates of MTCT occurred in women who received placebo (25.5%), which was followed by women who received monotherapy. Antenatal HAART was associated with the lowest rates of MTCT (Table 4.5).

4.4.6 ART and association with perinatal outcomes

In the following analysis, pairwise and network meta-analyses are used to investigate the association between ART and the following perinatal outcomes: PTB, sPTB, VPTB, LBW, VLBW, SGA and NND. The association is assessed between each perinatal outcome and individual ARTs as reported by the RCTs (e.g. AZT/3TC/ABC), the antiretroviral regimens reported (monotherapy, dual therapy, HAART), and antiretroviral class (NRTI, NNRTI, PI) of ART reported (see Table 4.5).

4.4.6.1 Preterm birth

Seven trials, including 5793 women, reported on the association between PTB and 8 ARTs (Figure 4.6). All comparisons had a high risk of bias, and the majority of participants received a HAART regimen containing AZT/3TC/LPV/r (n=2292; 39.6%) or AZT monotherapy (n=2287, 39,5%) (Figure 4.6).

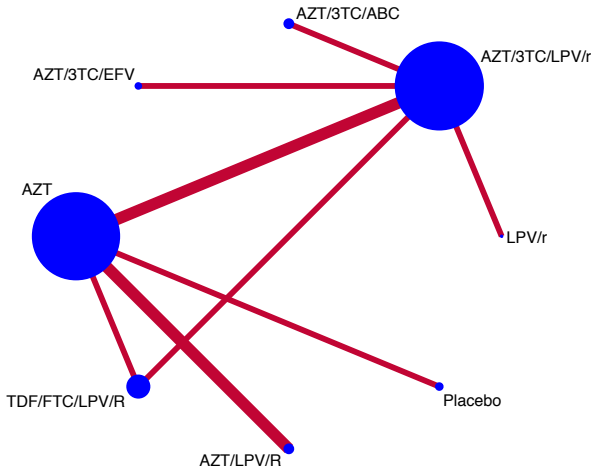


Figure 4.6: Network plot illustrating direct comparisons between ART for PTB outcome. Each circle represents a treatment group. The size of the circle represents the number of participants who received the ART. The lines connecting the circles represent direct comparisons of ART reported by the RCTs. The red colour of the connecting lines represents a high risk of bias in trials reporting the treatment comparison and the width of the lines represents the number of trials reporting on the treatment comparison.

4.4.6.1.1 Pairwise meta-analysis: association between ART and PTB

- Antenatal ART

Pairwise comparisons of the 8 ARTs showed a significant risk of PTB in participants who initiated AZT/3TC/LPV/r HAART, compared to those who initiated AZT monotherapy (RR1.43; 95%CI 1.12 – 1.84) or AZT/3TC/ABC HAART (RR1.52; 95%CI 1.07 – 2.17) (Figure 4.7). No statistical heterogeneity was observed.

- Antenatal antiretroviral regimen

When the 8 treatments were stratified according to their respective ART regimens (i.e. monotherapy, dual therapy or HAART), participants who initiated a HAART regimen were more likely to deliver preterm than those initiating monotherapy (RR1.46; 95%CI 1.27-1.68) (Figure 4.8). No statistical heterogeneity was observed.

- Antenatal antiretroviral class

Each ART was then classified according to the most complex antiretroviral class (e.g. NRTI, NNRTI, PI) included in the regimen. Women initiating a PI-based ART were 46% (RR1.46; 95%1.28 -1.66) more likely to deliver preterm than those who initiated a NRTI-based ART (Figure 4.9). There was no statistical heterogeneity between trials.

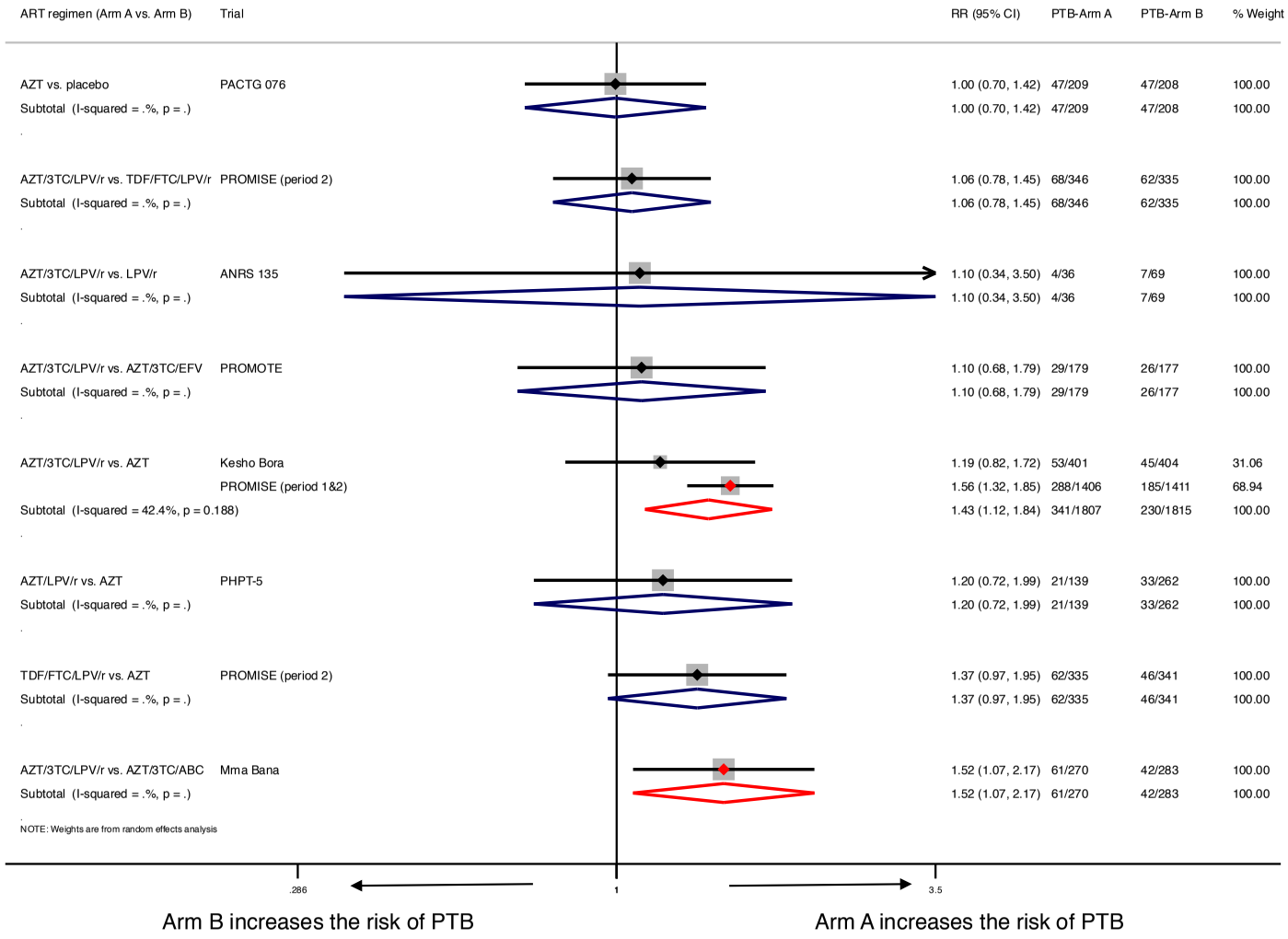


Figure 4.7: Random-effect pairwise meta-analysis results for the association between antenatal ART and PTB.
Statistically significant effects are presented in red. AZT/3TC/LPV/r HAART significantly increased the risk of PTB, compared to AZT monotherapy or AZT/3TC/ABC HAART. Abbreviations: 3TC = lamivudine, ABC = abacavir, ART = antiretroviral treatment, AZT = zidovudine, EFV = efavirenz, FTC = emtricitabine, LPV/r = lopinavir/ritonavir, PTB = preterm birth, RR = relative risk, TDF = tenofovir disoproxil fumarate.

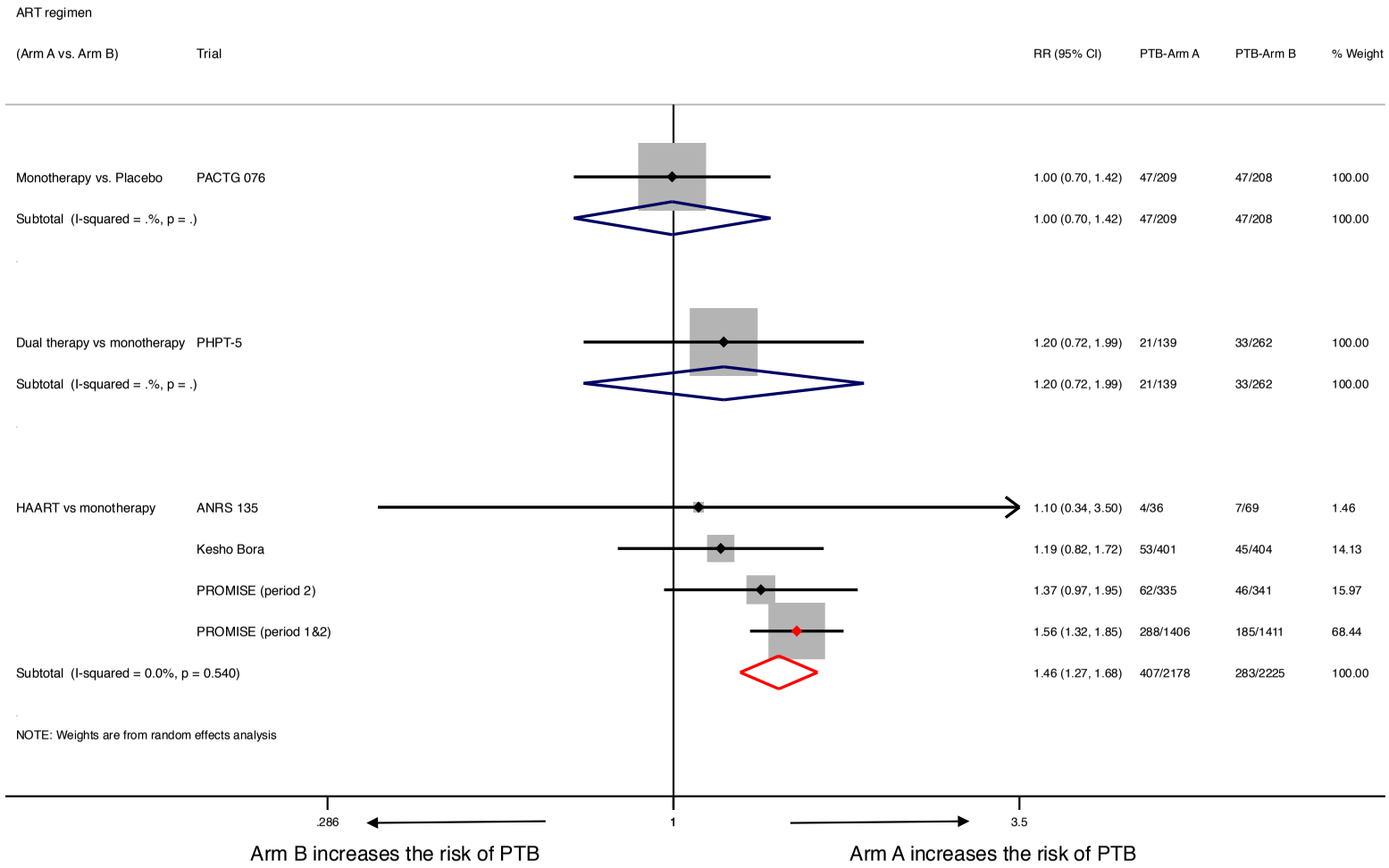


Figure 4.8: Random-effect pairwise meta-analysis results for the association between antenatal antiretroviral regimen and PTB. Statistically significant effects are presented in red. HAART significantly increased the risk of PTB, compared to monotherapy. Abbreviations: ART = antiretroviral treatment, HAART = Highly Active Antiretroviral Treatment, PTB = preterm birth, RR = relative risk.

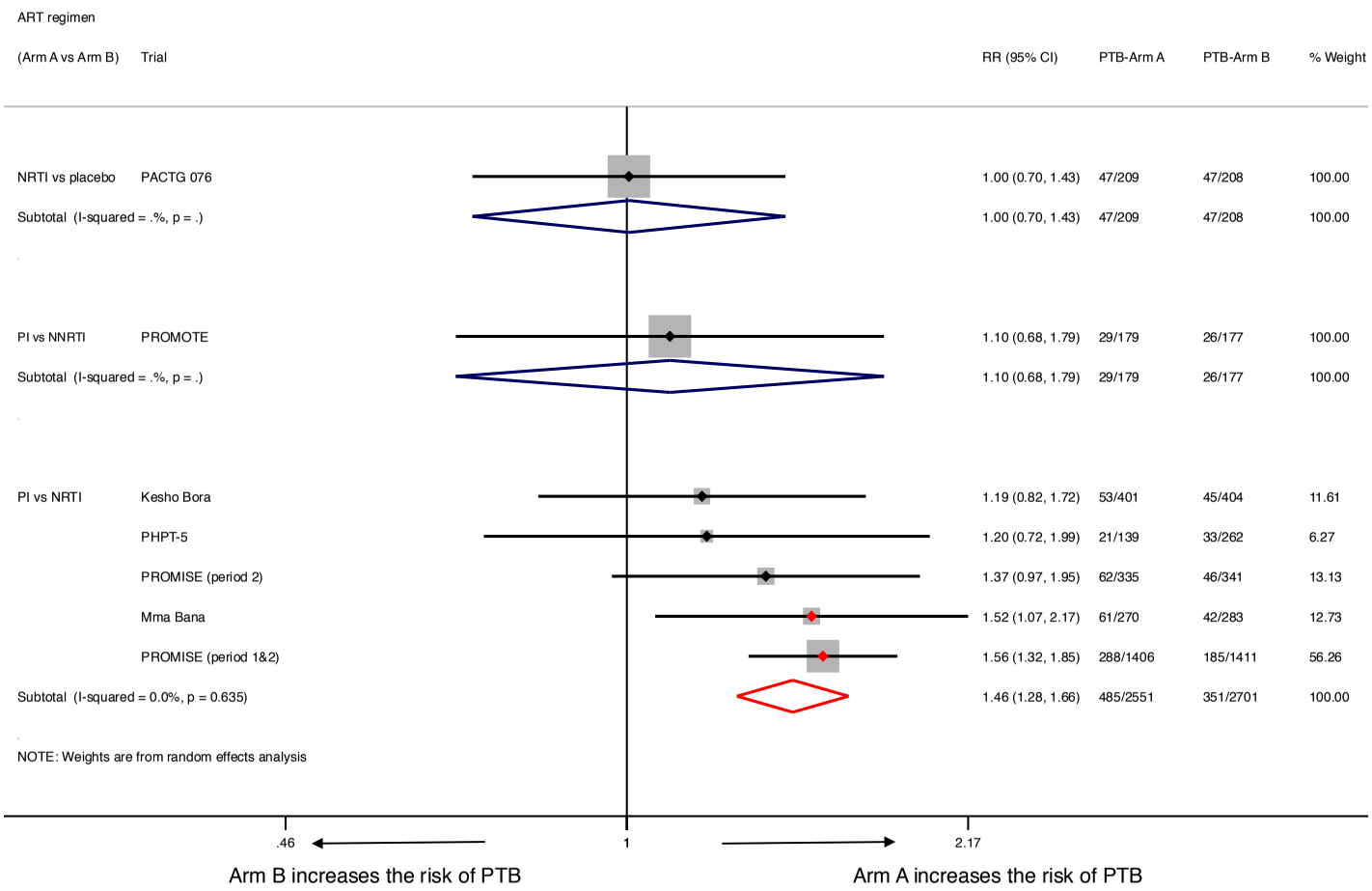


Figure 4.9: Random-effects pairwise meta-analysis results for the association between antenatal antiretroviral class and PTB.
Statistically significant effects are presented in red. PI-based ART significantly increased the risk of PTB compared to NRTI-based ART.
Abbreviations: ART = antiretroviral treatment, NNRTI = non-nucleoside reverse transcriptase inhibitor, NRTI = nucleoside reverse transcriptase inhibitor, PI = protease inhibitor, PTB = preterm birth, RR = relative risk.

4.4.6.1.2 Network meta-analysis: association between ART and PTB

- Antenatal ART

Treatment estimates from direct evidence in the network meta-analysis were consistent with those observed in the pairwise meta-analysis. Participants initiating AZT/3TC/LPV/r HAART were more likely to deliver preterm than those initiating AZT monotherapy (RR1.48; 95%CI 1.29-1.71), AZT/3TC/ABC HAART (RR1.52; 95%CI 1.07 – 2.17) or placebo (1.48; 95%CI 1.01-2.17) (Figure 4.10, Figure 4.11). Similarly, participants who initiated TDF/FTC/LPV/r HAART showed a significantly higher risk of PTB than those initiating AZT monotherapy (RR1.39; 95%CI 1.04-1.86) (Figure 4.10, Figure 4.11). There was no evidence of statistical inconsistency between direct and indirect estimates (global test for inconsistency p=0.95) or publication bias (Figure 4.12).

- Antenatal antiretroviral regimen

Participants initiating HAART displayed a significantly higher risk of PTB than those initiating monotherapy (RR 1.45; 1.17-1.79) (Figure 4.13, Figure 4.14). There was no evidence of statistical inconsistency (p=0.65) or publication bias (Figure 4.15).

- Antenatal antiretroviral class

PI-based ART significantly increased the risk of PTB compared to NRTI-based ART (RR1.46; 95%CI 1.28-1.66) (Figure 4.16, Figure 4.17). There was no evidence of statistical inconsistency (p=0.97) or publication bias (Figure 4.18).

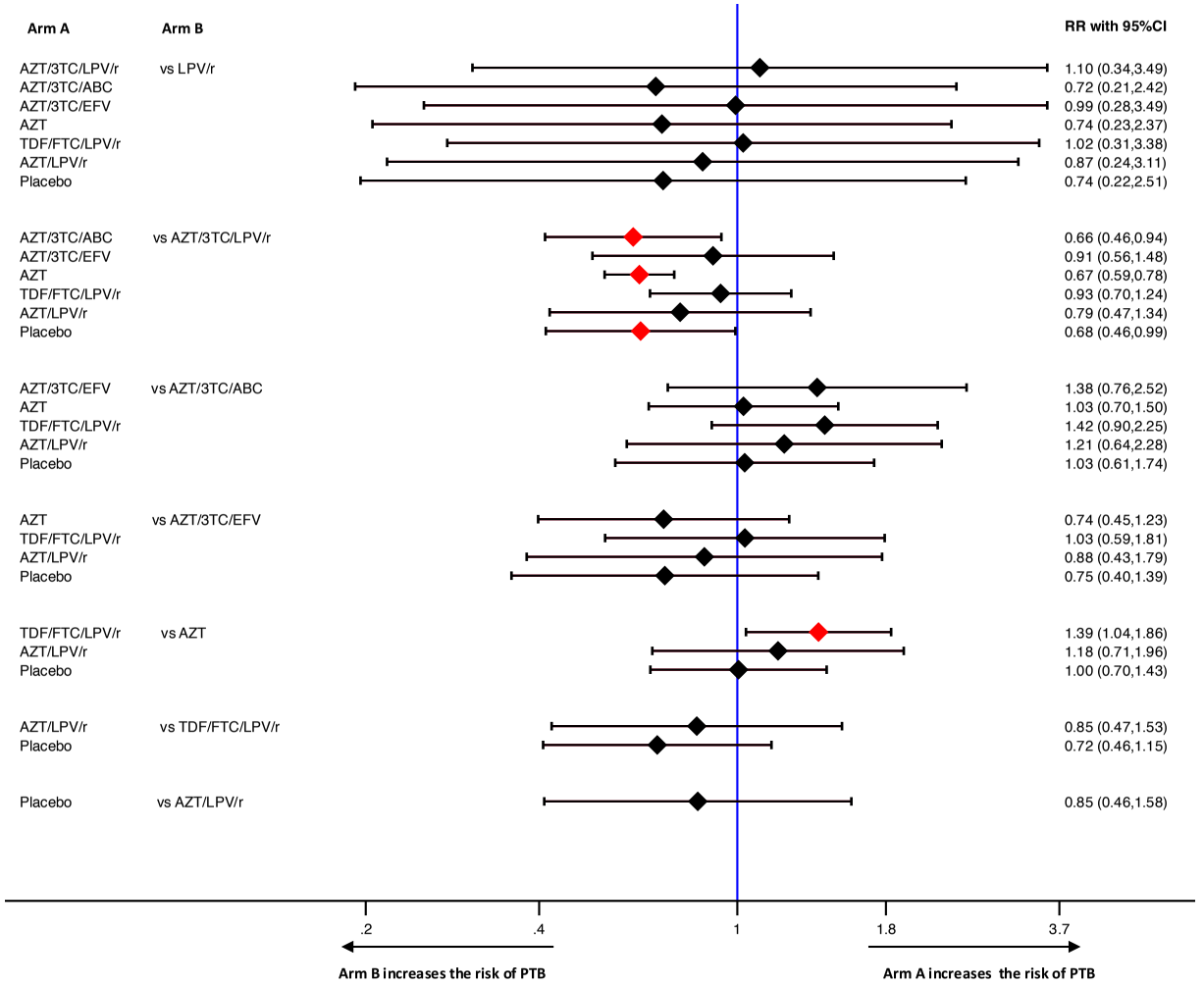


Figure 4.10: Random-effects network meta-analysis results for the association between antenatal ART and PTB.
Statistically significant effects are presented in red. AZT/3TC/LPV/r HAART significantly increased the risk of PTB compared to AZT monotherapy, AZT/3TC/ABC HAART and placebo. TDF/FTC/LPV/r HAART significantly increased the risk of PTB, compared to AZT monotherapy. Abbreviations: 3TC = lamivudine, ABC = abacavir, AZT = zidovudine, EFV = efavirenz, FTC = emtricitabine, LPV/r = lopinavir/ritonavir, PTB = preterm birth, RR = relative risk, TDF = tenofovir disoproxil fumarate.

AZT	1.00 (0.70 - 1.43)	1.36 (0.42 - 4.36)	1.18 (0.71 - 1.96)	1.35 (0.81 - 2.23)	0.98 (0.67 - 1.43)	**1.48 (1.29 - 1.71)**	**1.39 (1.04 - 1.86)**
1.00 (0.70 - 1.42)	**Placebo**	1.35 (0.40 - 4.58)	1.17 (0.63 - 2.18)	1.34 (0.72 - 2.49)	0.97 (0.58 - 1.64)	**1.48 (1.01 - 2.17)**	1.38 (0.87 - 2.19)
0.74 (0.23 - 2.37)	0.74 (0.22 - 2.51)	**LPV/r**	0.87 (0.24 - 3.11)	0.99 (0.28 - 3.49)	0.72 (0.21 - 2.42)	1.10 (0.34 - 3.49)	1.02 (0.31 - 3.38)
0.85 (0.51 - 1.41)	0.85 (0.46 - 1.58)	1.15 (0.32 - 4.11)	**AZT/LPV/r**	1.14 (0.56 - 2.34)	0.83 (0.44 - 1.56)	1.26 (0.74 - 2.13)	1.18 (0.66 - 2.12)
0.74 (0.45 - 1.23)	0.75 (0.40 - 1.39)	1.01 (0.29 - 3.54)	0.88 (0.43 - 1.79)	**AZT/3TC/EFV**	0.72 (0.40 - 1.32)	1.10 (0.68 - 1.79)	1.03 (0.59 - 1.81)
1.03 (0.70 - 1.50)	1.03 (0.61 - 1.74)	1.39 (0.41 - 4.68)	1.21 (0.64 - 2.28)	1.38 (0.76 - 2.52)	**AZT/3TC/ABC**	**1.52 (1.07 - 2.17)**	1.42 (0.90 - 2.25)
0.67 (0.59 - 0.78)	**0.68 (0.46 - 0.99)**	0.91 (0.29 - 2.91)	0.79 (0.47 - 1.34)	0.91 (0.56 - 1.48)	**0.66 (0.46 - 0.94)**	**AZT/3TC/LPV/r**	0.93 (0.70 - 1.24)
0.72 (0.54 - 0.97)	0.72 (0.46 - 1.15)	0.98 (0.30 - 3.23)	0.85 (0.47 - 1.53)	0.97 (0.55 - 1.71)	0.70 (0.45 - 1.11)	1.07 (0.80 - 1.42)	**TDF/FTC/LPV/r**

Figure 4.11: League table of random-effects network meta-analysis results for the association between antenatal ART and PTB.
This table reflects all possible pairwise comparisons generated from the random-effect network meta-analysis. Results are reported as a relative risk (RR) of the column ART (e.g. AZT/) relative to the row ART (AZT/3TC/LPV/r). Statistically significant effects are presented in red. AZT/3TC/LPV/r HAART significantly increased the risk of PTB, compared to AZT monotherapy, AZT/3TC/ABC HAART and placebo. TDF/FTC/LPV/r HAART significantly increased the risk of PTB compared to AZT monotherapy. Abbreviations: 3TC = lamivudine, ABC = abacavir, ART = antiretroviral treatment, AZT = zidovudine, EFV = efavirenz, FTC = emtricitabine, LPV/r = lopinavir/ritonavir, PTB = preterm birth, TDF = tenofovir disoproxil fumarate.

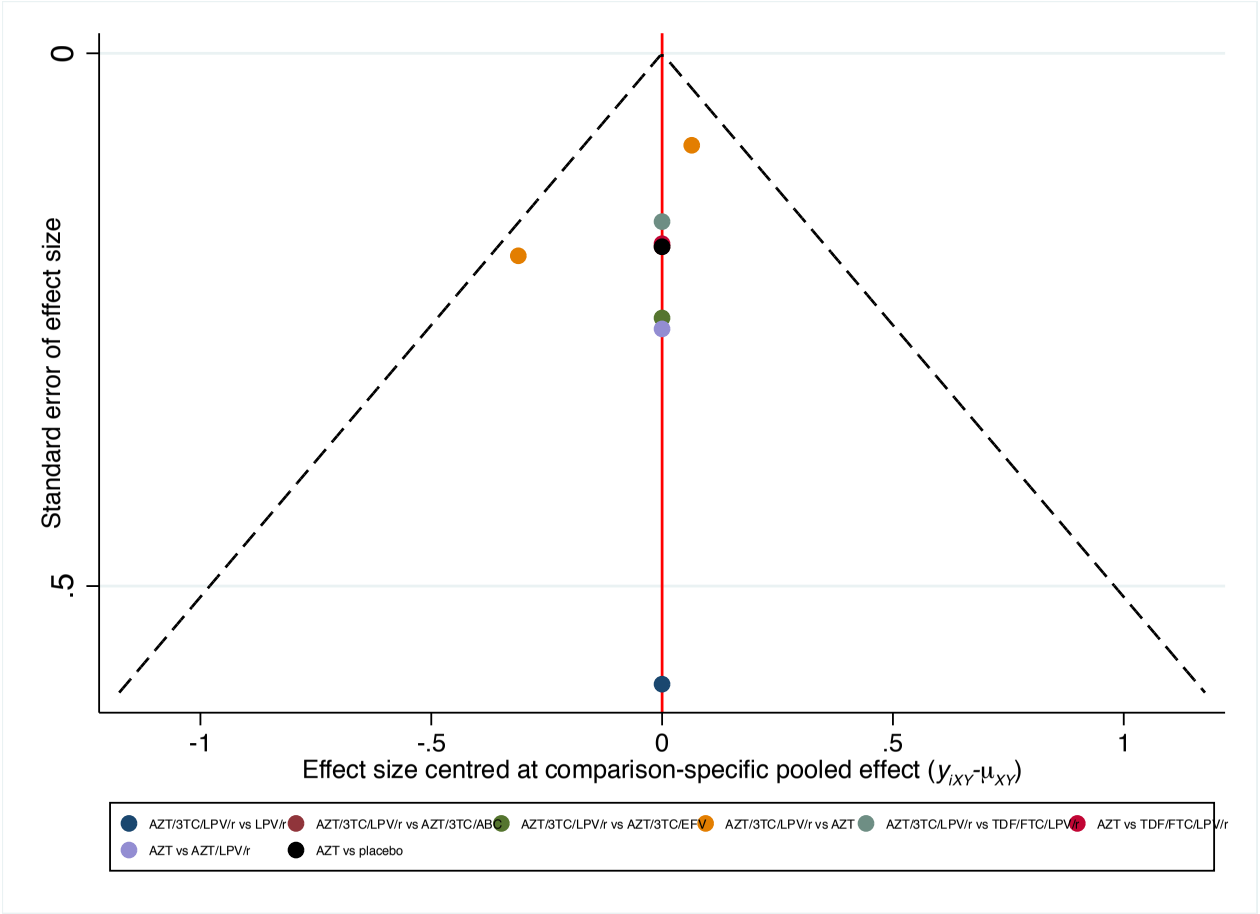


Figure 4.12: Comparison-adjusted funnel plot for the association between antenatal ART and PTB.
The vertical axis represents the measure of the dispersion of the effect size; the horizontal axis represents the difference between the study specific effect size and the comparison-specific summary effect size, and the red line represents the null hypothesis of no difference between the study specific and comparison specific effect estimates[50]. The colours represent individual comparisons. There was no evidence of small study effect.

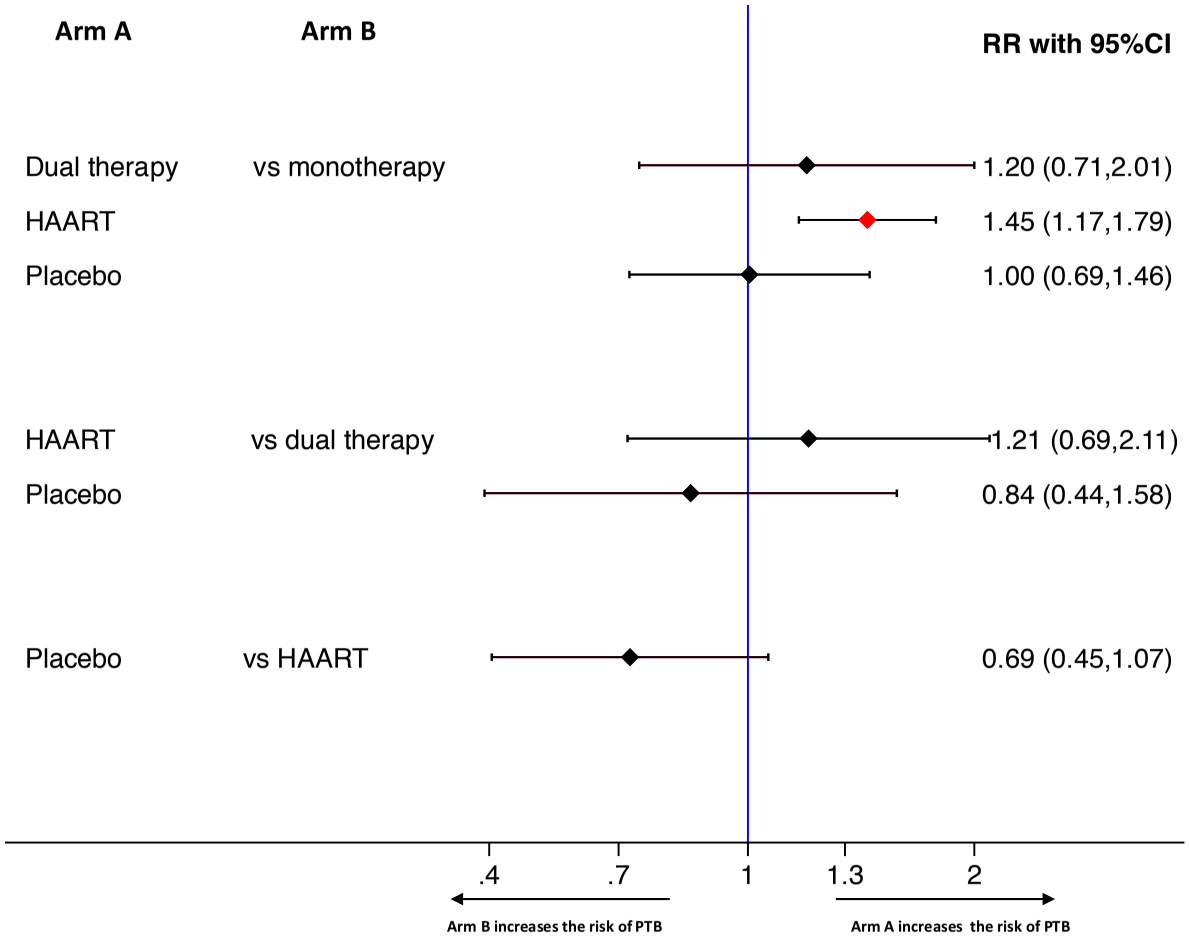


Figure 4.13: Random-effects network meta-analysis results for the association between antenatal antiretroviral regimens and PTB. Statistically significant effects are presented in red. HAART significantly increased the risk of PTB, compared to monotherapy. Abbreviations: HAART = Highly Active Antiretroviral Treatment, PTB = preterm birth, RR = relative risk.

Placebo	1.00 (0.69 - 1.44)	1.19 (0.63 - 2.26)	1.44 (0.94 - 2.21)
1.00 (0.69 - 1.46)	**Monotherapy**	1.20 (0.71 - 2.01)	**1.45** **(1.17 - 1.79)**
0.84 (0.44 - 1.58)	0.83 (0.50 - 1.40)	**Dual therapy**	1.21 (0.69 - 2.11)
0.69 (0.45 - 1.07)	**0.69** **(0.56 - 0.85)**	0.83 (0.47 - 1.45)	**HAART**

Figure 4.14: League table of random-effects network meta-analysis results for the association between antenatal antiretroviral regimen and PTB. This table reflects all possible pairwise comparisons generated from the random-effect network meta-analysis. Results are reported as a relative risk (RR) of the column regimen (e.g. Monotherapy) relative to the row regimen (HAART). Statistically significant effects are presented in red. HAART significantly increased the risk of PTB, compared to monotherapy. Abbreviations: HAART = Highly Active Antiretroviral Treatment, PTB = preterm birth.

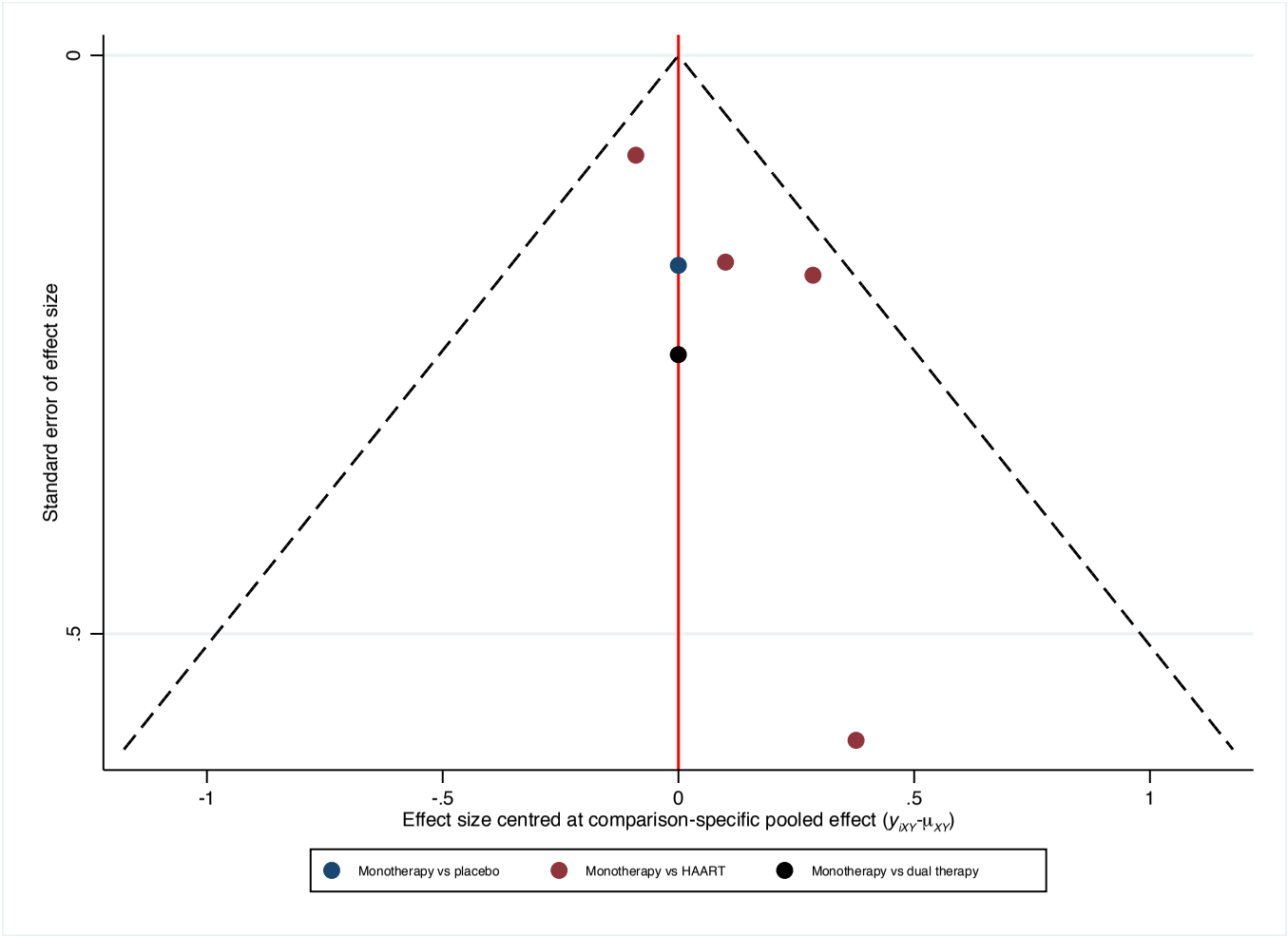


Figure 4.15: Comparison-adjusted funnel plot for the association between antenatal antiretroviral regimens and PTB.
The vertical axis represents the measure of the dispersion of the effect size; the horizontal axis represents the difference between the study specific effect size and the comparison-specific summary effect size, and the red line represents the null hypothesis of no difference between the study specific and comparison specific effect estimates[50]. The colours represent individual comparisons. There was no evidence of small study effect.

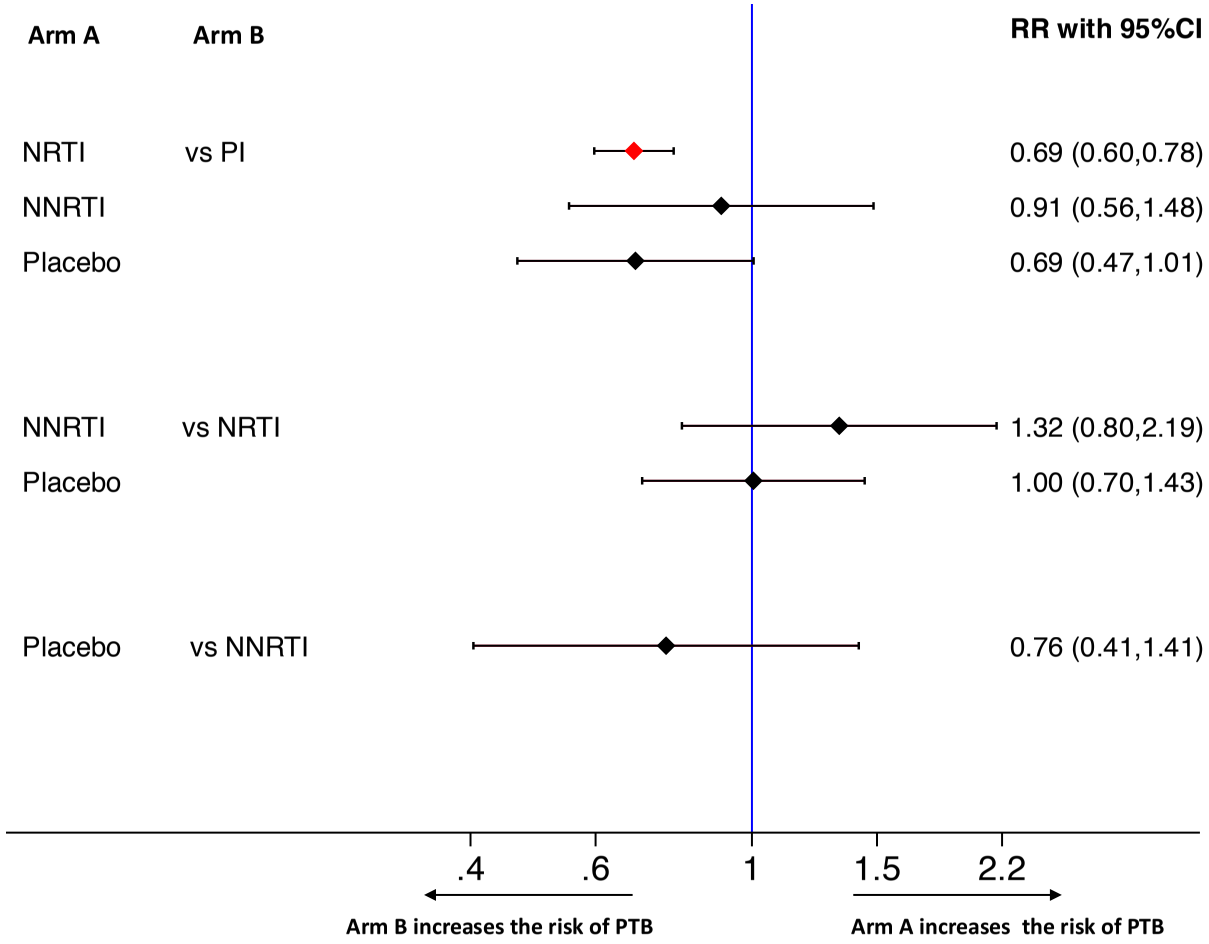


Figure 4.16: Random-effects network meta-analysis results for the association between antenatal antiretroviral class and PTB.
Statistically significant effects are presented in red. PI-based ART significantly increased the risk of PTB, compared to NRTI-based ART. Abbreviations: NNRTI = non-nucleoside reverse transcriptase inhibitor, NRTI = nucleoside reverse transcriptase inhibitor, PI = protease inhibitor, PTB = preterm birth, RR = relative risk.

Placebo	1.45 (0.99 - 2.12)	1.32 (0.71 - 2.44)	1.00 (0.70 - 1.42)
0.69 (0.47 - 1.01)	**PI**	0.91 (0.56 - 1.48)	**0.69** **(0.60 - 0.78)**
0.76 (0.41 - 1.41)	1.10 (0.68 - 1.79)	**NNRTI**	0.76 (0.46 - 1.25)
1.00 (0.70 - 1.43)	**1.46** **(1.28 - 1.66)**	1.32 (0.80 - 2.19)	**NRTI**

Figure 4.17: League table of random-effects network meta-analysis results for the association between antenatal antiretroviral class and PTB. This table reflects all possible pairwise comparisons generated from the random-effect network meta-analysis. Results are reported as a relative risk (RR) of the column regimen (e.g. PI) relative to the row regimen (NRTI). Statistically significant effects are presented in red. PI-based ART significantly increased the risk of PTB, compared to NRTI-based ART. Abbreviations: NNRTI = non-nucleoside reverse transcriptase inhibitor, NRTI = nucleoside reverse transcriptase inhibitor, PI = protease inhibitor, PTB = preterm birth.

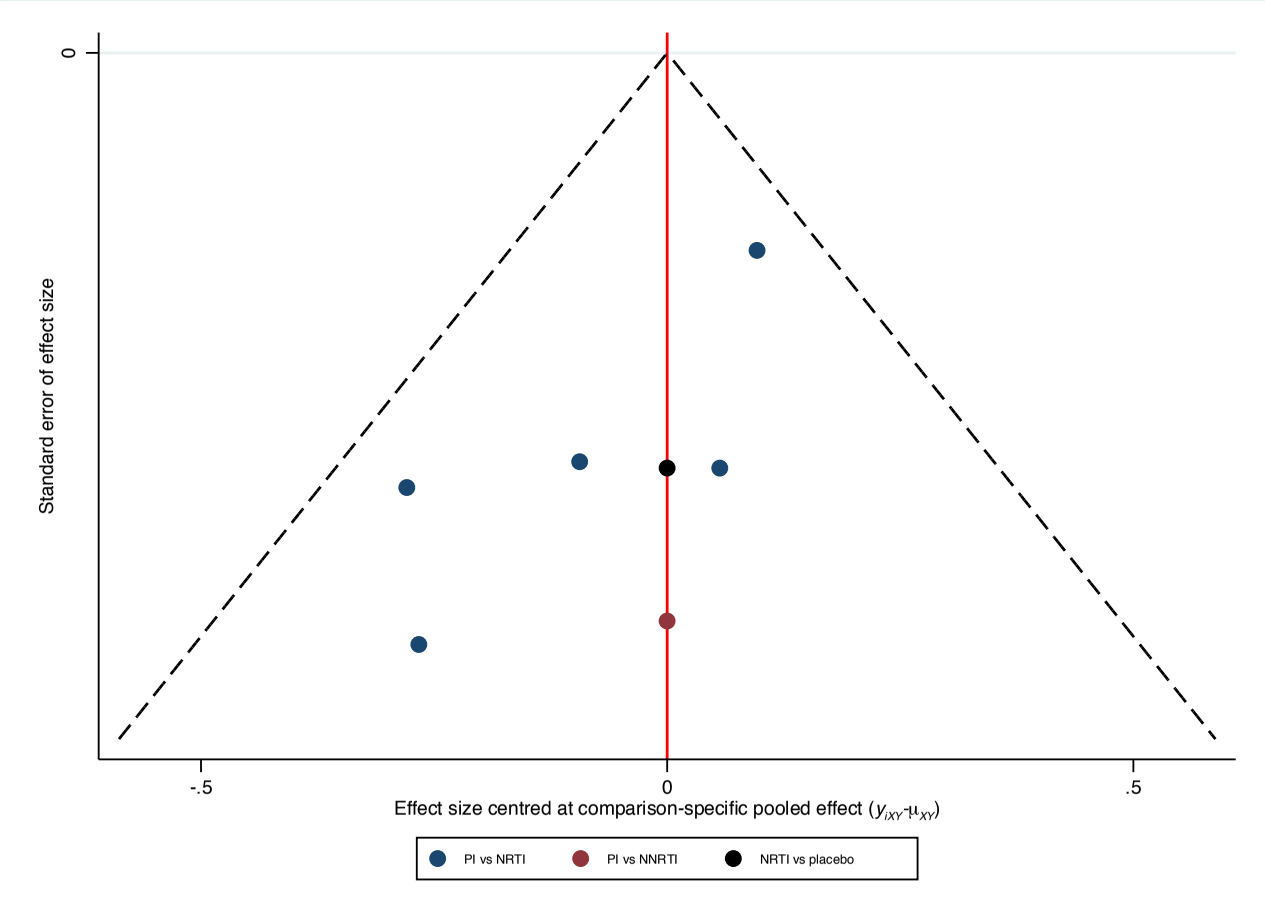


Figure 4.18: Comparison-adjusted funnel plot for the association between classes of antiretroviral and PTB.
The vertical axis represents the measure of the dispersion of the effect size; the horizontal axis represents the difference between the study specific effect size and the comparison-specific summary effect size, and the red line represents the null hypothesis of no difference between the study specific and comparison specific effect estimates[50]. The colours represent individual comparisons. There was no evidence of small study effect.

4.4.6.1.3 Network meta-analysis: SUCRA ranking of comparative effect of ART on PTB

- Antenatal ART

Of the 8 ARTs assessed AZT/3TC/ABC HAART (SUCRA = 75.4%) and AZT monotherapy (SUCRA = 75.0%) were associated with the lowest risk of PTB, and were ranked superior to all other treatments (Figure 4.19, Figure 4.20). AZT/3TC/LPV/r HAART (SUCRA = 19.1%) was associated with the highest risk of PTB, and was subsequently ranked inferior to all other ARTs (Figure 4.19, Figure 4.20).

- Antenatal antiretroviral regimen

Of the 4 antiretroviral regimens assessed monotherapy (SUCRA = 75.6%) was associated with the lowest risk of PTB, and was ranked superior to all other regimens. HAART (SUCRA = 10.2%) was associated with the highest risk of PTB and was subsequently ranked in 4th place (Figure 4.21, Figure 4.22).

- Antenatal antiretroviral class

Of the 4 classes of antiretroviral assessed – including placebo – NRTI-based ART (SUCRA = 79.2%) was associated with the lowest risk of PTB, and was ranked superior to all other regimens (Figure 4.23, Figure 4.24). PI-based ARTs (SUCRA = 12.5%) was associated with the highest risk of PTB, and was ranked lowest (4th place) (Figure 4.23, Figure 4.24).

Treatment	SUCRA	PrBest	MeanRank
LPV/r	41.9	22.2	5.1
AZT/3TC/LPV/r	19.1	0.0	6.7
AZT/3TC/ABC	75.4	28.4	2.7
AZT/3TC/EFV	35.4	4.0	5.5
AZT	75.0	10.8	2.7
TDF/FTC/LPV/r	29.5	0.3	5.9
AZT/LPV/r	51.3	11.4	4.4
Placebo	72.3	22.9	2.9

Figure 4.19: SUCRA ranking of antenatal ART for PTB outcome.
This figure provides a hierarchy of the 8 ARTs assessed in the network meta-analysis for the association between ART and PTB. The larger the SUCRA percentage, the lower the risk of PTB. SUCRA = surface under the cumulative rank curve, represented as a percentage. PrBest = probability that each treatment is the best i.e. associated with the lowest risk of PTB, represented as a percentage. MeanRank = mean rank of each ART. Abbreviations: 3TC = lamivudine, ABC = abacavir, ART = antiretroviral treatment, AZT = zidovudine, EFV = efavirenz, FTC = emtricitabine, LPV/r = lopinavir/ritonavir, PTB = preterm birth, TDF = tenofovir disoproxil fumarate.

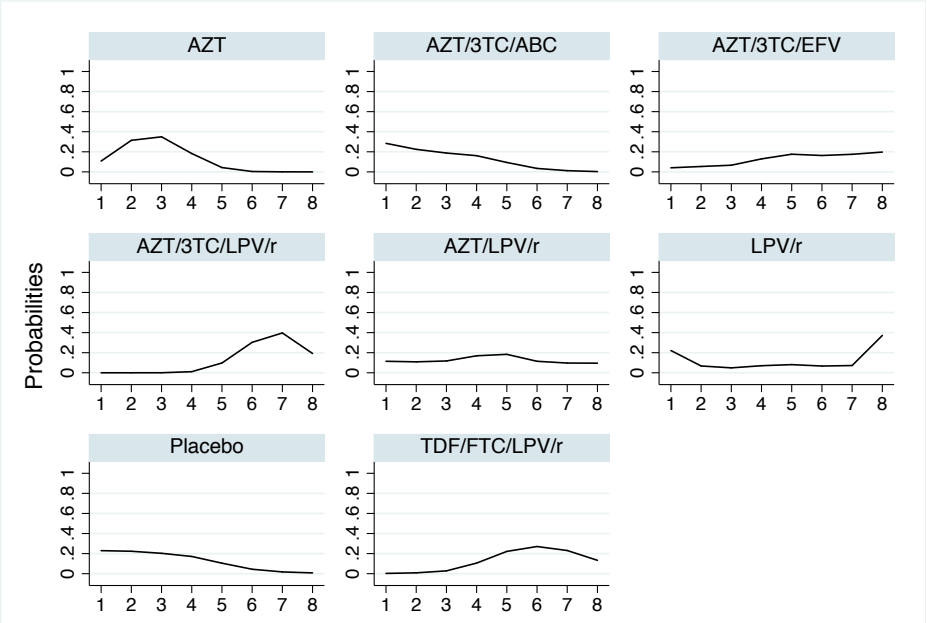


Figure 4.20: Rankograms for ARTs included in the network meta-analysis for the association between ART and PTB. This figure represents the surface under the cumulative ranking probabilities for each ART included in the network meta-analysis. The horizontal axis represents the 8 possible ranks that the ART can achieve and the vertical axis illustrates the probability of the ART to attain each rank[312]. Abbreviations: 3TC = lamivudine, ABC = abacavir, ART = antiretroviral treatment, AZT = zidovudine, EFV = efavirenz, FTC = emtricitabine, LPV/r = lopinavir/ritonavir, PTB = preterm birth, RR = relative risk, TDF = tenofovir disoproxil fumarate.

Treatment	SUCRA	PrBest	MeanRank
Monotherapy	75.6	38.4	1.7
Dual-therapy	42.4	18.6	2.7
HAART	10.2	0.0	3.7
Placebo	71.8	43.0	1.8

Figure 4.21: SUCRA ranking of antenatal antiretroviral regimens for PTB outcome. This figure provides a hierarchy of the 4 regimens assessed in the network meta-analysis for the association between antiretroviral regimen and PTB. The larger the SUCRA percentage, the lower the risk of PTB. SUCRA = surface under the cumulative rank curve, represented as a percentage. PrBest = probability that each regimen is the best i.e. associated with the lowest risk of PTB, represented as a percentage. MeanRank = mean rank of each ART. Abbreviation: HAART = Highly Active Antiretroviral Treatment.

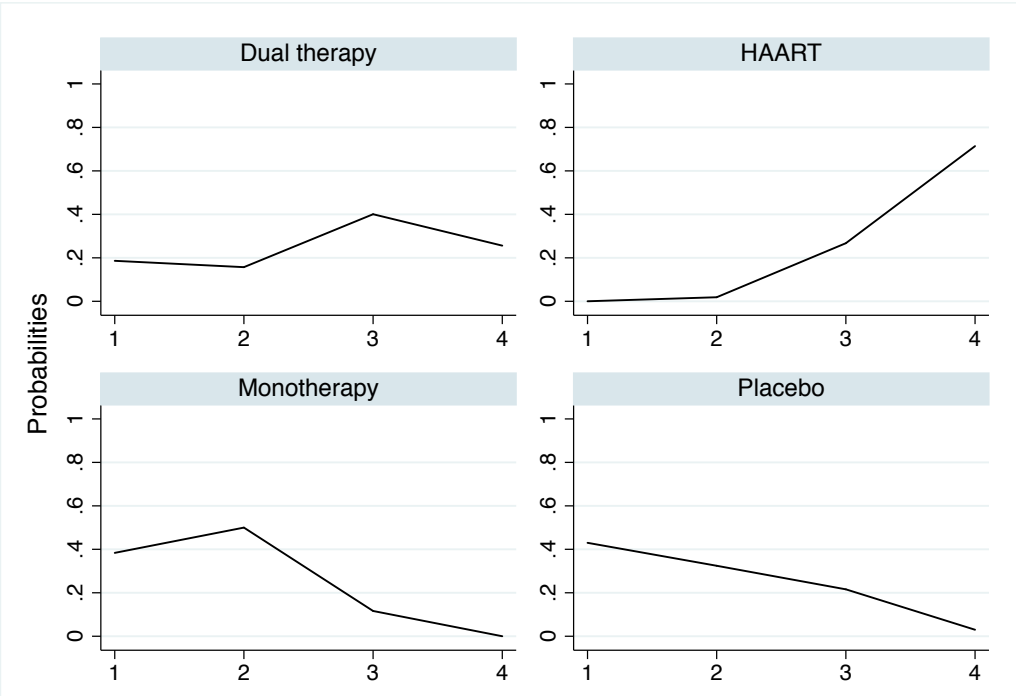


Figure 4.22: Rankograms for antiretroviral regimens included in the network meta-analysis for the association between antiretroviral regimens and PTB. This figure represents the surface under the cumulative ranking probabilities for each regimen included in the network meta-analysis. The horizontal axis represents the 4 possible ranks that the regimen can achieve and the vertical axis illustrates the probability of the regimen to attain each rank[308]. Abbreviation: HAART = Highly Active Antiretroviral Treatment.

Treatm~t	SUCRA	PrBest	MeanRank
PI	12.5	0.0	3.6
NRTI	79.2	44.0	1.6
NNRTI	32.9	10.2	3.0
Placebo	75.5	45.8	1.7

Figure 4.23: SUCRA ranking of antenatal antiretroviral class for PTB outcome.
This figure provides a hierarchy of the 4 classes of antiretrovirals (including placebo) assessed in the network meta-analysis for the association between antiretroviral class and PTB. The larger the SUCRA percentage, the lower the risk of PTB. SUCRA = surface under the cumulative rank curve, represented as a percentage. PrBest = probability that each treatment is the best i.e. associated with the lowest risk of PTB, represented as a percentage. MeanRank = mean rank of each ART. Abbreviations: NNRTI = non-nucleoside reverse transcriptase inhibitor, NRTI = nucleoside reverse transcriptase inhibitor, PI = protease inhibitor, PTB = preterm birth

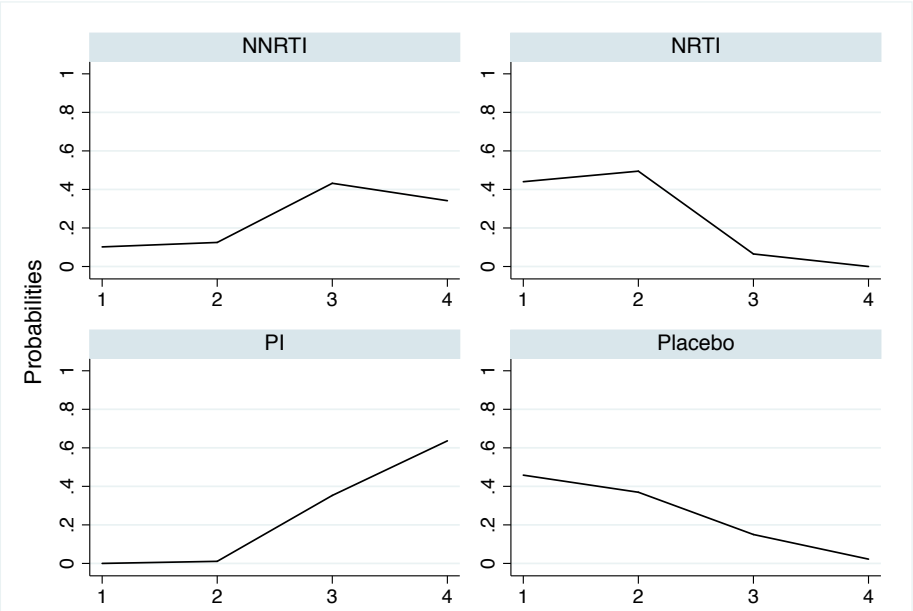


Figure 4.24: Rankograms for classes of antiretrovirals included in the network meta-analysis for the association between antiretroviral class and PTB. This figure represents the surface under the cumulative ranking probabilities for each antiretroviral class included in the network meta-analysis. The horizontal axis represents the 4 possible ranks that the class can achieve and the vertical axis illustrates the probability of the ART to attain each rank[312]. Abbreviations: NNRTI = non-nucleoside reverse transcriptase inhibitor, NRTI = nucleoside reverse transcriptase inhibitor, PI = protease inhibitor, PTB = preterm birth.

4.4.6.2 Spontaneous preterm birth

Three trials, including 991 women, reported on the association between sPTB and 4 ARTs. The most common treatment comparison was AZT/3TC/LPV/r HAART (n=482; 48.6%) and all the treatment comparisons were classified as having a high risk of bias (Figure 4.25).

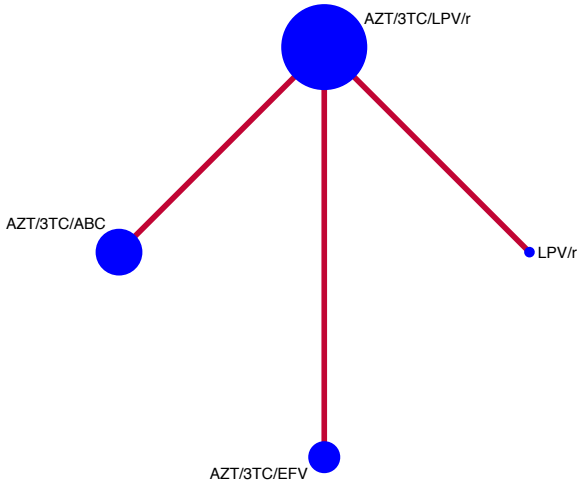


Figure 4.25: Network map illustrating direct comparisons between ART for sPTB outcome. Each circle represents a treatment group. The size of the circle represents the number of participants who received the ART. The lines connecting the circles represent direct comparisons of ART reported by the RCTs. The red colour of the connecting lines represents a high risk of bias in trials reporting the treatment comparison. The width of the lines represents the number of trials reporting on the treatment comparison.

4.4.6.2.1 Pairwise meta-analysis: association between ART and sPTB

- Antenatal ART

Participants initiating AZT/3TC/LPV/r HAART displayed a significantly higher risk of sPTB than those initiating AZT/3TC/ABC HAART (RR1.81; 95%CI1.21-2.71) (Figure 4.26).

- Antenatal antiretroviral regimens

Only one small study reported on the association between antiretroviral regimens and PTB (Figure 4.27). No significant difference was observed in the risk of sPTB in women initiating monotherapy compared to those initiating HAART (RR1.92, 95%CI 0.12-29.75) (Figure 4.27).

- Antenatal antiretroviral class

Two trials reported on the association between the class of antiretrovirals and sPTB (Figure 4.28). PI-based ART significantly increased the risk of sPTB compared to NRTI-based ART (RR1.81; 95%CI 1.21-2.71).

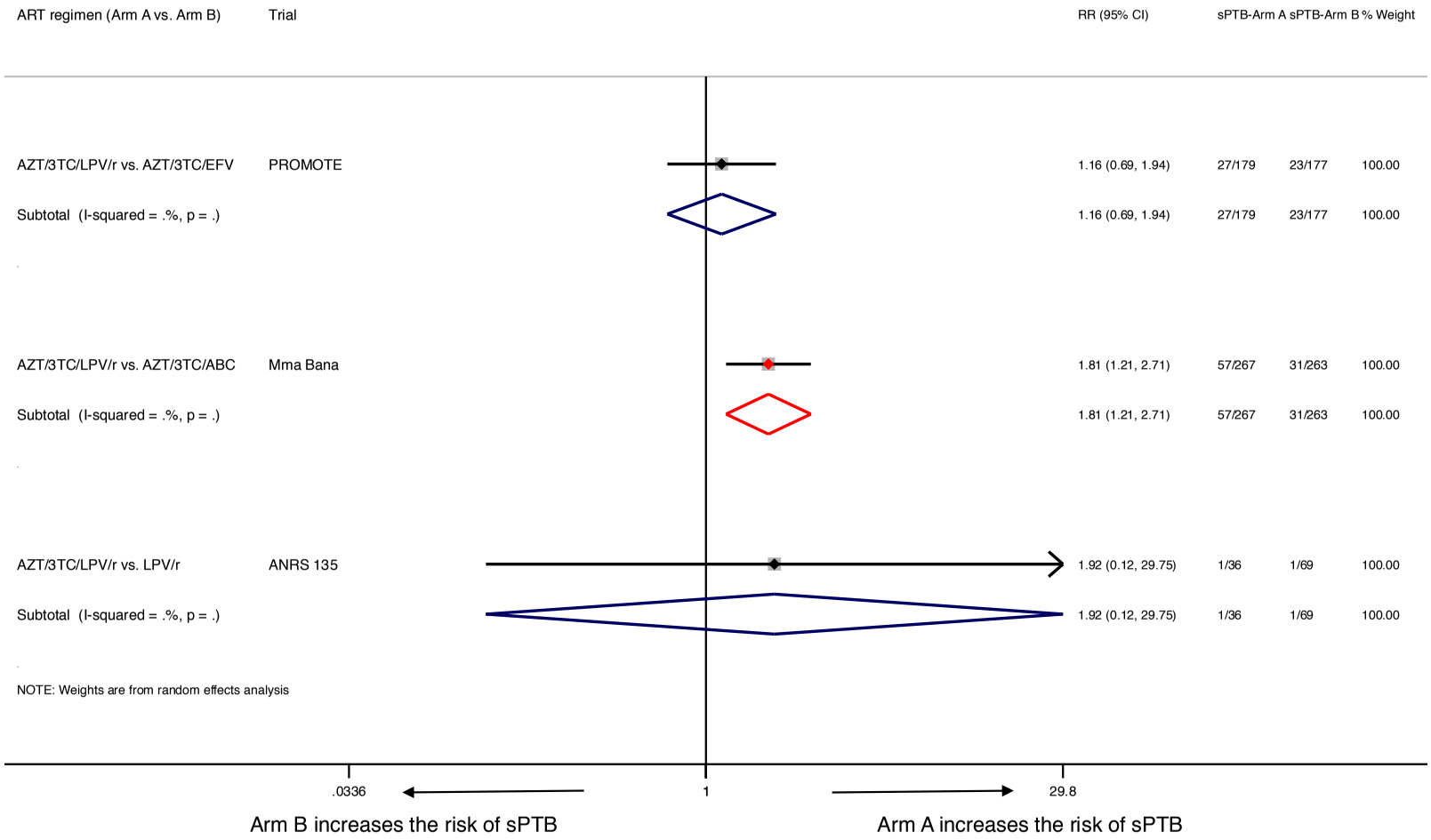


Figure 4.26: Random-effect pairwise meta-analysis results for the association between antenatal ART and sPTB.
Statistically significant effects are presented in red. AZT/3TC/LPV/r HAART significantly increased the risk of PTB, compared to AZT/3TC/ABC HAART. Abbreviations: 3TC = lamivudine, ABC = abacavir, ART = antiretroviral treatment, AZT = zidovudine, EFV = efavirenz, LPV/r = lopinavir/ritonavir, RR = relative risk, sPTB = spontaneous preterm birth.

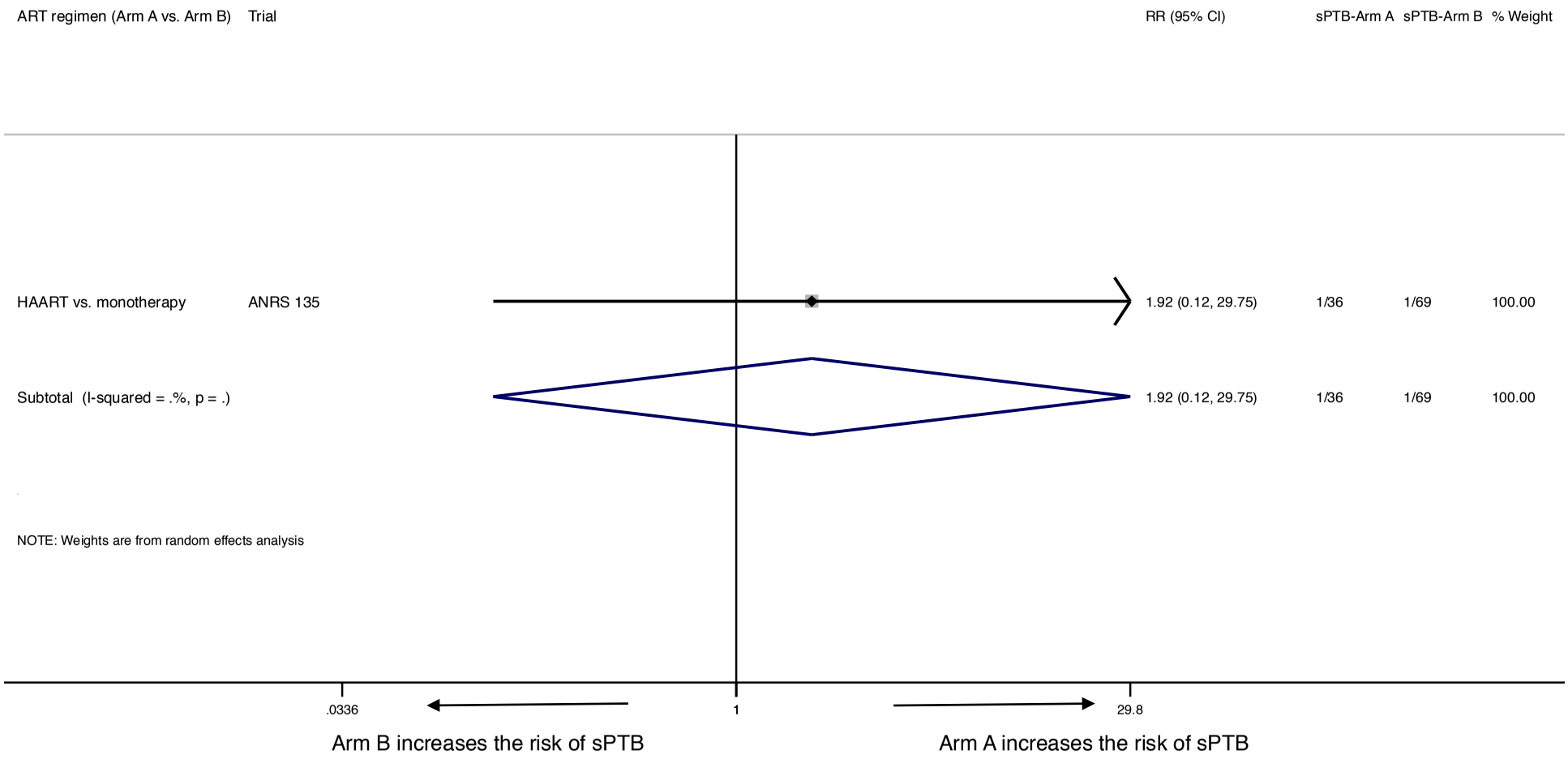


Figure 4.27: Random-effect pairwise meta-analysis results for the association between antenatal antiretroviral regimens and sPTB.
Statistically significant effects are presented in red. No significant difference observed in the risk of sPTB in women initiating HAART versus monotherapy. Abbreviations: ART = antiretroviral treatment, HAART = Highly Active Antiretroviral Treatment, RR = relative risk, sPTB = spontaneous preterm birth.

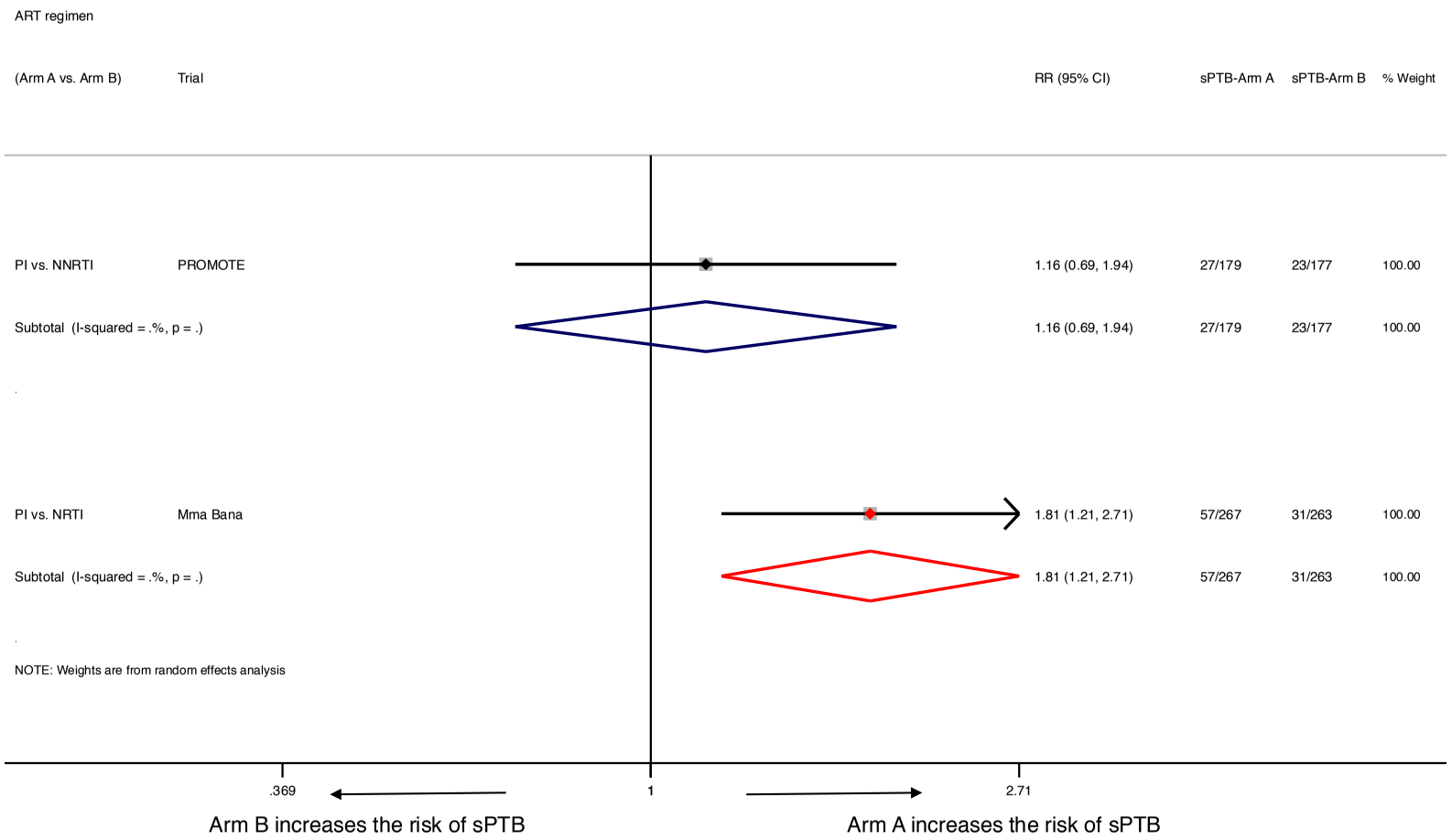


Figure 4.28: Random-effects pairwise meta-analysis results for the association between antenatal antiretroviral class and sPTB.
Statistically significant effects are presented in red. PI-based ART significantly increased the risk of sPTB compared to NRTI-based ART.
Abbreviations: ART = antiretroviral treatment, NNRTI = non-nucleoside reverse transcriptase inhibitor, NRTI = nucleoside reverse transcriptase inhibitor, PI = protease inhibitor, RR = relative risk, sPTB = spontaneous preterm birth.

4.4.6.2.2 Network meta-analysis: association between ART and sPTB

- Antenatal ART

Participants who initiated AZT/3TC/LPV/r HAART were almost twice as likely to deliver a PTB infant after spontaneous onset of labour, than those on AZT/3TC/ABC HAART (RR1.81; 95%CI1.21-2.71) (Figure 4.29; Figure 4.30). There was no evidence of statistical inconsistency (p=0.57) or publication bias (Figure 4.31).

The limited number of studies prohibited one from assessing networks according to the regimen or class of antiretrovirals.

4.4.6.2.3 Network meta-analysis: ranking of comparative effect of ART on sPTB

- Antenatal ART

Of the 4 treatment groups assessed, AZT/3TC/ABC HAART was associated with the lowest risk of sPTB and ranked superior to all treatments (SUCRA = 79.7%) (Figure 4.32, Figure 4.33). AZT/3TC/LPV/r HAART was associated with the highest risk for sPTB, and was ranked in 4th place (Figure 4.32, Figure 4.33).

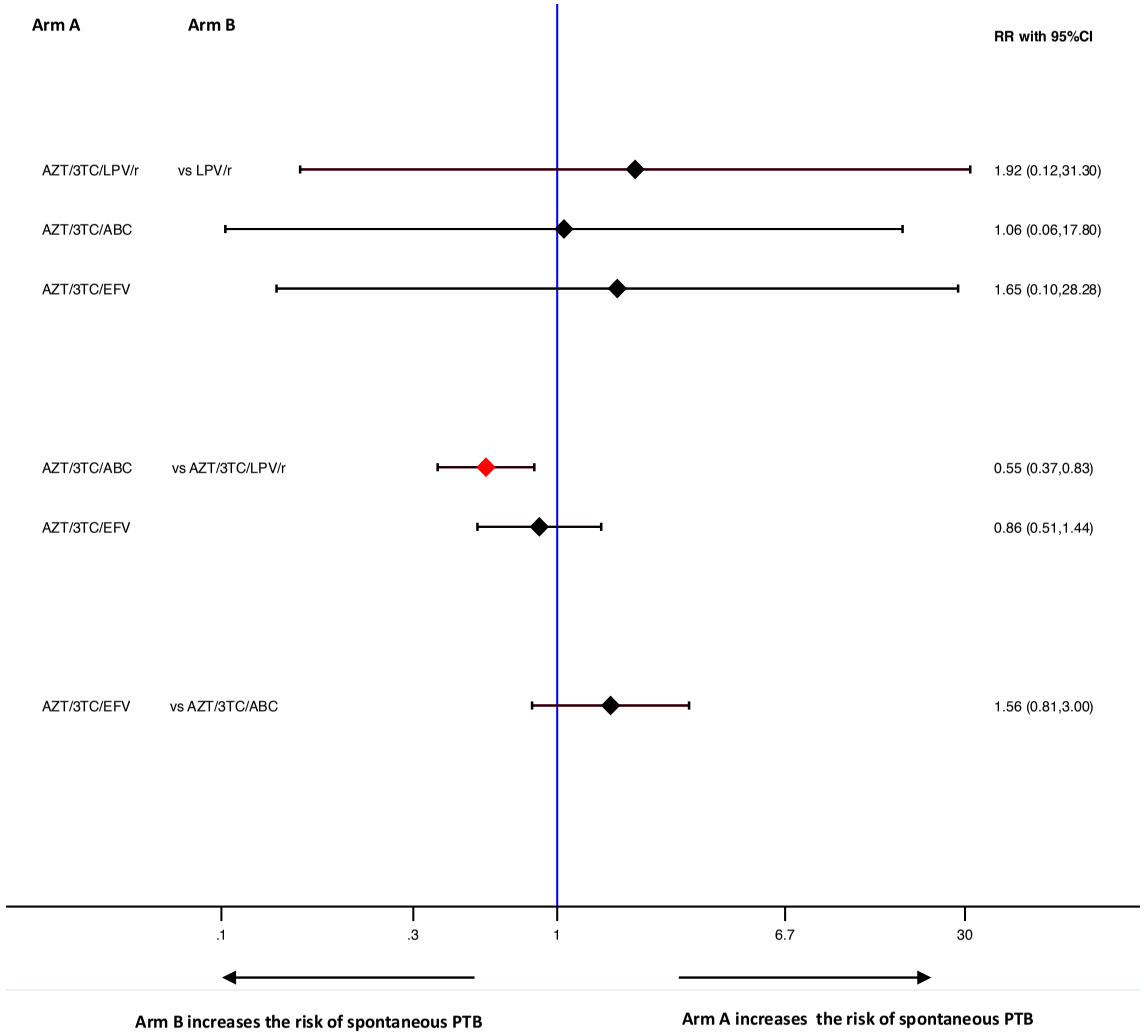


Figure 4.29: Random-effect network meta-analysis results for the association between antenatal ART and sPTB.
Statistically significant effects are presented in red. AZT/3TC/LPV/r HAART significantly increased the risk of sPTB compared to AZT/3TC/ABC HAART. Abbreviations: 3TC = lamivudine, ABC = abacavir, ART = antiretroviral treatment, AZT = zidovudine, EFV = efavirenz, LPV/r = lopinavir/ritonavir, RR = relative risk, sPTB = spontaneous preterm birth.

LPV/r	1.65 (0.10 - 28.28)	1.06 (0.06 - 17.80)	1.92 (0.12 - 31.30)
0.61 (0.04 - 10.38)	**AZT/3TC/EFV**	0.64 (0.33 - 1.23)	1.16 (0.69 - 1.94)
0.95 (0.06 - 15.91)	1.56 (0.81 - 3.00)	**AZT/3TC/ABC**	**1.81** **(1.21 - 2.71)**
0.52 (0.03 - 8.53)	0.86 (0.51 - 1.44)	**0.55** **(0.37 - 0.83)**	**AZT/3TC/LPV/r**

Figure 4.30: League table of random-effects network meta-analysis results for the association between antenatal ART and sPTB.
This table reflects all possible pairwise comparisons generated from the random-effect network meta-analysis. Results are reported as a relative risk (RR) of the column ART (e.g. AZT/3TC/LPV/r) relative to the row ART (AZT/3TC/ABC). Statistically significant effects are presented in red. AZT/3TC/LPV/r HAART significantly increased the risk of sPTB, compared to AZT/3TC/ABC HAART. Abbreviations: 3TC = lamivudine, ABC = abacavir, ART = antiretroviral treatment, AZT = zidovudine, EFV = efavirenz, LPV/r = lopinavir/ritonavir, sPTB = spontaneous preterm birth.

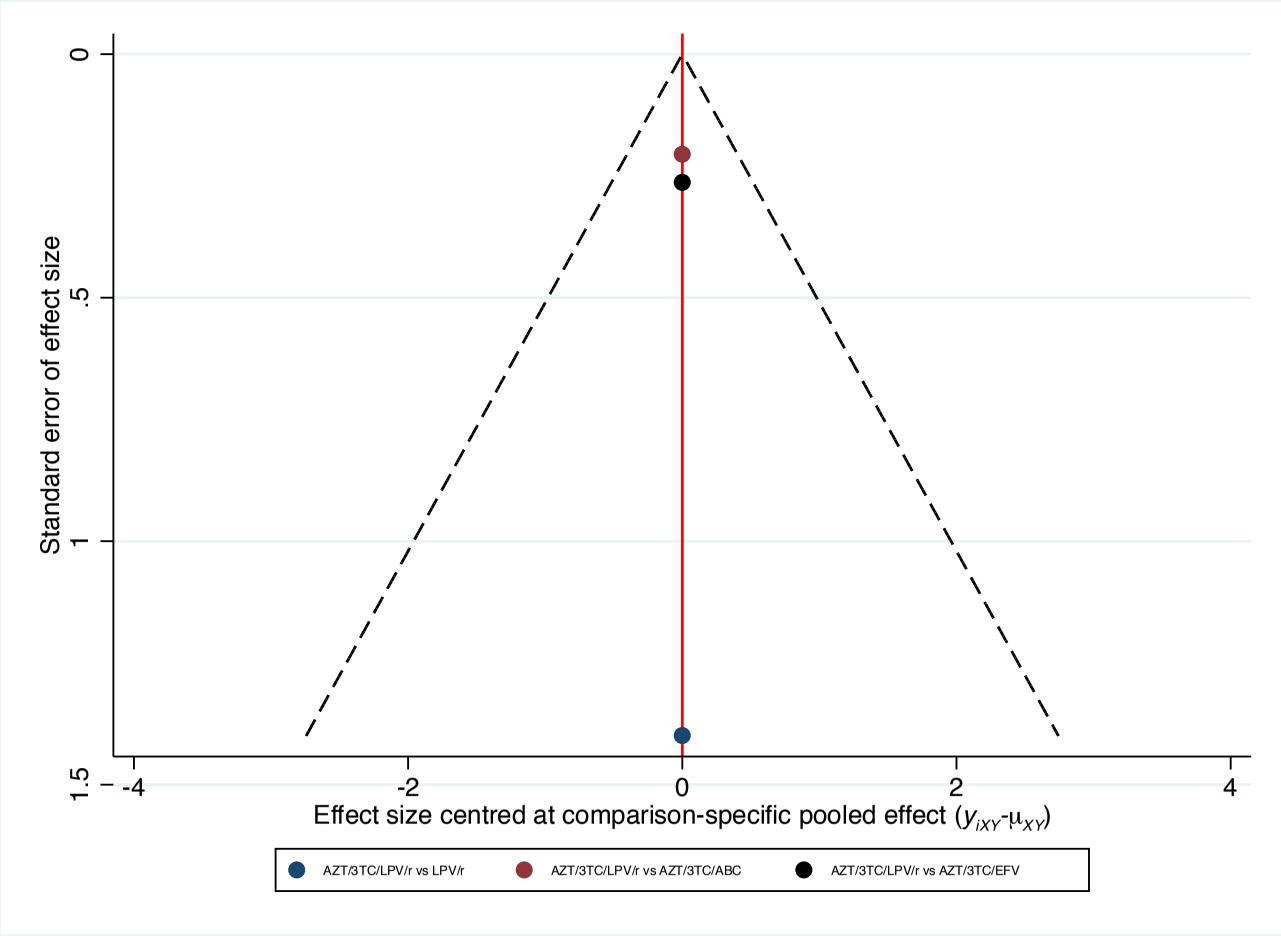


Figure 4.31: Comparison-adjusted funnel plot for the association between antenatal ART and sPTB.
The vertical axis represents the measure of the dispersion of the effect size; the horizontal axis represents the difference between the study specific effect size and the comparison-specific summary effect size, and the red line represents the null hypothesis of no difference between the study specific and comparison specific effect estimates. The colours represent individual comparisons. There was no evidence of small study effect.

Treatment	SUCRA	PrBest	MeanRank
LPV/r	60.8	51.2	2.2
AZT/3TC/LPV/r	20.4	0.0	3.4
AZT/3TC/ABC	79.7	44.6	1.6
AZT/3TC/EFV	39.0	4.1	2.8

Figure 4.32: SUCRA ranking of antenatal ART for sPTB outcome.
This figure provides a hierarchy of the 4 ARTs assessed in the network meta-analysis for the association between ART and sPTB. The larger the SUCRA percentage, the lower the risk of sPTB. SUCRA = surface under the cumulative rank curve, represented as a percentage. PrBest = probability that each treatment is the best i.e. associated with the lowest risk of sPTB, represented as a percentage. MeanRank = mean rank of each ART. Abbreviations: 3TC = lamivudine, ABC = abacavir, ART = antiretroviral treatment, AZT = zidovudine, EFV = efavirenz, LPV/r = lopinavir/ritonavir, sPTB = spontaneous preterm birth

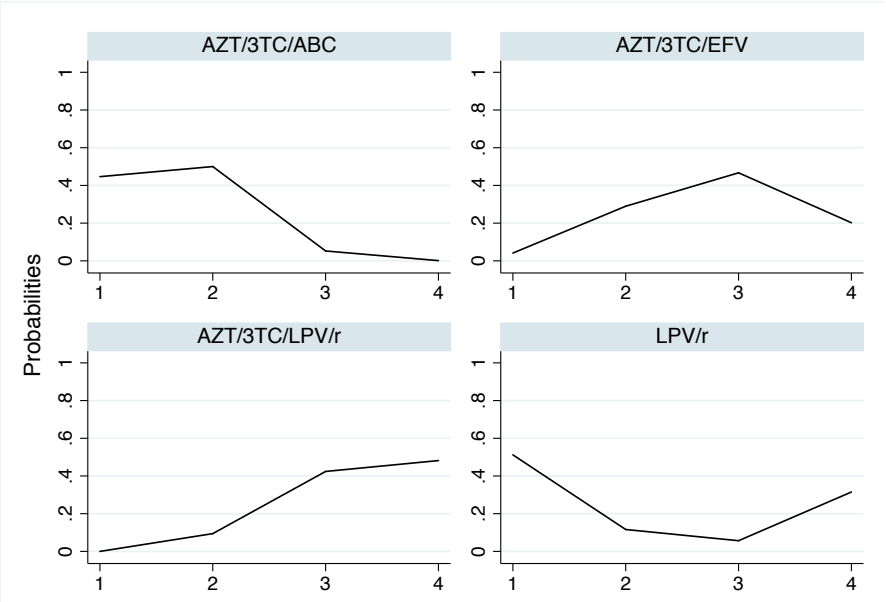


Figure 4.33: Rankograms for ARTs included in the network meta-analysis for the association between ART and sPTB. This figure represents the surface under the cumulative ranking probabilities for each ART included in the network meta-analysis. The horizontal axis represents the 4 possible ranks that the ART can achieve and the vertical axis illustrates the probability of the ART to attain each rank[312]. Abbreviations: 3TC = lamivudine, ABC = abacavir, ART = antiretroviral treatment, AZT = zidovudine, EFV = efavirenz, LPV/r = lopinavir/ritonavir, sPTB = spontaneous preterm birth

4.4.6.3 Very preterm birth

Four studies, including 1819 women, assessed the association between VPTB and 5 ARTs (Figure 4.34). The majority of participants were initiated on a AZT/3TC/LPV/r HAART (n = 886; 48.7%) and all comparisons were considered as containing a high risk of bias.

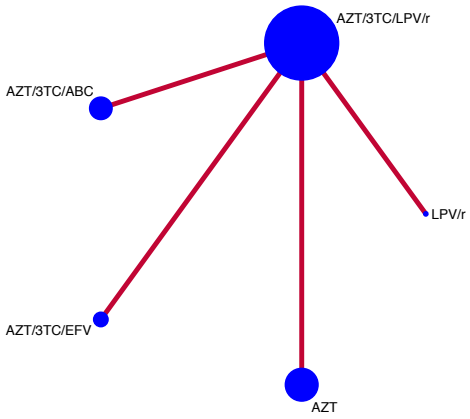


Figure 4.34: Network map illustrating direct comparisons between ART for VPTB outcome.
Each circle represents a treatment group. The size of the circle represents the number of participants who received the ART. The lines connecting the circles represent direct comparisons of ART reported by the RCTs. The red colour of the connecting lines represents a high risk of bias in trials reporting the treatment comparison. The width of the lines represents the number of trials reporting on the treatment comparison.

4.4.6.3.1 Pairwise meta-analysis: association between ART and VPTB

Pairwise meta-analyses of ARTs (Figure 4.35), regimens (Figure 4.36) and different classes of ART (4.37) showed no significant association with VPTB.

4.4.6.3.2 Network meta-analysis: association between ART and very preterm birth

Similarly, network meta-analyses did not show any significant association between ART and VPTB (Figure 4.38 to Figure 4.46). Furthermore, there was no evidence of inconsistency or publication bias.

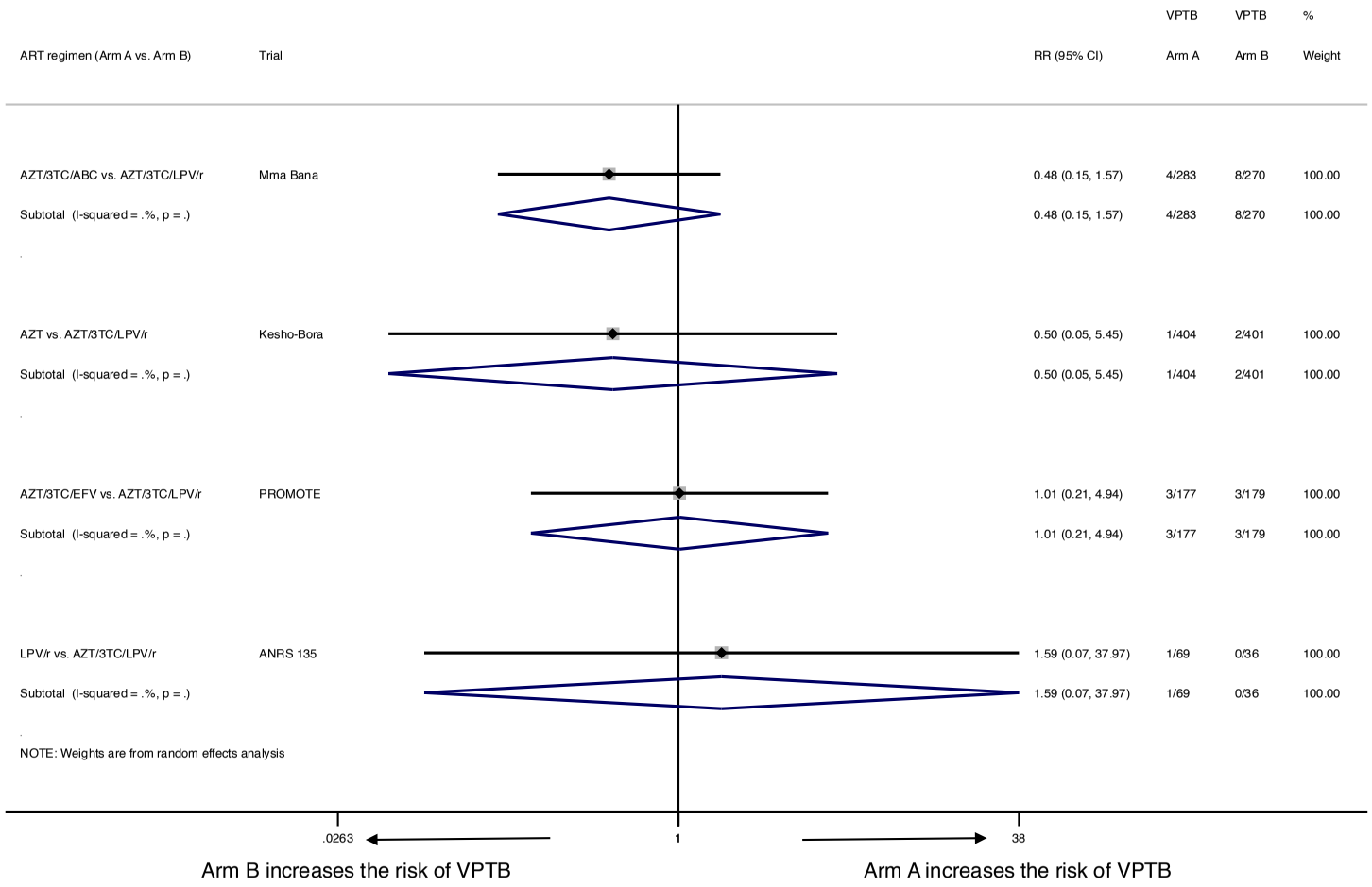


Figure 4.35: Random-effect pairwise meta-analysis results for the association between antenatal ART and VPTB.
There was no significant association between ART and VPTB. Abbreviations: 3TC = lamivudine, ABC = abacavir, ART = antiretroviral treatment, AZT = zidovudine, EFV = efavirenz, LPV/r = lopinavir/ritonavir, RR = relative risk, VPTB = very preterm birth.

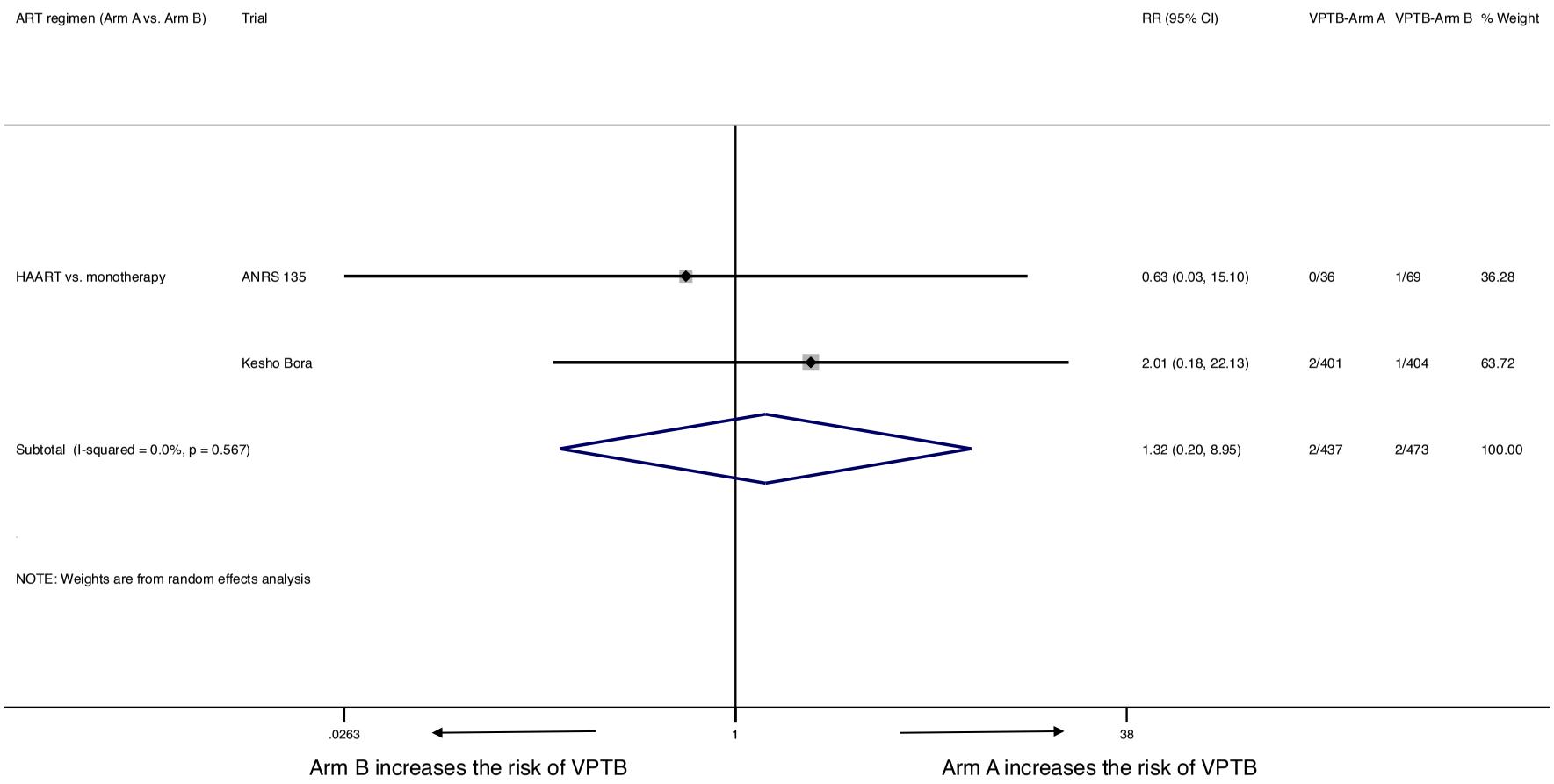


Figure 4.36: Random-effect pairwise meta-analysis results for the association between antenatal antiretroviral regimen and VPTB.
No significant difference observed in the risk of VPTB in women initiating HAART or monotherapy. Abbreviations: ART = antiretroviral treatment, HAART = Highly Active Antiretroviral Treatment, RR = relative risk, VPTB = very preterm birth.

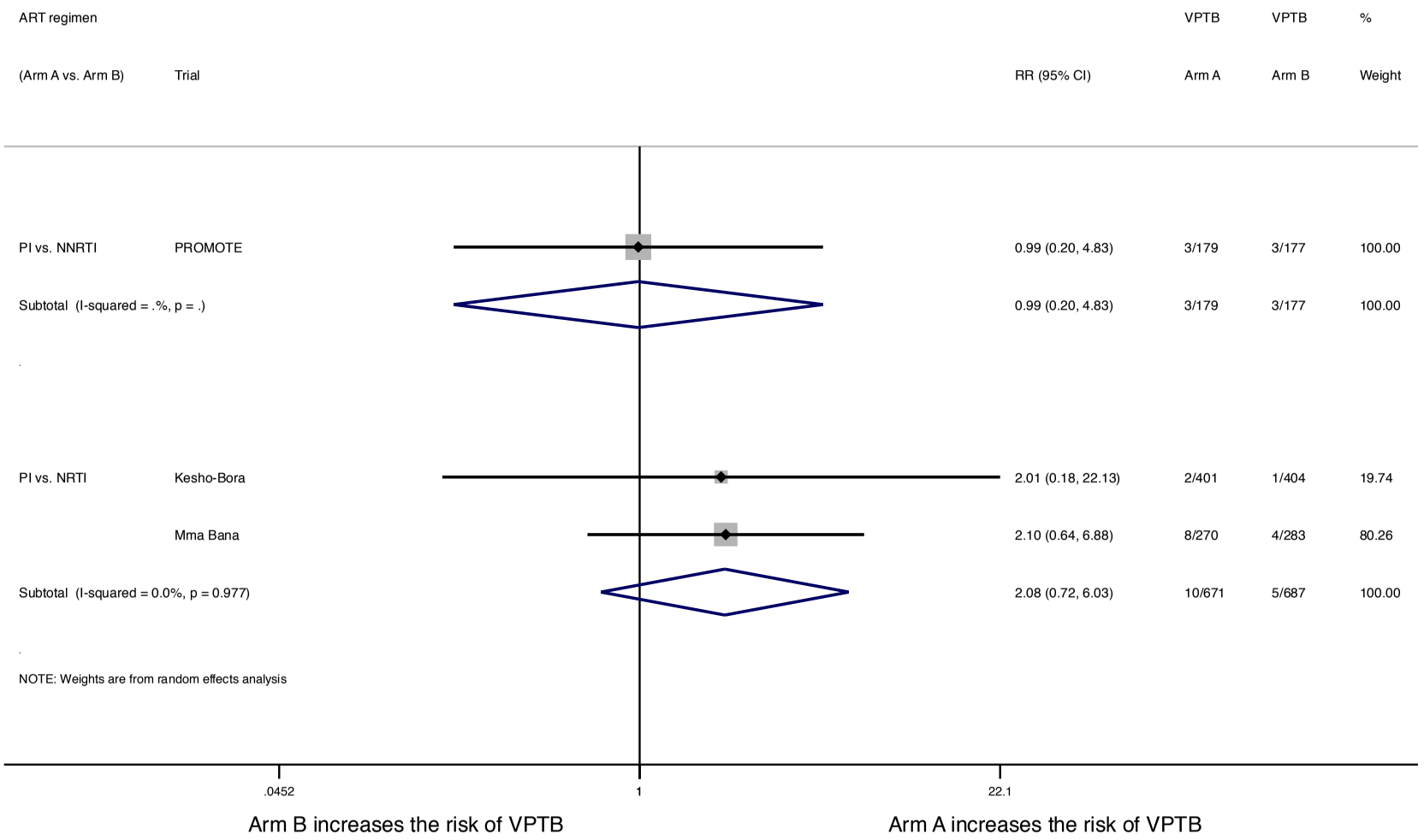


Figure 4.37: Random-effects pairwise meta-analysis results for the association between antenatal antiretroviral class and VPTB. There was no significant association between antiretroviral class and VPTB. Abbreviations: ART = antiretroviral treatment, NNRTI = non-nucleoside reverse transcriptase inhibitor, NRTI = nucleoside reverse transcriptase inhibitor, PI = protease inhibitor, RR = relative risk, VPTB = very preterm birth.

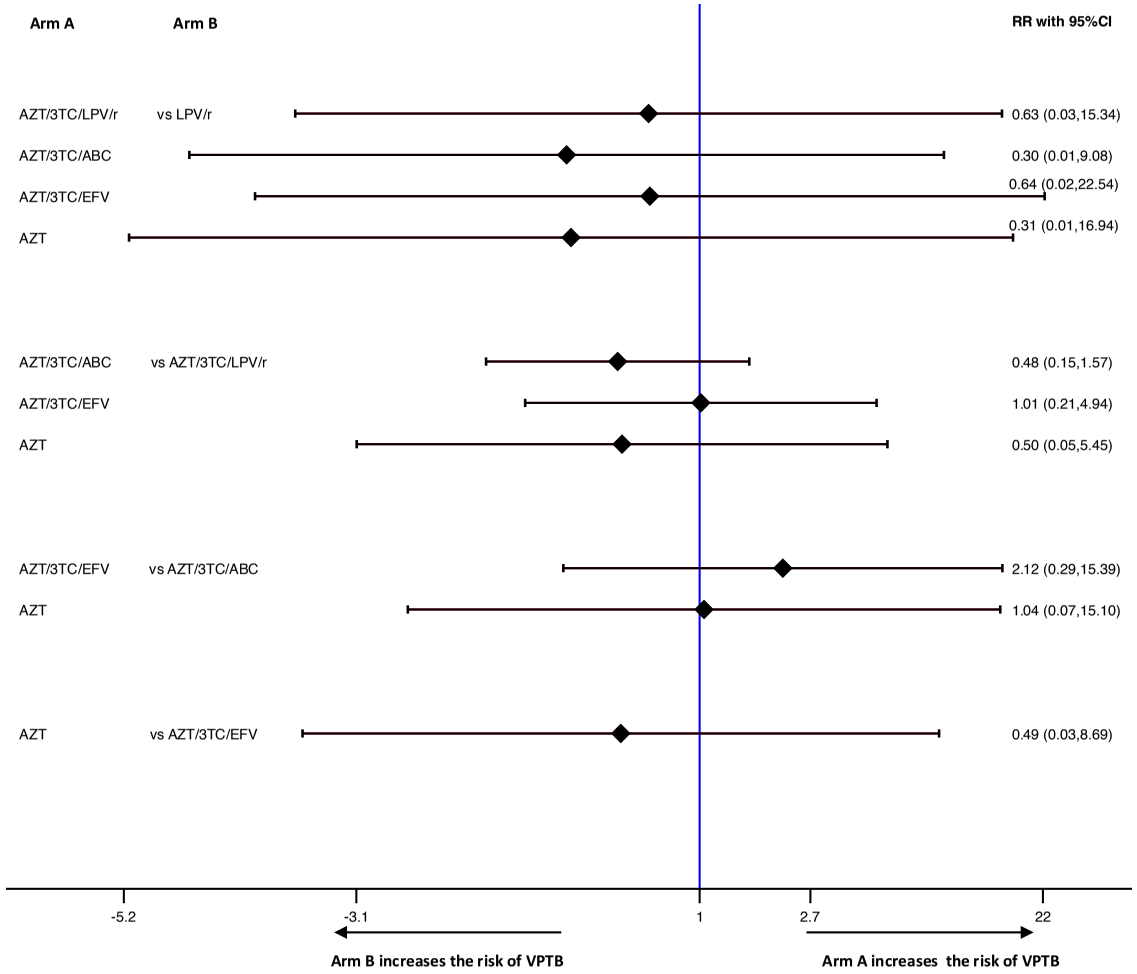


Figure 4.38: Random-effect network meta-analysis results for the association between antenatal ART and VPTB.
There was no significant association between ART and VPTB. Abbreviations: 3TC = lamivudine, ABC = abacavir, ART = antiretroviral treatment, AZT = zidovudine, EFV = efavirenz, LPV/r = lopinavir/ritonavir, RR = relative risk, VPTB = very preterm birth.

AZT	3.19 (0.06 - 172.95)	2.04 (0.12 - 36.09)	0.96 (0.07 - 13.95)	2.01 (0.18 - 22.13)
0.31 (0.01 - 16.94)	**LPV/r**	0.64 (0.02 - 22.54)	0.30 (0.01 - 9.08)	0.63 (0.03 - 15.34)
0.49 (0.03 - 8.69)	1.57 (0.04 - 55.41)	**AZT/3TC/EFV**	0.47 (0.06 - 3.43)	0.99 (0.20 - 4.83)
1.04 (0.07 - 15.10)	3.32 (0.11 - 100.31)	2.12 (0.29 - 15.39)	**AZT/3TC/ABC**	2.10 (0.64 - 6.88)
0.50 (0.05 - 5.45)	1.59 (0.07 - 38.55)	1.01 (0.21 - 4.94)	0.48 (0.15 - 1.57)	**AZT/3TC/LPV/r**

Figure 4.39: League table of random-effects network meta-analysis results for the association between antenatal ART and VPTB.
This table reflects all possible pairwise comparisons generated from the random-effect network meta-analysis. Results are reported as a relative risk (RR) of the column ART (e.g. AZT/3TC/LPV/r) relative to the row ART (AZT/3TC/ABC). There was no significant association between ART and VPTB. Abbreviations: 3TC = lamivudine, ABC = abacavir, ART = antiretroviral treatment, AZT = zidovudine, EFV = efavirenz, LPV/r = lopinavir/ritonavir, VPTB = very preterm birth.

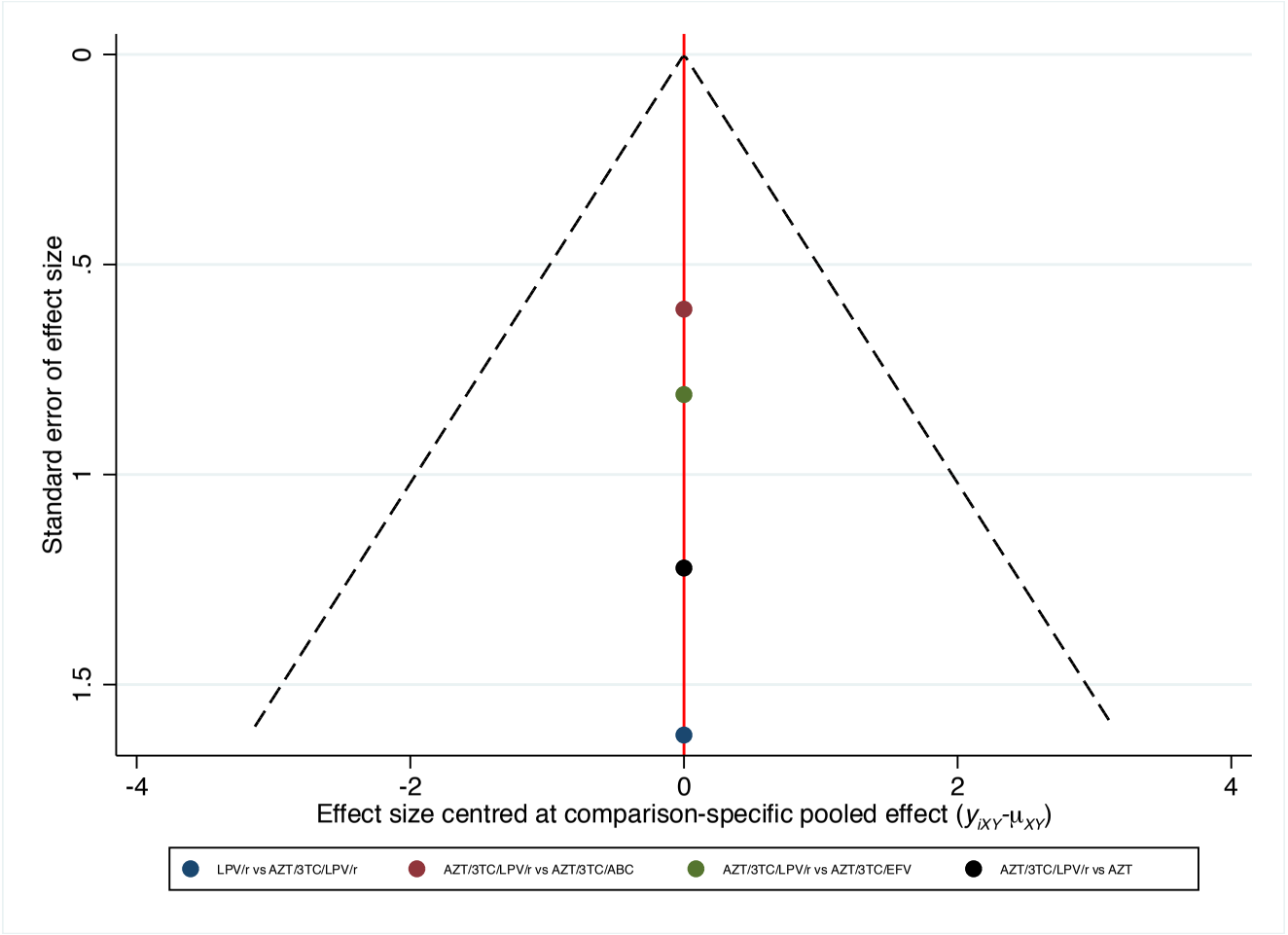


Figure 4.40: Comparison-adjusted funnel plot for the association between antenatal ART and VPTB.
The vertical axis represents the measure of the dispersion of the effect size; the horizontal axis represents the difference between the study specific effect size and the comparison-specific summary effect size, and the red line represents the null hypothesis of no difference between the study specific and comparison specific effect estimates. The colours represent individual comparisons. There was no evidence of small study effect.

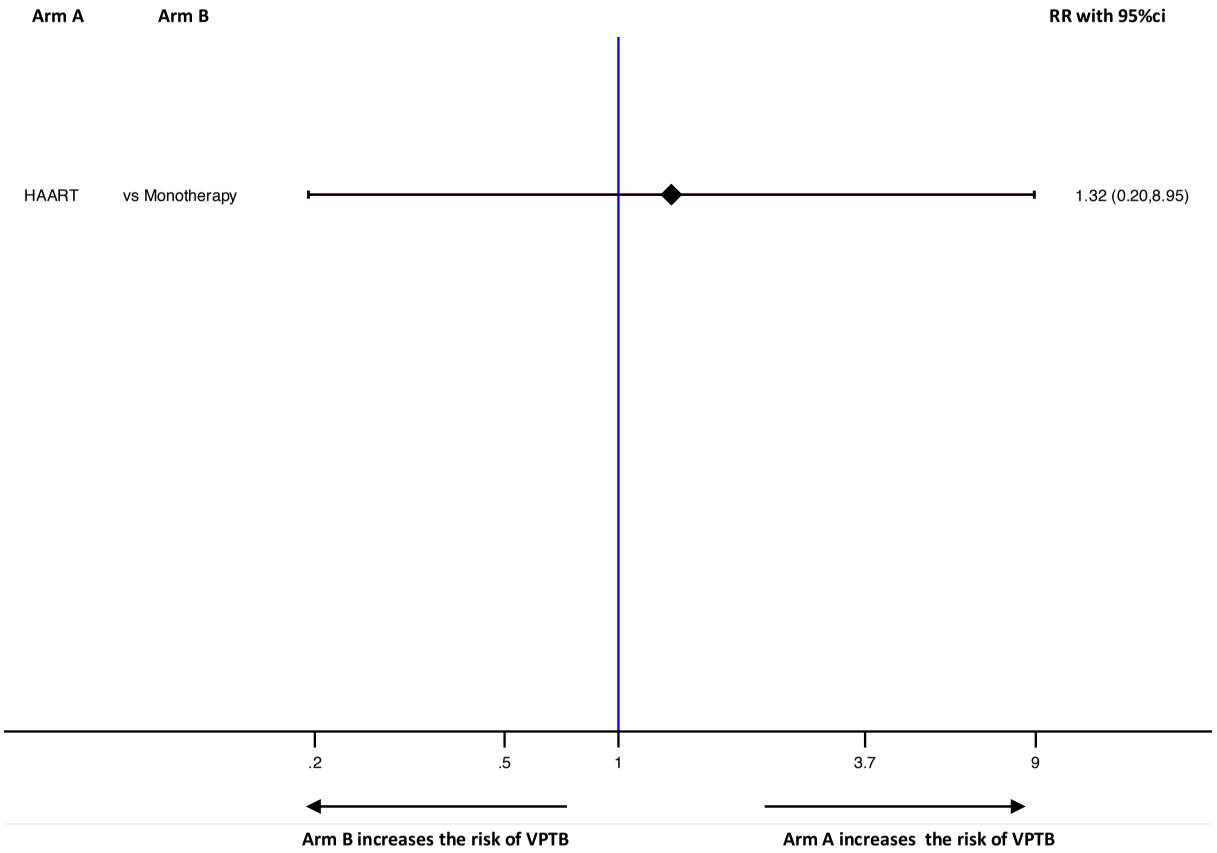


Figure 4.41: Random-effect network meta-analysis results for the association between antenatal antiretroviral regimen and VPTB.
No significant difference observed in the risk of VPTB in women initiating HAART versus those initiating monotherapy. Abbreviations: HAART = Highly Active Antiretroviral Treatment, RR = relative risk, VPTB = very preterm birth.

Monotherapy	1.32 (0.20 - 8.95)
0.76 (0.11 - 5.12)	**HAART**

Figure 4.42: League table of random-effects network meta-analysis results for the association between antenatal antiretroviral regimen and VPTB. This table reflects all possible pairwise comparisons generated from the random-effect network meta-analysis. Results are reported as a relative risk (RR) of the column ART (e.g. HAART) relative to the row ART (Monotherapy). No significant difference was observed in the risk of VPTB in women initiating HAART versus those initiating monotherapy. Abbreviations: ART = antiretroviral treatment, HAART = Highly

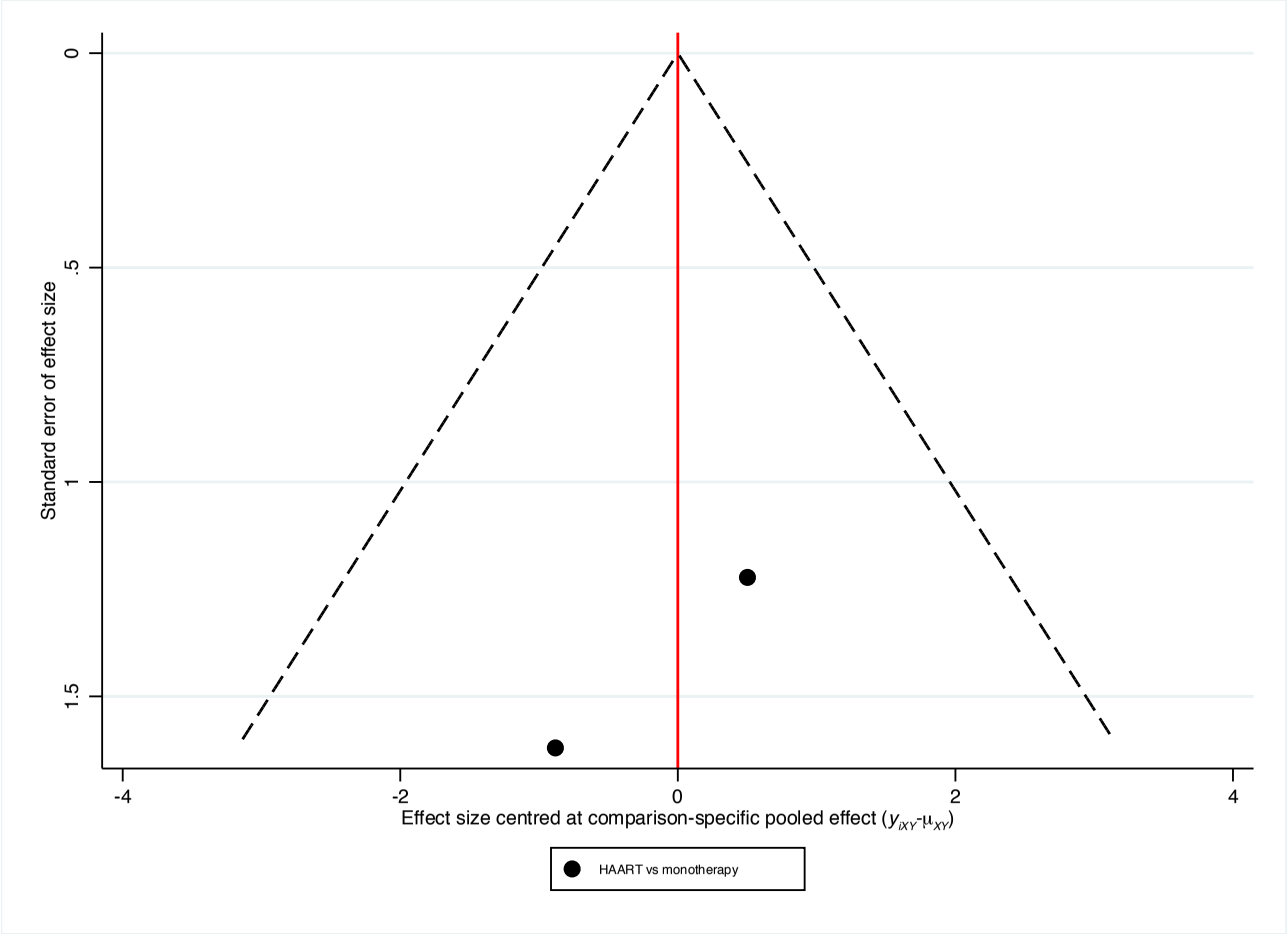


Figure 4.43: Comparison-adjusted funnel plot for the association between antenatal antiretroviral regimens and VPTB.
The vertical axis represents the measure of the dispersion of the effect size; the horizontal axis represents the difference between the study specific effect size and the comparison-specific summary effect size, and the red line represents the null hypothesis of no difference between the study specific and comparison specific effect estimates. The colours represent individual comparisons. There was no evidence of small study effect.

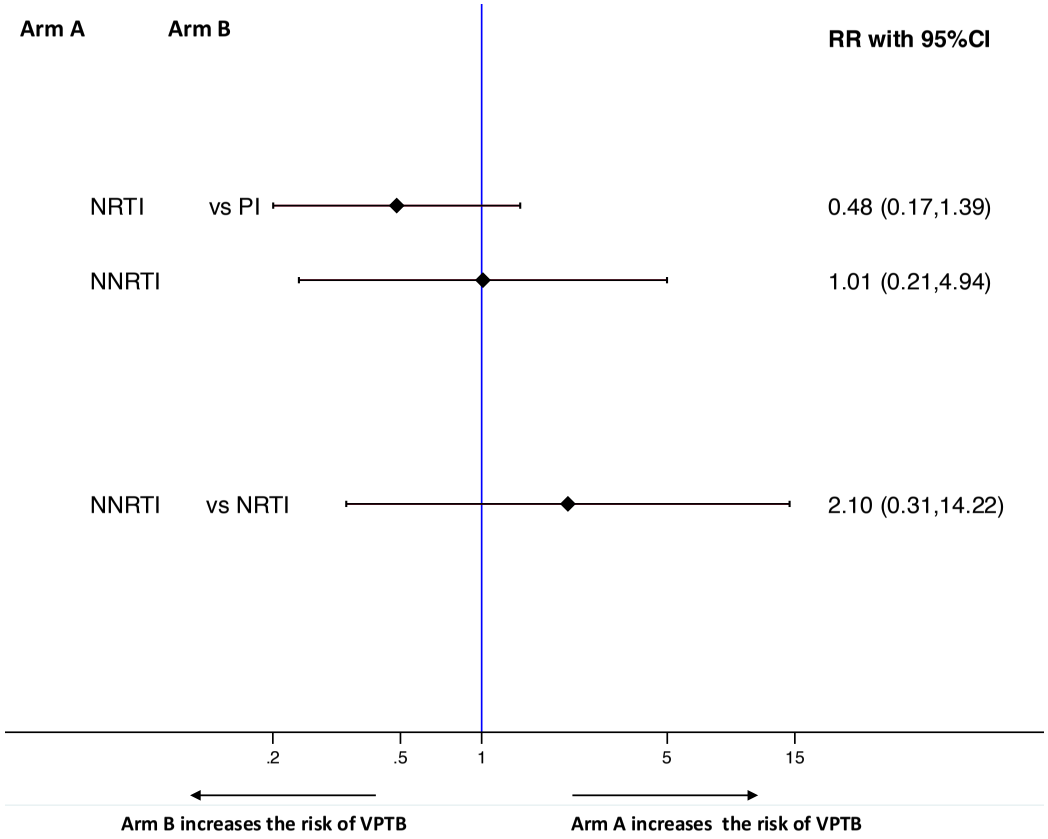


Figure 4.44: Random-effects network meta-analysis results for the association between antenatal antiretroviral class and VPTB.
No significant difference was observed in the risk of VPTB in women initiating different classes of antiretrovirals. Abbreviations: NNRTI = non-nucleoside reverse transcriptase inhibitor, NRTI = nucleoside reverse transcriptase inhibitor, PI = protease inhibitor, RR = relative risk, VPTB = very preterm birth.

PI	1.01 (0.21 - 4.94)	0.48 (0.17 - 1.39)
0.99 (0.20 - 4.83)	**NNRTI**	0.48 (0.07 - 3.21)
2.08 (0.72 - 6.03)	2.10 (0.31 - 14.22)	**NRTI**

Figure 4.45: League table of random-effects network meta-analysis results for the association between antenatal antiretroviral class and VPTB. This table reflects all possible pairwise comparisons generated from the random-effect network meta-analysis. Results are reported as a relative risk (RR) of the column ART (e.g. PI) relative to the row ART (NNRTI). No significant difference observed in the risk of VPTB in women initiating different classes of antiretrovirals. Abbreviations: ART = antiretroviral treatment, NNRTI = non-nucleoside reverse transcriptase inhibitor, NRTI = nucleoside reverse transcriptase inhibitor, PI = protease inhibitor, PTB = preterm birth.

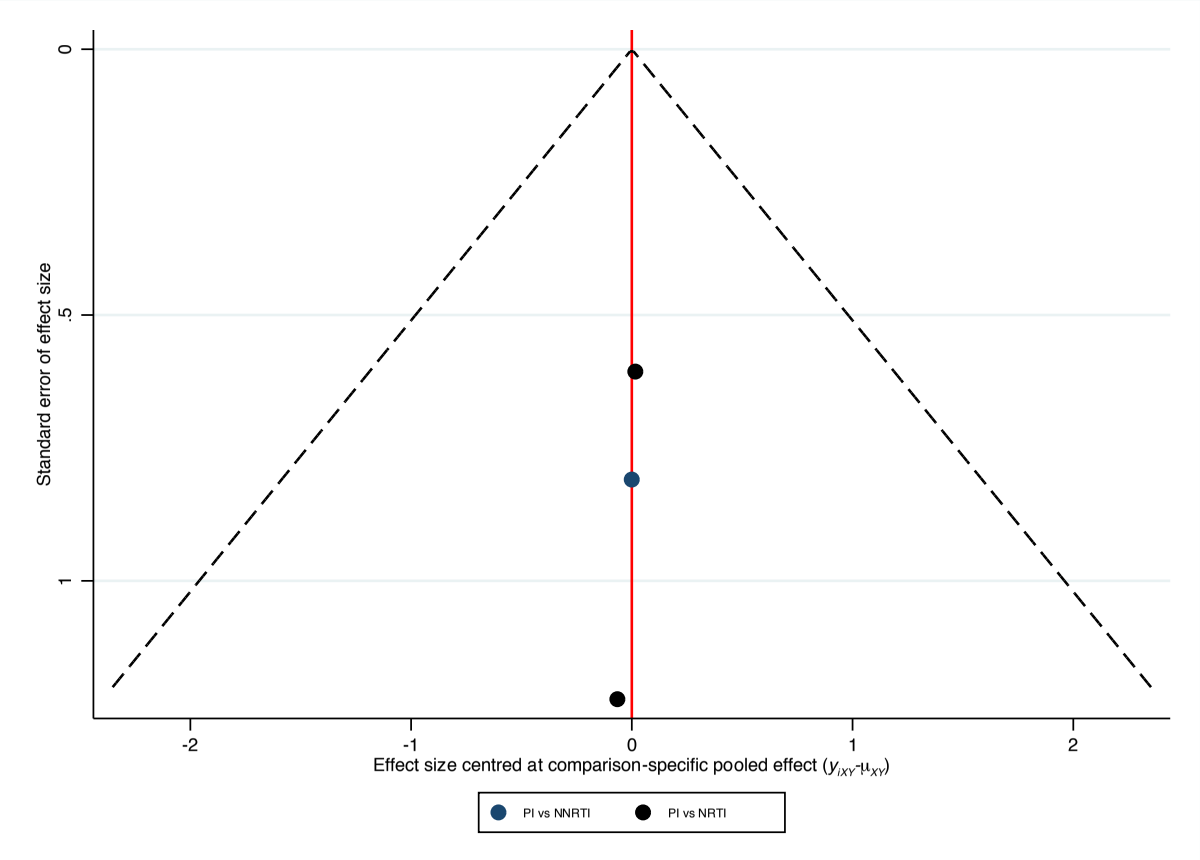


Figure 4.46: Comparison-adjusted funnel plot for the association between antenatal classes of antiretrovirals and VPTB.
The vertical axis represents the measure of the dispersion of the effect size; the horizontal axis represents the difference between the study specific effect size and the comparison-specific summary effect size, and the red line represents the null hypothesis of no difference between the study specific and comparison specific effect estimates. The colours represent individual comparisons. There was no evidence of small study effect.

4.4.6.3.3 Network meta-analysis: ranking of comparative effect of ART on VPTB

- Antenatal ART

Of the five ARTs assessed, AZT/3TC/ABC HAART (SUCRA = 73.3%) and AZT monotherapy (SUCRA = 65.3%) were associated with the lowest risk of VPTB, and were ranked superior to all other treatments (Figure 4.47, Figure 4.48). ARTs containing a PI drug – including LPV/r monotherapy (SUCRA = 33.1%) and AZT/3TC/LPV/r HAART (37.9%) – were associated with the highest risk for VPTB and received the lowest ranking (Figure 4.47, Figure 4.48).

- Antenatal antiretroviral regimen

Of the 2 antiretroviral regimens assessed, monotherapy (SUCRA = 61.5%) was associated with the lowest risk of VPTB and was ranked superior to all other regimens. HAART (SUCRA = 38.5%) was associated with the highest risk of VPTB (Figure 4.49, Figure 4.50).

- Antenatal antiretroviral class

Of the 3 classes of antiretrovirals assessed, NRTI-based ART (SUCRA = 84.7%) was associated with the lowest risk of VPTB and was ranked superior to all other regimens (Figure 4.51, Figure 4.52). PI-based ARTs (SUCRA = 29.58%) was associated with the highest risk of VPTB and was ranked lowest (Figure 4.51, Figure 4.52).

Treatment	SUCRA	PrBest	MeanRank
LPV/r	33.1	15.9	3.7
AZT/3TC/LPV/r	37.9	1.2	3.5
AZT/3TC/ABC	73.3	33.7	2.1
AZT/3TC/EFV	40.5	9.6	3.4
AZT	65.3	39.6	2.4

Figure 4.47: SUCRA ranking of antenatal ART for VPTB outcome.
This figure provides a hierarchy of the 5 ARTs assessed in the network meta-analysis for the association between ART and VPTB. The larger the SUCRA percentage, the lower the risk of VPTB. SUCRA = surface under the cumulative rank curve, represented as a percentage. PrBest = probability that each treatment is the best i.e. associated with the lowest risk of VPTB, represented as a percentage. MeanRank = mean rank of each ART. Abbreviations: 3TC = lamivudine, ABC = abacavir, ART = antiretroviral treatment, AZT = zidovudine, EFV = efavirenz, LPV/r = lopinavir/ritonavir, VPTB = very preterm birth.

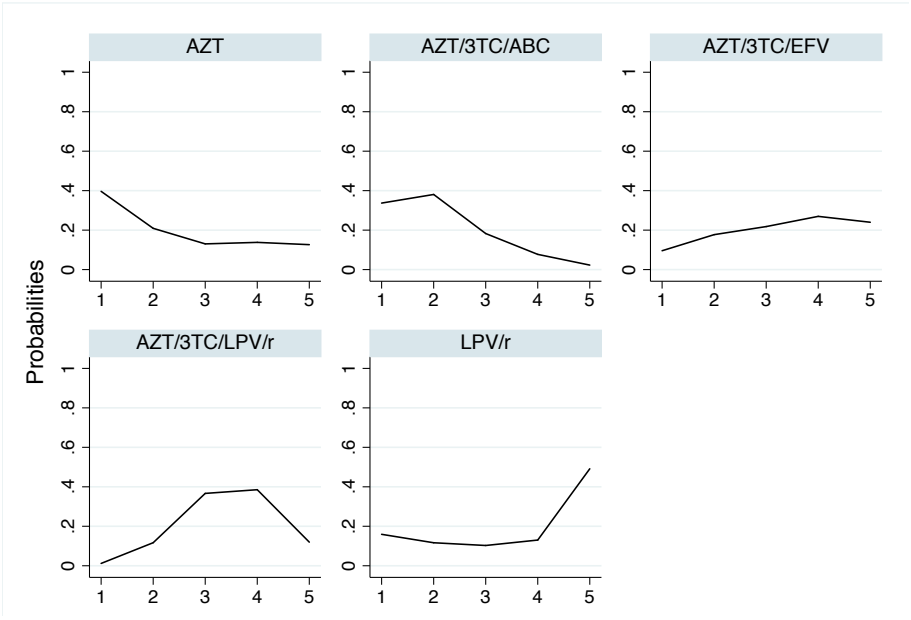


Figure 4.48: Rankograms for ARTs included in the network meta-analysis for the association between ART and VPTB. This figure represents the surface under the cumulative ranking probabilities for each ART included in the network meta-analysis. The horizontal axis represents the 5 possible ranks that the ART can achieve and the vertical axis illustrates the probability of the ART to attain each rank[312]. Abbreviations: 3TC = lamivudine, ABC = abacavir, ART = antiretroviral treatment, AZT = zidovudine, EFV = efavirenz, LPV/r = lopinavir/ritonavir, VPTB = very preterm birth.

Treatment	SUCRA	PrBest	MeanRank
Monotherapy	61.5	61.5	1.4
HAART	38.5	38.5	1.6

Figure 4.49: SUCRA ranking of antenatal antiretroviral regimens for VPTB outcome. This figure provides a hierarchy of the 2 regimens assessed in the network meta-analysis for the association between antiretroviral regimen and VPTB. The larger the SUCRA percentage, the lower the risk of VPTB. SUCRA = surface under the cumulative rank curve, represented as a percentage. PrBest = probability that each treatment is the best i.e. associated with the lowest risk of VPTB, represented as a percentage. MeanRank = mean rank of each ART. Abbreviation: HAART = Highly Active Antiretroviral Treatment.

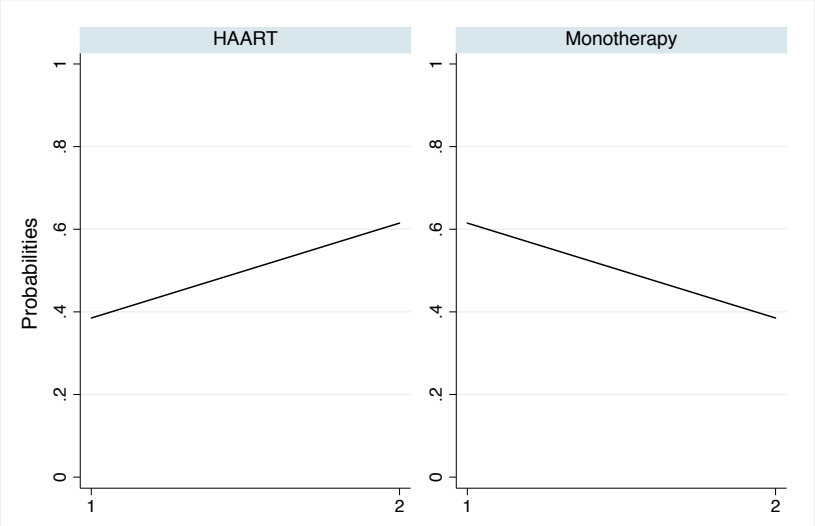


Figure 4.50: Rankograms for antiretroviral regimens included in the network meta-analysis for the association between ART and VPTB. This figure represents the surface under the cumulative ranking probabilities for each regimen included in the network meta-analysis. The horizontal axis represents the 2 possible ranks that the ART can achieve and the vertical axis illustrates the probability of the ART to attain each rank[312]. Abbreviation: HAART = Highly Active Antiretroviral Treatment.

Treatm~t	SUCRA	PrBest	MeanRank
PI	29.8	4.6	2.4
NRTI	84.7	74.7	1.3
NNRTI	35.5	20.8	2.3

Figure 4.51: SUCRA ranking for antiretroviral classes for VPTB outcome. This figure provides a hierarchy of the 3 classes of antiretrovirals assessed in the network meta-analysis for the association between antiretroviral classes and VPTB. The larger the SUCRA percentage, the lower the risk of VPTB. SUCRA = surface under the cumulative rank curve, represented as a percentage. PrBest = probability that each treatment is the best i.e. associated with the lowest risk of VPTB, represented as a percentage. MeanRank = mean rank of each ART. Abbreviation: NNRTI = non-nucleoside reverse transcriptase inhibitor, NRTI = nucleoside reverse transcriptase inhibitor, PI = protease inhibitor, VPTB = very preterm birth.

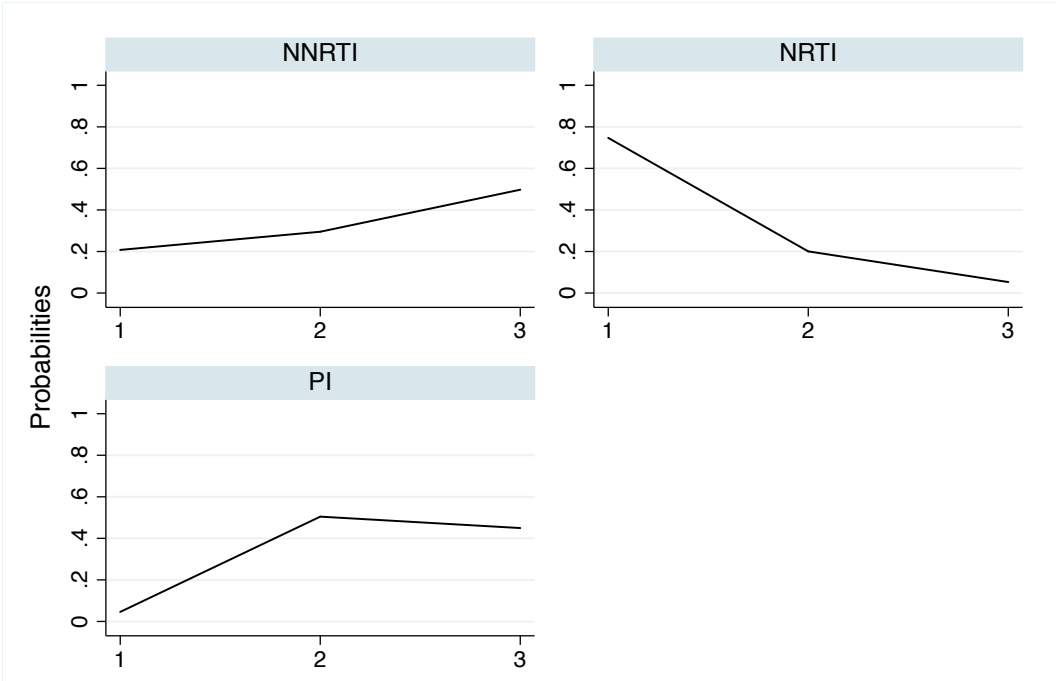


Figure 4.52: Rankograms for antiretroviral classes included in the network meta-analysis for the association between antiretroviral class and VPTB. This figure represents the surface under the cumulative ranking probabilities for each antiretroviral class included in the network meta-analysis. The horizontal axis represents the 3 possible ranks that the ART can achieve and the vertical axis illustrates the probability of the ART to attain each rank[312]. Abbreviations: NNRTI = non-nucleoside reverse transcriptase inhibitor, NRTI = nucleoside reverse transcriptase inhibitor, PI = protease inhibitor, VPTB = very preterm birth

4.4.6.4 Low birthweight

Six trials, including 5471 pregnant women, reported on the association between LBW and 7 ARTs (Figure 4.53). The majority of women were initiated on AZT/3TC/LPV/r HAART (n = 2167; 39.6%) and AZT monotherapy (n = 2194; 40.1%). All trials included in this analysis were regarded as having a high risk of bias (Figure 4.53).

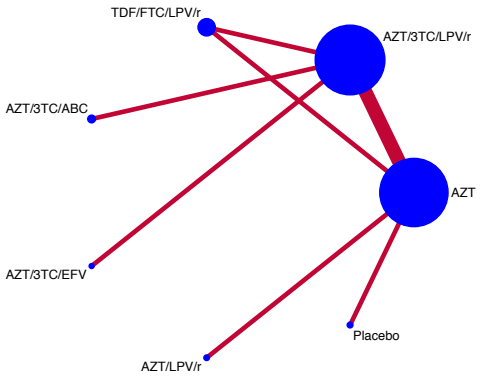


Figure 4.53: Network plot illustrating direct comparisons between ART for LBW outcome. Each circle represents a treatment group. The size of the circle represents the number of participants who received the ART. The lines connecting the circles represent direct comparisons of ART reported by the RCTs. The red colour of the connecting lines represents a high risk of bias in trials reporting the treatment comparison. The width of the lines represents the number of trials reporting on the treatment comparison.

4.4.6.4.1 Pairwise meta-analysis: association between ART and LBW

- Antenatal ART

Women who initiated AZT/3TC/LPV/r HAART (RR1.87; 95%CI1.59-2.20) or TDF/FTC/LPV/r HAART (RR1.91; 95%CI1.24-2.94) were almost twice as likely to deliver a LBW infant as those initiating AZT monotherapy (Figure 4.54). There was no statistical heterogeneity between trials.

- Antenatal antiretroviral regimens

Women who initiated HAART (RR1.87; 95%CI 1.61-2.18) were more likely to deliver a LBW infant than those on monotherapy (Figure 4.55).

- Antenatal antiretroviral class

PI-based ART significantly increased the risk of giving birth to a LBW infant (RR1.61; 95%CI 1.30-1.99) compared to NRTI-based ART (Figure 4.56).

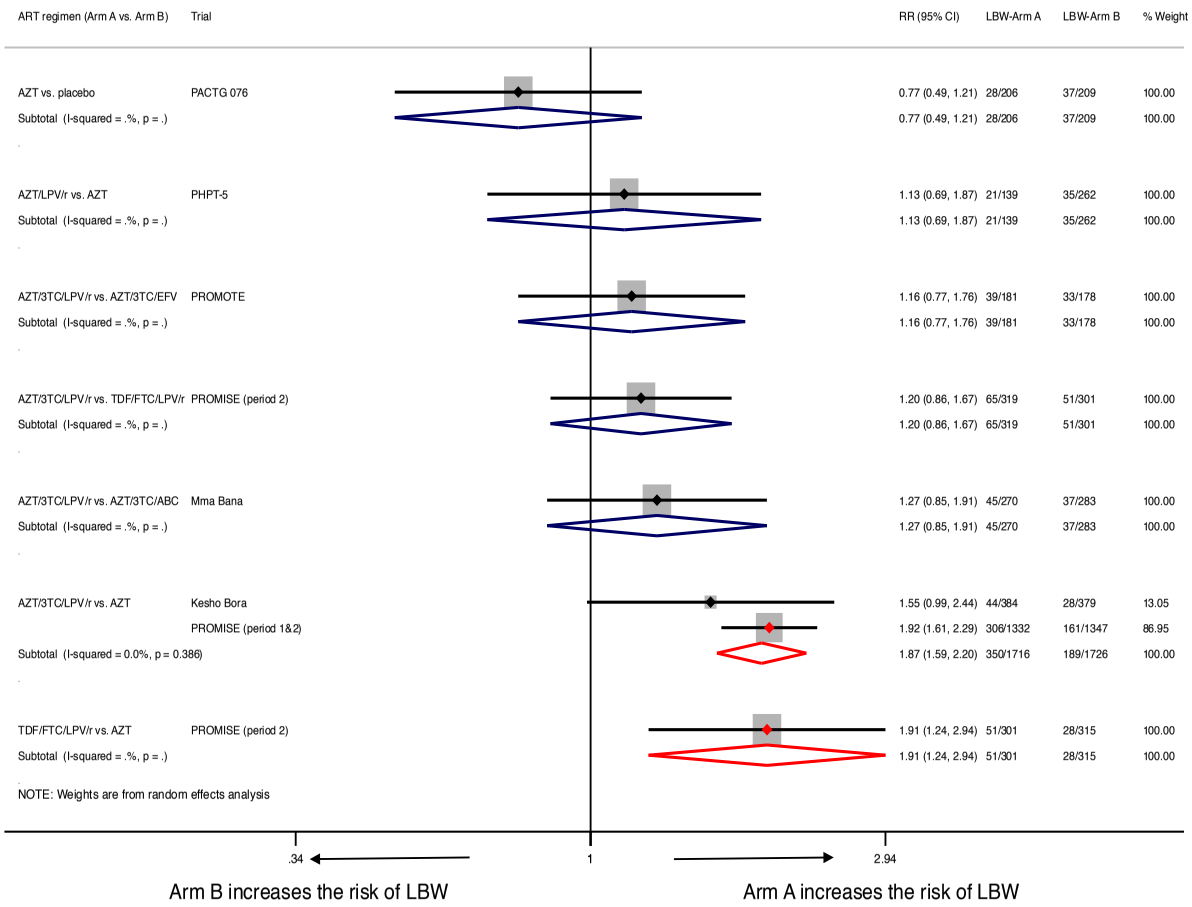


Figure 4.54: Random-effect pairwise meta-analysis results for the association between antenatal ART and LBW.
Statistically significant effects are presented in red. AZT/3TC/LPV/r HAART significantly increased the risk of LBW, compared to AZT monotherapy or AZT/3TC/ABC HAART. TDF/FTC/LPV/r HAART significantly increased the risk of LBW, compared to AZT monotherapy. Abbreviations: 3TC = lamivudine, ABC = abacavir, ART = antiretroviral treatment, AZT = zidovudine, EFV = efavirenz, FTC = emtricitabine, LBW = low birthweight, LPV/r = lopinavir/ritonavir, RR = relative risk, TDF = tenofovir disoproxil fumarate.

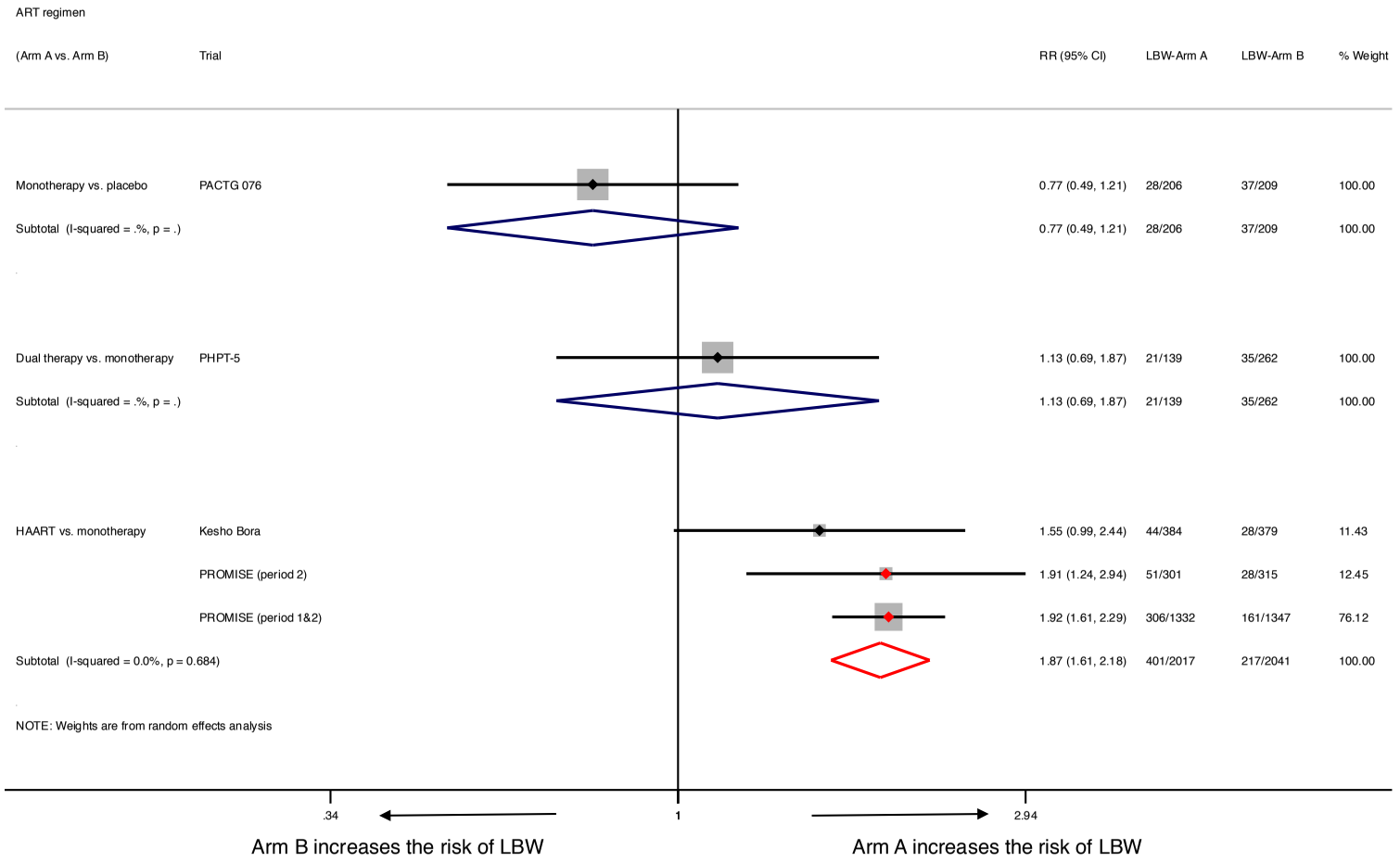


Figure 4.55: Random-effect pairwise meta-analysis results for the association between antenatal antiretroviral regimen and LBW. Statistically significant effects are presented in red. HAART significantly increased the risk of LBW, compared to monotherapy. Abbreviations: ART = antiretroviral treatment, HAART = Highly Active Antiretroviral Treatment, LBW = low birthweight, RR = relative risk.

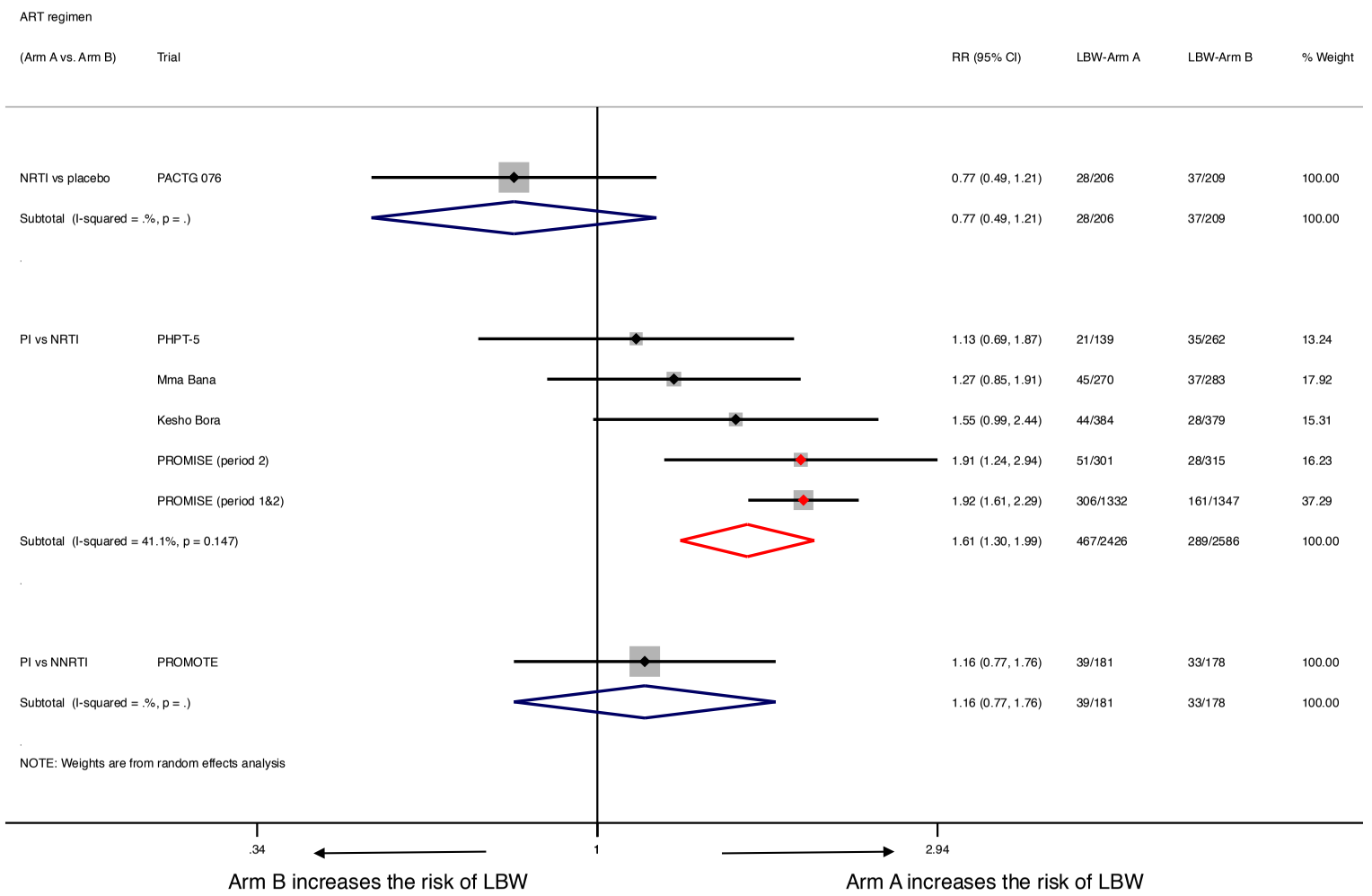


Figure 4.56: Random-effects pairwise meta-analysis results for the association between antenatal antiretroviral class and LBW. Statistically significant effects are presented in red. PI-based ART significantly increased the risk of LBW compared to NRTI-based ART. Abbreviations: ART = antiretroviral treatment, LBW = Low birthweight, NNRTI = non-nucleoside reverse transcriptase inhibitor, NRTI = nucleoside reverse transcriptase inhibitor, PI = protease inhibitor, RR = relative risk.

4.4.6.4.2 Network meta-analysis: association between ART and LBW

- Antenatal ART

Women who initiated AZT/3TC/LPV/r (RR1.92; 95%CI1.65-2.24), AZT/3TC/EFV HAART (RR1.65; 95%CI 1.06-2.57) and TDF/FTC/LPV/r (RR1.68; 95%CI 1.21-2.33) displayed a significantly higher risk of giving birth to a LBW infant than those on AZT monotherapy (Figure 4.57, Figure 4.58). Furthermore, women who initiated AZT/3TC/LPV/r HAART were also at higher risk of giving birth to a LBW infant than those initiating AZT/LPV/r dual therapy (RR1.70; 95%CI 1.01-2.87) (Figure 4.57, Figure 4.58). There was no evidence of statistical inconsistency between direct and indirect estimates (p=0.43) or publication bias (Figure 4.59).

- Antenatal antiretroviral regimen

The initiation of a HAART regimen significantly increased the risk of LBW compared to monotherapy (RR1.87; 95%CI 1.61-2.18) (Figure 4.60, Figure 4.61). While the global test for inconsistency did suggest that some inconsistency was present (p<0.001), no evidence of inconsistency between direct and indirect evidence was found when the inconsistency was explored by side splitting (side A vs B: p=1.00, side B vs C: p=0.998; side B vs D: p=0.998). There was no evidence of publication bias (Figure 4.62)

- Antenatal antiretroviral class

The initiation of a PI-based ART significantly increased the risk of LBW, compared to NRTI-based HAART (RR1.62; 95%CI 1.27 -2.01) (Figure 4.63, Figure 4.64). There was no evidence of statistical inconsistency between direct and indirect estimates (p=0.43); however, there was some evidence of publication bias (Figure 4.65).

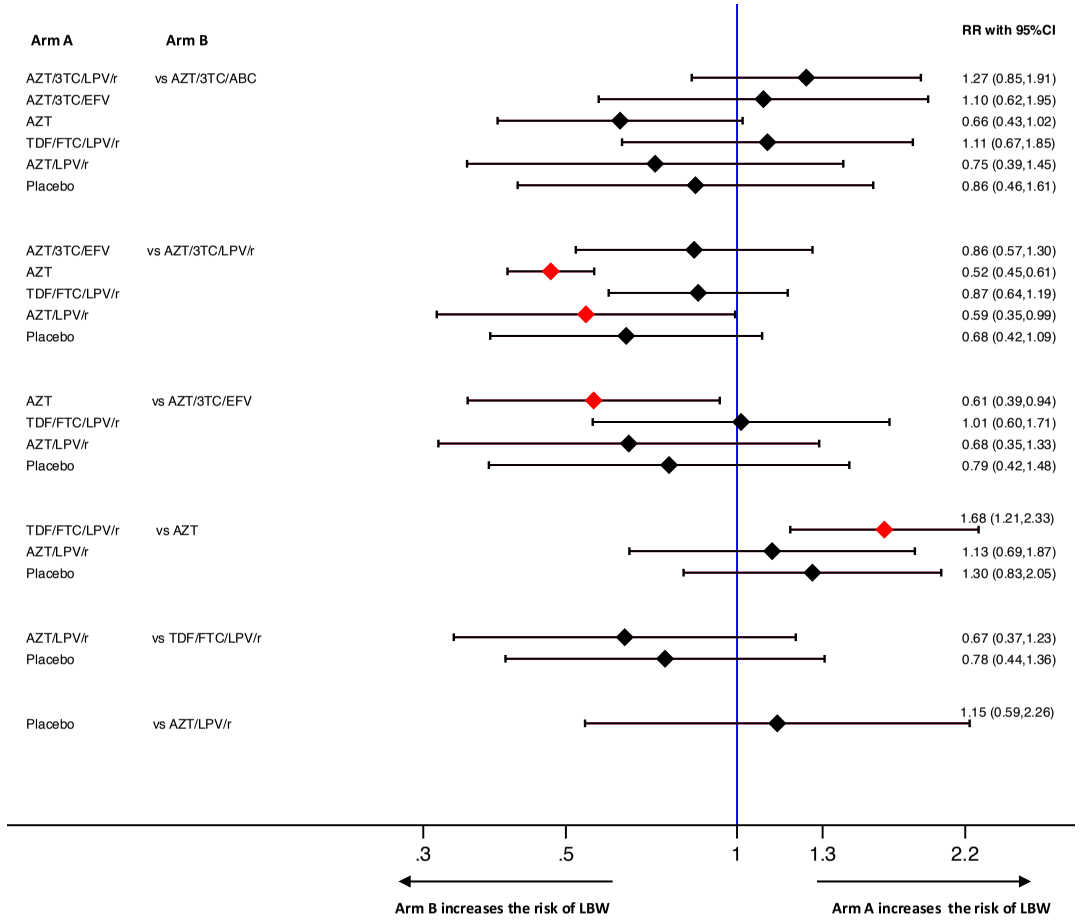


Figure 4.57: Random-effects network meta-analysis results for the association between antenatal ART and LBW.
Statistically significant effects are presented in red. AZT/3TC/LPV/r HAART, AZT/3TC/EFV HAART and TDF/FTC/LPV/r HAART significantly increased the risk of LBW compared to AZT monotherapy. AZT/3TC/LPV/r HAART significantly increased the risk of LBW, compared to AZT/LPV/r dual therapy. Abbreviations: 3TC = lamivudine, ABC = abacavir, ART = antiretroviral treatment, AZT = zidovudine, EFV = efavirenz, FTC = emtricitabine, LBW = low birthweight, LPV/r = lopinavir/ritonavir, RR = relative risk, TDF = tenofovir disoproxil fumarate.

AZT	1.30 (0.83 - 2.05)	1.13 (0.69 - 1.87)	**1.65 (1.06 - 2.57)**	1.51 (0.98 - 2.32)	**1.92 (1.65 - 2.24)**	**1.68 (1.21 - 2.33)**
0.77 (0.49,1.21)	**Placebo**	0.87 (0.44 - 1.70)	1.27 (0.67 - 2.39)	1.16 (0.62 - 2.16)	1.47 (0.92 - 2.38)	1.29 (0.74 - 2.25)
0.88 (0.54,1.46)	1.15 (0.59 - 2.26)	**AZT/LPV/r**	1.46 (0.75 - 2.85)	1.33 (0.69 - 2.58)	**1.70 (1.01 - 2.87)**	1.48 (0.81 - 2.70)
0.61 (0.39 - 0.94)	0.79 (0.42 - 1.48)	0.68 (0.35 - 1.33)	**AZT/3TC/EFV**	0.91 (0.51 - 1.62)	1.16 (0.77 - 1.76)	1.01 (0.60 - 1.71)
0.66 (0.43 - 1.02)	0.86 (0.46 - 1.61)	0.75 (0.39 - 1.45)	1.10 (0.62 - 1.95)	**AZT/3TC/ABC**	1.27 (0.85 - 1.91)	1.11 (0.67 - 1.85)
0.52 (0.45 - 0.61)	0.68 (0.42 - 1.09)	**0.59 (0.35 - 0.99)**	0.86 (0.57 - 1.30)	0.78 (0.52 - 1.17)	**AZT/3TC/LPV/r**	0.87 (0.64 - 1.19)
0.60 (0.43,0.83)	0.78 (0.44 - 1.36)	0.67 (0.37 - 1.23)	0.99 (0.59 - 1.66)	0.90 (0.54 - 1.50)	1.15 (0.84 - 1.57)	**TDF/FTC/LPV/r**

Figure 4.58: League table of random-effects network meta-analysis results for the association between antenatal ART and LBW. This table reflects all possible pairwise comparisons generated from the random-effect network meta-analysis. Results are reported as a relative risk (RR) of the column ART (e.g. AZT) relative to the row ART (AZT/3TC/EFV). Statistically significant effects are presented in red. AZT/3TC/LPV/r HAART, AZT/3TC/EFV HAART and TDF/FTC/LPV/r HAART significantly increased the risk of LBW compared to AZT monotherapy. AZT/3TC/LPV/r HAART significantly increased the risk of LBW, compared to AZT/LPV/r dual therapy. Abbreviations: 3TC = lamivudine, ABC = abacavir, ART = antiretroviral treatment, AZT = zidovudine, EFV = efavirenz, FTC = emtricitabine, LBW = low birthweight, LPV/r = lopinavir/ritonavir, RR = relative risk, TDF = tenofovir disoproxil fumarate.

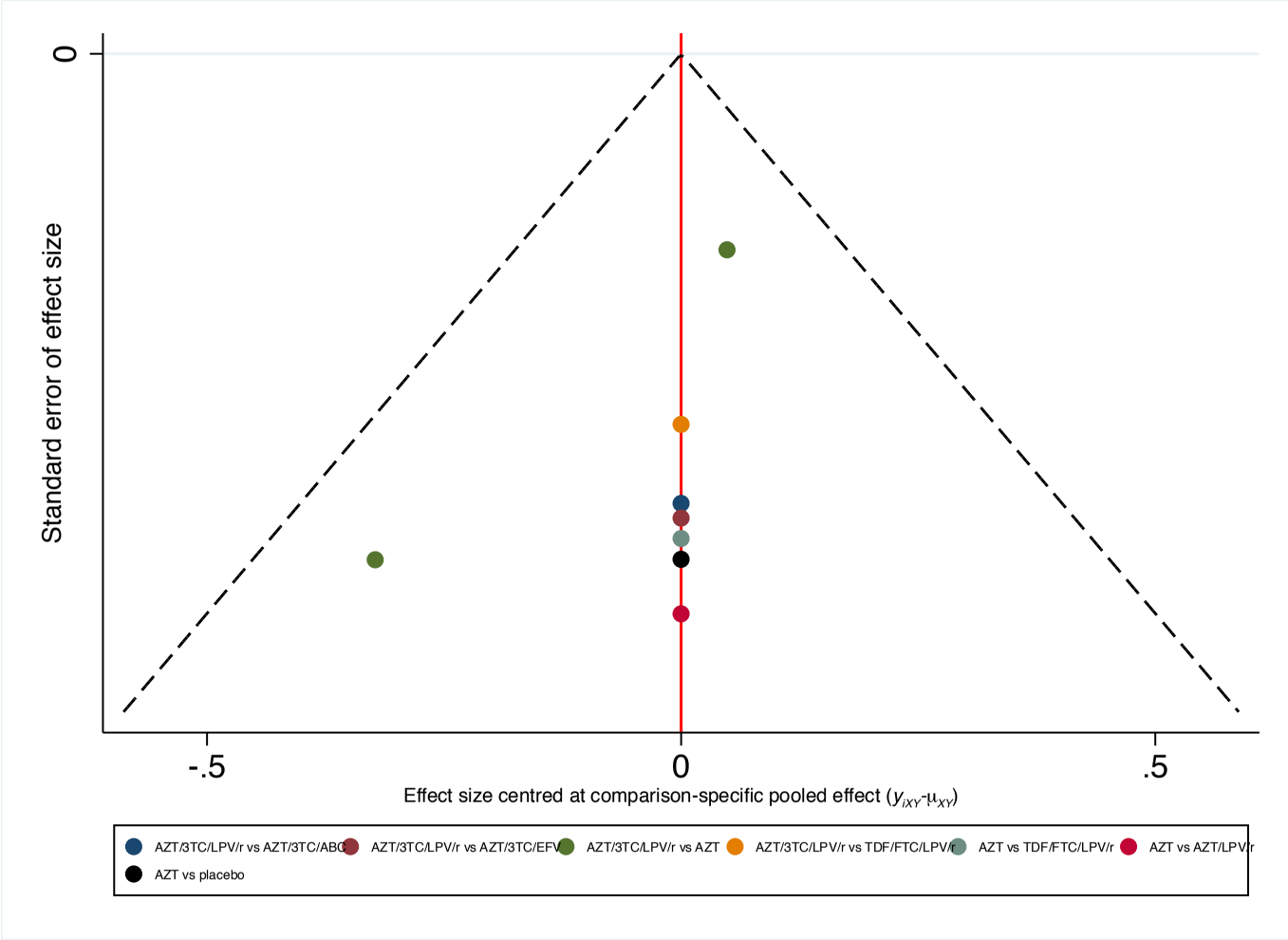


Figure 4.59: Comparison-adjusted funnel plot for the association between antenatal ART and LBW.
The vertical axis represents the measure of the dispersion of the effect size; the horizontal axis represents the difference between the study specific effect size and the comparison-specific summary effect size, and the red line represents the null hypothesis of no difference between the study specific and comparison specific effect estimates. The colours represent individual comparisons. There was no evidence of small study effect.

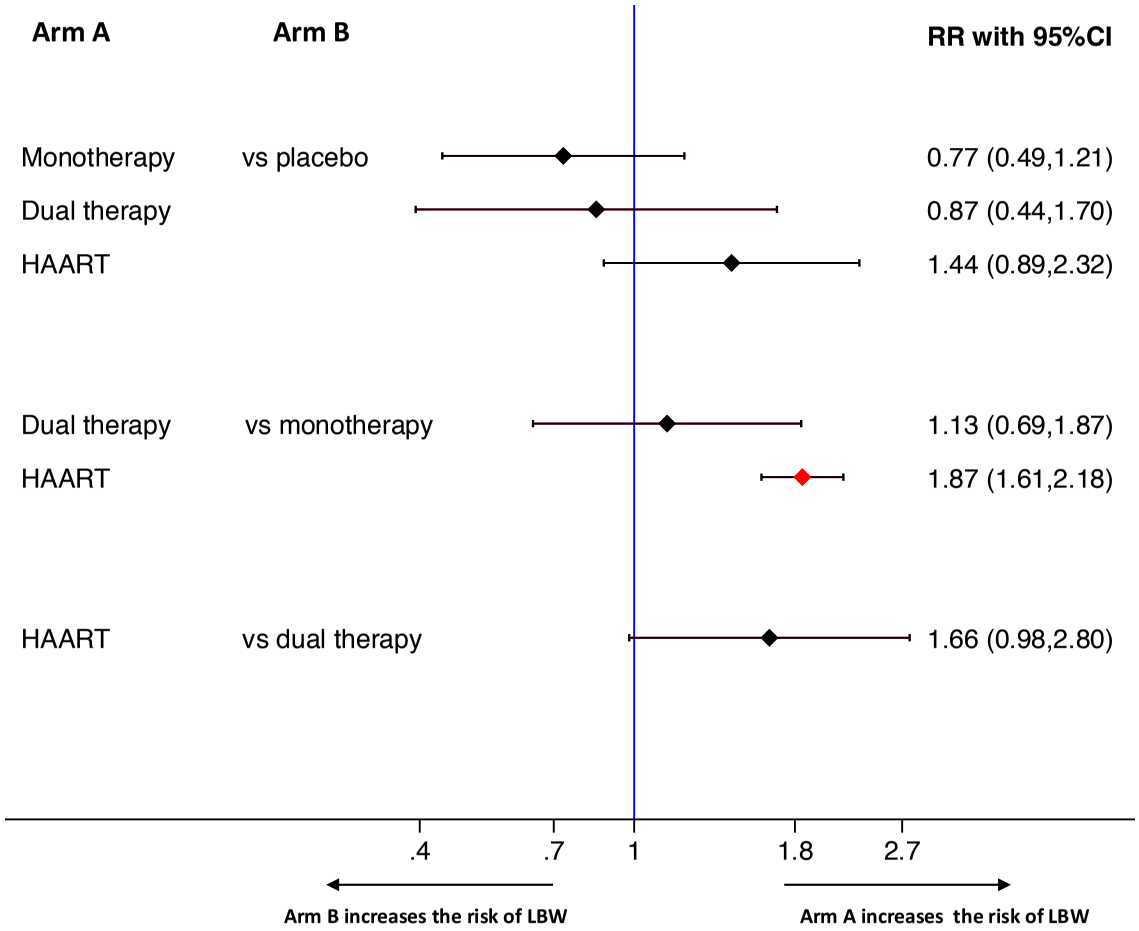


Figure 4.60: Random-effects network meta-analysis results for the association between antenatal antiretroviral regimens and LBW. Statistically significant effects are presented in red. HAART significantly increased the risk of LBW, compared to monotherapy. Abbreviations: HAART = Highly Active Antiretroviral Treatment, LBW = low birthweight, RR = relative risk.

Placebo	0.77 (0.49 - 1.21)	0.87 (0.44 - 1.70)	1.44 (0.89 - 2.32)
1.30 (0.83 - 2.05)	**Monotherapy**	1.13 (0.69 - 1.87)	**1.87** **(1.61 - 2.18)**
1.15 (0.59 - 2.26)	0.88 (0.54 - 1.46)	**Dual therapy**	1.66 (0.98 - 2.80)
0.70 (0.43 - 1.12)	**0.53** **(0.46 - 0.62)**	0.60 (0.36 - 1.02)	**HAART**

Figure 4.61: League table of random-effects network meta-analysis results for the association between antenatal antiretroviral regimen and LBW. This table reflects all possible pairwise comparisons generated from the random-effect network meta-analysis. Results are reported as a relative risk (RR) of the column regimen (e.g. Monotherapy) relative to the row regimen (HAART). Statistically significant effects are presented in red. HAART significantly increased the risk of LBW, compared to monotherapy. Abbreviations: HAART = Highly Active Antiretroviral Treatment, LBW = low birthweight.

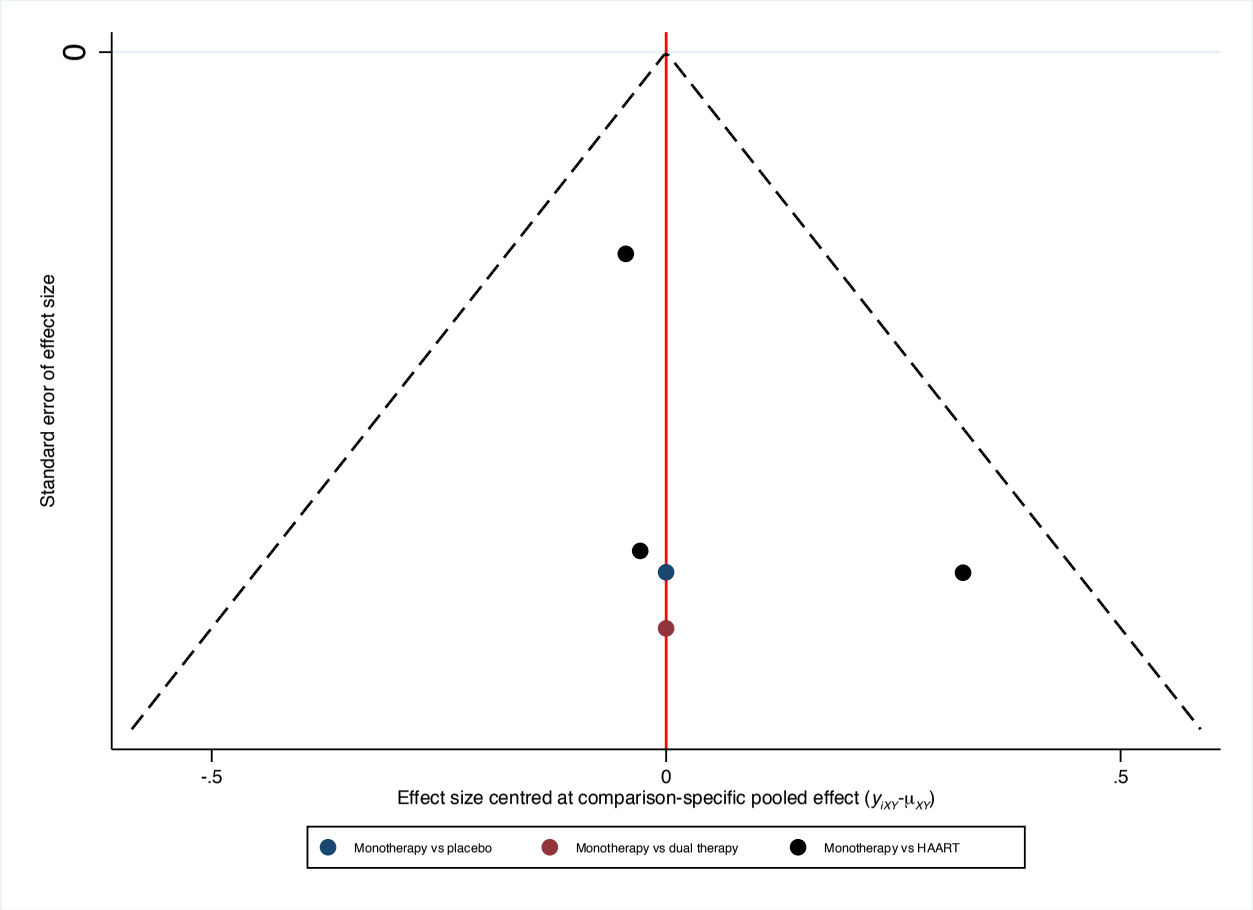


Figure 4.62: Comparison-adjusted funnel plot for the association between antenatal antiretroviral regimen and LBW.
The vertical axis represents the measure of the dispersion of the effect size; the horizontal axis represents the difference between the study specific effect size and the comparison-specific summary effect size, and the red line represents the null hypothesis of no difference between the study specific and comparison specific effect estimates. The colours represent individual comparisons. There was no evidence of small study effect.

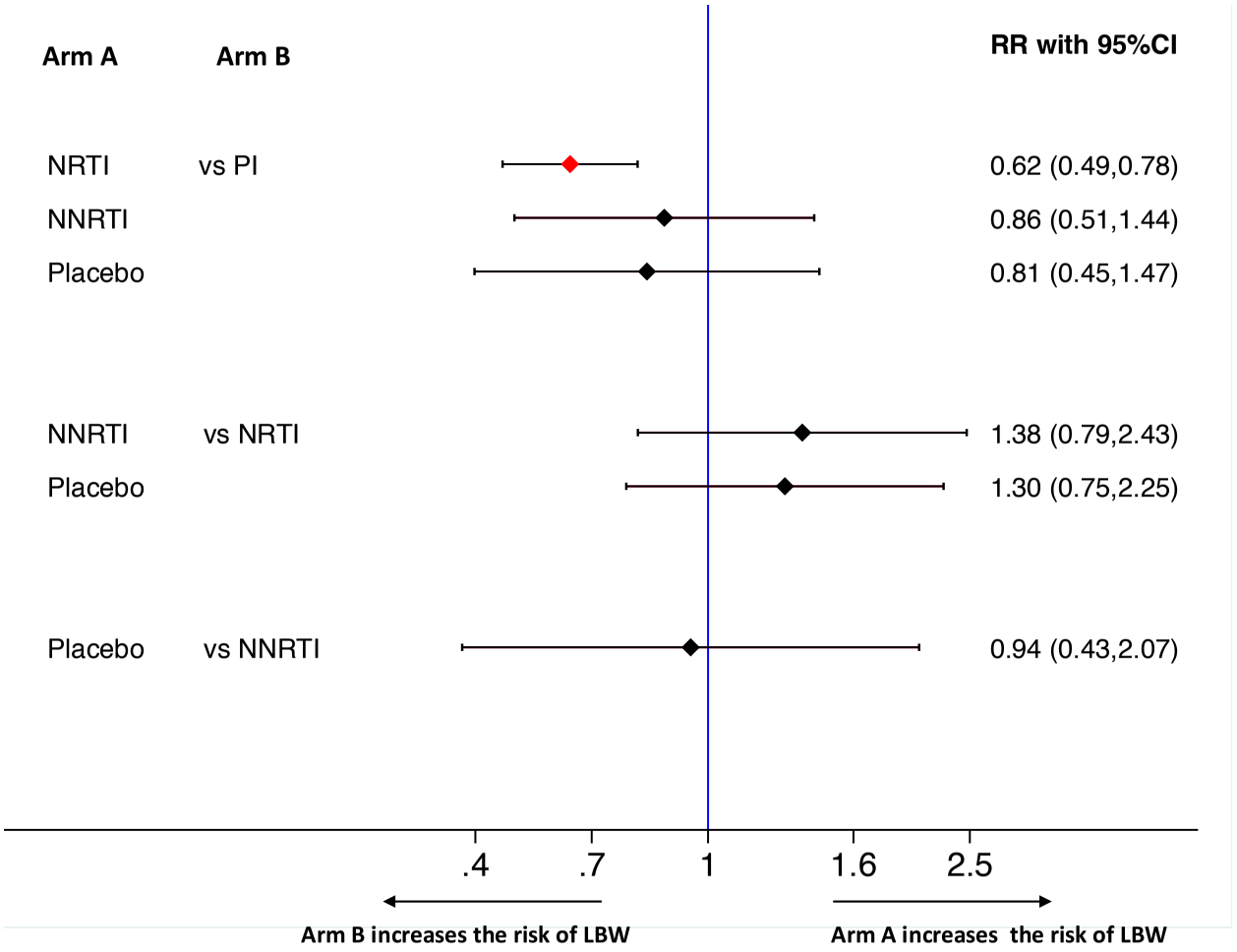


Figure 4.63: Random-effects network meta-analysis results for the association between antenatal antiretroviral class and LBW.
Statistically significant effects are presented in red. PI-based ART significantly increased the risk of LBW, compared to NRTI-based ART. Abbreviations: LBW = low birthweight, NNRTI = non-nucleoside reverse transcriptase inhibitor, NRTI = nucleoside reverse transcriptase inhibitor, PI = protease inhibitor, RR = relative risk.

Placebo	1.23 (0.68 - 2.23)	1.06 (0.48 - 2.33)	0.77 (0.44 - 1.32)
0.81 (0.45 - 1.47)	**PI**	0.86 (0.51 - 1.44)	**0.62** **(0.49 - 0.78)**
0.94 (0.43 - 2.07)	1.16 (0.69 - 1.95)	**NNRTI**	0.72 (0.41 - 1.27)
1.30 (0.75 - 2.25)	**1.61** **(1.27 - 2.03)**	1.38 (0.79 - 2.43)	**NRTI**

Figure 4.64: League table of random-effects network meta-analysis results for the association between antenatal ART class and LBW. This table reflects all possible pairwise comparisons generated from the random-effect network meta-analysis. Results are reported as a relative risk (RR) of the column antiretroviral class (e.g. PI) relative to the row antiretroviral class (NTRI). Statistically significant effects are presented in red. PI containing ART significantly increased the risk of PTB, compared to NRTI-based regimens. Abbreviations: ART = antiretroviral treatment, NNRTI = non-nucleoside reverse transcriptase inhibitor, NRTI = nucleoside reverse transcriptase inhibitor, PI = protease inhibitor, LBW = low birthweight.

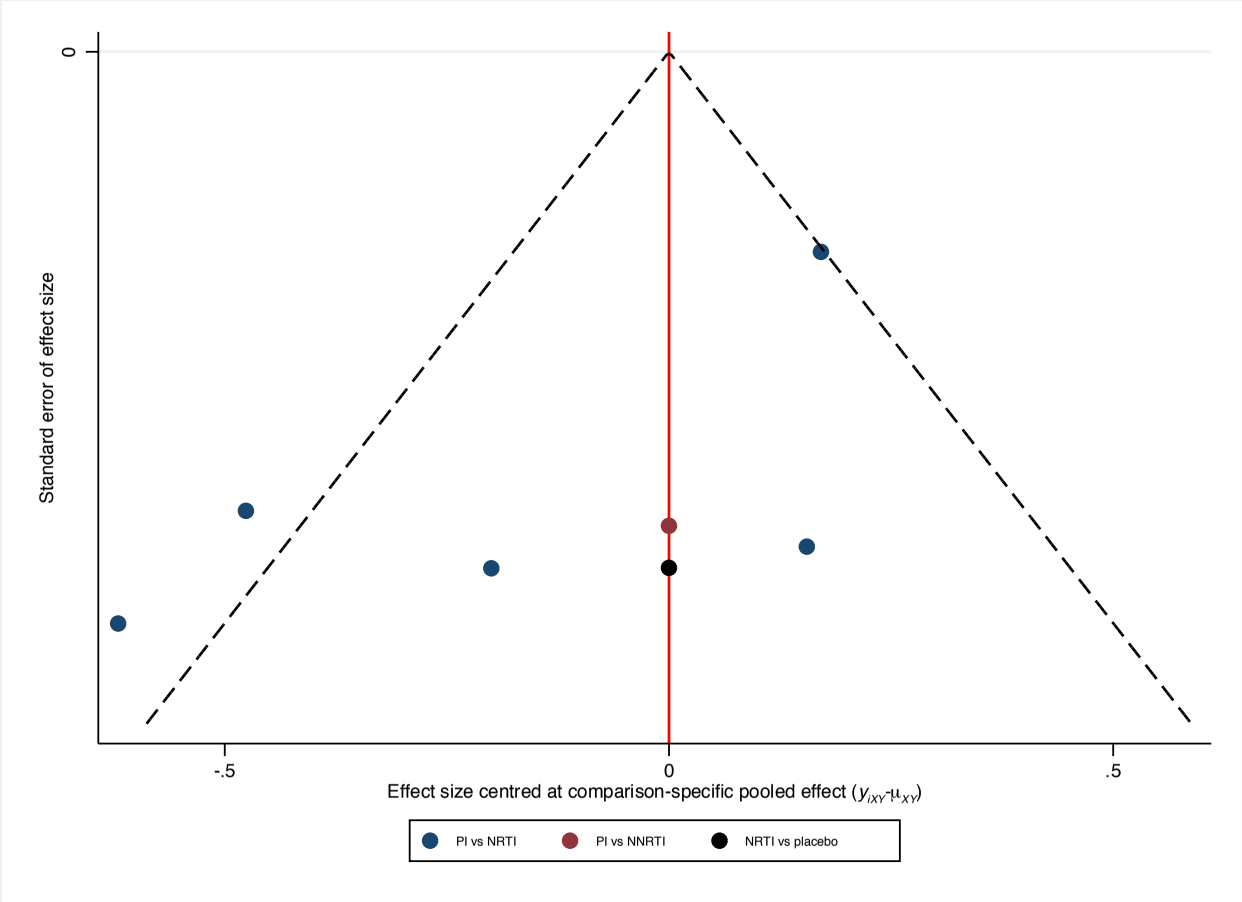


Figure 4.65: Comparison-adjusted funnel plot for the association between classes of antiretroviral regimen and LBW

The vertical axis represents the measure of the dispersion of the effect size; the horizontal axis represents the difference between the study specific effect size and the comparison-specific summary effect size, and the red line represents the null hypothesis of no difference between the study specific and comparison specific effect estimates. The colours represent individual comparisons. There was some evidence of small study effect.

4.4.6.4.3 Network meta-analysis: ranking of comparative effect of ART on LBW

- Antenatal ART

Of the 7 ARTs assessed, AZT monotherapy was associated with the lowest risk of LBW, and ranked superior to all other treatments (SUCRA = 91.8%). Conversely, AZT/3TC/LPV/r HAART was associated with the highest risk of LBW and was ranked in 7th place (SUCRA = 10.7%); it was preceded by TDF/FTC/LPV/r HAART (31.0%) (Figure 4.66, Figure 4.67).

- Antenatal antiretroviral regimen

Of the 4 regimens assessed, monotherapy was associated with the lowest risk of LBW and was ranked superior to all other treatments regimens (SUCRA = 85.1%). HAART was associated with the highest risk of LBW and received the lowest ranking (SUCRA = 3.3%) (Figure 4.68, Figure 4.69).

- Antenatal antiretroviral class

Of the 4 class of antiretrovirals assessed, NRTI-based ART was associated with the lowest risk of LBW and was ranked superior to the other antiretrovirals (SUCRA = 90.1%). PI-based ART was associated with the highest risk of LBW and was ranked in 4th place (SUCRA = 17.5%) (Figure 4.70, Figure 4.71).

Treatment	SUCRA	PrBest	MeanRank
AZT/3TC/ABC	45.3	2.3	4.3
AZT/3TC/LPV/r	10.7	0.0	6.4
AZT/3TC/EFV	33.8	0.8	5.0
AZT	91.8	56.7	1.5
TDF/FTC/LPV/r	31.4	0.1	5.1
AZT/LPV/r	75.4	30.1	2.5
Placebo	61.5	10.0	3.3

Figure 4.66: SUCRA ranking of antenatal ART for LBW outcome.
This figure provides a hierarchy of the 7 ARTs assessed in the network meta-analysis for the association between ART and LBW. The larger the SUCRA percentage, the lower the risk of LBW. SUCRA = surface under the cumulative rank curve, represented as a percentage. PrBest = probability that each treatment is the best i.e. associated with the lowest risk of LBW, represented as a percentage. MeanRank = mean rank of each ART. Abbreviations: 3TC = lamivudine, ABC = abacavir, ART = antiretroviral treatment, AZT = zidovudine, EFV = efavirenz, FTC = emtricitabine, LBW = low birthweight, LPV/r = lopinavir/ritonavir, TDF = tenofovir disoproxil fumarate.

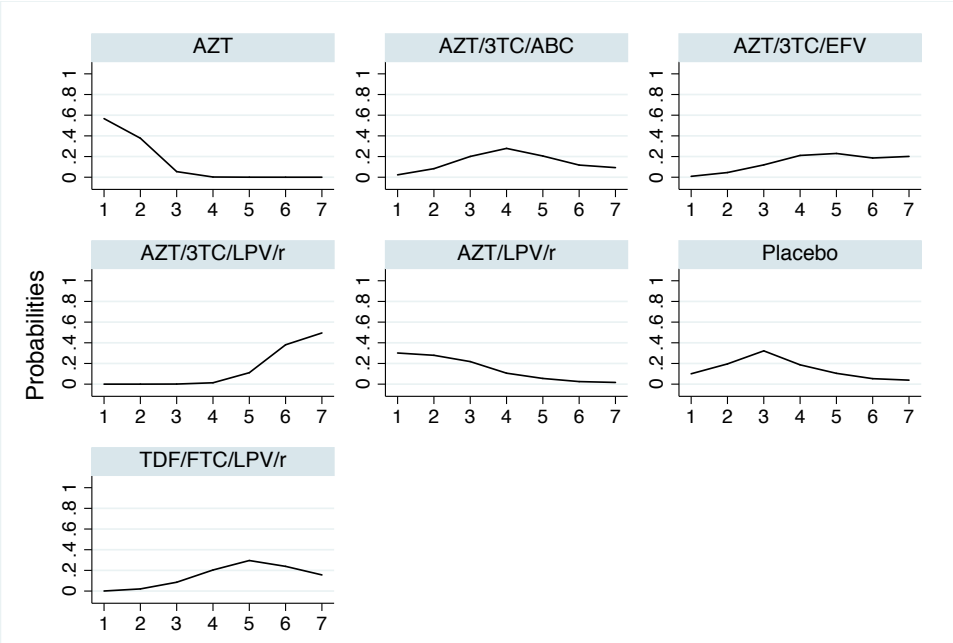


Figure 4.67: Rankograms for ARTs included in the network meta-analysis for the association between ART and LBW This figure represents the surface under the cumulative ranking probabilities for each ART included in the network meta-analysis. The horizontal axis represents the 7 possible ranks that the ART can achieve and the vertical axis illustrates the probability of the ART to attain each rank[312]. Abbreviations: 3TC = lamivudine, ABC = abacavir, ART = antiretroviral treatment, AZT = zidovudine, EFV = efavirenz, FTC = emtricitabine, LBW = low birthweight, LPV/r = lopinavir/ritonavir, RR = relative risk, TDF = tenofovir disoproxil fumarate.

Treatment	SUCRA	PrBest	MeanRank
Placebo	46.8	10.3	2.6
Monotherapy	85.1	59.2	1.4
Dual-therapy	64.8	30.5	2.1
HAART	3.3	0.0	3.9

Figure 4.68: SUCRA ranking of antenatal antiretroviral regimens for LBW outcome. This figure provides a hierarchy of the 4 regimens assessed in the network meta-analysis for the association between antiretroviral regimen and LBW. The larger the SUCRA percentage, the lower the risk of LBW. SUCRA = surface under the cumulative rank curve, represented as a percentage. PrBest = probability that each treatment is the best i.e. associated with the lowest risk of LBW, represented as a percentage. MeanRank = mean rank of each ART. Abbreviation: HAART = Highly Active Antiretroviral Treatment, LBW = low birthweight.

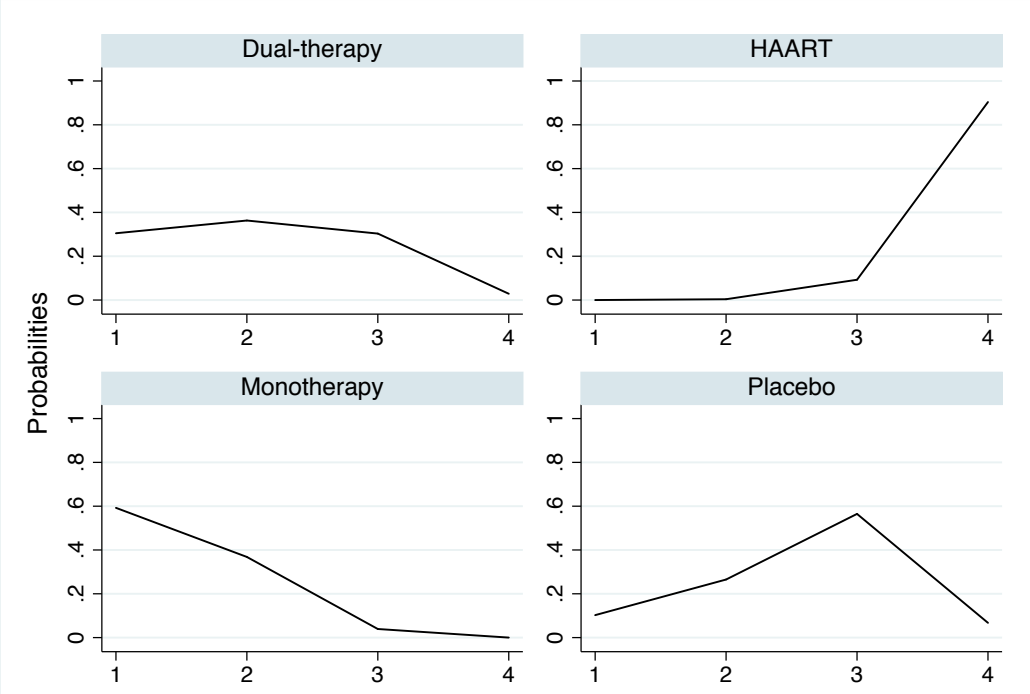


Figure 4.69: Rankograms for antiretroviral regimens included in the network meta-analysis for the association between antiretroviral regimens and LBW. This figure represents the surface under the cumulative ranking probabilities for each regimen included in the network meta-analysis. The horizontal axis represents the 4 possible ranks that the regimen can achieve and the vertical axis illustrates the probability of the regimen to attain each rank[312]. Abbreviation: HAART = Highly Active Antiretroviral Treatment, LBW = low birthweight.

Treatm~t	SUCRA	PrBest	MeanRank
PI	17.5	0.0	3.5
NRTI	90.1	72.2	1.3
NNRTI	42.8	12.2	2.7
Placebo	49.6	15.6	2.5

Figure 4.70: SUCRA ranking of antenatal antiretroviral class for LBW outcome.
This figure provides a hierarchy of the 4 classes of antiretrovirals assessed in the network meta-analysis for the association between antiretroviral class and LBW. The larger the SUCRA percentage, the lower the risk of LBW. SUCRA = surface under the cumulative rank curve, represented as a percentage. PrBest = probability that each treatment is the best i.e. associated with the lowest risk of LBW, represented as a percentage. MeanRank = mean rank of each ART. Abbreviations: LBW = low birthweight, NNRTI = non-nucleoside reverse transcriptase inhibitor, NRTI = nucleoside reverse transcriptase inhibitor, PI = protease inhibitor.

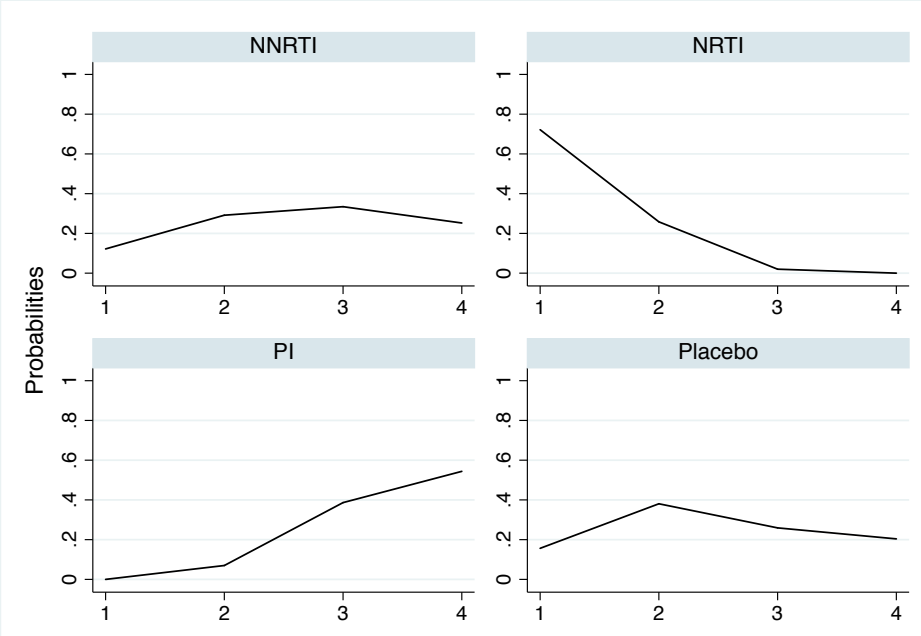


Figure 4.71: Rankograms for antiretroviral classes included in the network meta-analysis for the association between antiretroviral class and LBW. This figure represents the surface under the cumulative ranking probabilities for each antiretroviral class included in the network meta-analysis. The horizontal axis represents the 4 possible ranks that the ART can achieve and the vertical axis illustrates the probability of the ART to attain each rank[312]. LBW = low birthweight, NNRTI = non-nucleoside reverse transcriptase inhibitor, NRTI = nucleoside reverse transcriptase inhibitor, PI = protease inhibitor.

4.4.6.5 Very low birthweight

Four trials, including 4711 women, reported on the association between VLBW and 5 ARTs. The majority of participants were on AZT/3TC/LPV/r HAART (n=1986; 42.2%) or zidovudine monotherapy (n=1932; 41.0%) (Figure 4.72), and all treatment comparisons had a high risk of bias.

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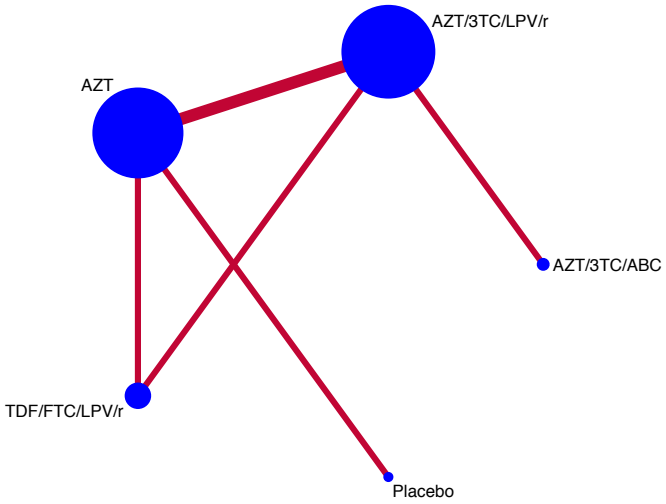

Figure 4.72: Network plot illustrating direct comparisons between ART for VLBW outcome. Each circle represents a treatment group. The size of the circle represents the number of participants who received the ART. The lines connecting the circles represent direct comparisons of ART reported by the RCTs. The red colour of the connecting lines represents a high risk of bias in trials reporting the treatment comparison. The width of the lines represents the number of trials reporting on the treatment comparison.

4.4.6.5.1 Pairwise meta-analysis: association between ART and VLBW

There were no significant associations between VLBW and ARTs (Figure 4.73), antiretroviral regimens (Figure 4.74) or classes of antiretrovirals (Figure 4.75).

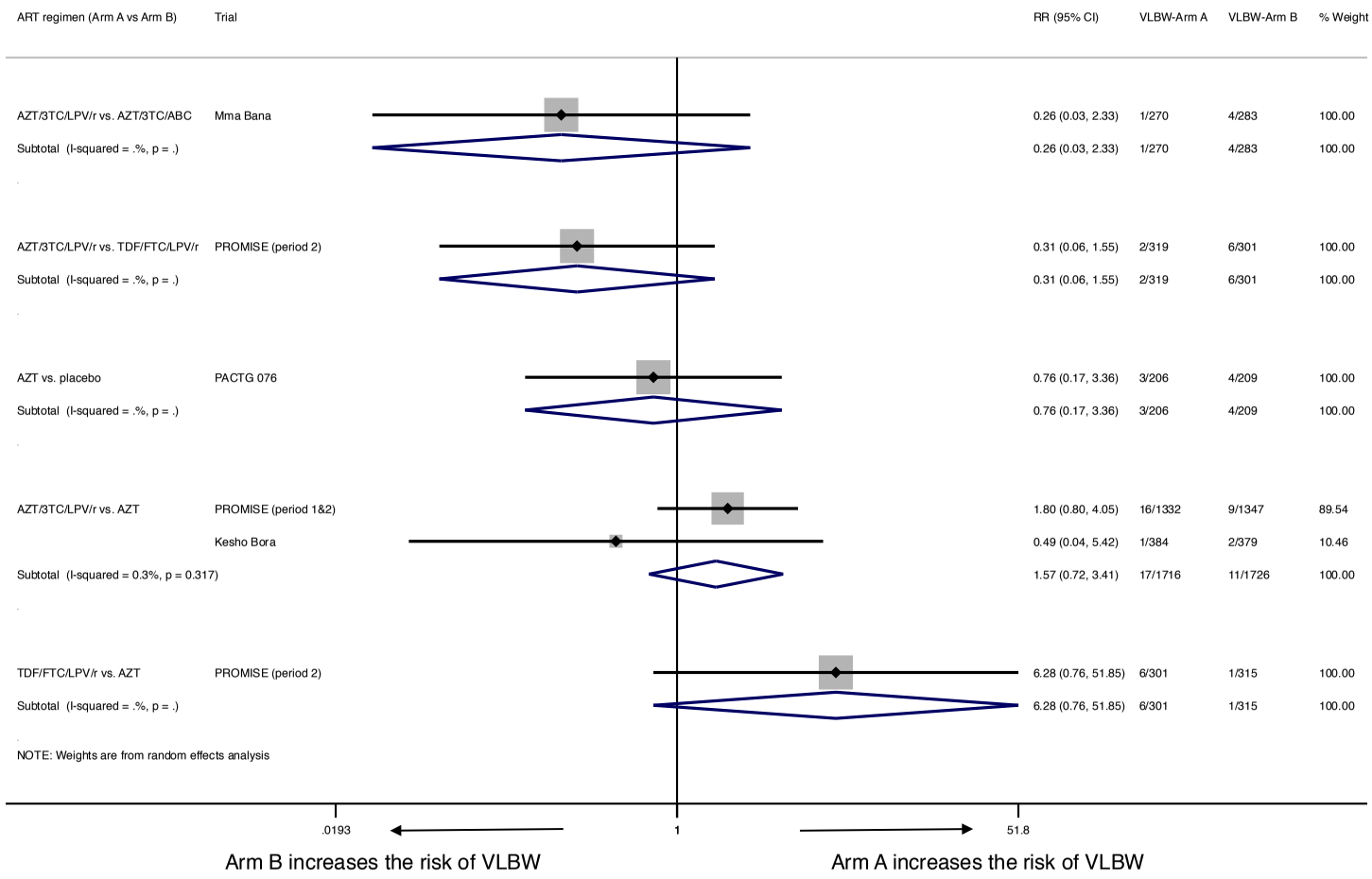

Figure 4.73: Random-effect pairwise meta-analysis results for the association between antenatal ART and VLBW.
There was no significant association between ART and VLBW. Abbreviations: 3TC = lamivudine, ABC = abacavir, ART = antiretroviral treatment, AZT = zidovudine, FTC = emtricitabine, LPV/r = lopinavir/ritonavir, RR = relative risk, TDF = tenofovir disoproxil fumarate, VLBW = very low birthweight.

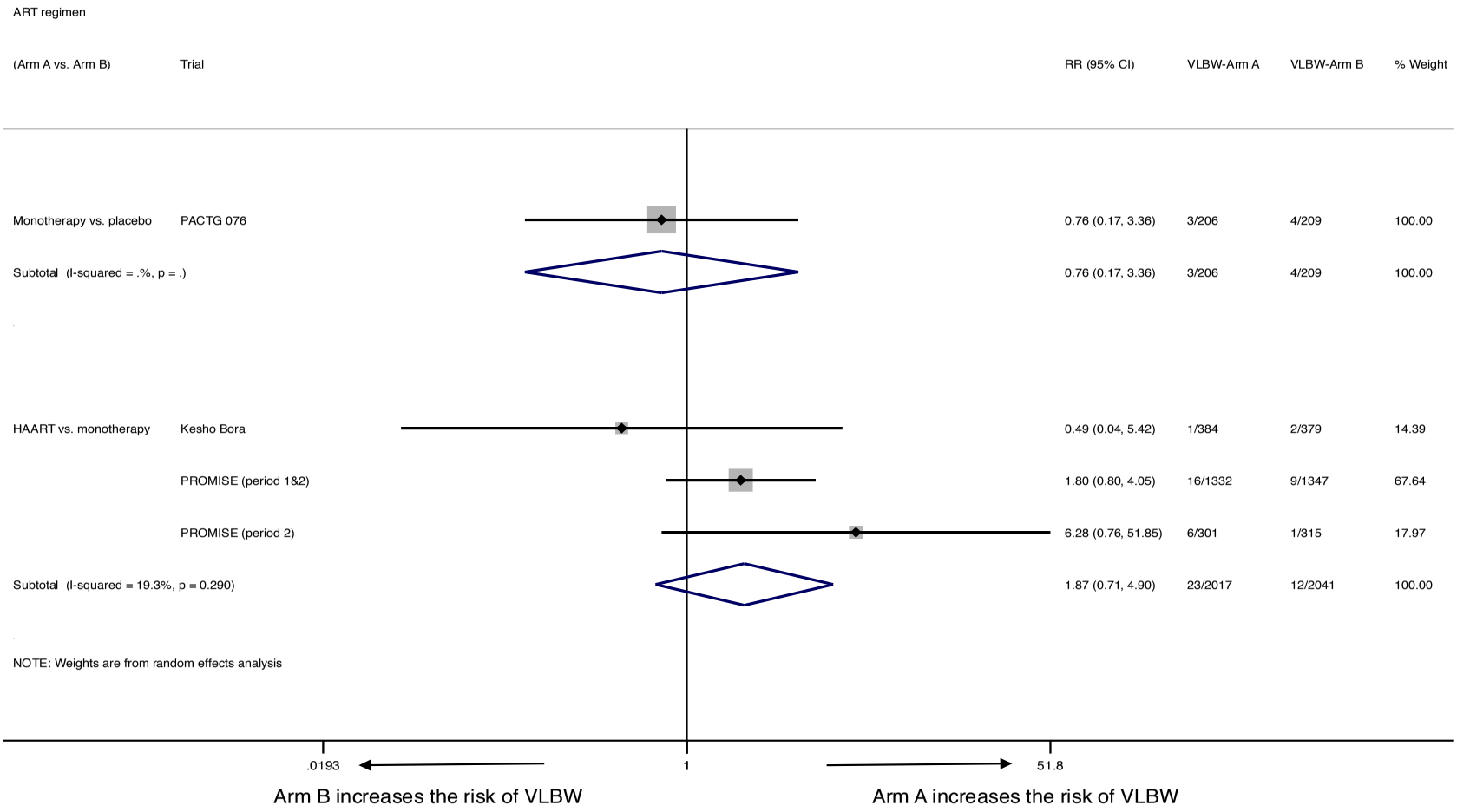

Figure 4.74: Random-effect pairwise meta-analysis results for the association between antenatal antiretroviral regimen and VLBW. No significant difference observed between VLBW and antiretroviral regimens. Abbreviations: ART = antiretroviral treatment, HAART = Highly Active Antiretroviral Treatment, RR = relative risk, VLBW = very low birthweight.

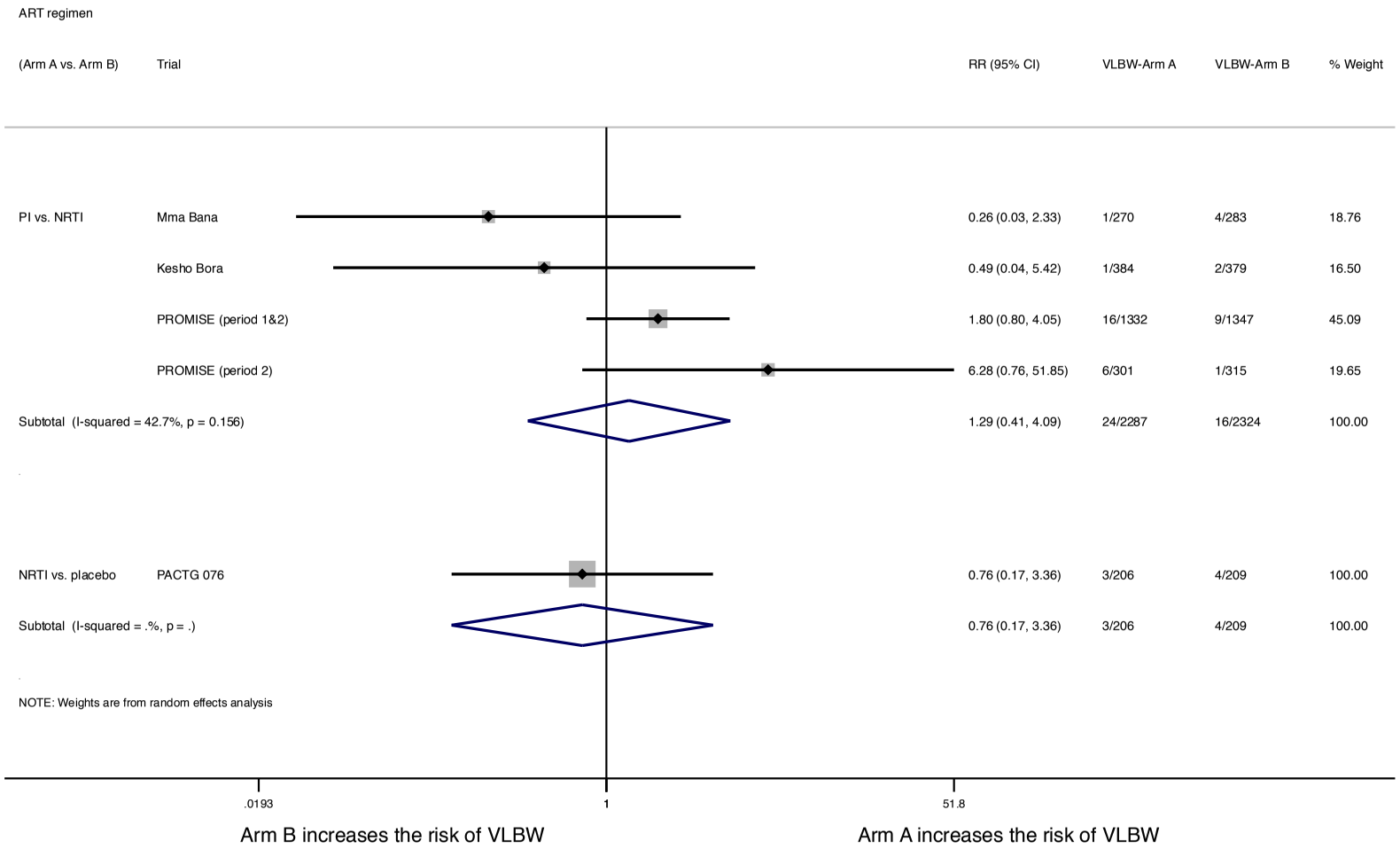

Figure 4.75: Random-effects pairwise meta-analysis results for the association between antenatal antiretroviral class and VLBW. There was no significant association between antiretroviral class and VLBW. Abbreviations: ART = antiretroviral treatment, NRTI = nucleoside reverse transcriptase inhibitor, PI = protease inhibitor, RR = relative risk, VLBW = very low birthweight.

4.4.6.5.2 Network meta-analysis: association between ART and VLBW.

- Antenatal ART

Women initiating TDF/FTC/LPV/r HAART were five times more likely to deliver a VLBW infant than those initiating AZT monotherapy (RR5.47; 95%CI 1.27 – 23.63) (Figure 4.76, Figure 4.77). The global test for inconsistency was non-significant (P=0.87) and there was no evidence of publication bias (Figure 4.78).

- Antenatal antiretroviral regimen and class of antiretrovirals.

There was no significant association observed between antiretroviral regimens or classes of antiretrovirals and VLBW (Figure 4.79 to 4.80, Figure 4.82 to 4.83) and there was no evidence of inconsistency. However, there was some evidence of publication bias (Figure 4.81 and Figure 4.84).

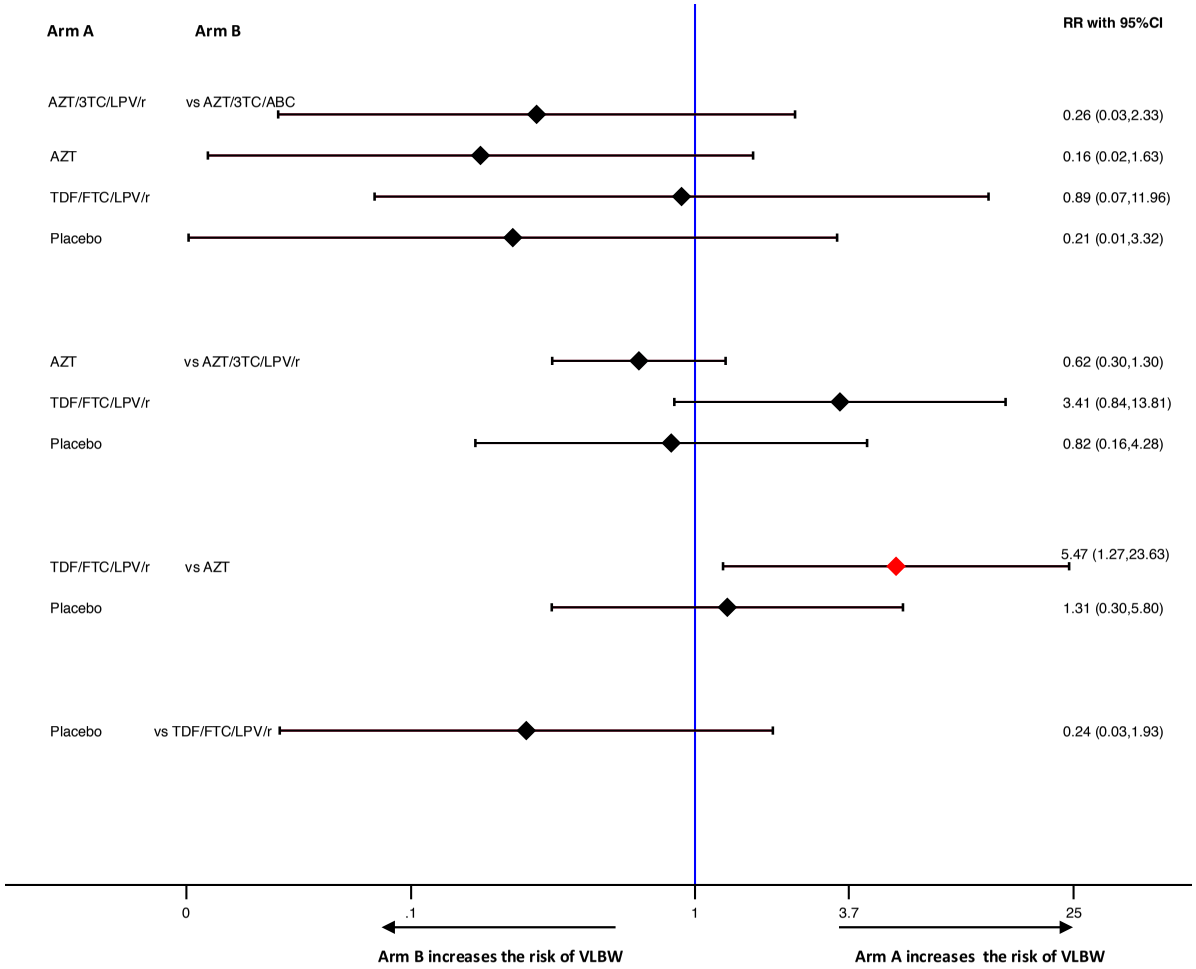

Figure 4.76: Random-effects network meta-analysis results for the association between antenatal ART and VLBW.
Statistically significant effects are presented in red. TDF/FTC/LPV/r HAART significantly increased the risk of VLBW compared to AZT monotherapy. Abbreviations: 3TC = lamivudine, ABC = abacavir, ART = antiretroviral treatment, AZT = zidovudine, FTC = emtricitabine, LPV/r = lopinavir/ritonavir, RR = relative risk, TDF = tenofovir disoproxil fumarate, VLBW = very low birthweight.

AZT	1.31 (0.30 - 5.80)	6.13 (0.61 - 61.45)	1.61 (0.77 - 3.34)	**5.47 (1.27 - 23.63)**
0.76 (0.17 - 3.36)	**Placebo**	4.67 (0.30 - 72.36)	1.22 (0.23 - 6.40)	4.16 (0.52 - 33.47)
0.16 (0.02 - 1.63)	0.21 (0.01 - 3.32)	**AZT/3TC/ABC**	0.26 (0.03 - 2.33)	0.89 (0.07 - 11.96)
0.62 (0.30 - 1.30)	0.82 (0.16 - 4.28)	3.82 (0.43 - 33.92)	**AZT/3TC/LPV/r**	3.41 (0.84 - 13.81)
0.18 (0.04 - 0.79)	0.24 (0.03 - 1.93)	1.12 (0.08 - 15.01)	0.29 (0.07 - 1.19)	**TDF/FTC/LPV/r**

Figure 4.77: League table of random-effects network meta-analysis results for the association between antenatal ART and VLBW. This table reflects all possible pairwise comparisons generated from the random-effect network meta-analysis. Results are reported as a relative risk (RR) of the column ART (e.g. TDF/FTC/LPV/r) relative to the row ART (AZT). Statistically significant effects are presented in red. TDF/FTC/LPV/r HAART significantly increased the risk of VLBW compared to AZT monotherapy. Abbreviations: 3TC = lamivudine, ABC = abacavir, ART = antiretroviral treatment, AZT = zidovudine, FTC = emtricitabine, LPV/r = lopinavir/ritonavir, RR = relative risk, TDF = tenofovir disoproxil fumarate, VLBW = very low birthweight.

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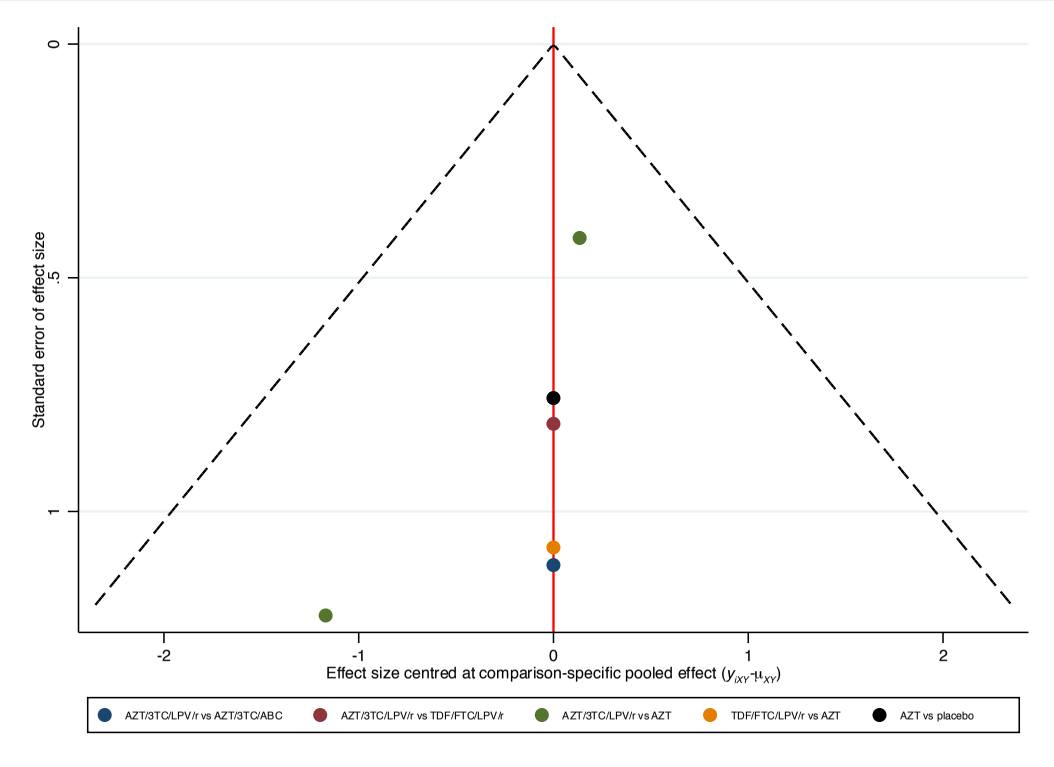

Figure 4.78: Comparison-adjusted funnel plot for the association between antenatal ART and VLBW.
The vertical axis represents the measure of the dispersion of the effect size; the horizontal axis represents the difference between the study specific effect size and the comparison-specific summary effect size, and the red line represents the null hypothesis of no difference between the study specific and comparison specific effect estimates[50]. The colours represent individual comparisons. There was no evidence of small study effect.

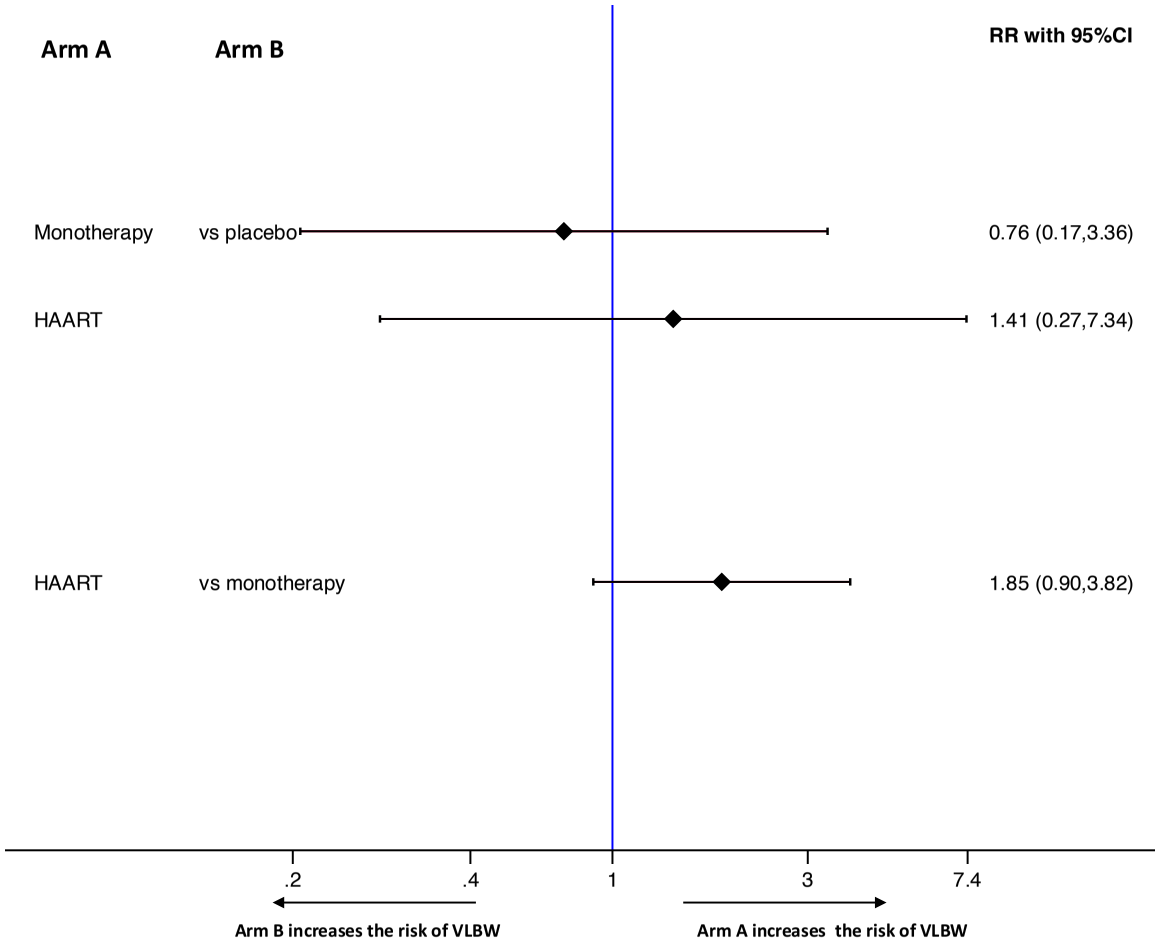

Figure 4.79: Random-effects network meta-analysis results for the association between antenatal antiretroviral regimens and VLBW. This table reflects all possible pairwise comparisons generated from the random-effect network meta-analysis, reported as a relative risk (RR). No significant difference was observed between VLBW and antiretroviral regimens. Abbreviations: HAART = Highly Active Antiretroviral Treatment, RR = relative risk, VLBW = very low birthweight.

Placebo	0.76 (0.17 - 3.36)	1.41 (0.27 - 7.34)
1.31 (0.30 - 5.80)	**Monotherapy**	1.85 (0.90 - 3.82)
0.71 (0.14 - 3.70)	0.54 (0.26 - 1.11)	**HAART**

Figure 4.80: League table of random-effects network meta-analysis results for the association between antiretroviral regimens and VLBW. This table reflects all possible pairwise comparisons generated from the random-effect network meta-analysis. Results are reported as a relative risk (RR) of the column antiretroviral regimen (e.g. Placebo) relative to the row antiretroviral regimen (monotherapy). No significant difference observed between VLBW and antiretroviral regimens. Abbreviations: HAART = Highly Active Antiretroviral Treatment, VLBW = very low birthweight.

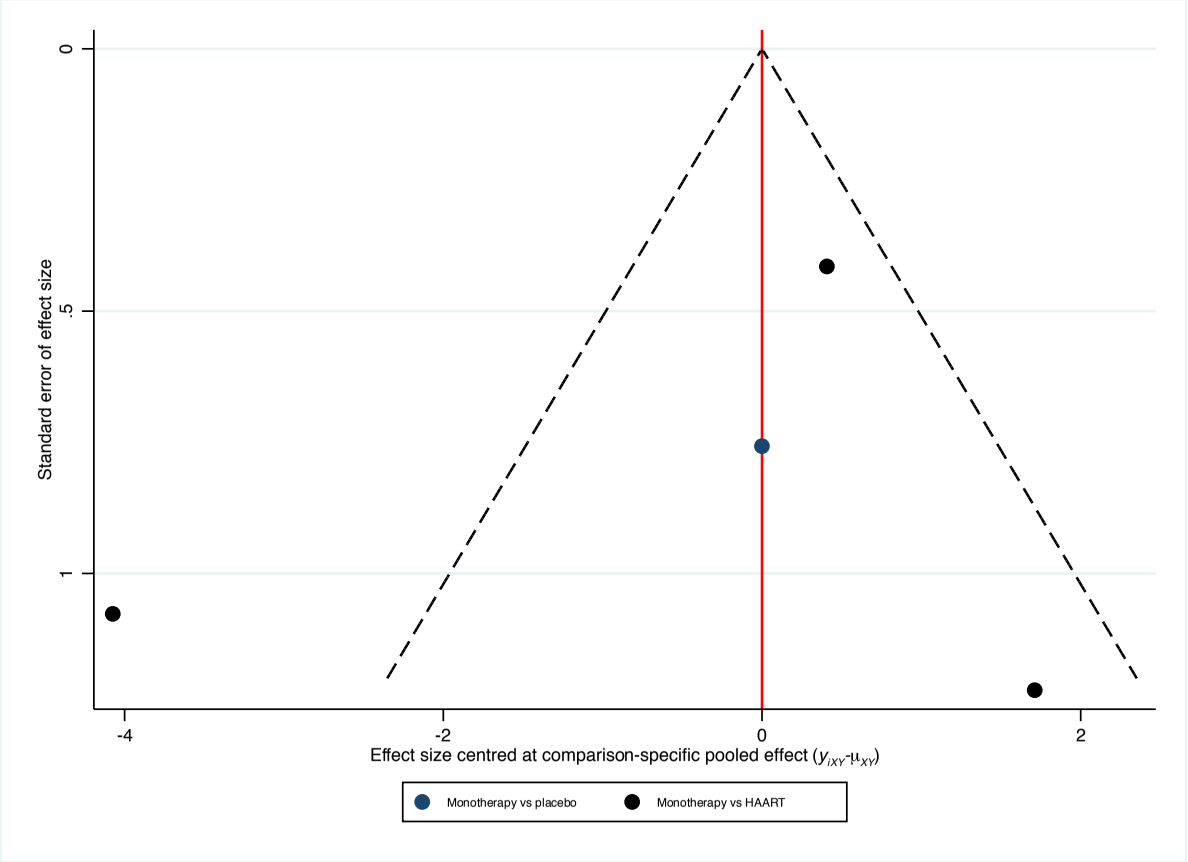

Figure 4.81: Comparison-adjusted funnel plot for the association between antenatal antiretroviral regimen and VLBW.
The vertical axis represents the measure of the dispersion of the effect size; the horizontal axis represents the difference between the study specific effect size and the comparison-specific summary effect size, and the red line represents the null hypothesis of no difference between the study specific and comparison specific effect estimates[50]. The colours represent individual comparisons. There was some evidence of small study effect.

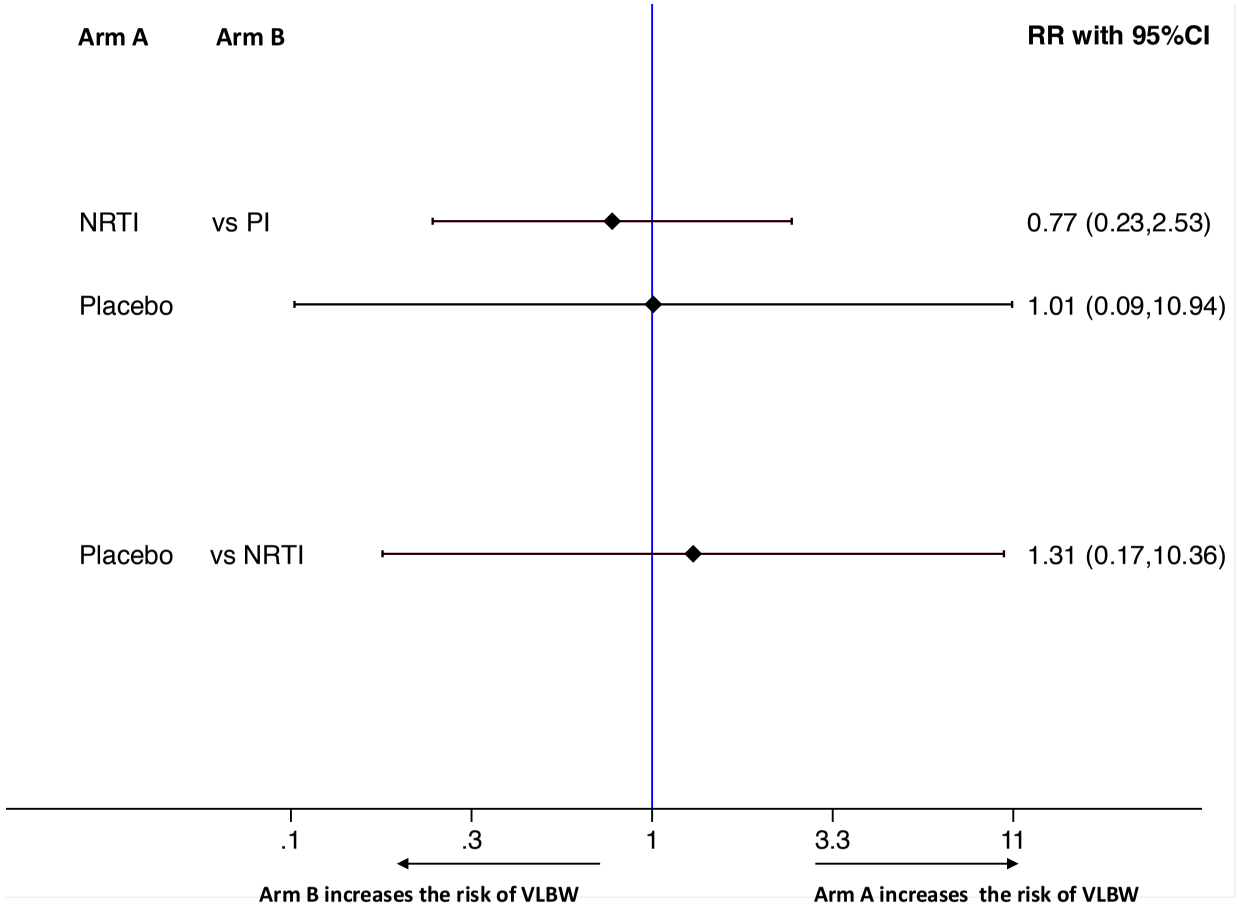

Figure 4.82: Random-effects network meta-analysis results for the association between antenatal antiretroviral class and VLBW. No significant difference observed between VLBW and antiretroviral class. Abbreviations: NRTI = nucleoside reverse transcriptase inhibitor, PI = protease inhibitor, RR = relative risk, VLBW = very low birthweight.

Placebo	0.99 (0.09 - 10.77)	0.76 (0.10 - 6.00)
1.01 (0.09 - 10.94)	**PI**	0.77 (0.23 - 2.53)
1.31 (0.17 - 10.36)	1.30 (0.40 - 4.30)	**NRTI**

Figure 4.83: League table of random-effects network meta-analysis results for the association between antiretroviral class and VLBW. This table reflects all possible pairwise comparisons generated from the random-effect network meta-analysis. Results are reported as a relative risk (RR) of the column antiretroviral class (e.g. Placebo) relative to the row antiretroviral class (PI). No significant difference observed between VLBW and antiretroviral class. Abbreviations: NRTI = nucleoside reverse transcriptase inhibitor, PI = protease inhibitor, VLBW = very low birthweight.

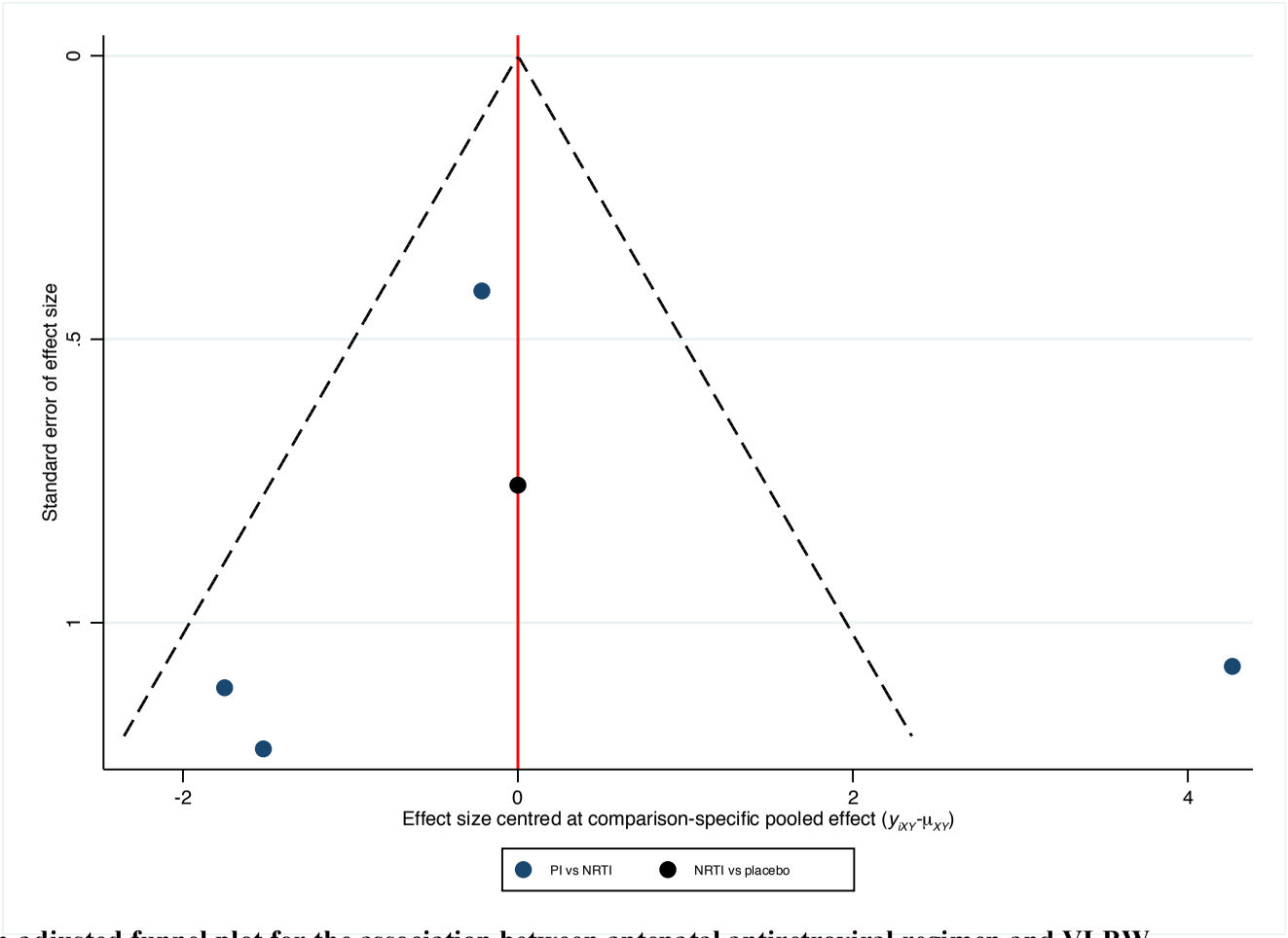

Figure 4.84: Comparison-adjusted funnel plot for the association between antenatal antiretroviral regimen and VLBW.
The vertical axis represents the measure of the dispersion of the effect size; the horizontal axis represents the difference between the study specific effect size and the comparison-specific summary effect size, and the red line represents the null hypothesis of no difference between the study specific and comparison specific effect estimates[50]. The colours represent individual comparisons. There was some evidence of small study effect.

4.4.6.5.3 Network meta-analysis: ranking of comparative effect of ART on VLBW

- Antenatal ART

Of the 5 ARTs assessed, AZT monotherapy was associated with the lowest risk of VLBW, and was ranked superior to the other ARTs (SUCRA = 86.7%) (Figure 4.85, Figure 4.86). TDF/FTC/LPV/r HAART was associated with the highest risk of VLBW; consequently, it received the lowest ranking (SUCRA = 16.7%) (Figure 4.85, Figure 4.86).

- Antenatal antiretroviral regimen

Of the 3 antiretroviral regimens assessed, monotherapy was associated with the lowest risk of VLBW, and was ranked superior to the other 2 regimens (SUCRA = 79.7%) (Figure 4.87, Figure 4.88). HAART was associated with the highest risk of VLBW, and was ranked in 3rd place (SUCRA = 19.4%) (Figure 4.87, Figure 4.88).

- Antenatal antiretroviral class

Of the 3 antiretroviral classes assessed, NRTI-based ART was associated with the lowest risk of VLBW, and was ranked in 1st place, while PI-based ART was ranked in last place (Figure 4.89, Figure. 4.90).

Treatment	SUCRA	PrBest	MeanRank
AZT/3TC/ABC	19.7	4.8	4.2
AZT/3TC/LPV/r	59.0	6.2	2.6
AZT	86.7	54.3	1.5
TDF/FTC/LPV/r	16.7	0.7	4.3
Placebo	67.9	34.0	2.3

Figure 4.85: SUCRA ranking of antenatal ART for VLBW outcome.
This figure provides a hierarchy of the 5 ARTs assessed in the network meta-analysis for the association between ART and VLBW. The larger the SUCRA percentage, the lower the risk of VLBW. SUCRA = surface under the cumulative rank curve, represented as a percentage. PrBest = probability that each treatment is the best i.e. associated with the lowest risk of VLBW, represented as a percentage. MeanRank = mean rank of each ART. Abbreviations: 3TC = lamivudine, ABC = abacavir, ART = antiretroviral treatment, AZT = zidovudine, EFV = efavirenz, FTC = emtricitabine, LPV/r = lopinavir/ritonavir, TDF = tenofovir disoproxil fumarate, VLBW = very low birthweight.

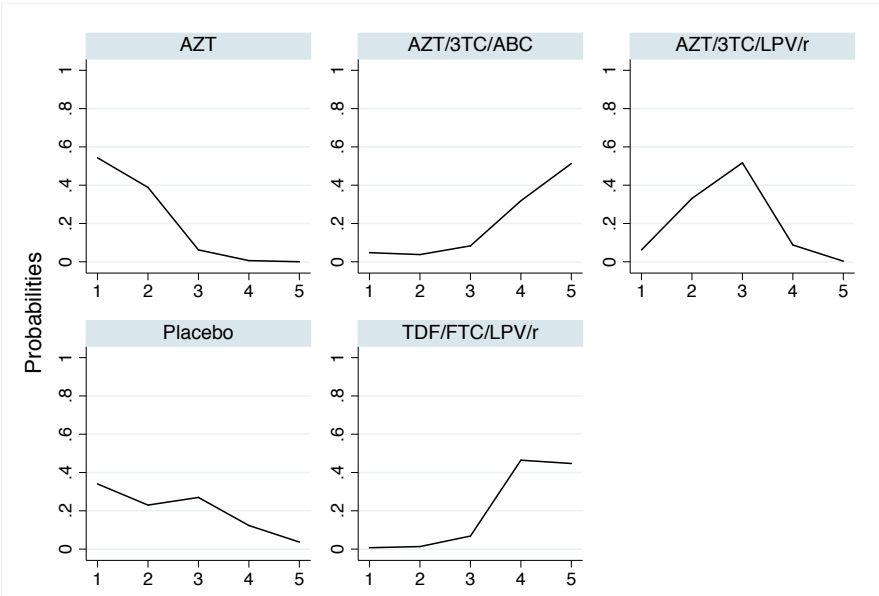

Figure 4.86: Rankograms for ARTs included in the network meta-analysis for the association between ART and VLBW. This figure represents the surface under the cumulative ranking probabilities for each ART included in the network meta-analysis. The horizontal axis represents the 5 possible ranks that the ART can achieve and the vertical axis illustrates the probability of the ART to attain each rank[312]. Abbreviations: 3TC = lamivudine, ABC = abacavir, ART = antiretroviral treatment, AZT = zidovudine, EFV = efavirenz, FTC = emtricitabine, LPV/r = lopinavir/ritonavir, RR = relative risk, TDF = tenofovir disoproxil fumarate, VLBW = very low birthweight.

Treatment	SUCRA	PrBest	MeanRank
Placebo	51.0	35.7	2.0
Monotherapy	79.7	61.0	1.4
HAART	19.4	3.3	2.6

Figure 4.87: SUCRA ranking of antenatal antiretroviral regimens for VLBW outcome. This figure provides a hierarchy of the 3 regimens assessed in the network meta-analysis for the association between antiretroviral regimen and VLBW. The larger the SUCRA percentage, the lower the risk of VLBW. SUCRA = surface under the cumulative rank curve, represented as a percentage. PrBest = probability that each treatment is the best i.e. associated with the lowest risk of VLBW, represented as a percentage. MeanRank = mean rank of each ART. Abbreviation: HAART = Highly Active Antiretroviral Treatment, VLBW = very low birthweight.

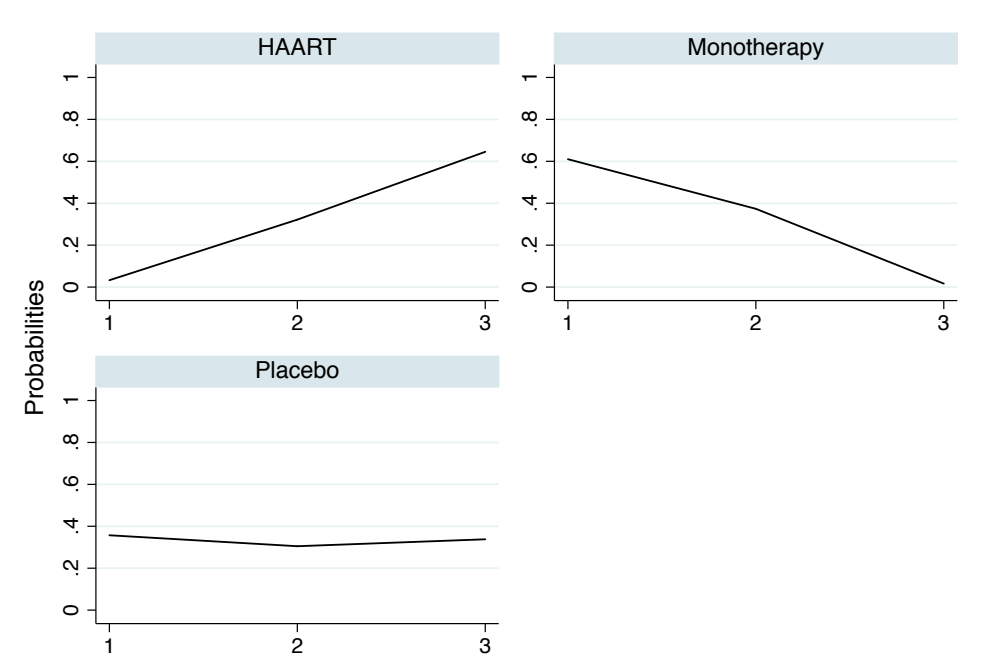

Figure 4.88: Rankograms for antiretroviral regimens included in the network meta-analysis for the association between antiretroviral regimens and VLBW. This figure represents the surface under the cumulative ranking probabilities for each regimen included in the network meta-analysis. The horizontal axis represents the 3 possible ranks that the regimen can achieve and the vertical axis illustrates the probability of the regimen to attain each rank[312]. Abbreviation: HAART = Highly Active Antiretroviral Treatment, VLBW = very low birthweight.

Treatm~t	SUCRA	PrBest	MeanRank
PI	41.7	23.9	2.2
NRTI	63.4	40.4	1.7
Placebo	44.9	35.7	2.1

Figure 4.89: SUCRA ranking of antenatal antiretroviral class for VLBW outcome.
This figure provides a hierarchy of the 3 classes of antiretrovirals assessed in the network meta-analysis for the association between antiretroviral class and VLBW. The larger the SUCRA percentage, the lower the risk of VLBW. SUCRA = surface under the cumulative rank curve, represented as a percentage. PrBest = probability that each treatment is the best i.e. associated with the lowest risk of VLBW, represented as a percentage. MeanRank = mean rank of each ART. Abbreviations: NRTI = nucleoside reverse transcriptase inhibitor, PI = protease inhibitor, VLBW = very low birthweight.

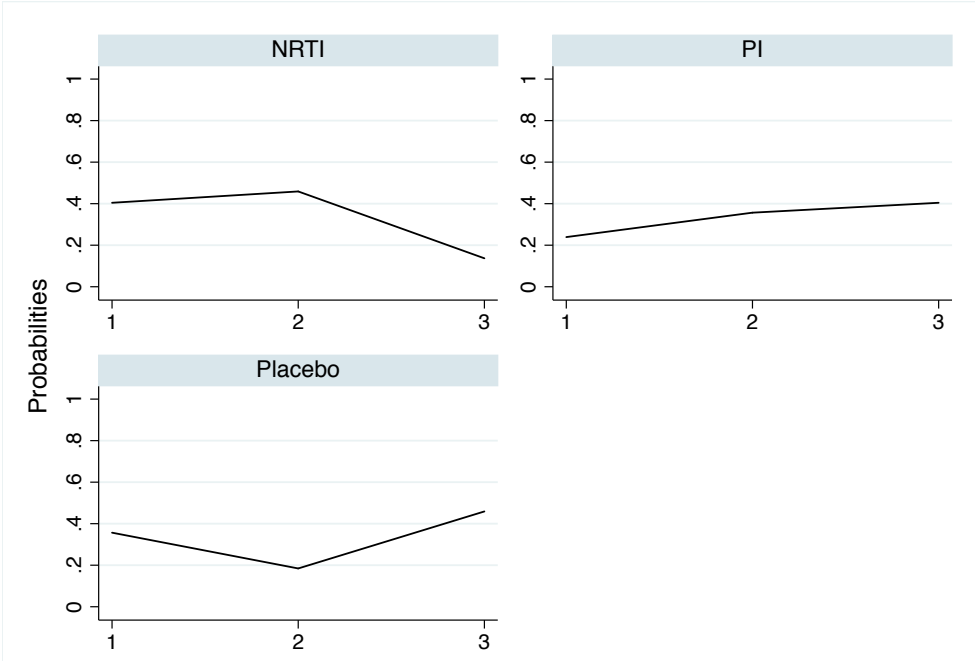

Figure 4.90: Rankograms for antiretroviral classes included in the network meta-analysis for the association between antiretroviral class and VLBW. This figure represents the surface under the cumulative ranking probabilities for each antiretroviral class included in the network meta-analysis. The horizontal axis represents the 3 possible ranks that the ART can achieve and the vertical axis illustrates the probability of the ART to attain each rank[312]. Abbreviations: NRTI = nucleoside reverse transcriptase inhibitor, PI = protease inhibitor, VLBW = very low birthweight.

4.4.6.6 Small for gestational age

Two trials, including 818 women, reported on the association between SGA and 3 antiretroviral treatments. The majority of participants were initiated on zidovudine monotherapy (n = 471; 57.6%) and all treatment comparisons were considered to be at high risk of bias (Figure 4.91).

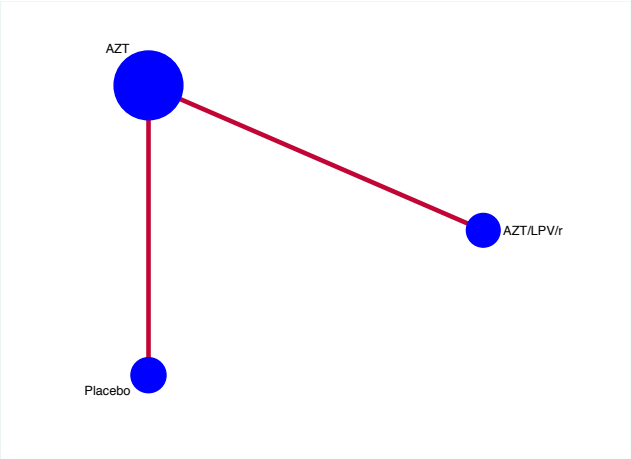

Figure 4.91: Network plot illustrating direct comparisons between ART for SGA outcome. Each circle represents a treatment group. The size of the circle represents the number of participants who received the ART. The lines connecting the circles represent direct comparisons of ART reported by the RCTs. The red colour of the connecting lines represents a high risk of bias in trials reporting the treatment comparison. The width of the lines represents the number of trials reporting on the treatment comparison.

4.4.6.6.1 Pairwise meta-analysis: association between ART and small for gestational age

There were no significant associations between SGA and ARTs (Figure 4.92), antiretroviral regimens (Figure 4.93) and classes of antiretrovirals (Figure 4.94).

4.4.6.6.2 Network meta-analysis: association between ART and SGA

Similarly, network meta-analyses showed no significant associations between SGA and ARTs (Figure 4.95, Figure 4.96) or classes of antiretrovirals (Figure 4.98, Figure 4.99). There were insufficient data to assess the effect of antiretroviral regimens on SGA. There was no evidence of inconsistency or publication bias (Figure 4.97, Figure 4.100).

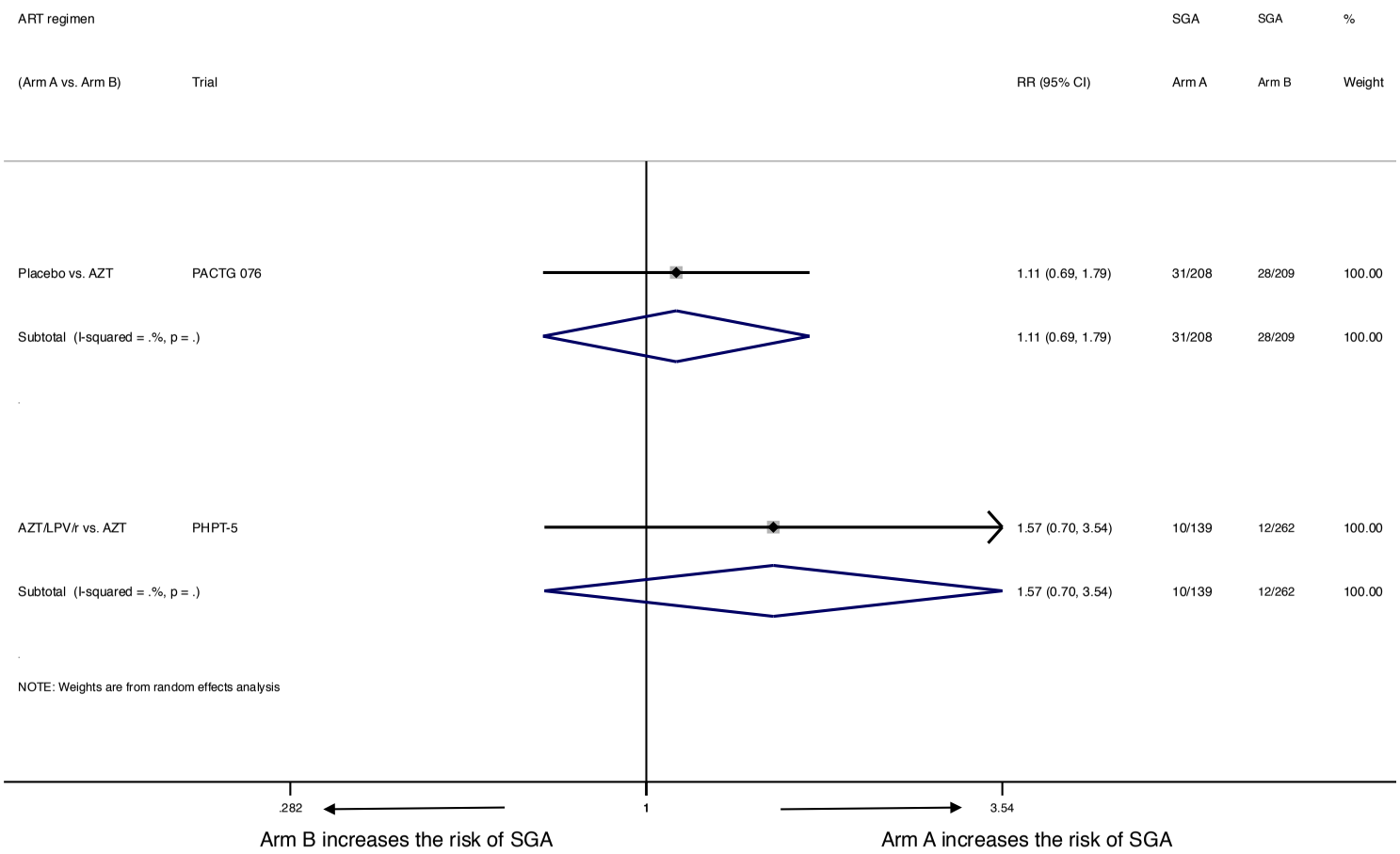

Figure 4.92: Random-effect pairwise meta-analysis results for the association between antenatal ART and SGA.
There was no significant association between ART and SGA. Abbreviations: ART = antiretroviral treatment, AZT = zidovudine, LPV/r = lopinavir/ritonavir, RR = relative risk, SGA = small for gestational age.

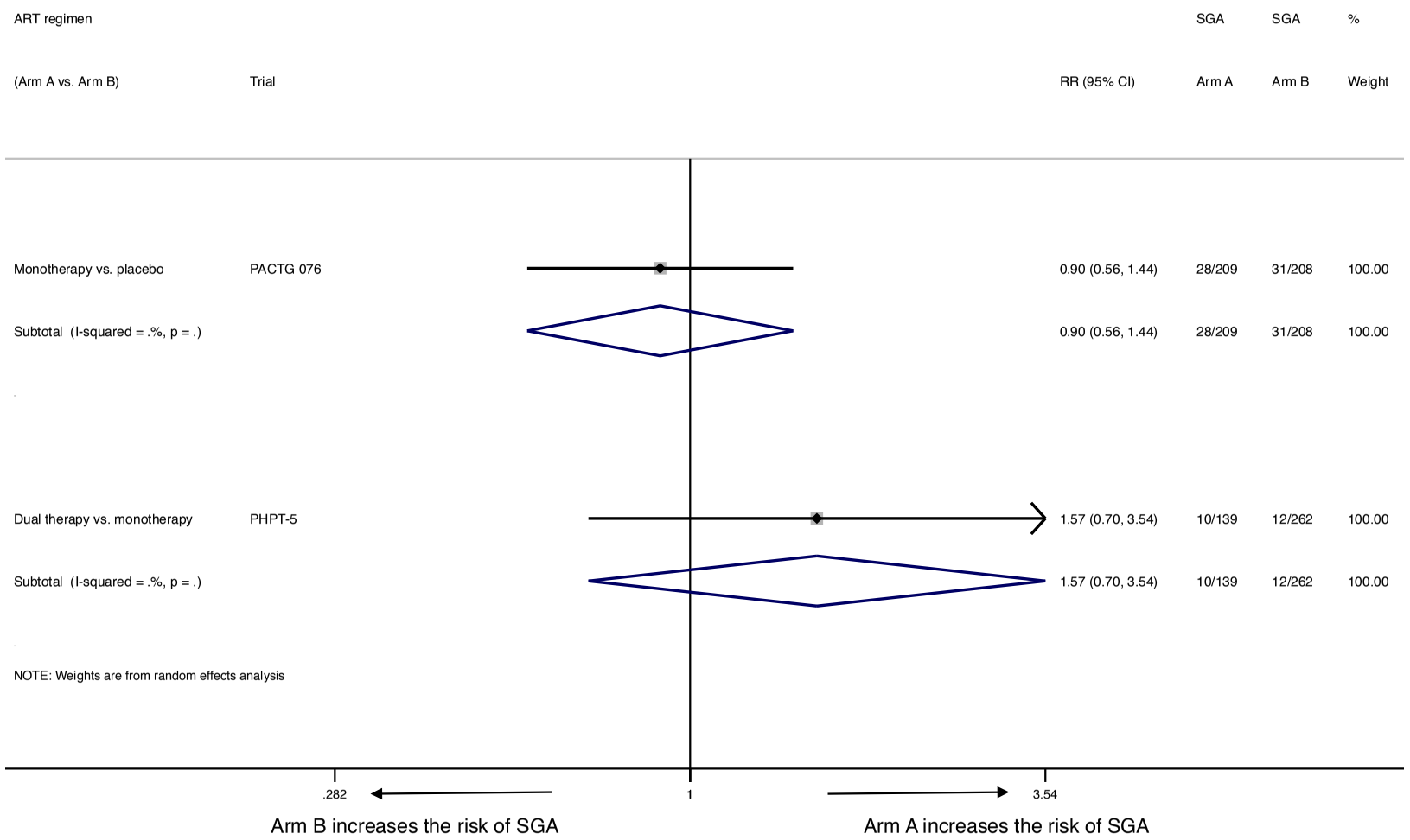

Figure 4.93: Random-effect pairwise meta-analysis results for the association between antenatal antiretroviral regimen and SGA. No significant difference observed between SGA and antiretroviral regimens. Abbreviations: ART = antiretroviral treatment, RR = relative risk, SGA = small for gestational age.

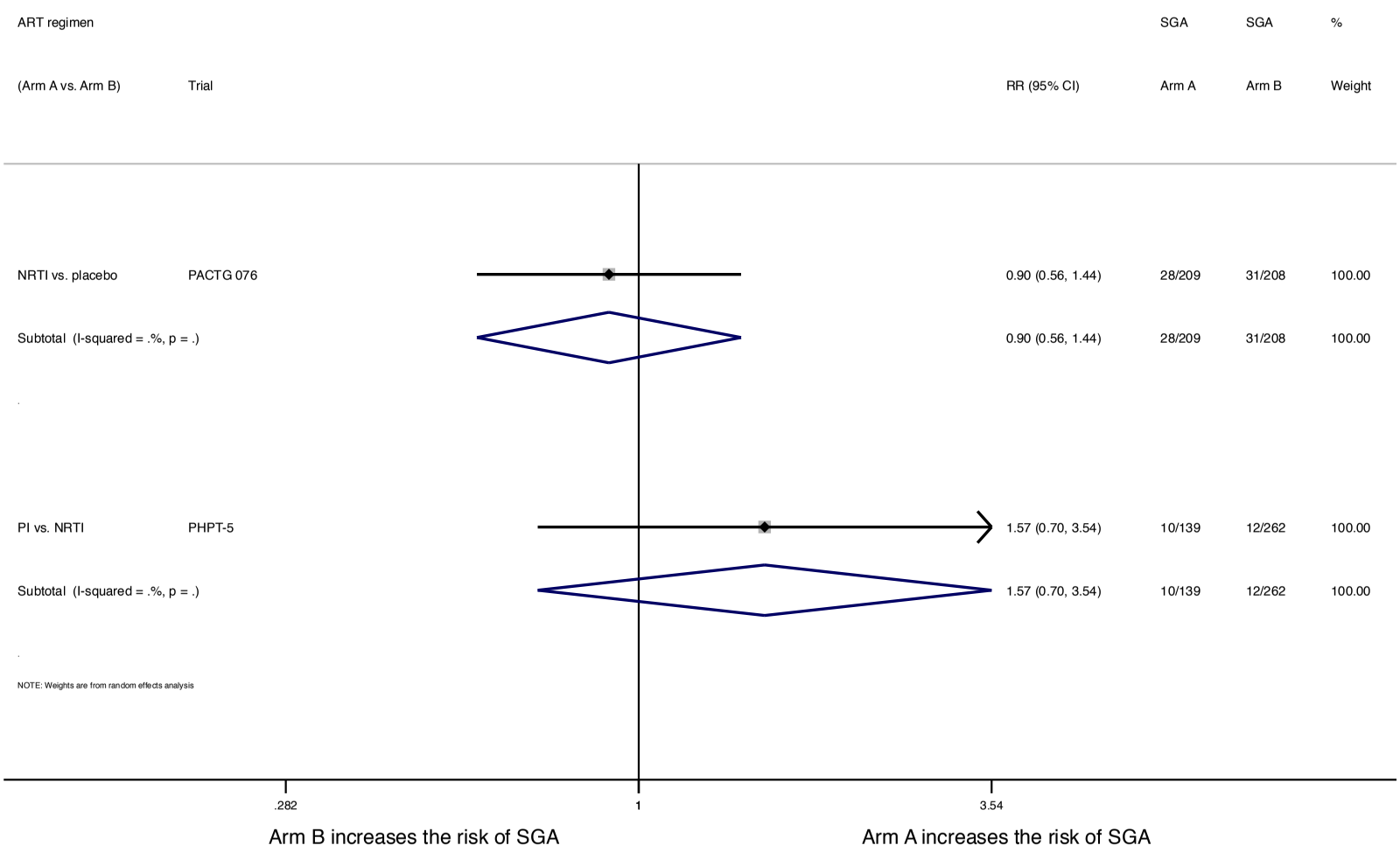

Figure 4.94: Random-effects pairwise meta-analysis results for the association between antenatal antiretroviral class and SGA. There was no significant association between antiretroviral class and SGA. Abbreviations: ART = antiretroviral treatment, NRTI = nucleoside reverse transcriptase inhibitor, PI = protease inhibitor, RR = relative risk, SGA = small for gestational age.

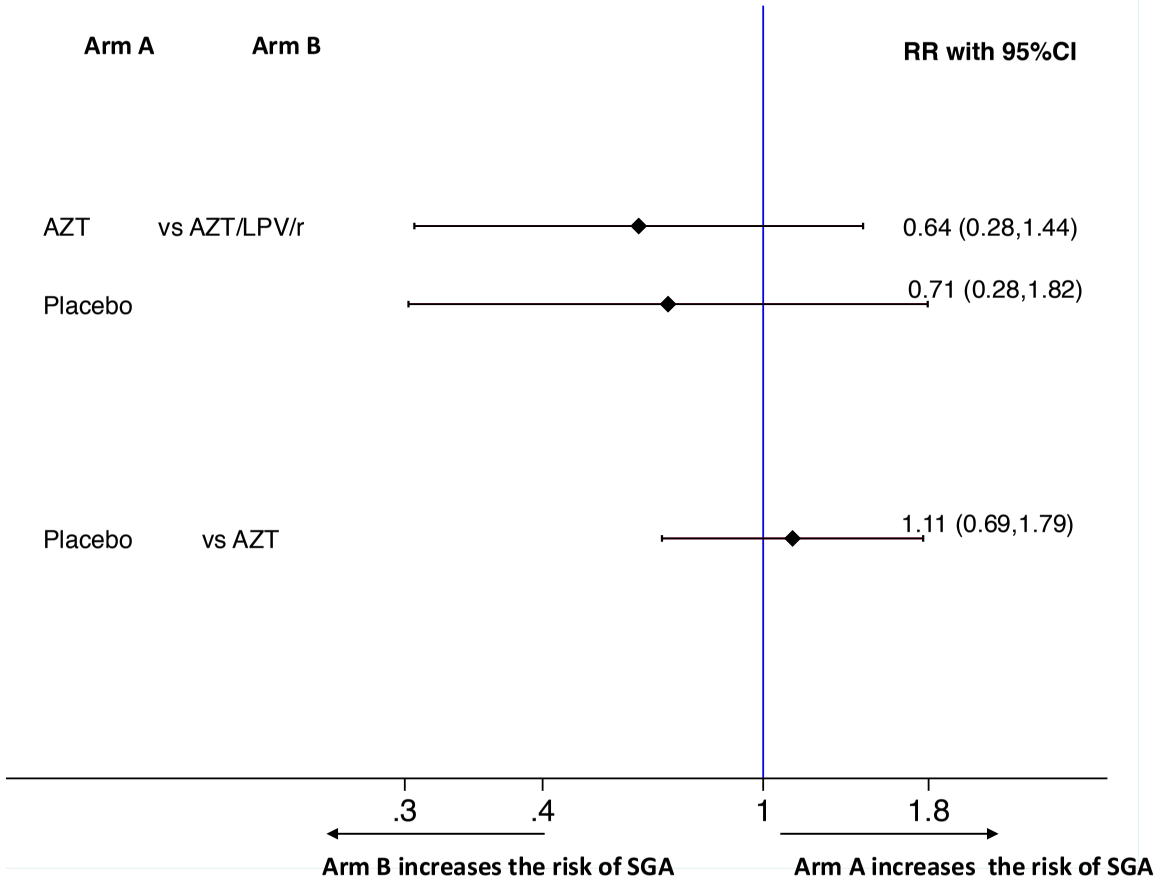

Figure 4.95: Random-effects network meta-analysis results for the association between antenatal ART and SGA.
Statistically significant effects are presented in red. There was no significant association between ART and SGA. Abbreviations: ART = antiretroviral treatment, AZT = zidovudine, LPV/r = lopinavir/ritonavir, RR = relative risk, SGA = small for gestational age.

AZT	1.11 (0.69 - 1.79)	1.57 (0.70 - 3.54)
0.90 (0.56 - 1.44)	**Placebo**	1.41 (0.55 - 3.62)
0.64 (0.28 - 1.44)	0.71 (0.28 - 1.682)	**AZT/LPV/r**

Figure 4.96: League table of random-effects network meta-analysis results for the association between antenatal ART and SGA. This table reflects all possible pairwise comparisons generated from the random-effect network meta-analysis. Results are reported as a relative risk (RR) of the column ART (e.g. ART) relative to the row ART (Placebo). Statistically significant effects are presented in red. There was no significant association between ART and SGA. Abbreviations: AZT = zidovudine, FTC = emtricitabine, LPV/r = lopinavir/ritonavir, SGA = small for gestational age.

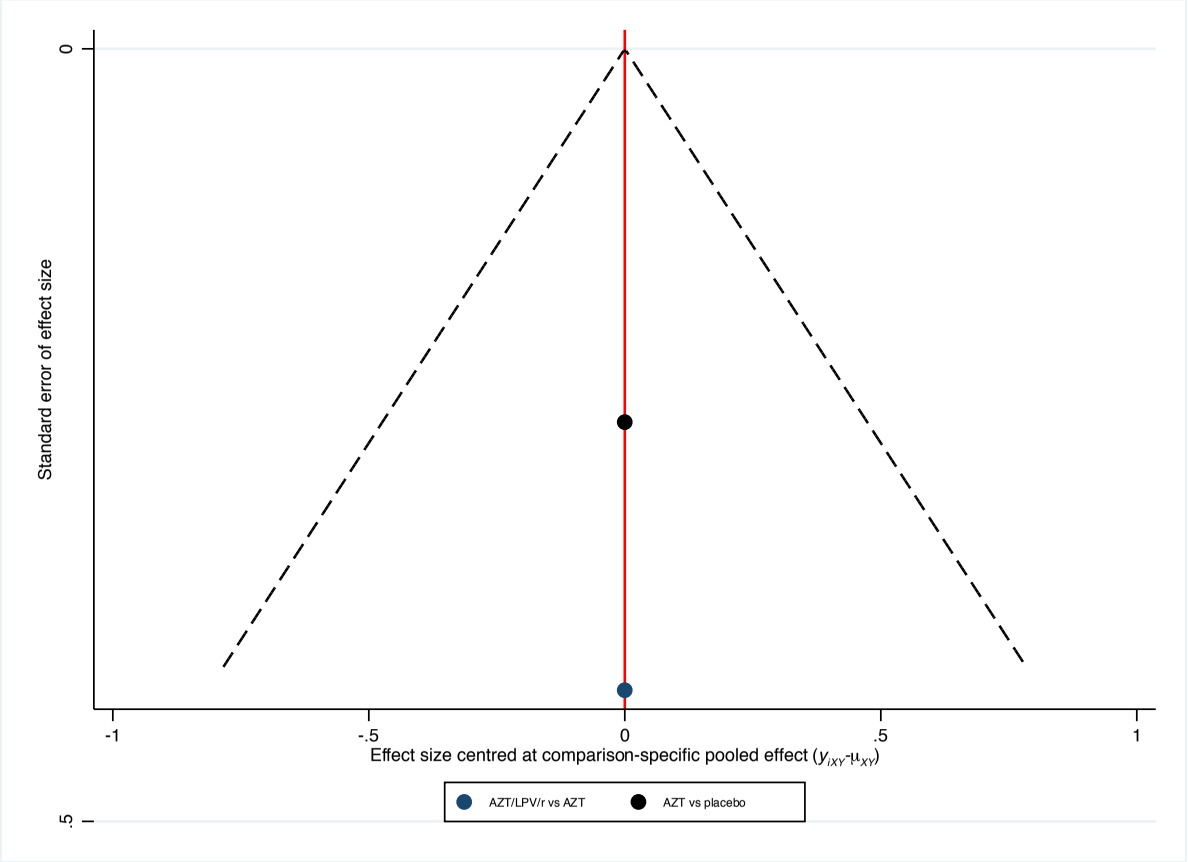

Figure 4.97: Comparison-adjusted funnel plot for the association between antenatal ART and SGA.

The vertical axis represents the measure of the dispersion of the effect size; the horizontal axis represents the difference between the study specific effect size and the comparison-specific summary effect size, and the red line represents the null hypothesis of no difference between the study specific and comparison specific effect estimates. The colours represent individual comparisons. There was no evidence of small study effect

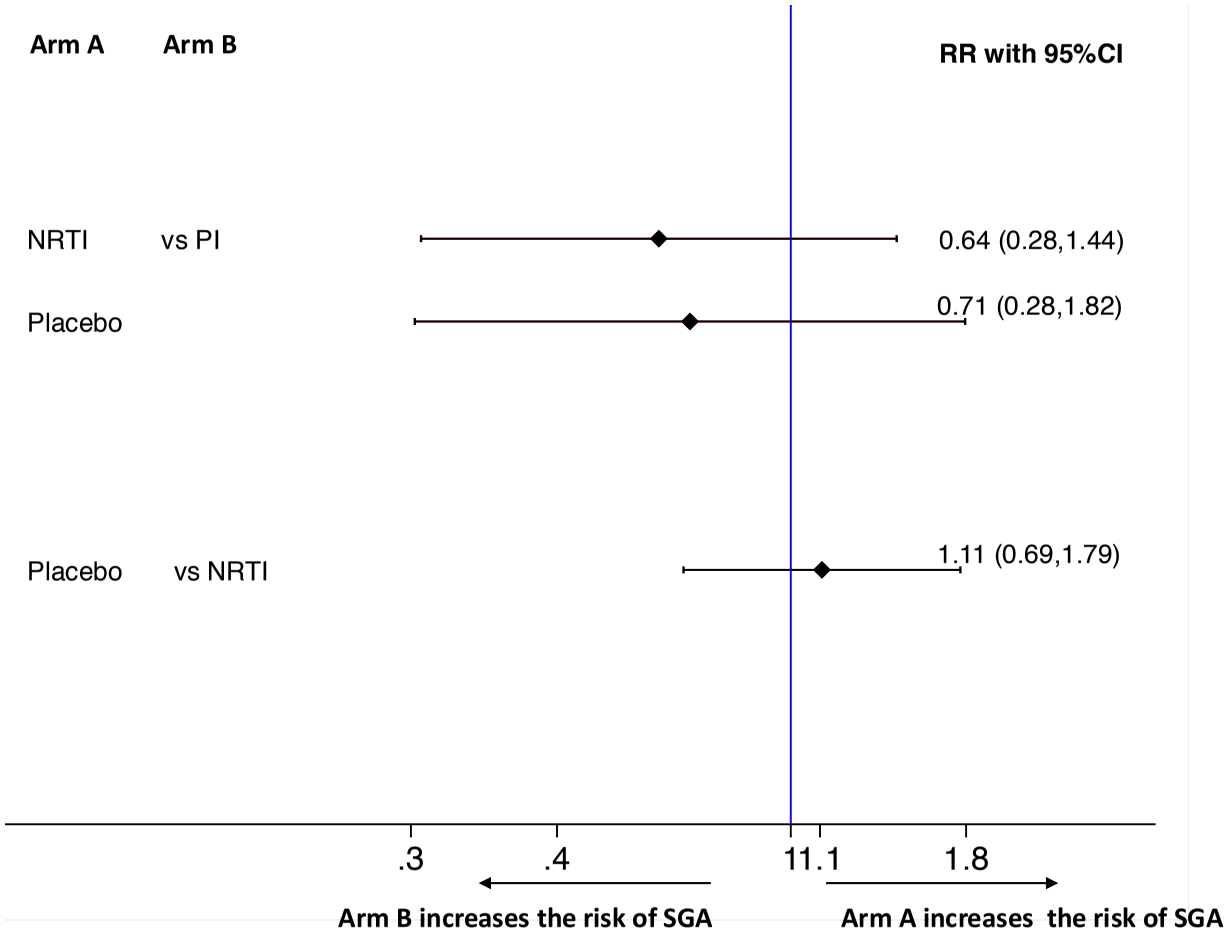

Figure 4.98: Random-effects network meta-analysis results for the association between antenatal antiretroviral class and SGA. No significant difference was observed between SGA and antiretroviral class. Abbreviations: NRTI = nucleoside reverse transcriptase inhibitor, PI = protease inhibitor, RR = relative risk, SGA = small for gestational age.

Placebo	1.41 (0.55 - 3.62)	0.90 (0.56 - 1.44)
0.71 (0.28 - 1.82)	**PI**	0.64 (0.28 - 1.44)
1.11 (0.69 - 1.79)	1.57 (0.70 - 3.54)	**NRTI**

Figure 4.99: League table of random-effects network meta-analysis results for the association between antiretroviral class and SGA.
This table reflects all possible pairwise comparisons generated from the random-effect network meta-analysis, reported as a relative risk (RR). No significant difference was observed between SGA and antiretroviral class. Abbreviations: NRTI = nucleoside reverse transcriptase inhibitor, PI = protease inhibitor, SGA = small for gestational age

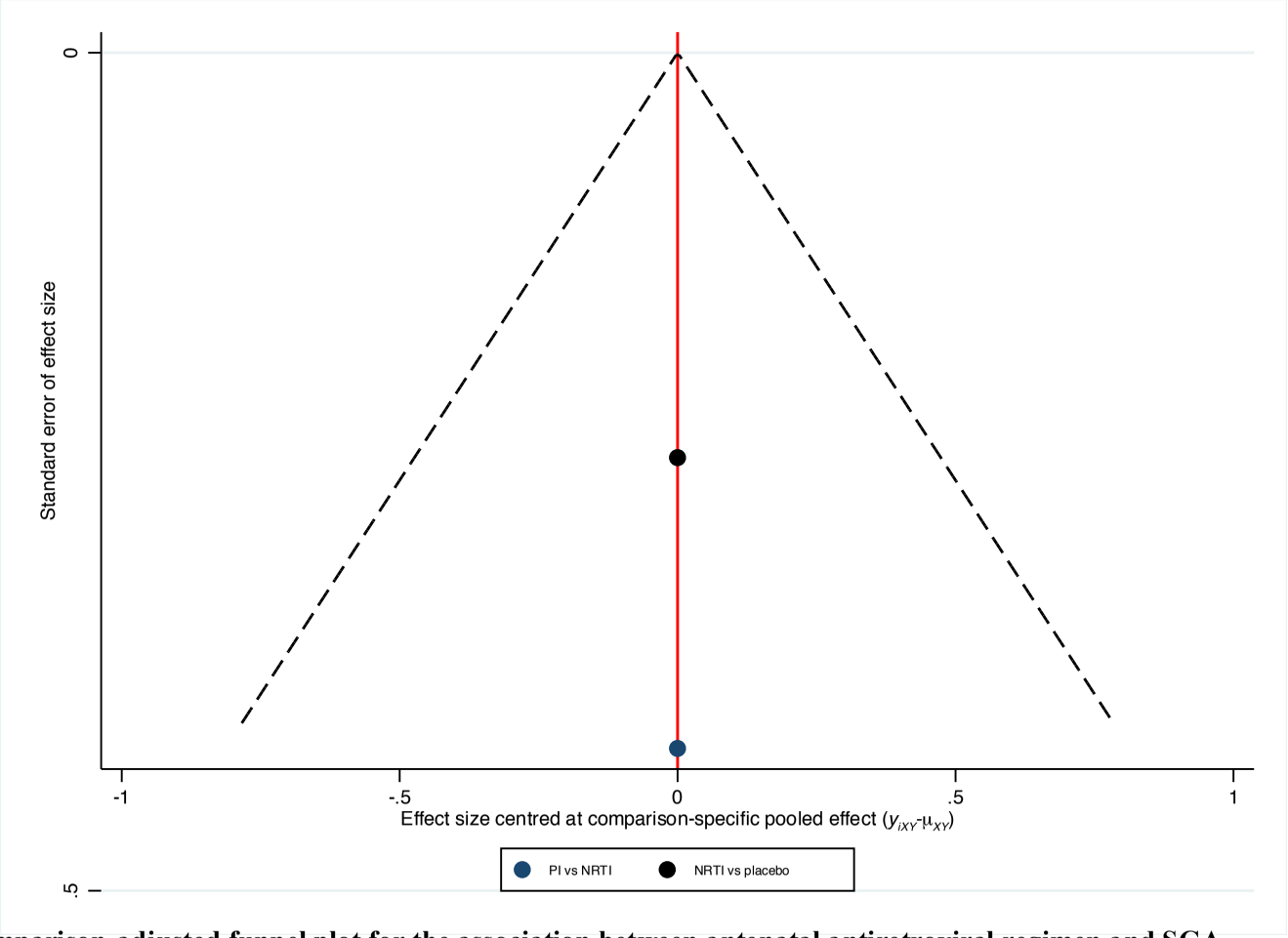

Figure 4.100: Comparison-adjusted funnel plot for the association between antenatal antiretroviral regimen and SGA.
The vertical axis represents the measure of the dispersion of the effect size; the horizontal axis represents the difference between the study specific effect size and the comparison-specific summary effect size, and the red line represents the null hypothesis of no difference between the study specific and comparison specific effect estimates. The colours represent individual comparisons. There was no evidence of small

4.4.6.6.3 Network meta-analysis: ranking of comparative effect of ART on SGA

- Antenatal ART

Of the 3 ARTs assessed, AZT monotherapy was ranked superior to the other treatments (SUCRA = 78.4%) and AZT/LPV/r dual therapy was ranked lowest (SUCRA = 15.4%) (Figure 4.101, Figure 4.102).

- Antenatal antiretroviral class

Of the 3 classes of antiretrovirals assessed, NRTI-based ART was associated with the lowest risk of SGA and was ranked superior to all other treatments (SUCRA = 76.4%), while PI-based ART was associated with the highest risk of SGA and was ranked lowest (SUCRA = 18.6%) (Figure 4.103; Figure 4.104).

Treatment	SUCRA	PrBest	MeanRank
AZT/LPV/r	15.4	8.2	2.7
AZT	78.4	60.3	1.4
Placebo	56.3	31.5	1.9

Figure 4.101: SUCRA ranking of antenatal ART for SGA outcome.
This figure provides a hierarchy of the 3 ARTs assessed in the network meta-analysis for the association between ART and SGA. The larger the SUCRA percentage, the lower the risk of SGA. SUCRA = surface under the cumulative rank curve, represented as a percentage. PrBest = probability that each treatment is the best i.e. associated with the lowest risk of SGA, represented as a percentage. MeanRank = mean rank of each ART. Abbreviations, ART = antiretroviral treatment, AZT = zidovudine, LPV/r = lopinavir/ritonavir, SGA = small for gestational age.
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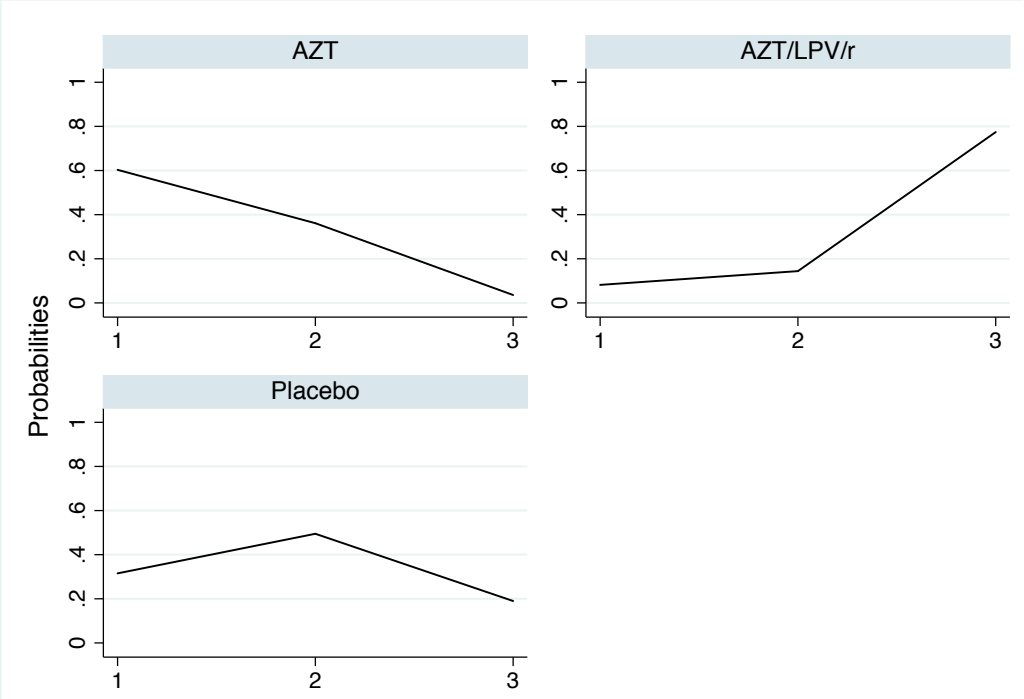

Figure 4.102: Rankograms for ARTs included in the network meta-analysis for the association between ART and SGA. This figure represents the surface under the cumulative ranking probabilities for each ART included in the network meta-analysis. The horizontal axis represents the 3 possible ranks that the ART can achieve and the vertical axis illustrates the probability of the ART to attain each rank[312]. Abbreviations: ART = antiretroviral treatment, AZT = zidovudine, LPV/r = lopinavir/ritonavir, SGA = small for gestational age

Treatm~t	SUCRA	PrBest	MeanRank
PI	18.6	11.7	2.6
NRTI	76.4	57.8	1.5
Placebo	54.9	30.5	1.9

Figure 4.103: SUCRA ranking of antenatal antiretroviral class for SGA outcome.
This figure provides a hierarchy of the 3 classes of antiretrovirals assessed in the network meta-analysis for the association between antiretroviral class and SGA. The larger the SUCRA percentage, the lower the risk of SGA. SUCRA = surface under the cumulative rank curve, represented as a percentage. PrBest = probability that each treatment is the best i.e. associated with the lowest risk of SGA, represented as a percentage. MeanRank = mean rank of each ART. Abbreviations: NRTI = nucleoside reverse transcriptase inhibitor, PI = protease inhibitor, SGA = small for gestational age

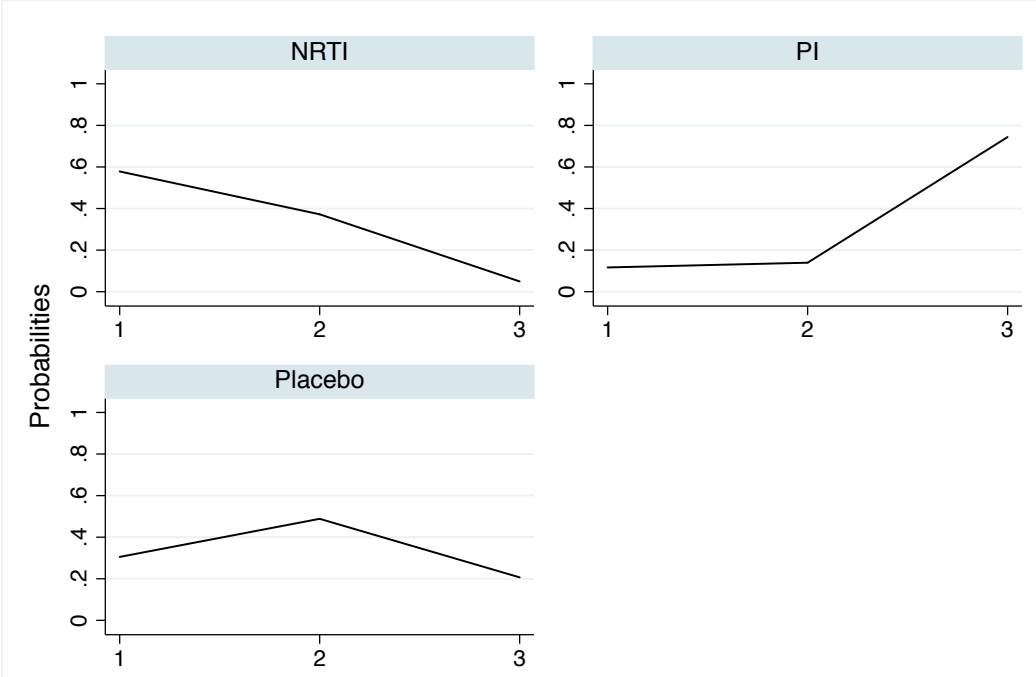

Figure 4.104: Rankograms for antiretroviral classes included in the network meta-analysis for the association between antiretroviral class and SGA. This figure represents the surface under the cumulative ranking probabilities for each antiretroviral class included in the network meta-analysis. The horizontal axis represents the 3 possible ranks that the ART can achieve and the vertical axis illustrates the probability of the ART to attain each rank[312]. NRTI = nucleoside reverse transcriptase inhibitor, PI = protease inhibitor, SGA = small for gestational age

4.4.6.7 Neonatal death

Only 1 study reported on the association between ART and neonatal death. It showed a non-significant increase in neonatal deaths in women initiating AZT/3TC/LPV/r HAART compared to women initiating AZT/3TC/EFV HAART (Figure 4.105).

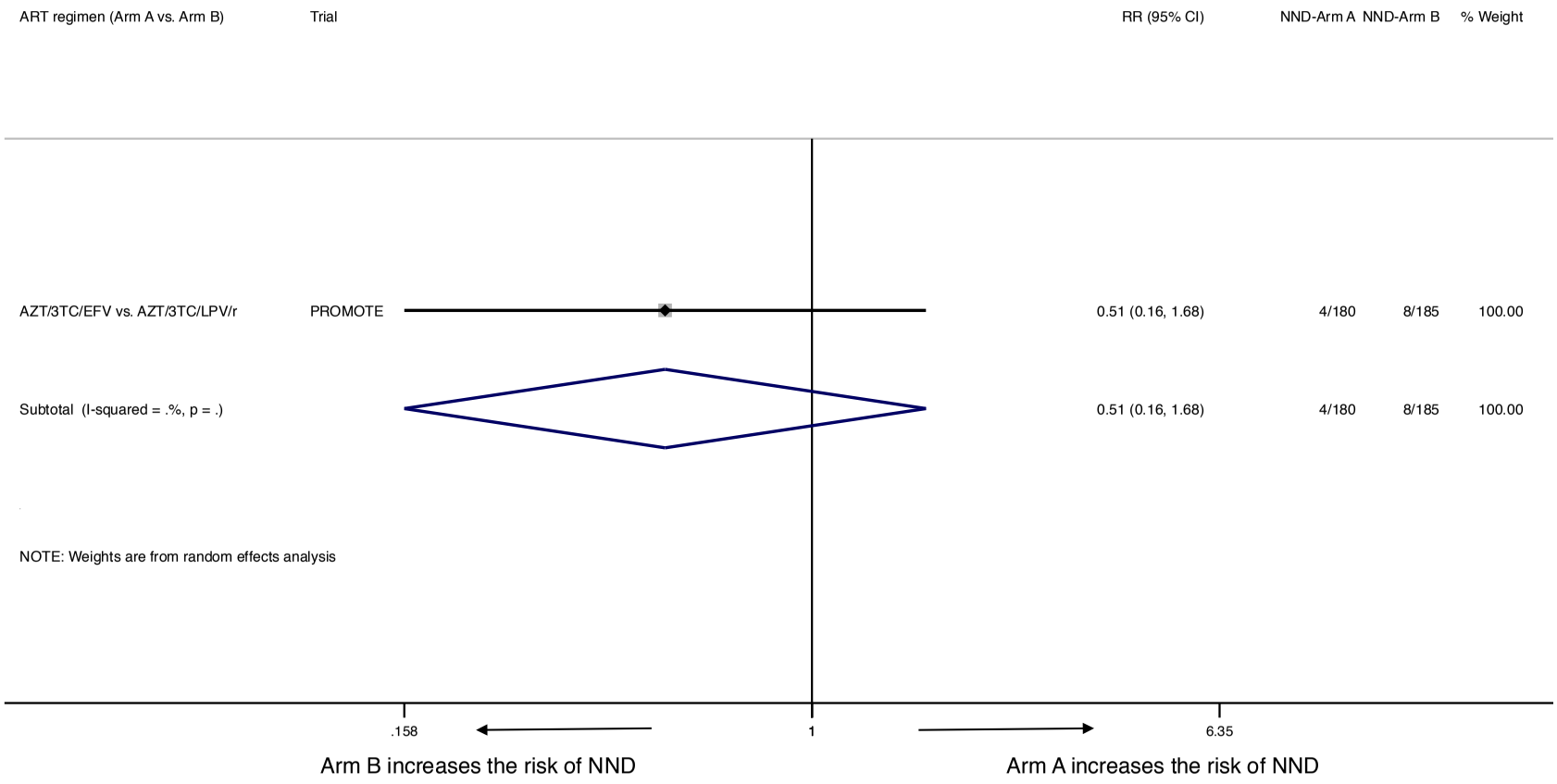

Figure 4.105: Random-effect pairwise meta-analysis results for the association between antenatal ART and NND.
There was no significant association between ART and NND. Abbreviations: Abbreviations: 3TC = lamivudine, ART = antiretroviral treatment, AZT = zidovudine, EFV = efavirenz, LPV/r = lopinavir/ritonavir, NND = neonatal death.

4.4.6.8 Summary points of perinatal outcome results

A summary of the significant findings in the pairwise and network meta-analyses is presented in Figure 4.106 and Figure 4.107. ART was significantly associated with PTB, spontaneous PTB and LBW. There were no significant associations observed between ART and VPTB, SGA or NND.

*4.4.6.8.1 Significant associations observed in pairwise and network meta-analysis results for the association between **ART** and perinatal outcomes.*

PTB

- Pairwise meta-analyses showed that AZT/3TC/LPV/r HAART significantly increased the risk of PTB compared to AZT monotherapy and AZT/3TC/ABC HAART (Figure 4.106).
- Network meta-analyses showed a significant increase in PTB in women initiating AZT/3TC/LPV/r HAART compared to those initiating AZT monotherapy, AZT/3TC/ABC HAART or placebo (Figure 4.107). Furthermore, in comparison to AZT monotherapy, TDF/FTC/LPV/r HAART significantly increased the risk of PTB (Figure 4.107).
- Of the 8 ARTs assessed, AZT/3TC/ABC HAART and AZT monotherapy were associated with the lowest risk of PTB, and ranked as the 1st and 2nd safest ARTs. AZT/3TC/LPV/r HAART was associated with the highest risk of PTB and ranked in 8th place, and was directly preceded by TDF/FTC/LPV/r HAART (ranked in 7th place).

sPTB

- Pairwise and network meta-analyses showed a significantly increased risk of sPTB in women initiating AZT/3TC/LPV/r HAART compared to those initiating AZT/3TC/ABC HAART (Figure 4.106, Figure 4.107).
- Of the 4 ARTs assessed, AZT/3TC/ABC HAART was ranked as the safest ART (ranked in 1st place) and AZT/3TC/LPV/r HAART was ranked as the least safe ART (ranked in 4th place).

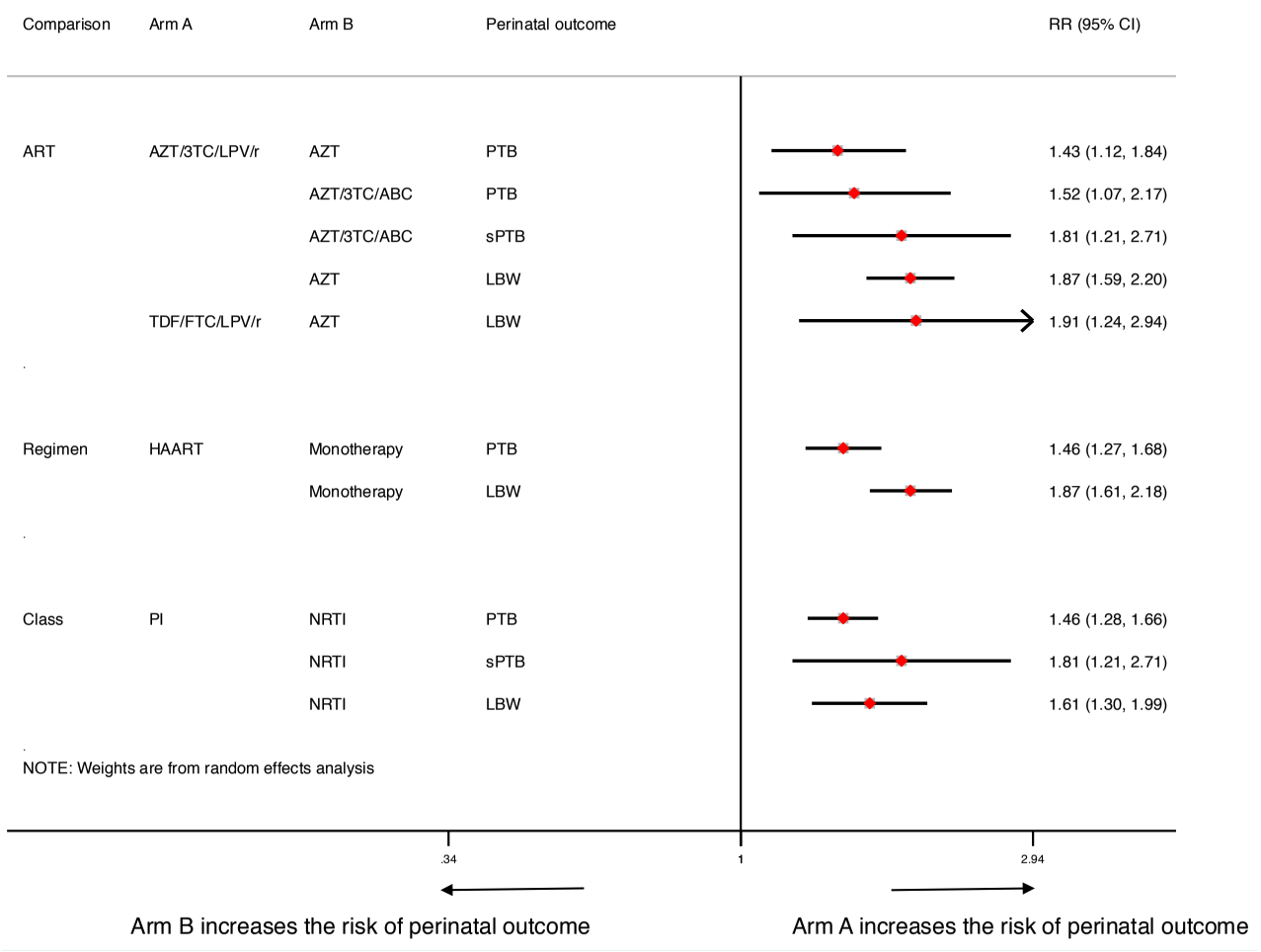

Figure 4.106: Random-effect pairwise meta-analysis results of significant associations between perinatal outcomes and antenatal ART, antiretroviral regimens and classes of antiretrovirals. Statistically significant effects are presented in red. Abbreviations: 3TC = lamivudine, ABC = abacavir, ART = antiretroviral treatment, AZT = zidovudine, FTC = emtricitabine, HAART = Highly Active Antiretroviral Therapy, LBW = low birthweight, LPV/r = lopinavir/ritonavir, NRTI = nucleoside reverse transcriptase inhibitor, PTB = preterm birth, RR = relative risk, sPTB = spontaneous PTB, TDF = tenofovir disoproxil fumarate.

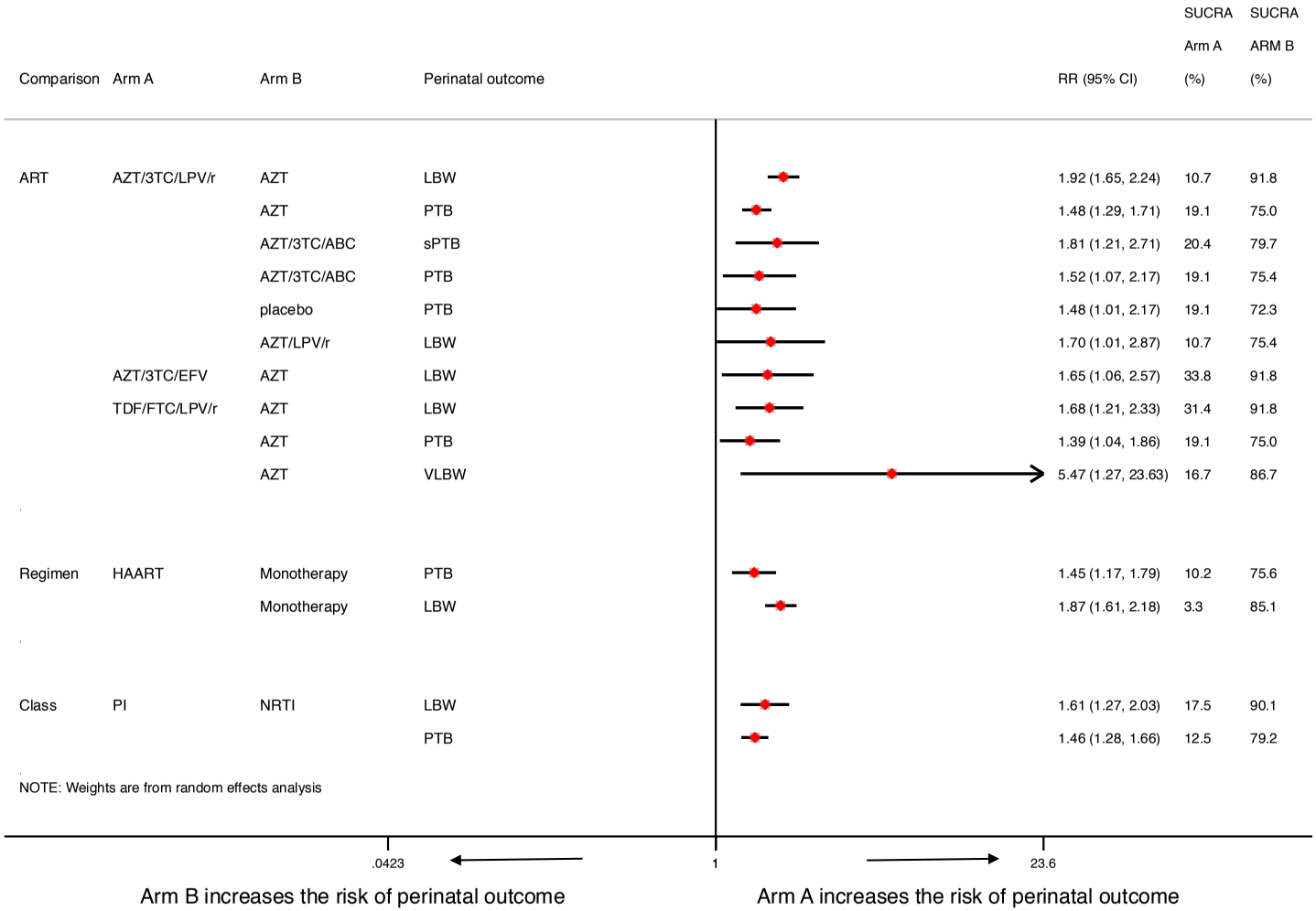

Figure 4.107: Random-effect network meta-analysis results of significant associations between perinatal outcomes and antenatal ART, antiretroviral regimens and classes of antiretrovirals This figure presents statistically significant results from random-effects network meta-analyses of the association between perinatal outcomes and antenatal ART, antiretroviral regimens and classes of antiretrovirals. Statistically significant effects are presented in red. Abbreviations: 3TC = lamivudine, ABC = abacavir, ART = antiretroviral treatment, AZT = zidovudine, EFV = efavirenz, FTC = emtricitabine, HAART = Highly Active Antiretroviral Therapy, LBW = low birthweight, LPV/r = lopinavir/ritonavir, NRTI = nucleoside reverse transcriptase inhibitor, PTB = preterm birth, RR = relative risk, sPTB = spontaneous PTB, SUCRA = surface under the cumulative rank curve, TDF = tenofovir disoproxil fumarate.

LBW

- Pairwise meta-analyses showed that, in comparison to women who initiated AZT monotherapy, women initiating AZT/3TC/LPV/r HAART or TDF/FTC/LPV/r HAART were at a significantly higher risk of giving birth to a LBW infant (Figure 4.106).
- Network meta-analyses showed that women initiating AZT/3TC/LPV/r HAART, AZT/3TC/EFV HAART or TDF/FTC/LPV/r HAART were at a significantly higher risk of giving birth to a LBW infant compared to women who initiated AZT monotherapy (Figure 4.107).
- Of the 7 ARTs assessed, AZT monotherapy was associated with the least risk of LBW, and ranked in 1st place, while AZT/3TC/LPV/r HAART was associated with the highest risk of LBW, and ranked in 7th place.

VLBW

- Network meta-analyses showed that in comparison to women initiating AZT monotherapy, women initiating TDF/FTC/LPV/r HAART were at a significantly higher risk of giving birth to a VLBW infant (Figure 4.107).

*4.4.6.8.2 Significant associations observed in pairwise and network meta-analysis results for the association between **antiretroviral regimens** and perinatal outcomes.*

PTB

- Pairwise and network meta-analyses showed that HAART significantly increased the risk of PTB compared to monotherapy (Figure 4.106, Figure 4.107).
- Of the 4 regimens assessed, monotherapy was ranked 1st, while HAART was ranked as the least safe ART regimen for PTB outcome (ranked in 4th place).

LBW

- Both pairwise and network meta-analyses showed a significantly increased risk of LBW in women initiating HAART compared to those initiating monotherapy (Figure 4.106, Figure 4.107)

- Of the 4 regimens assessed, monotherapy was associated with the lowest risk of LBW and was ranked 1st, and HAART was associated with the highest risk of LBW and was ranked 4th.

*4.4.6.8.3 Significant associations observed in pairwise and network meta-analysis results for the association between **antiretroviral classes** and perinatal outcomes.*

PTB

- Pairwise and network meta-analyses showed a significantly increased risk of PTB in women initiating a PI-based ART compared to those initiating a NRTI-based ART (Figure 4.106, Figure 4.107).
- Of the 4 classes of ART assessed, NRTI was associated with the least risk of PTB and was ranked in 1st place, and PI-based ART was associated with the highest risk of PTB and was ranked in 4th place.

sPTB

- Pairwise meta-analyses showed that PI-based ART significantly increased the risk of spontaneous PTB compared to NRTI-based ART (Figure 4.106).

LBW

- Pairwise and network meta-analyses showed a significantly higher risk of LBW in women initiating a PI-based ART compared to those initiating a NRTI-based ART (Figure 4.106, Figure 4.107).
- Of the 4 classes of ART assessed, NRTI-based ART was associated with the least risk of LBW and was ranked 1st, while PI-based ART was associated with the highest risk of LBW and was ranked in 4th place.

4.4.7 ART and efficacy for PMTCT

This analysis assessed the efficacy of individual ARTs, antiretroviral regimens and classes of antiretrovirals for PMTCT. Seven trials, including 5568 HIV-positive pregnant women, reported on the association between MTCT and 8 ARTs (Figure 4.108). All the treatment comparisons had a high risk of bias, and the majority of participants assessed received AZT monotherapy (n=2167; 38.9%) or a AZT/3TC/LPV/r HAART (n=2276; 40.8%) (Figure 4.108).

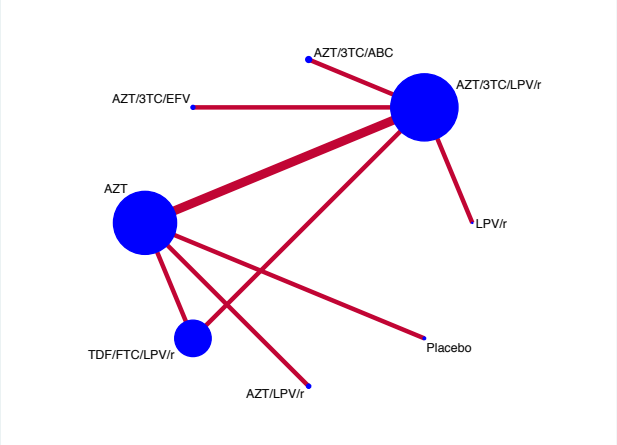

Figure 4.108: Network plot illustrating direct comparisons between ART for MTCT outcome. Each circle represents a treatment group. The size of the circle represents the number of participants who received the ART. The lines connecting the circles represent direct comparisons of ART reported by the RCTs. The red colour of the connecting lines represents a high risk of bias in trials reporting the treatment comparison and the width of the lines represents the number of trials reporting on the treatment comparison.

4.4.7.1.1 Pairwise meta-analysis: association between ART and MTCT

- Antenatal ART

Women initiating AZT monotherapy displayed a significantly lower risk of MTCT than women who initiated placebo (0.30;95%CI 0.15-0.61) (Figure 4.109). There was no evidence of heterogeneity.

- Antenatal regimen

In comparison to placebo, monotherapy significantly reduced the risk of MTCT (RR0.30; 95%CI 0.15-0.61) (Figure 4.110). There was no evidence of heterogeneity.

- Antenatal class of ART

Women initiating a PI-based ART showed a significantly lower risk of MTCT than those initiating NRTI (RR 0.41; 95%CI 0.24-0.71) (Figure 4. 111). There was no evidence of heterogeneity.

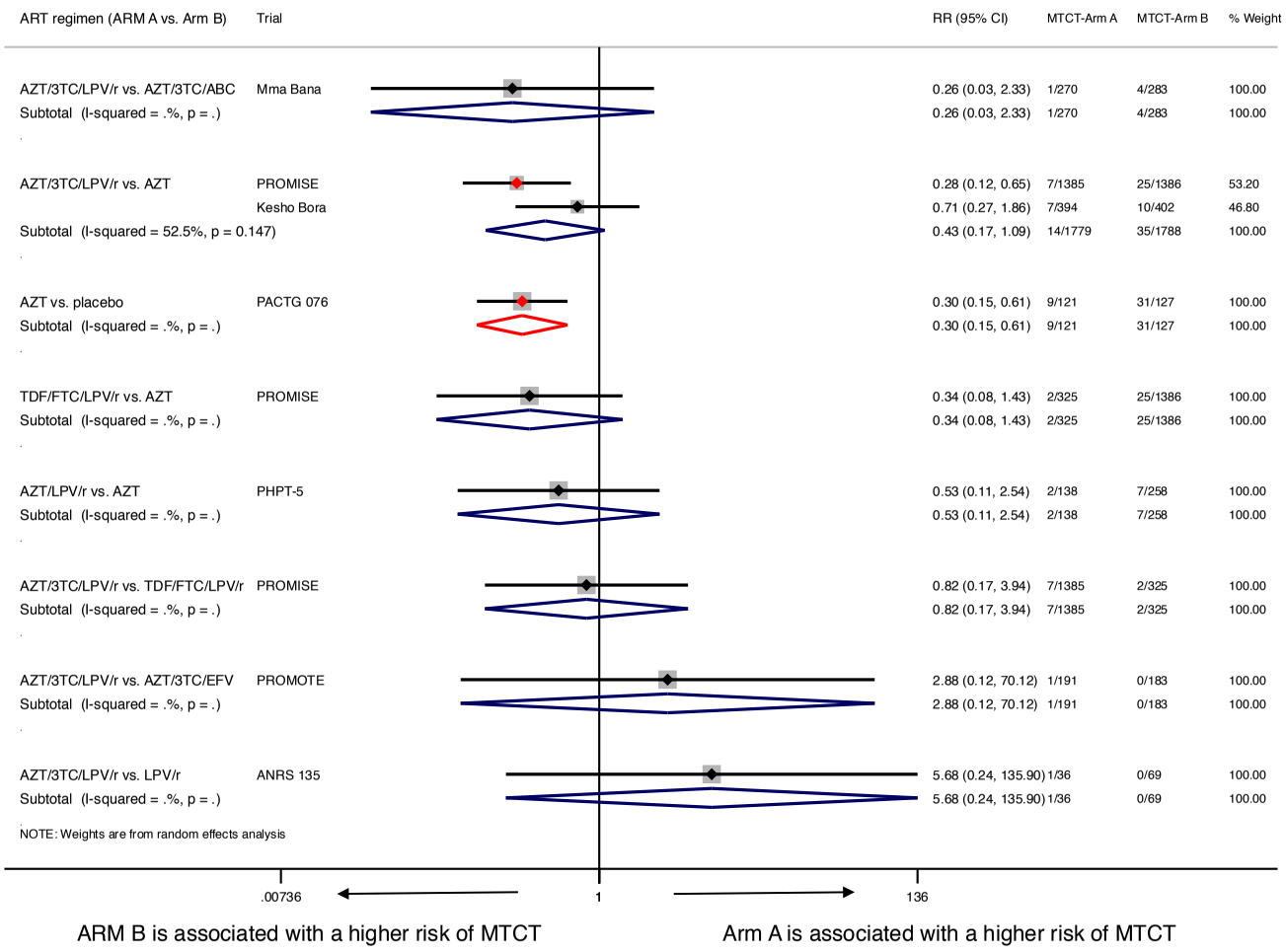

Figure 4.109: Random-effect pairwise meta-analysis results for the association between antenatal ART and MTCT.
Statistically significant effects are presented in red. AZT monotherapy significantly reduced the risk of MTCT, compared to placebo. Abbreviations: 3TC = lamivudine, ABC = abacavir, ART = antiretroviral treatment, AZT = zidovudine, EFV = efavirenz, FTC = emtricitabine, LPV/r = lopinavir/ritonavir, MTCT = mother to child transmission, RR = relative risk, TDF = tenofovir disoproxil fumarate.

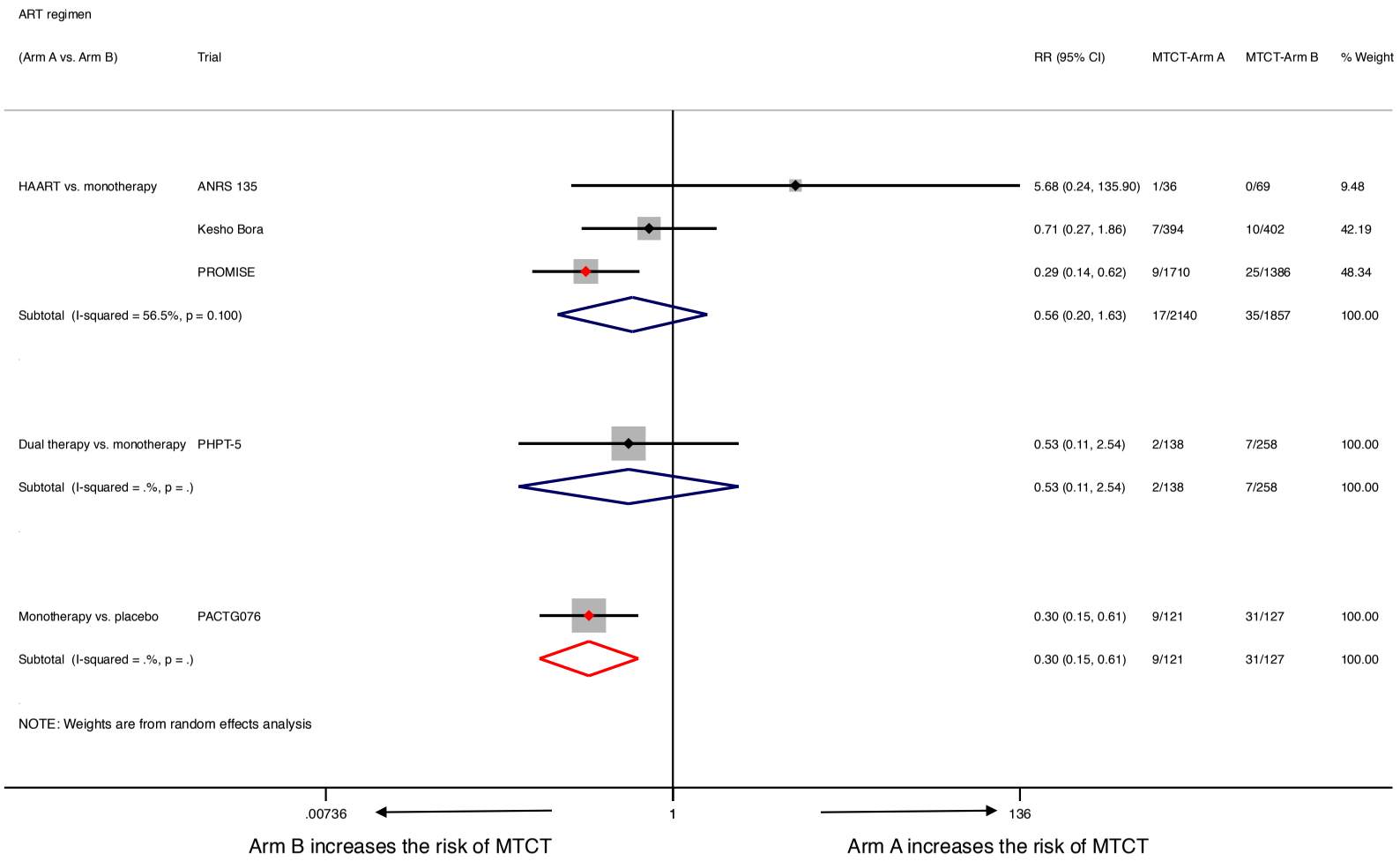

Figure 4.110: Random-effect pairwise meta-analysis results for the association between antenatal antiretroviral regimen and MTCT. Statistically significant effects are presented in red. Monotherapy significantly reduced the risk of MTCT, compared to placebo. Abbreviations: ART = antiretroviral treatment, HAART = Highly Active Antiretroviral Treatment, MTCT = mother to child transmission, RR = relative risk.

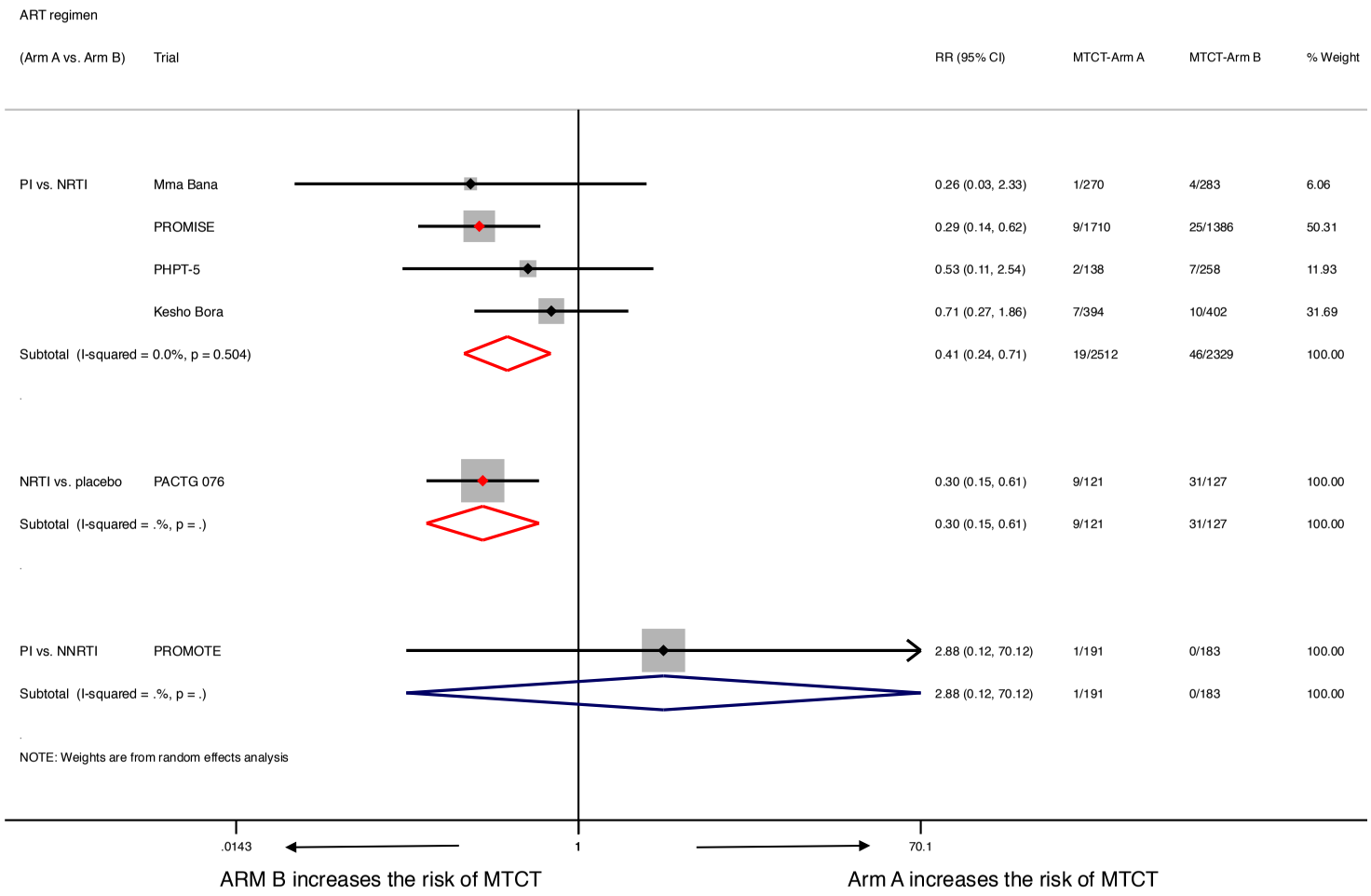

Figure 4.111: Random-effects pairwise meta-analysis results for the association between antenatal antiretroviral class and MTCT. Statistically significant effects are presented in red. PI-based ART significantly reduced the risk of MTCT compared to NRTI-based ART. NRTI-based ART significantly reduced the risk of MTCT compared to placebo. Abbreviations: ART = antiretroviral treatment, MTCT = mother to child transmission, NNRTI = non-nucleoside reverse transcriptase inhibitor, NRTI = nucleoside reverse transcriptase inhibitor, PI = protease inhibitor, RR = relative risk.

4.4.7.1.2 Network meta-analysis: association between ART and MTCT

- Antenatal ART

Women who initiated placebo displayed a significantly higher risk of MTCT than those who initiated LPV/r monotherapy (RR42.80; 95%CI 1.24-1610.29), AZT/3TC/LPV/r HAART (RR7.56; 95%CI 1.71-33.41), AZT monotherapy (RR3.28; 95%CI 1.02-10.55) and TDF/3TC/LPV/r HAART (RR8.16; 95%CI1.04-63.92) (Figure 4.112, Figure 4.113) Furthermore, lopinavir-based HAART was associated with a lower risk of MTCT than zidovudine monotherapy (RR2.32;95%CI 1.01-5.32) (Figure 4.112, Figure 4.113). There was no evidence of inconsistency (p=0.15) or publication bias (Figure 4.114).

- Antenatal regimen

No significant association was observed between antiretroviral regimens and MTCT (Figure 4.115, Figure 4.116). There was no evidence of inconsistency (P=0.33); however, there was some evidence of publication bias (Figure 4.117)

- Antenatal class

PI-based ART significantly reduced the risk of MTCT compared to NRTI-based ART (RR0.42; 95%CI0.23-0.77) and placebo (RR0.13; 95%CI 0.05-0.36) (Figure 4.118, Figure 4.119). Furthermore, NRTI-based ART significantly reduced the risk of MTCT compared to placebo (RR 0.30; 95%CI 0.13-0.69). The global test for inconsistency was statistically significant (p=0.002). However, the comparison of direct and indirect evidence by side-splitting show no statistically significant evidence of inconsistency (A vs. B: p= 0.98 and B vs. D: p=0.98). There was no evidence of publication bias (Figure 4.120).

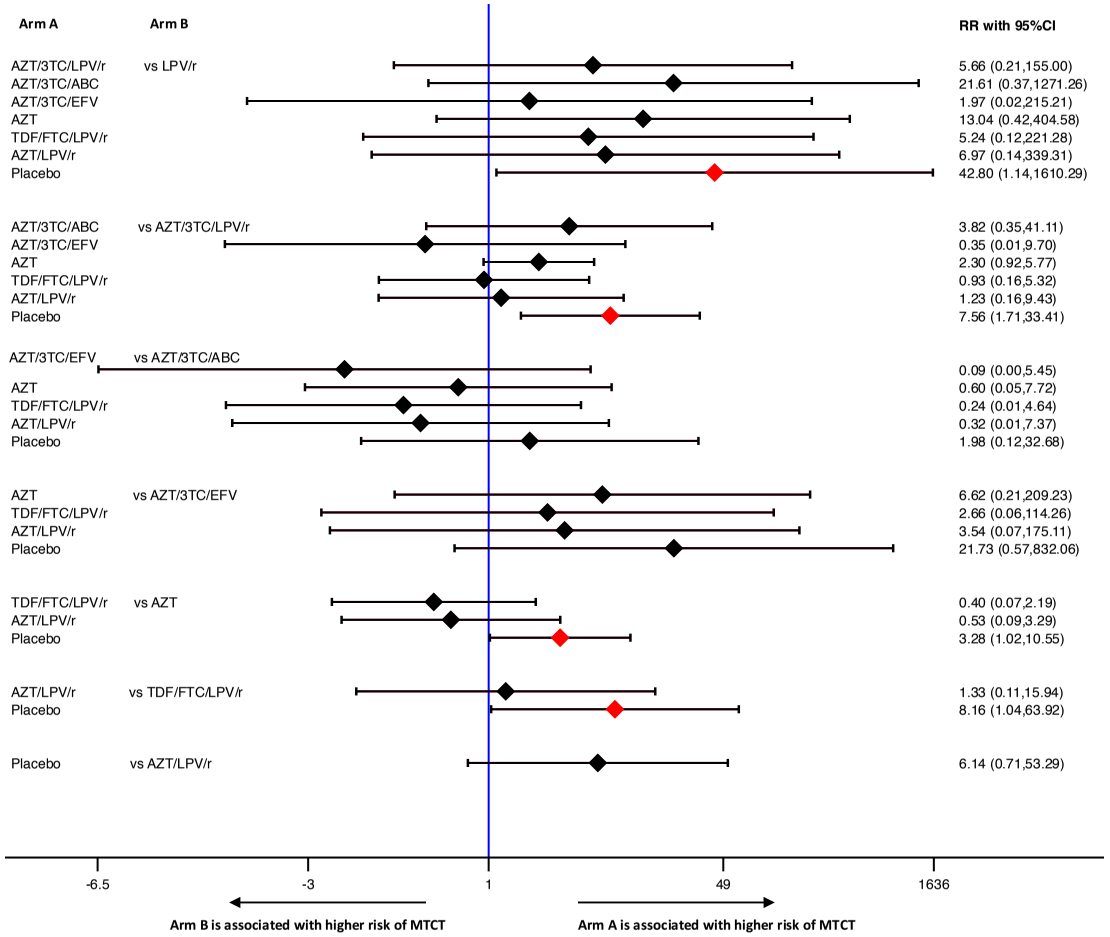

Figure 4.112: Random-effects network meta-analysis results for the association between antenatal ART and MTCT.
Statistically significant effects are presented in red. Placebo was associated with a significantly higher risk of MTCT than LPV/r monotherapy, AZT/3TC/LPV/r HAART, AZT monotherapy and TDF/FTC/LPV/r HAART. Abbreviations: 3TC = lamivudine, ABC = abacavir, ART = antiretroviral treatment, AZT = zidovudine, EFV = efavirenz, FTC = emtricitabine, LPV/r = lopinavir/ritonavir, MTCT = mother to child transmission, RR = relative risk, TDF = tenofovir disoproxil fumarate.

AZT	**3.28 (1.02 - 10.55)**	0.08 (0.00 - 2.38)	0.53 (0.09 - 3.29)	0.15 (0.00 - 4.77)	1.66 (0.13 - 21.18)	0.43 (0.17 - 1.09)	0.40 (0.07 - 2.19)
0.30 (0.09 - 0.98)	**Placebo**	**0.02 (0.00 - 0.88)**	0.16 (0.02 - 1.41)	0.05 (0.00 - 1.76)	0.50 (0.03 - 8.33)	**0.13 (0.03 - 0.58)**	**0.12 (0.02 - 0.96)**
13.04 (0.42 - 404.58)	**42.80 (1.14 - 1610.29)**	**LPV/r**	6.97 (0.14 - 339.31)	1.97 (0.02 - 215.21)	21.61 (0.37 - 1271.26)	5.66 (0.21 - 155.00)	5.24 (0.12 - 221.28)
1.87 (0.30 - 11.52)	6.14 (0.71 - 53.29)	0.14 (0.00 - 6.99)	**AZT/LPV/r**	0.28 (0.01 - 13.99)	3.10 (0.14 - 70.94)	0.81 (0.11 - 6.23)	0.75 (0.06,9.03)
6.62 (0.21 - 209.23)	21.73 (0.57 - 832.06)	0.51 (0.00 - 55.48)	3.54 (0.07 - 175.11)	**AZT/3TC/EFV**	10.97 (0.18 - 655.49)	2.88 (0.10 - 80.20)	2.66 (0.06 - 114.26)
0.60 (0.05 - 7.72)	1.98 (0.12 - 32.68)	0.05 (0.00 - 2.72)	0.32 (0.01 - 7.37)	0.09 (0.00 - 5.45)	**AZT/3TC/ABC**	0.26 (0.02 - 2.82)	0.24 (0.01 - 4.64)
2.30 (0.92 - 5.77)	**7.56 (1.71 - 33.41)**	0.18 (0.01 - 4.83)	1.23 (0.16 - 9.43)	0.35 (0.01 - 9.70)	3.82 (0.35 - 41.11)	**AZT/3TC/LPV/r**	0.93 (0.16 - 5.32)
2.49 (0.46 - 13.54)	**8.16 (1.04 - 63.92)**	0.19 (0.00 - 8.05)	1.33 (0.11 - 15.94)	0.38 (0.01 - 16.13)	4.12 (0.22 - 78.78)	1.08 (0.19 - 6.20)	**TDF/FTC/LPV/r**

Figure 4.113: League table of random-effects network meta-analysis results for the association between antenatal ART and MTCT.
This table reflects all possible pairwise comparisons generated from the random-effect network meta-analysis. Results are reported as a relative risk (RR) of the column ART (e.g. AZT) relative to the row ART (AZT/3TC/LPV/r). Statistically significant effects are presented in red. Placebo was associated with a significantly higher risk of MTCT than LPV/r monotherapy, AZT/3TC/LPV/r HAART, AZT monotherapy and TDF/FTC/LPV/r HAART. Abbreviations: 3TC = lamivudine, ABC = abacavir, ART = antiretroviral treatment, AZT = zidovudine, EFV = efavirenz, FTC = emtricitabine, LPV/r = lopinavir/ritonavir, MTCT = mother to child transmission, RR = relative risk, TDF = tenofovir disoproxil fumarate.

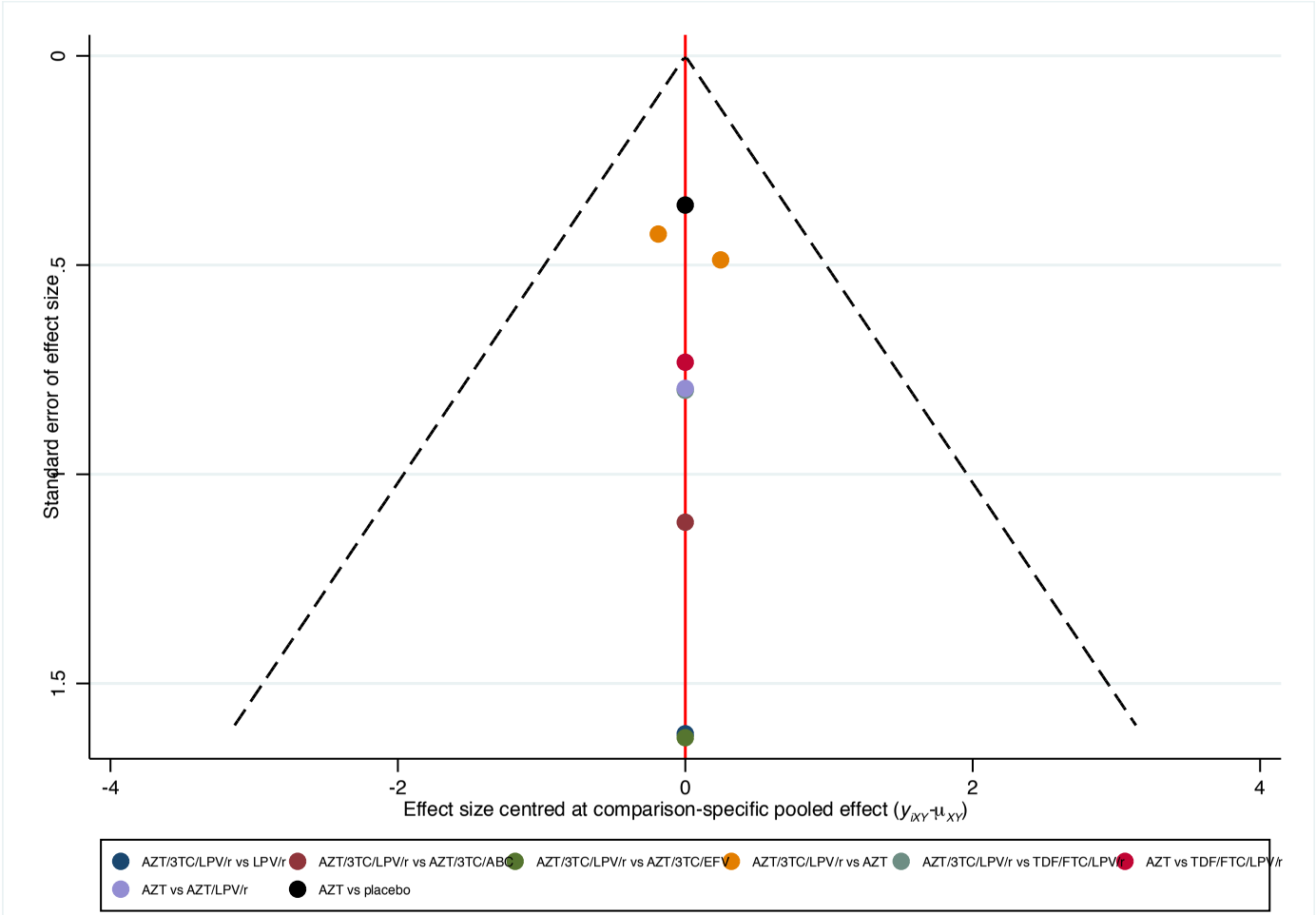

Figure 4.114: Comparison-adjusted funnel plot for the association between antenatal antiretroviral regimen and MTCT.
The vertical axis represents the measure of the dispersion of the effect size; the horizontal axis represents the difference between the study specific effect size and the comparison-specific summary effect size, and the red line represents the null hypothesis of no difference between the study specific and comparison specific effect estimates. The colours represent individual comparisons. There was no evidence of small study effect.

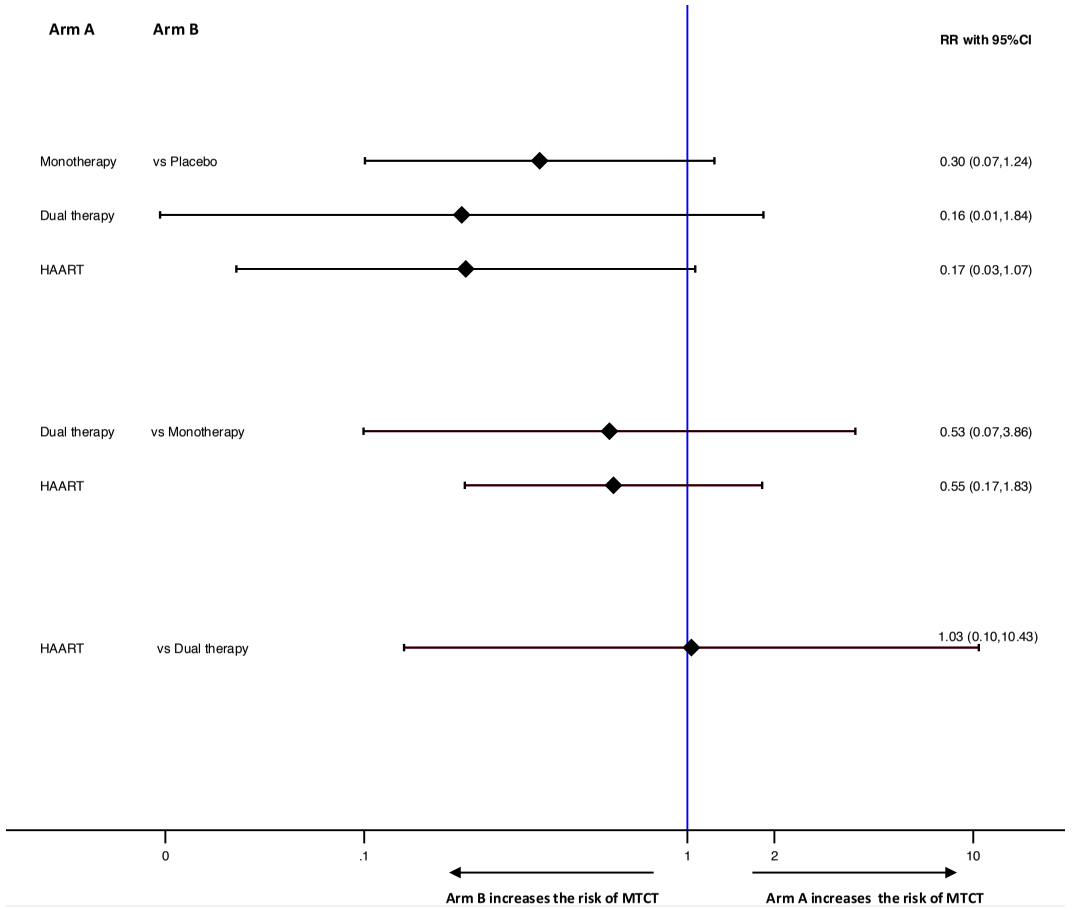

Figure 4.115: Random-effects network meta-analysis results for the association between antenatal antiretroviral regimens and MTCT. Statistically significant effects are presented in red. No significant association was observed between MTCT and antiretroviral regimens. Abbreviations: HAART = Highly Active Antiretroviral Treatment, MTCT = mother to child transmission, RR = relative risk.

Placebo	0.30 (0.07 - 1.24)	0.16 (0.01 - 1.84)	0.17 (0.03 - 1.07)
3.28 (0.80 - 13.39)	**Monotherapy**	0.53 (0.07 - 3.86)	0.55 (0.17 - 1.83)
6.14 (0.54 - 69.58)	1.87 (0.26 - 13.54)	**Dual therapy**	1.03 (0.10 - 10.43)
5.95 (0.94 - 37.67)	1.81 (0.55 - 5.99)	0.97 (0.10 - 9.77)	**HAART**

Figure 4.116: League table of random-effects network meta-analysis results for the association between antenatal antiretroviral regimen and MTCT. This table reflects all possible pairwise comparisons generated from the random-effect network meta-analysis. Results are reported as a relative risk (RR) of the column regimen (e.g. Monotherapy) relative to the row regimen (HAART). Statistically significant effects are presented in red. No significant association was observed between antiretroviral regimens and MTCT. Abbreviations: HAART = Highly Active Antiretroviral Treatment, MTCT = mother to child transmission.

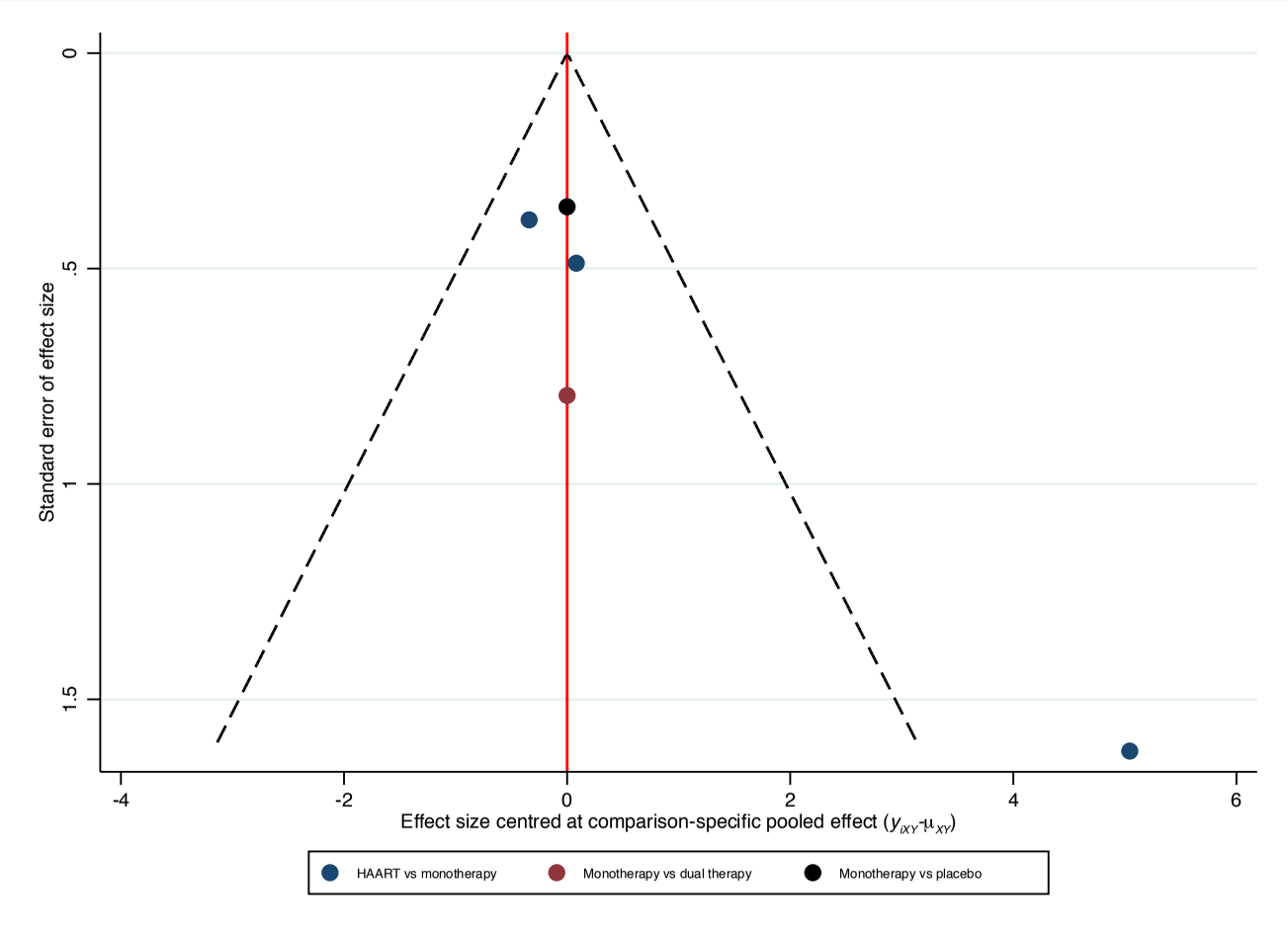

Figure 4.117: Comparison-adjusted funnel plot for the association between antenatal antiretroviral regimen and MTCT.
The vertical axis represents the measure of the dispersion of the effect size; the horizontal axis represents the difference between the study specific effect size and the comparison-specific summary effect size, and the red line represents the null hypothesis of no difference between the study specific and comparison specific effect estimates. The colours represent individual comparisons. There was some evidence of small study effect.

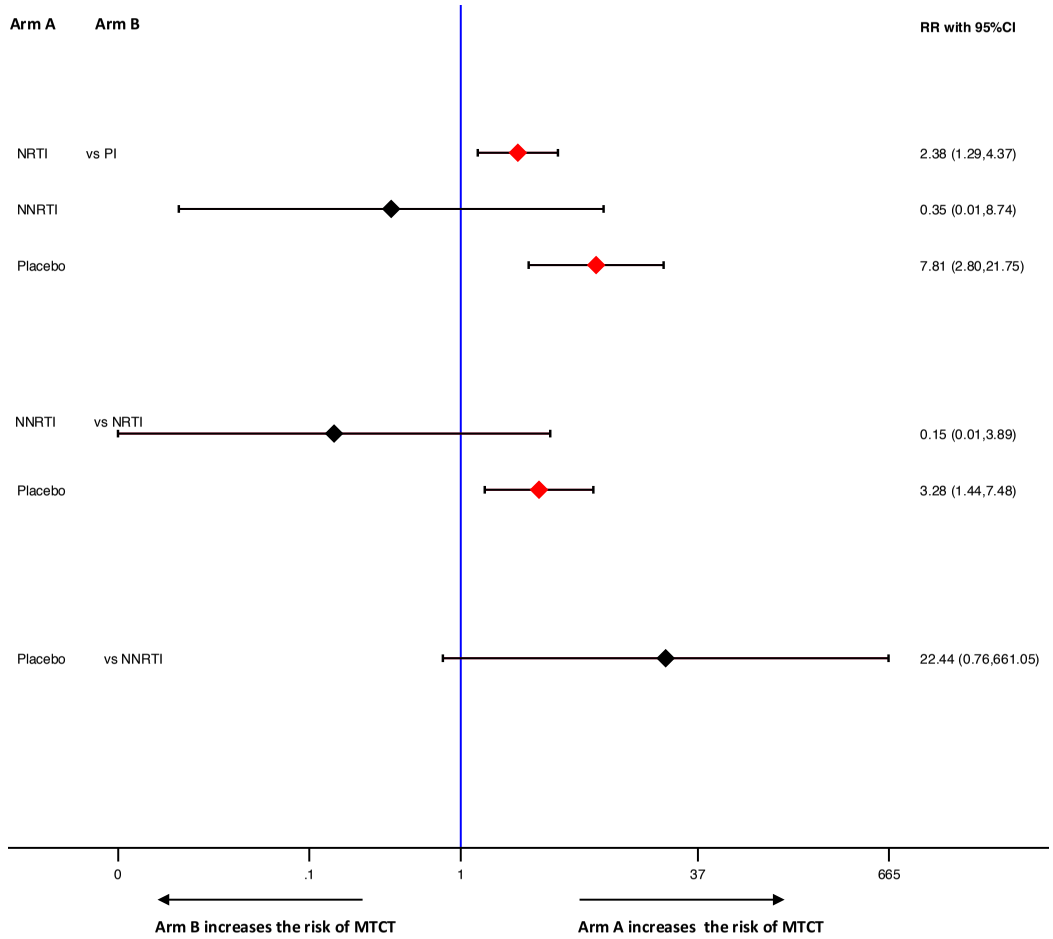

Figure 4.118: Random-effects network meta-analysis results for the association between antenatal antiretroviral class and MTCT. Statistically significant effects are presented in red. PI-based ART significantly reduced the risk of MTCT, compared to NRTI-based ART and placebo. NRTI-based ART significantly reduced the risk of MTCT compared to placebo. Abbreviations: NNRTI = non-nucleoside reverse transcriptase inhibitor, NRTI = nucleoside reverse transcriptase inhibitor, MTCT = mother to child transmission, PI = protease inhibitor, RR = relative risk.

Placebo	**0.13 (0.05 - 0.36)**	0.04 (0.00 - 1.31)	**0.30 (0.13 - 0.69)**
7.81 (2.80 - 21.75)	**PI**	0.35 (0.01 - 8.74)	**2.38 (1.29 - 4.37)**
22.44 (0.76 - 661.05)	2.88 (0.11 - 72.24)	**NNRTI**	6.84 (0.26 - 181.92)
3.28 (1.44 - 7.48)	**0.42 (0.23 - 0.77)**	0.15 (0.01 - 3.89)	**NRTI**

Figure 4.119: League table of random-effects network meta-analysis results for the association between antenatal antiretroviral class and MTCT. This table reflects all possible pairwise comparisons generated from the random-effect network meta-analysis. Results are reported as a relative risk (RR) of the column regimen (e.g. PI) relative to the row regimen (NRTI). Statistically significant effects are presented in red. PI-based ART significantly reduced the risk of MTCT, compared to NRTI-based ART and placebo. NRTI-based ART significantly reduced the risk of MTCT compared to placebo. Abbreviations: NNRTI = non-nucleoside reverse transcriptase inhibitor, NRTI = nucleoside reverse transcriptase inhibitor, MTCT = mother to child transmission, PI = protease inhibitor, RR = relative risk.

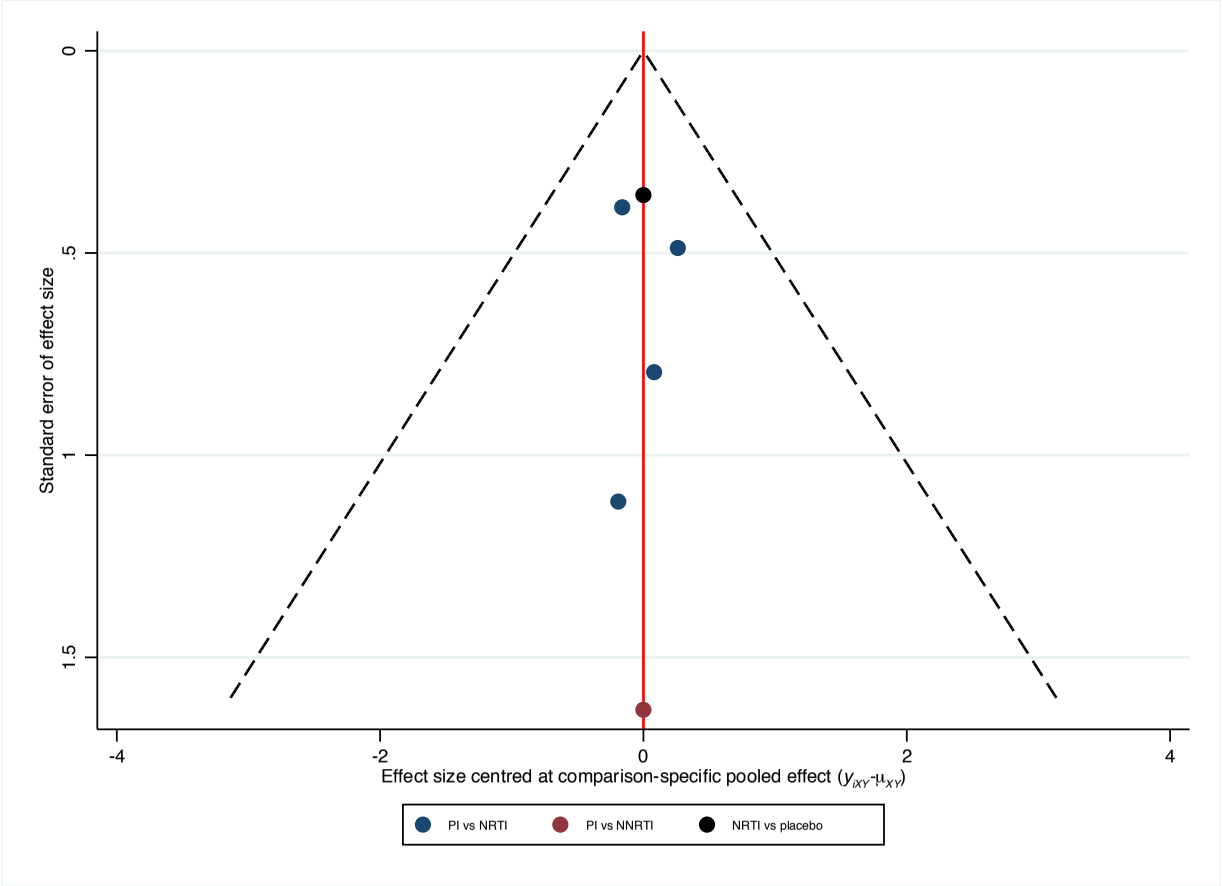

Figure 4.120: Comparison-adjusted funnel plot for the association between classes of antiretrovirals and MTCT.
The vertical axis represents the measure of the dispersion of the effect size; the horizontal axis represents the difference between the study specific effect size and the comparison-specific summary effect size, and the red line represents the null hypothesis of no difference between the study specific and comparison specific effect estimates. The colours represent individual comparisons. There was no evidence of small study effect.

4.4.7.1.3 Network meta-analysis: ranking of comparative effect of ART on MTCT

- Antenatal ART

Of the 8 ARTs assessed, LPV/r monotherapy (SUCRA = 84.7%), AZT/3TC/EFV HAART (74.4%), AZT/3TC/LPV/r HAART (61.6%) and TDF/FTC/LPV/r HAART (61.0%) were associated with the lowest risk of MTCT and were ranked as the most efficacious ARTs for PMTCT (Figure 4.121, Figure 4.122). Conversely, placebo (SUCRA = 7.2%), AZT/3TC/ABC HAART (SUCRA = 25.0%) and AZT monotherapy (SUCRA = 32.6%) were associated with the highest risk of MTCT and were ranked as the least efficacious ARTs for PMTCT (Figure 4.121, Figure 4.122).

- Antenatal antiretroviral regimen

Of the 4 antiretroviral regimens assessed, HAART (SUCRA = 76.6%) was associated with the lowest risk of MTCT and was ranked as the most efficacious regimen (Figure 4.123, Figure 4.124). Placebo (SUCRA = 5.1%), followed by monotherapy (SUCRA = 46.2%), were associated with the highest risk of MTCT and were subsequently ranked as the least efficacious regimens (Figure 4.123, Figure 4.124).

- Antenatal antiretroviral class

Of the 4 classes of antiretrovirals assessed, NNRTI-based ART (SUCRA = 86.0%) and PI-based ART (SUCRA = 75.3%) were associated with the lowest risk of MTCT and were ranked as the most efficacious class of antiretrovirals for PMTCT (Figure 4.125, Figure 4.126). Conversely, placebo (SUCRA = 1.2%) and NRTI-based ART (SUCRA = 37.5%), were associated with the highest risk of MTCT and were ranked as the least efficacious classes of antiretrovirals (Figure 4.125, Figure 4.126).

Treatment	SUCRA	PrBest	MeanRank
LPV/r	84.7	54.1	2.1
AZT/3TC/LPV/r	61.6	1.0	3.7
AZT/3TC/ABC	25.0	1.0	6.3
AZT/3TC/EFV	74.4	33.1	2.8
AZT	32.6	0.0	5.7
TDF/FTC/LPV/r	61.0	5.9	3.7
AZT/LPV/r	53.5	4.8	4.3
Placebo	7.2	0.0	7.5

Figure 4.121: SUCRA ranking of antenatal ART for MTCT outcome.
This figure provides a hierarchy of the 8 ARTs assessed in the network meta-analysis for the association between ART and MTCT. The larger the SUCRA percentage, the lower the risk of MTCT. SUCRA = surface under the cumulative rank curve, represented as a percentage. PrBest = probability that each treatment is the best i.e. associated with the lowest risk of PTB, represented as a percentage. MeanRank = mean rank of each ART. Abbreviations: 3TC = lamivudine, ABC = abacavir, ART = antiretroviral treatment, AZT = zidovudine, EFV = efavirenz, FTC = emtricitabine, LPV/r = lopinavir/ritonavir, MTCT = mother to child transmission, RR = relative risk, TDF = tenofovir disoproxil fumarate.

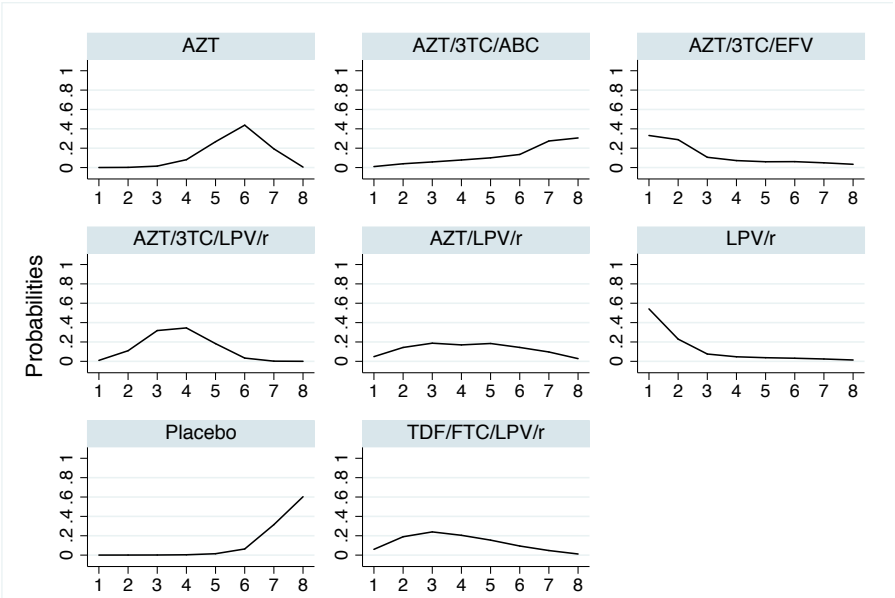

Figure 4.122: Rankograms for ARTs included in the network meta-analysis for the association between ART and MTCT. This figure represents the surface under the cumulative ranking probabilities for each ART included in the network meta-analysis. The horizontal axis represents the 8 possible ranks that the ART can achieve and the vertical axis illustrates the probability of the ART to attain each rank[312]. Abbreviations: 3TC = lamivudine, ABC = abacavir, ART = antiretroviral treatment, AZT = zidovudine, EFV = efavirenz, FTC = emtricitabine, LPV/r = lopinavir/ritonavir, MTCT = mother to child transmission, RR = relative risk, TDF = tenofovir disoproxil fumarate.

Treatment	SUCRA	PrBest	MeanRank
Placebo	5.1	0.6	3.8
Monotherapy	46.2	4.3	2.6
Dual-therapy	72.0	49.4	1.8
HAART	76.6	45.7	1.7

Figure 4.123: SUCRA ranking of antenatal antiretroviral regimens for MTCT outcome. This figure provides a hierarchy of the 4 regimens assessed in the network meta-analysis for the association between antiretroviral regimen and MTCT. The larger the SUCRA percentage, the lower the risk of MTCT. SUCRA = surface under the cumulative rank curve, represented as a percentage. PrBest = probability that each regimen is the best i.e. associated with the lowest risk of MTCT, represented as a percentage. MeanRank = mean rank of each ART. Abbreviation: HAART = Highly Active Antiretroviral Treatment, MTCT = mother to child transmission.

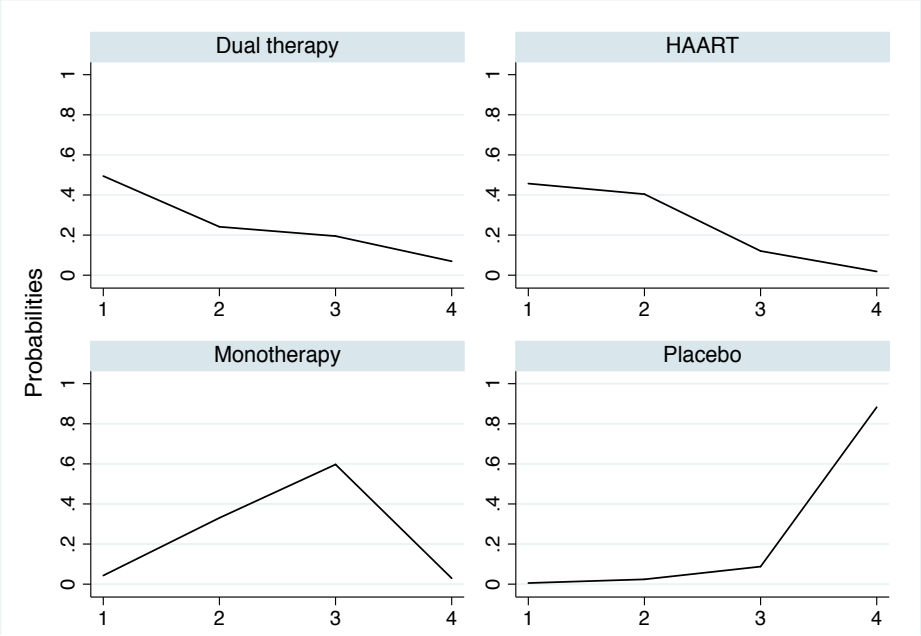

Figure 4.124: Rankograms for ARTs included in the network meta-analysis for the association between antiretroviral regimens and MTCT. This figure represents the surface under the cumulative ranking probabilities for each ART included in the network meta-analysis. The horizontal axis represents the 4 possible ranks that the ART can achieve and the vertical axis illustrates the probability of the ART to attain each rank[312]. Abbreviation: HAART = Highly Active Antiretroviral Treatment, MTCT = mother to child transmission.

Treatm~t	SUCRA	PrBest	MeanRank
PI	75.3	26.2	1.7
NRTI	37.5	0.0	2.9
NNRTI	86.0	73.8	1.4
Placebo	1.2	0.0	4.0

Figure 4.125: SUCRA ranking of antenatal antiretroviral class for MTCT outcome.
This figure provides a hierarchy of the 4 classes of antiretrovirals (including placebo) assessed in the network meta-analysis for the association between antiretroviral class and MTCT. The larger the SUCRA percentage, the lower the risk of MTCT. SUCRA = surface under the cumulative rank curve, represented as a percentage. PrBest = probability that each treatment is the best i.e. associated with the lowest risk of MTCT, represented as a percentage. MeanRank = mean rank of each ART. Abbreviations: MTCT = mother to child transmission, NNRTI = non-nucleoside reverse transcriptase inhibitor, NRTI = nucleoside reverse transcriptase inhibitor, PI = protease inhibitor.

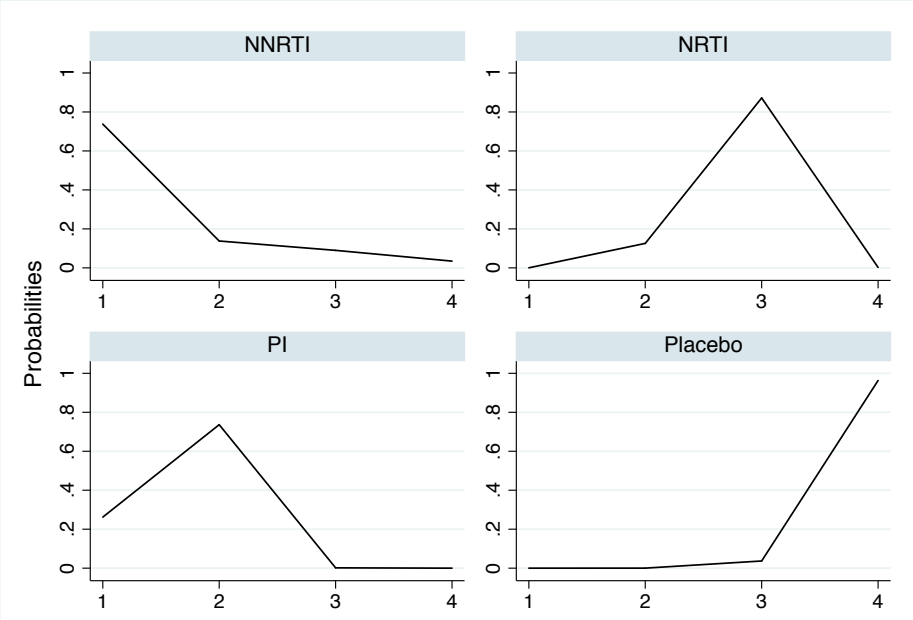

Figure 4.126: Rankograms for classes of antiretrovirals included in the network meta-analysis for the association between antiretroviral class and MTCT. This figure represents the surface under the cumulative ranking probabilities for each antiretroviral class included in the network meta-analysis. The horizontal axis represents the 4 possible ranks that the class can achieve and the vertical axis illustrates the probability of the ART to attain each rank[312]. Abbreviations: MTCT = mother to child transmission, NNRTI = non-nucleoside reverse transcriptase inhibitor, NRTI = nucleoside reverse transcriptase inhibitor, PI = protease inhibitor.

4.4.7.2 Summary points of PMTCT results

Figure 4.127 reports the significant results observed in the pairwise and network meta-analyses of the association between ART and MTCT. The initiation of ART during the antenatal period significantly reduced the risk of MTCT.

*4.4.7.2.1 Significant associations observed in pairwise and network meta-analysis for the association between **ART** and MTCT*

- Pairwise comparisons showed a significant reduction in the risk of MTCT in women initiating AZT monotherapy compared to those who initiated placebo
- Network comparisons showed a significant reduction in the risk of MTCT in women initiating AZT monotherapy, LPV/r monotherapy, AZT/3TC/LPV/r HAART and TDF/FTC/LPV/r HAART, compared to those initiating placebo.
- LPV/r monotherapy, AZT/3TC/EFV HAART, AZT/3TC/LPV/r HAART and TDF/FTC/LPV/r were the most efficacious ARTs.
- Placebo, AZT/3TC/ABC HAART, and AZT monotherapy were the least efficacious ARTs.

*4.4.7.2.2 Significant associations observed in pairwise and network meta-analysis for the association between **antiretroviral regimens** and MTCT*

- Pairwise comparisons showed a significant reduction in MTCT in women initiating monotherapy versus those initiating placebo.
- The network meta-analysis did not show any significant associations.
- HAART was the most efficacious regimen.
- Placebo, followed by monotherapy was the least efficacious regimen.

*4.4.7.2.3 Significant associations observed in pairwise and network meta-analysis for the association between **antiretroviral class** and MTCT*

- Pairwise comparisons showed a significant reduction in MTCT in women who initiated PI-based ART compared to those who initiated NRTI-based ART. Furthermore, women initiating placebo had a significantly higher risk of MTCT than those initiating NRTI.
- Network comparisons showed a significant reduction in the risk of MTCT in women initiating PI-based ART versus those initiating placebo or NRTI-based ART. Furthermore, women initiating NRTI-based ART were at a significantly lower risk of MTCT than those initiating placebo.

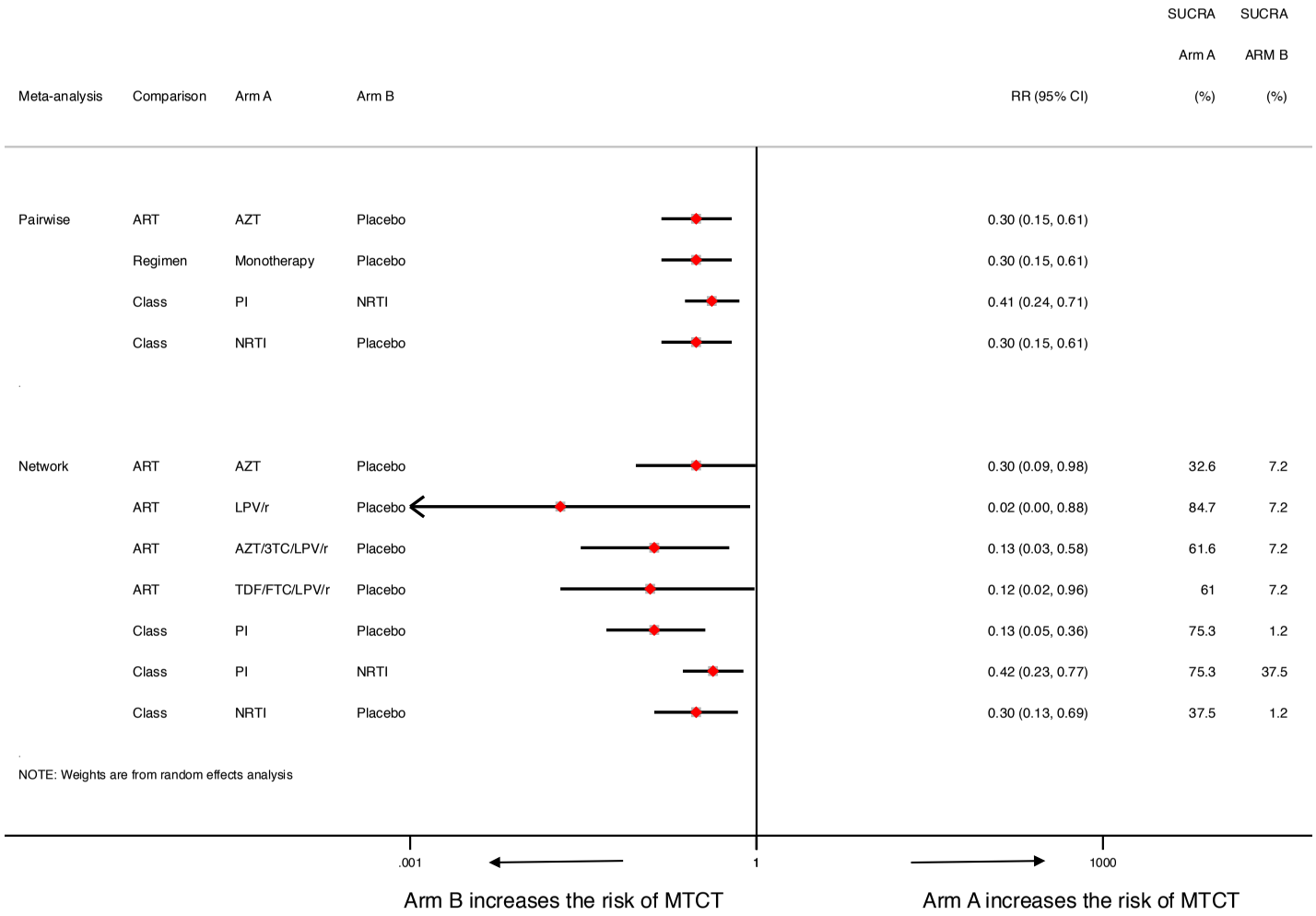

Figure 4.127: Random-effect pairwise and network meta-analysis results of significant associations between MTCT and antenatal ART, antiretroviral regimens and classes of antiretrovirals This figure presents statistically significant results from random-effects pairwise and network meta-analyses of the association between MTCT and antenatal ART, antiretroviral regimens and classes of antiretrovirals. Statistically significant effects are presented in red. Abbreviations: 3TC = lamivudine, ABC = abacavir, ART = antiretroviral treatment, AZT = zidovudine, FTC = emtricitabine, HAART = Highly Active Antiretroviral Therapy, LPV/r = lopinavir/ritonavir, MTCT = mother to child transmission, NRTI = nucleoside reverse transcriptase inhibitor, RR = relative risk, SUCRA = surface under the cumulative rank curve, TDF = tenofovir disoproxil fumarate.

4.4.8 Comparative analysis of ART safety versus efficacy for PMTCT

This analysis used cluster ranking plots to assess the relative safety of each ART (i.e. risk of adverse perinatal outcome) against their efficacy for PMTCT (i.e. risk of MTCT).

4.4.8.1 Comparative risk of PTB versus MTCT

- Antenatal ART

None of the ARTs were both safe and effective (upper right corner) (Figure 4.128). The most effective ARTs – brown cluster including LPV/r monotherapy, AZT/3TC/EFV HAART, TDF/FTC/EFV HAART and AZT/3TC/LPV/r HAART– were associated with the highest risk of PTB (Figure 4.128), while ARTs associated with the lowest risk of PTB – blue cluster AZT/3TC/ABC HAART; placebo, AZT monotherapy – were also the least efficacious.

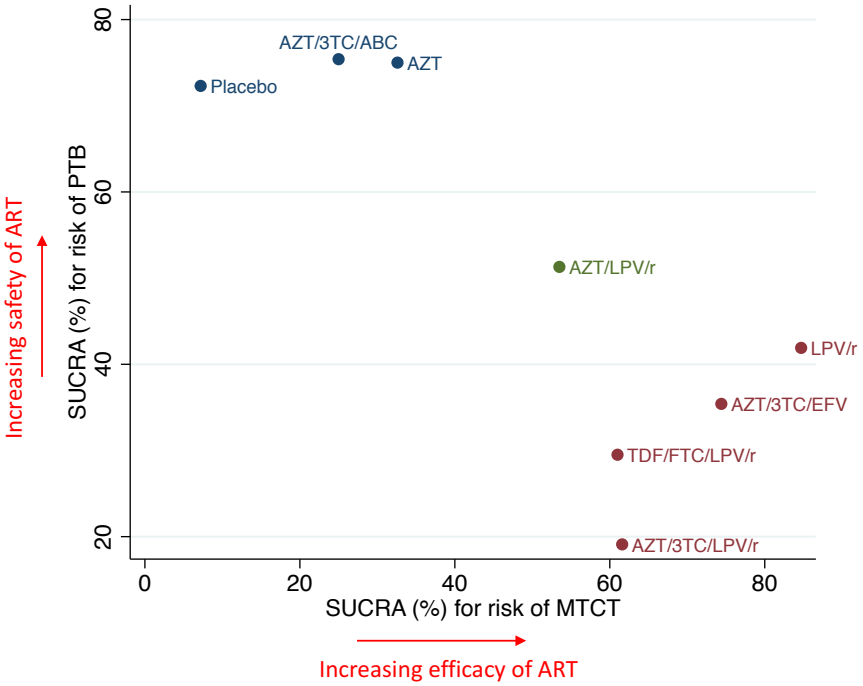

Figure 4.128: Cluster analysis of the comparative risk of PTB and MTCT of ARTs. Clusters are represented by different colours (e.g. blue cluster = placebo, AZT, AZT/3TC/ABC). ART situated in the upper right corner represent the safest (i.e. lowest risk of PTB) and most effective ART for PMTCT (i.e. lowest risk of MTCT). Brown cluster represents the most effective ART. Blue cluster represent the safest ART. Abbreviations: 3TC = lamivudine, ABC = abacavir, ART = antiretroviral treatment, AZT = zidovudine, EFV = efavirenz, FTC = emtricitabine, LPV/r = lopinavir/ritonavir, MTCT = mother to child transmission, PTB = preterm birth, SUCRA = surface under the cumulative rank curve, represented as a percentage, TDF = tenofovir disoproxil fumarate.

- Antenatal antiretroviral regimen

None of the antiretroviral regimens were both effective and safe (upper right corner) (Figure 4.129). HAART, which was the most effective regimen, was associated with the highest risk of PTB. Placebo and monotherapy – the least efficacious regimens – were associated with the lowest risk of PTB (Figure 4.129). Monotherapy was associated with the lowest risk of PTB and with reasonable efficacy (Figure 4.129).

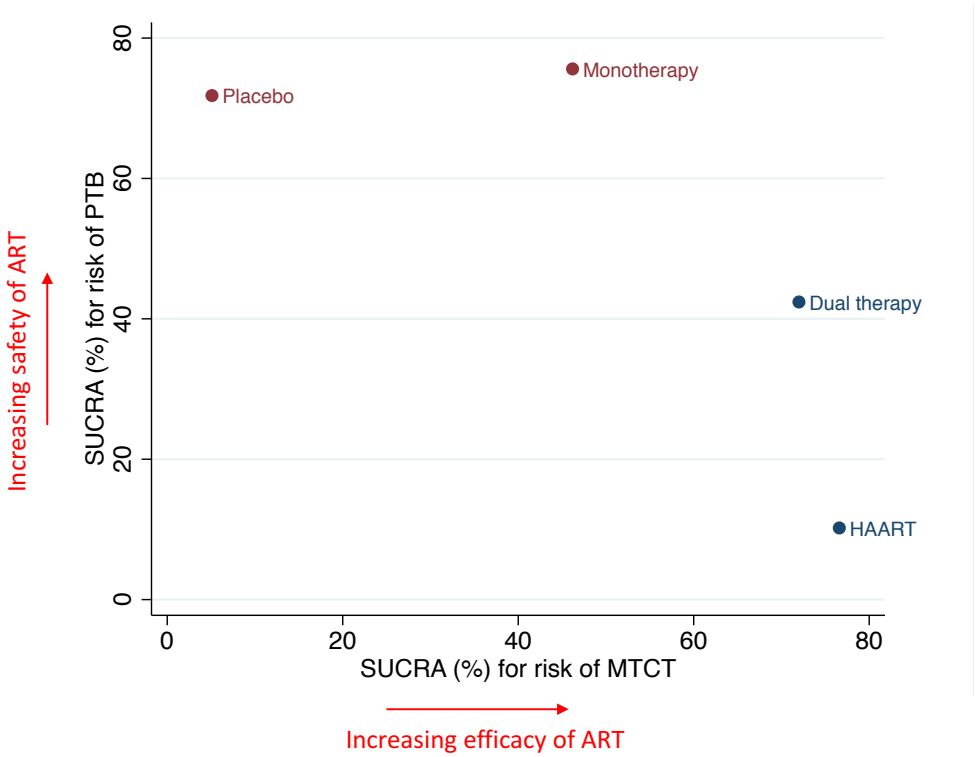

Figure 4.129: Cluster analysis of the comparative risk of PTB and MTCT of antiretroviral regimen. Clusters are represented by different colours (e.g. blue cluster = dual therapy, HAART). Antiretroviral regimens situated in the upper right corner represent the safest (i.e. lowest risk of PTB) and most effective regimen for PMTCT (i.e. lowest risk of MTCT). Blue cluster represents the most effective antiretroviral regimen. Brown cluster represent the safest antiretroviral regimen. Abbreviations: ART = antiretroviral treatment, HAART = Highly Active Antiretroviral Treatment, MTCT = mother to child transmission, PTB = preterm birth, SUCRA = surface under the cumulative rank curve, represented as a percentage.

- Antenatal Antiretroviral class

None of the classes of antiretrovirals were both safe and efficient (upper right corner) (Figure 4.130). The most effective classes of antiretrovirals – blue cluster including PI and NNRTI-based ART – were associated with the highest risk of PTB. Furthermore, placebo and NRTI-based ART, which were associated with the lowest risk of PTB, were the least efficacious (Figure 4.130).

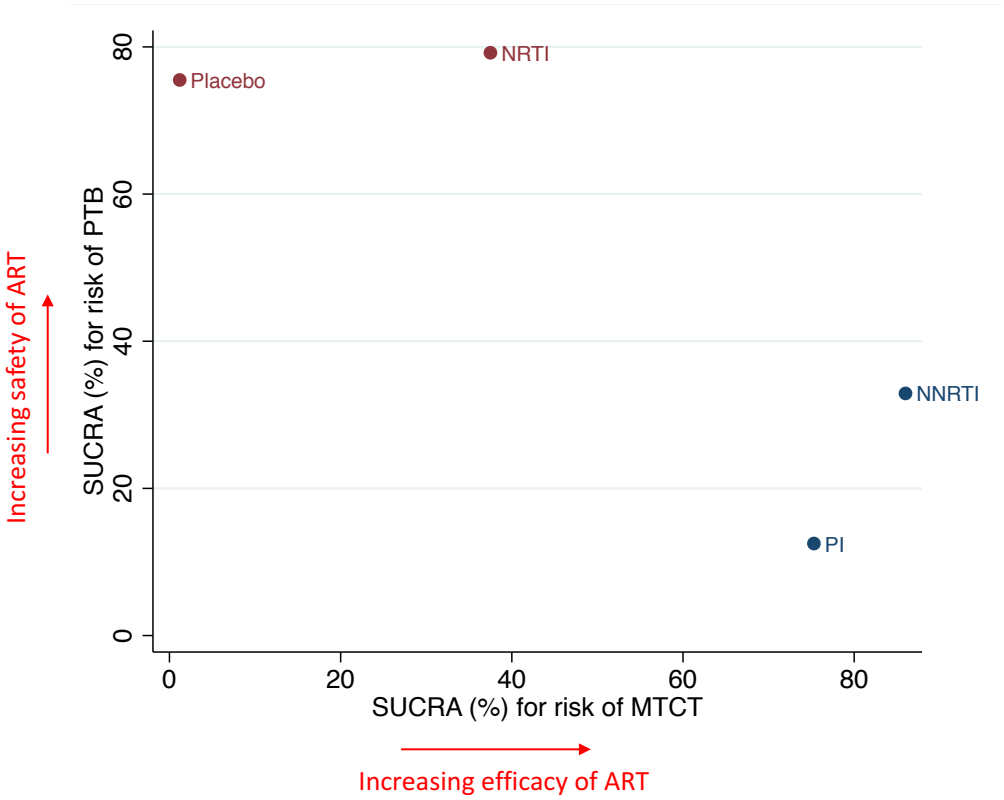

Figure 4.130: Cluster analysis of the comparative risk of PTB and MTCT of antiretroviral classes. Clusters are represented by different colours (e.g. blue cluster = NNRTI, PI). Classes of antiretrovirals situated in the upper right corner represent the safest (i.e. lowest risk of PTB) and most effective antiretroviral class for PMTCT (i.e. lowest risk of MTCT). Blue cluster represents the most effective class of antiretrovirals. Brown cluster represents the safest antiretroviral class. Abbreviations: ART = antiretroviral treatment, MTCT = mother to child transmission, NNRTI = non-nucleoside reverse transcriptase inhibitor, NRTI = nucleoside reverse transcriptase inhibitor, PI = protease inhibitor, PTB = preterm birth, SUCRA = surface under the cumulative rank curve, represented as a percentage.

4.4.8.2 Comparative risk of sPTB versus MTCT

- Antenatal ART

None of the ARTs were both safe and effective (upper right corner) (Figure 4.131). The most effective ARTs – blue cluster including LPV/r monotherapy, AZT/3TC/EFV HAART, and AZT/3TC/LPV/r HAART– were associated with the highest risk of sPTB (Figure 4.131) while AZT/3TC/ABC HAART, which was associated with the lowest risk of sPTB, was also the least efficacious (Figure 4.131).

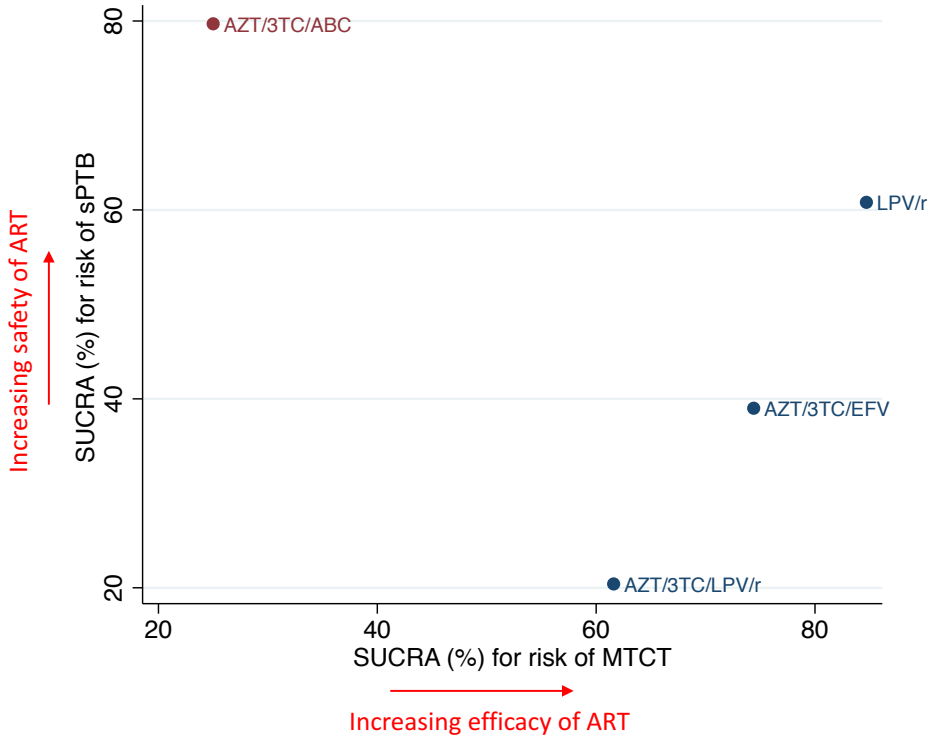

Figure 4.131: Cluster analysis of the comparative risk of sPTB and MTCT of ARTs. Clusters are represented by different colours (e.g. blue cluster = LPV/r, AZT/3TC/EFV, AZT/3TC/LPV/r). ART situated in the upper right corner represent the safest (i.e. lowest risk of sPTB) and most effective ART for PMTCT (i.e. lowest risk of MTCT). Blue cluster represents the most effective ARTs. Brown cluster represents the safest ART. Abbreviations: 3TC = lamivudine, ABC = abacavir, ART = antiretroviral treatment, AZT = zidovudine, EFV = efavirenz, FTC = emtricitabine, LPV/r = lopinavir/ritonavir, MTCT = mother to child transmission, sPTB = spontaneous preterm birth, SUCRA = surface under the cumulative rank curve, represented as a percentage, TDF = tenofovir disoproxil fumarate.

4.4.8.3 Comparative risk of VPTB versus MTCT

- Antenatal ART

None of the ARTs were both safe and effective (upper right corner) (Figure 4.132). The most effective ARTs – blue cluster including LPV/r monotherapy, AZT/3TC/EFV HAART and AZT/3TC/LPV/r HAART– were associated with the highest risk of VPTB (Figure 4.132) while ARTs associated with the lowest risk of VPTB – brown cluster AZT/3TC/ABC and AZT monotherapy – were also the least efficacious (Figure 4.132).

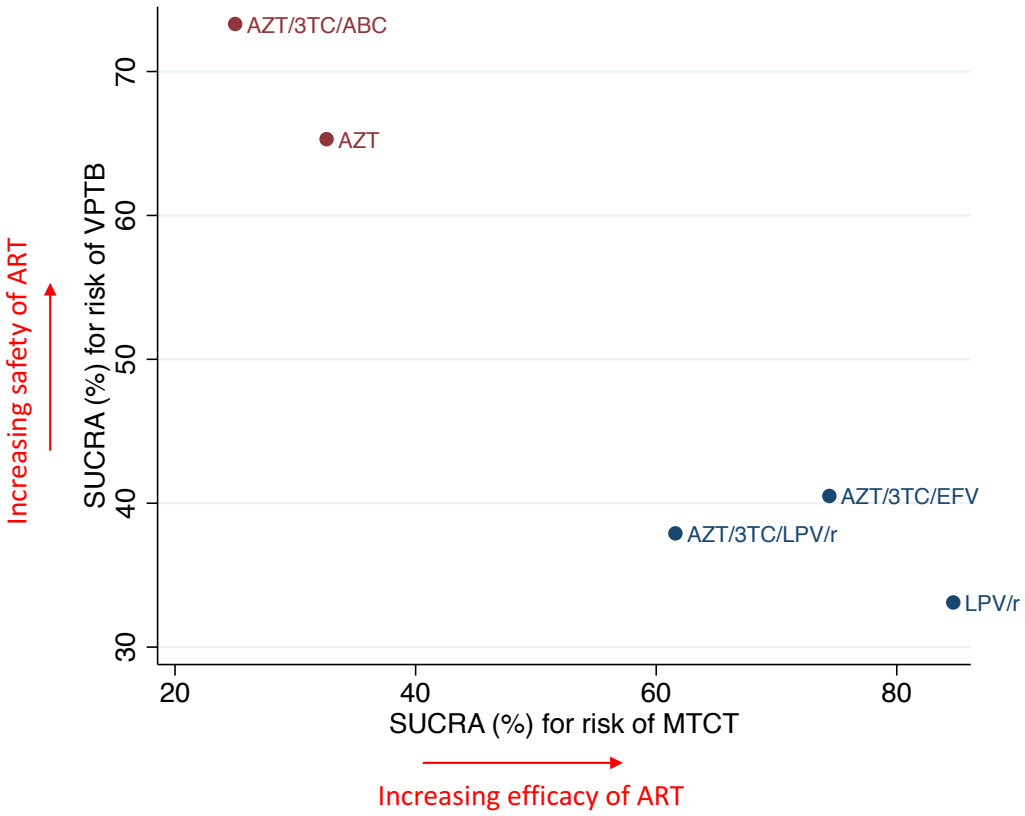

Figure 4.132: Cluster analysis of the comparative risk of VPTB and MTCT of ARTs. Clusters are represented by different colours (e.g. blue cluster = placebo, AZT, AZT/3TC/ABC). ART situated in the upper right corner represent the safest (i.e. lowest risk of VPTB) and most effective ART for PMTCT (i.e. lowest risk of MTCT). Brown cluster represents the most effective ART. Blue cluster represent the safest ART. Abbreviations: 3TC = lamivudine, ABC = abacavir, ART = antiretroviral treatment, AZT = zidovudine, EFV = efavirenz, FTC = emtricitabine, LPV/r = lopinavir/ritonavir, MTCT = mother to child transmission, SUCRA = surface under the cumulative rank curve, represented as a percentage, TDF = tenofovir disoproxil fumarate, VPTB = very preterm birth

- Antenatal Antiretroviral class

Of the 3 classes of ART assessed, none were both safe and efficient (upper right corner) (Figure 4.133). The most effective classes of antiretrovirals – blue cluster including PI and NNRTI-based ART – were associated with the highest risk of VPTB. NRTI-based ART, which was associated with the lowest risk of VPTB, was the least effective (Figure 4.133).

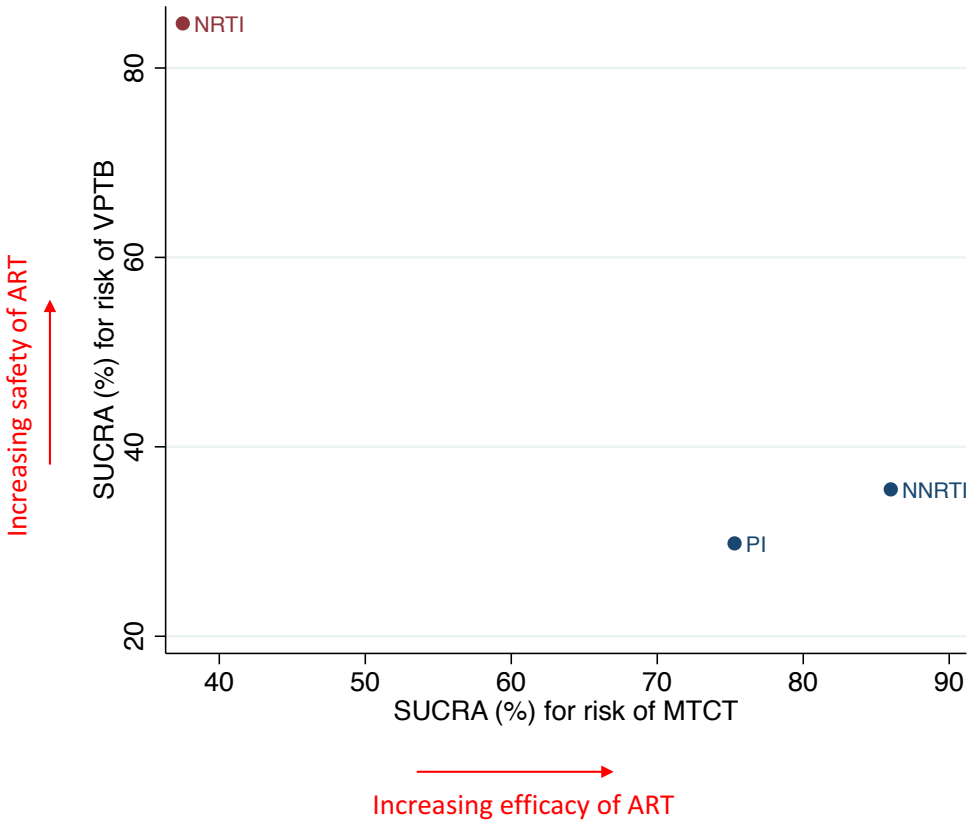

Figure 4.133: Cluster analysis of the comparative risk of VPTB and MTCT of antiretroviral classes. Clusters are represented by different colours (e.g. blue cluster = NNRTI, PI). Classes of antiretrovirals situated in the upper right corner represent the safest (i.e. lowest risk of VPTB) and most effective antiretroviral class for PMTCT (i.e. lowest risk of MTCT). Blue cluster represents the most effective class of antiretrovirals. Brown cluster represents the safest antiretroviral class. Abbreviations: ART = antiretroviral treatment, MTCT = mother to child transmission, NNRTI = non-nucleoside reverse transcriptase inhibitor, NRTI = nucleoside reverse transcriptase inhibitor, PI = protease inhibitor, SUCRA = surface under the cumulative rank curve, represented as a percentage, VPTB = very preterm birth.

4.4.8.4 Comparative risk of LBW versus MTCT

- Antenatal ART

None of the ARTs were both safe and effective (upper right corner) (Figure 4.134). The most effective ARTs – blue cluster including AZT/3TC/EFV HAART, TDF/FTC/EFV HAART and AZT/3TC/LPV/r HAART– were associated with the highest risk of LBW (Figure 4.134). ARTs associated with the low risk of LBW – brown cluster including AZT monotherapy and AZT/LPV/r dual therapy – had a low to moderate efficacy. Placebo and AZT/3TC/ABC were associated with the lowest efficacy, and moderate risk of LBW.

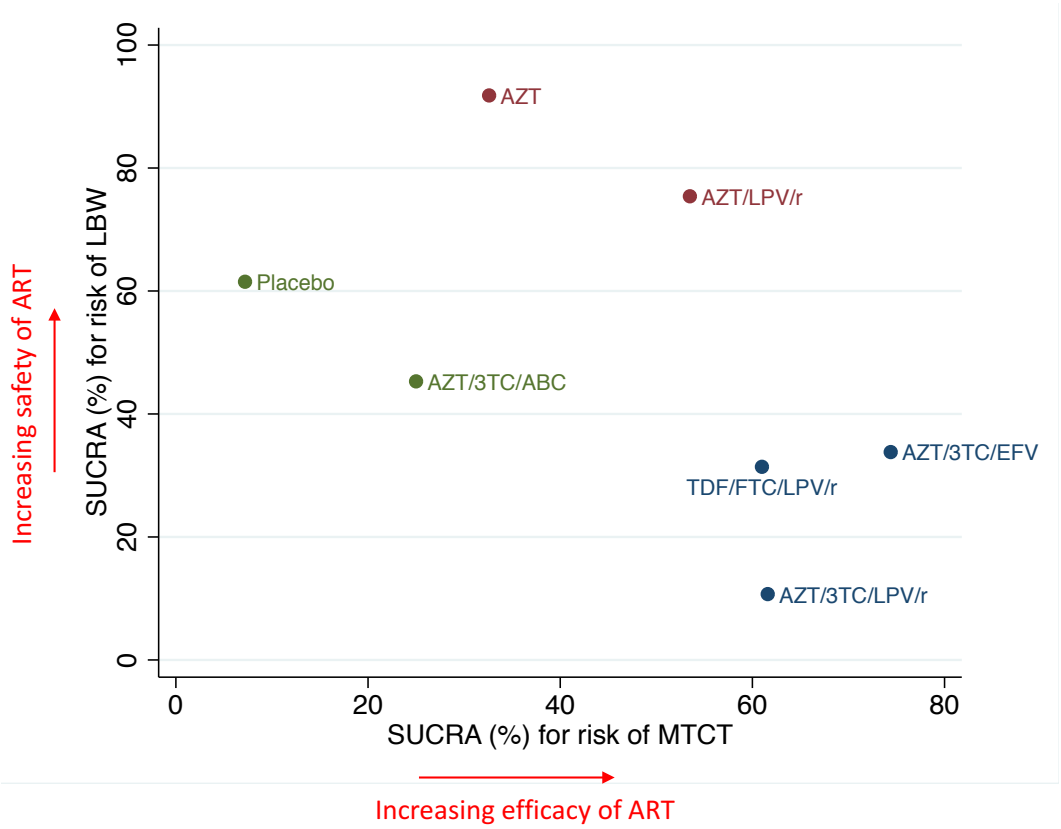

Figure 4.134: Cluster analysis of the comparative risk of LBW and MTCT of ARTs. Clusters are represented by different colours (e.g. blue cluster = AZT/3TC/EFV, AZT/3TC/LPV/r, TDF/FTC/LPV/r). ART situated in the upper right corner represent the safest (i.e. lowest risk of LBW) and most effective ART for PMTCT (i.e. lowest risk of MTCT). Blue cluster represents the most effective ART. Blue cluster represent the safest ART. Abbreviations: 3TC = lamivudine, ABC = abacavir, ART = antiretroviral treatment, AZT = zidovudine, EFV = efavirenz, FTC = emtricitabine, LPV/r = lopinavir/ritonavir, MTCT = mother to child transmission, PTB = preterm birth, SUCRA = surface under the cumulative rank curve, represented as a percentage, TDF = tenofovir disoproxil fumarate.

- Antenatal antiretroviral regimen

Monotherapy and dual therapy were found to have a relatively low risk of LBW and moderate efficacy (Figure 4.135). HAART, which was the most effective regimen, was associated with the highest risk of LBW. Placebo was the least efficacious regimen and was associated with a moderate risk of LBW (Figure 4.135).

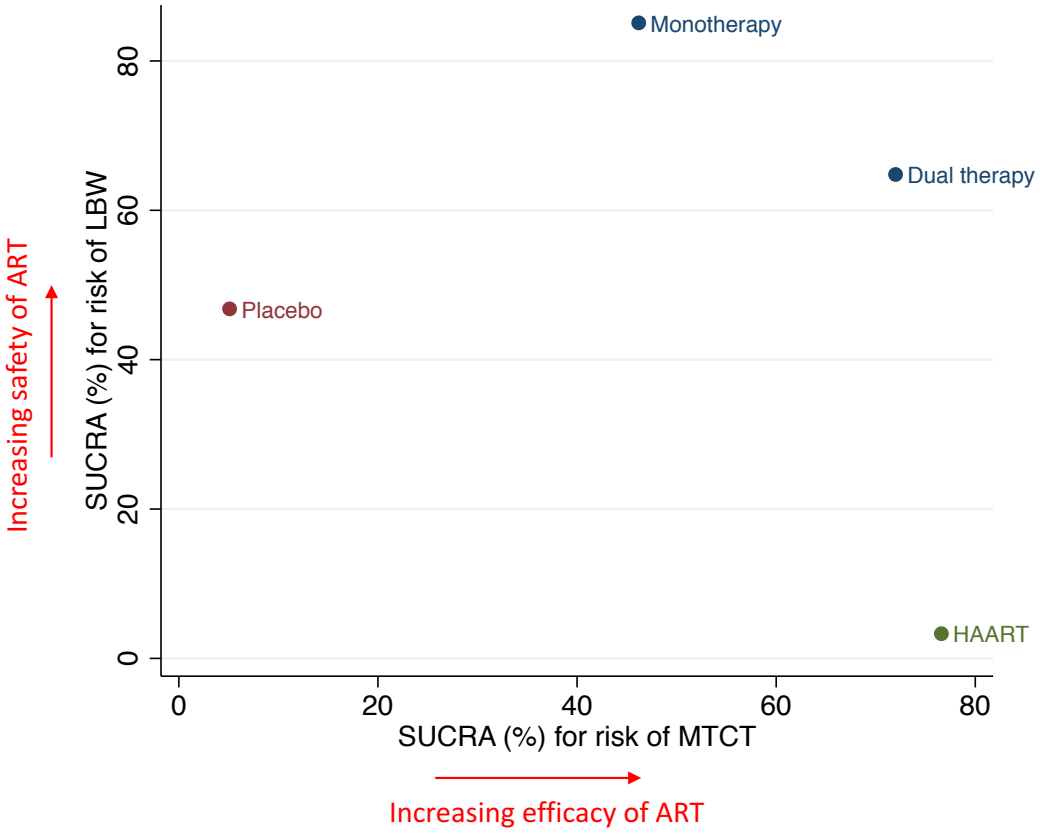

Figure 4.135: Cluster analysis of the comparative risk of LBW and MTCT of antiretroviral regimen. Clusters are represented by different colours (e.g. blue cluster = monotherapy, dual therapy). Antiretroviral regimens situated in the upper right corner represent the safest (i.e. lowest risk of LBW) and most effective regimen for PMTCT (i.e. lowest risk of MTCT). Green cluster represents the most effective antiretroviral regimen. Blue cluster represents regimens that are relatively safe and effective. Abbreviations: ART = antiretroviral treatment, HAART = Highly Active Antiretroviral Treatment, LBW = low birthweight, MTCT = mother to child transmission, SUCRA = surface under the cumulative rank curve, represented as a percentage.

- Antenatal antiretroviral class

Of the 3 classes of ART assessed, none were both safe and efficient (upper right corner) (Figure 4.136). The most effective classes of antiretrovirals – blue cluster including PI and NNRTI-based ART – were associated with the highest risk of LBW. NRTI-based ART and placebo, which was associated with the lowest risk of LBW, were the least effective (Figure 4.136).

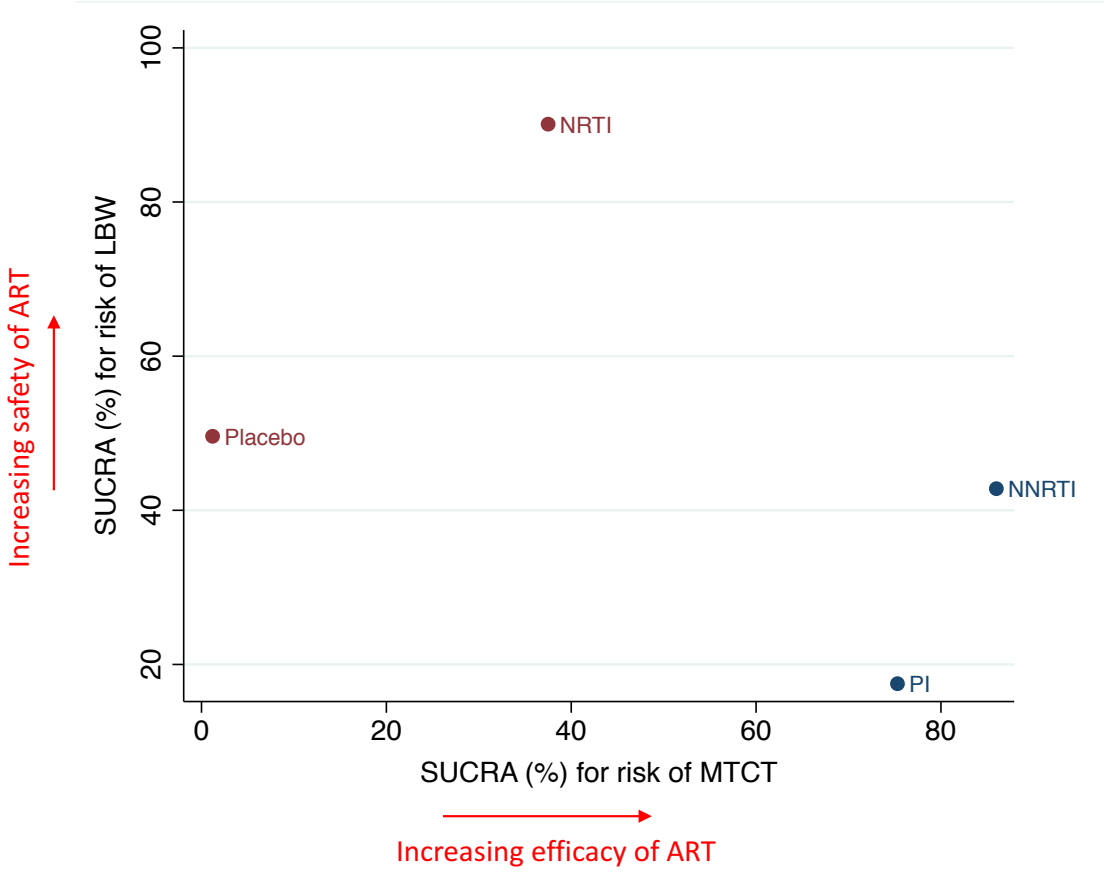

Figure 4.136: Cluster analysis of the comparative risk of LBW and MTCT of antiretroviral classes. Clusters are represented by different colours (e.g. blue cluster = NNRTI). Classes of antiretrovirals situated in the upper right corner represent the safest (i.e. lowest risk of LBW) and most effective antiretroviral class for PMTCT (i.e. lowest risk of MTCT). Blue cluster represents the most effective class of antiretrovirals. Brown cluster represents the safest antiretroviral class. Abbreviations: ART = antiretroviral treatment, LBW = Low birthweight, MTCT = mother to child transmission, NNRTI = non-nucleoside reverse transcriptase inhibitor, NRTI = nucleoside reverse transcriptase inhibitor, PI = protease inhibitor, SUCRA = surface under the cumulative rank curve, represented as a percentage.

4.4.8.5 Comparative risk of VLBW versus MTCT

- Antenatal ART

None of the ARTs were both safe and effective (upper right corner) (Figure 4.137). TDF/FTC/EFV HAART was the most effective ART, but was also associated with the highest risk of VLBW (Figure 4.137). AZT/3TC/LPV/r HAART, which was one of the most effective ARTs, showed a moderate risk of VLBW (Figure 4.137). ARTs associated with the lowest risk of VLBW – brown cluster including AZT monotherapy and placebo – had a low efficacy for PMTCT.

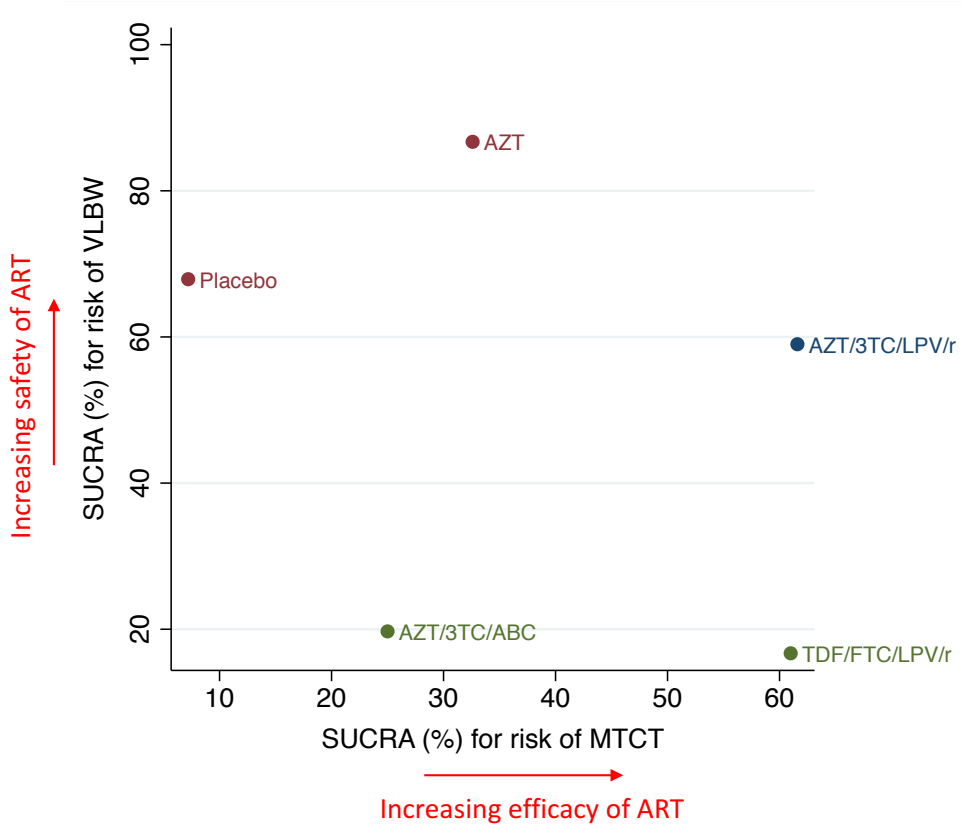

Figure 4.137: Cluster analysis of the comparative risk of VLBW and MTCT of ARTs. Clusters are represented by different colours (e.g. brown cluster = AZT and placebo). ART situated in the upper right corner represent the safest (i.e. lowest risk of VLBW) and most effective ART for PMTCT (i.e. lowest risk of MTCT). Blue cluster represents the most effective ART. Blue cluster represents the safest ART. Abbreviations: 3TC = lamivudine, ABC = abacavir, ART = antiretroviral treatment, AZT = zidovudine, FTC = emtricitabine, LPV/r = lopinavir/ritonavir, MTCT = mother to child transmission, SUCRA = surface under the cumulative rank curve, represented as a percentage, TDF = tenofovir disoproxil fumarate, VLBW = very low birthweight.

- Antenatal antiretroviral regimen

None of the antiretroviral regimens were both effective and safe (upper right corner) (Figure 4.138). HAART, which was the most effective regimen, was associated with the highest risk of VLBW. Placebo and monotherapy – the least efficacious regimens – were associated with the lowest risk of VLBW (Figure 4.138). Monotherapy was associated with the lowest risk of VLBW and with reasonable efficacy (Figure 4.138).

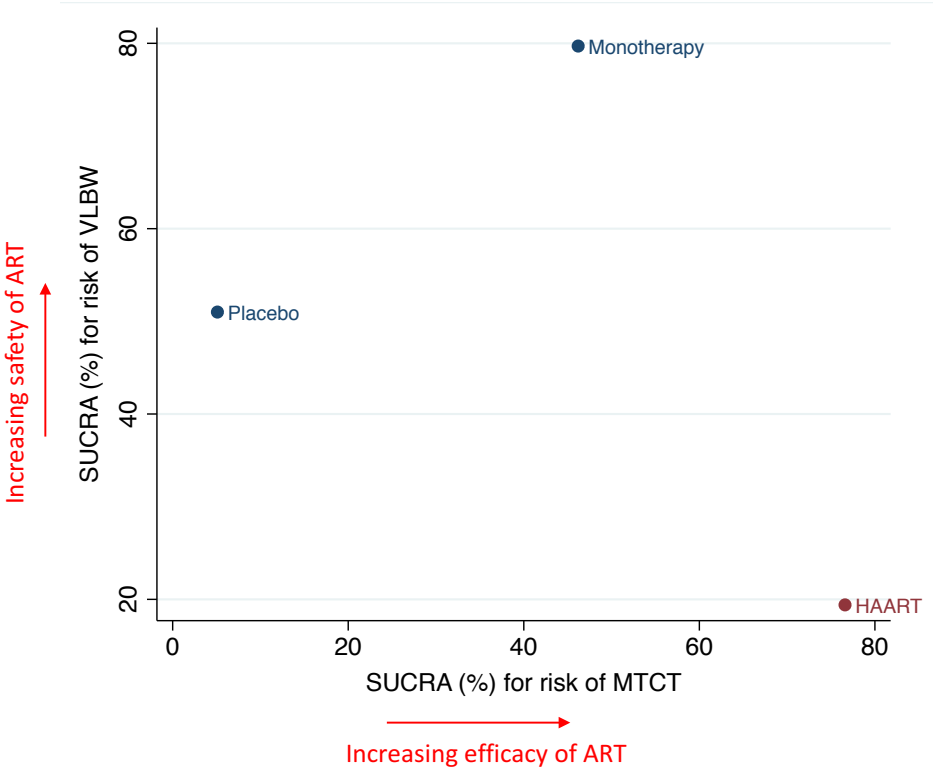

Figure 4.138: Cluster analysis of the comparative risk of VLBW and MTCT of antiretroviral regimen. Clusters are represented by different colours (e.g. blue cluster = monotherapy, dual therapy). Antiretroviral regimens situated in the upper right corner represent the safest (i.e. lowest risk of VLBW) and most effective regimen for PMTCT (i.e. lowest risk of MTCT). Brown cluster represents the most effective antiretroviral regimen. Blue cluster represents regimens that are associated with the lowest risk of VLBW. Abbreviations: ART = antiretroviral treatment, HAART = Highly Active Antiretroviral Treatment, MTCT = mother to child transmission, SUCRA = surface under the cumulative rank curve, represented as a percentage, VLBW = very low birthweight.

- Antenatal antiretroviral class

Of the 3 classes of ART assessed, none were both safe and efficient (upper right corner) (Figure 4.139). PI-based ART was the most effective class of antiretroviral but it was also associated with highest risk of VLBW (Figure 4.139). NRTI-based ART, which was associated with the lowest risk of VLBW, showed a moderate efficacy for PMTCT (Figure 4.139). Placebo was associated with a high risk of VLBW and was the least efficacious (Figure 4.139).

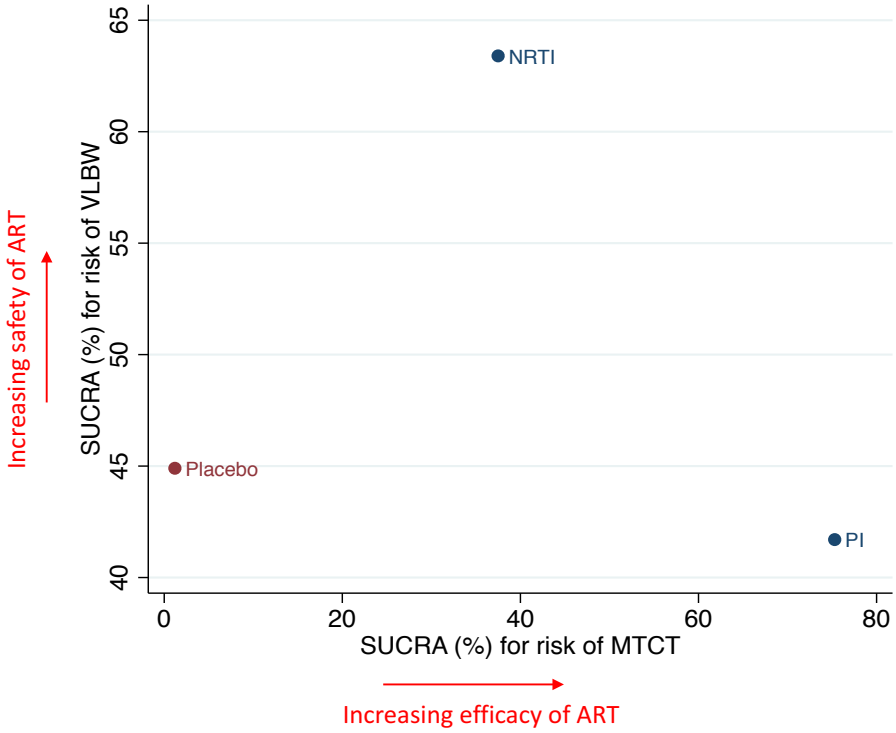

Figure 4.139: Cluster analysis of the comparative risk of VLBW and MTCT of antiretroviral classes. Clusters are represented by different colours (e.g. blue cluster = NNRTI, PI). Classes of antiretrovirals situated in the upper right corner represent the safest (i.e. lowest risk of VLBW) and most effective antiretroviral class for PMTCT (i.e. lowest risk of MTCT). Blue cluster represents the most effective class of antiretrovirals. Brown cluster represents the least efficacious antiretroviral class. Abbreviations: ART = antiretroviral treatment, MTCT = mother to child transmission, NRTI = nucleoside reverse transcriptase inhibitor, PI = protease inhibitor, SUCRA = surface under the cumulative rank curve, represented as a percentage, VLBW = very low birthweight.

4.4.8.6 Comparative risk of SGA versus MTCT

- Antenatal ART

None of the ARTs were both safe and effective (upper right corner) (Figure 4.140). AZT/LPV/r dual therapy was the most effective ART but was also associated with the highest risk of SGA (Figure 4.140). ARTs associated with the lowest risk of SGA – blue cluster including AZT monotherapy and placebo – had a low efficacy for PMTCT.

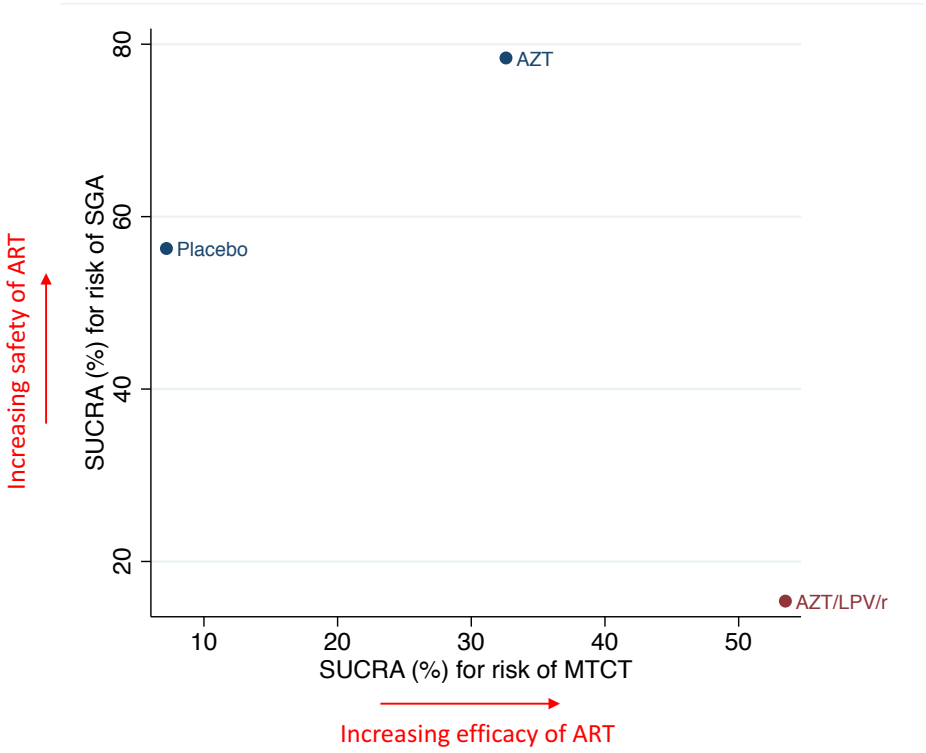

Figure 4.140: Cluster analysis of the comparative risk of SGA and MTCT of ARTs. Clusters are represented by different colours (e.g. brown cluster = AZT/LPV/r). ART situated in the upper right corner represent the safest (i.e. lowest risk of SGA) and most effective ART for PMTCT (i.e. lowest risk of MTCT). Blue cluster represents the safest ART. Brown cluster represents the most effective ART. Abbreviations: ART = antiretroviral treatment, AZT = zidovudine, LPV/r = lopinavir/ritonavir, MTCT = mother to child transmission, SGA = small for gestational age. SUCRA = surface under the cumulative rank curve, represented as a percentage, VLBW = very low birthweight.

- Antenatal antiretroviral class

Of the 3 classes of ART assessed, none were both safe and effective (upper right corner) (Figure 4.140). PI-based ART was the most effective class of antiretroviral but it was also associated with highest risk of SGA (Figure 4.140). NRTI-based ART, which was associated with the lowest risk of SGA, but showed a moderate efficacy for PMTCT (Figure 4.140). Placebo was the least efficacious but was associated with a moderate risk of SGA (Figure 4.140).

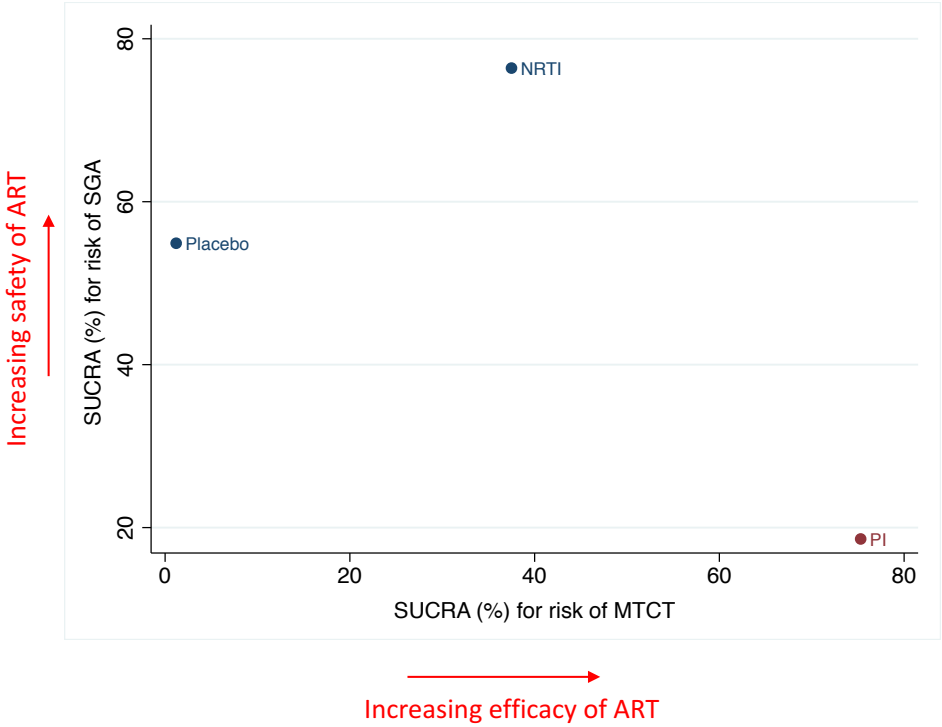

Figure 4.140: Cluster analysis of the comparative risk of SGA and MTCT of antiretroviral classes. Clusters are represented by different colours (e.g. brown cluster = PI). Classes of antiretrovirals situated in the upper right corner represent the safest (i.e. lowest risk of SGA) and most effective antiretroviral class for PMTCT (i.e. lowest risk of MTCT). Brown cluster represents the most effective class of antiretrovirals. Blue cluster represents the safest class of antiretrovirals. Abbreviations: ART = antiretroviral treatment, MTCT = mother to child transmission, NRTI = nucleoside reverse transcriptase inhibitor, PI = protease inhibitor, SUCRA = surface under the cumulative rank curve, represented as a percentage, VLBW = very low birthweight.

4.5 Discussion

The aims of this study were to a) determine whether antenatal ART increased the risk of 11 adverse perinatal outcomes in HIV-positive women and b) provide an evidence-based hierarchy for the safety (i.e. risk of adverse perinatal outcomes) and efficacy – for PMTCT – of antenatal ART.

4.5.1 Summary of findings

The evidence presented shows that ART is associated with adverse perinatal outcomes and significantly increases the risk of PTB, sPTB, LBW and VLBW. There was no evidence to suggest an association between ART and VPTB, SGA or NND. The literature search did not yield any RCT data on the association between ART and sVPTB, preterm-LBW, term-LBW, miscarriage or stillbirth; therefore, no conclusions could be made on these outcomes.

The association between ART and perinatal outcomes varied depending on the drugs, regimen and class of treatment used. AZT/3TC/LPV/r HAART significantly increased the risk of PTB, sPTB and LBW. TDF/3TC/LPV/r HAART significantly increased the risk of PTB, LBW and VLBW, and the initiation of AZT/3TC/EFV HAART significantly increased the risk of giving birth to a LBW infant. The initiation of a HAART regimen was significantly associated with PTB and LBW. Furthermore, PI-based ARTs significantly increased the risk of PTB and LBW.

Overall, the evidence suggests that ART, antiretroviral regimens and classes of ART with the highest efficacy for PMTCT are associated with the highest risk of adverse perinatal outcomes. Of the 8 antenatal ARTs assessed, LPV/r monotherapy, AZT/3TC/EFV HAART, AZT/3TC/LPV/r HAART and TDF/FTC/LPV/r HAART were the most efficacious. However, barring LPV/r monotherapy, they were all significantly associated with adverse perinatal outcomes. AZT/3TC/LPV/r HAART was associated with the highest risk of PTB, sPTB and LBW, while TDF/FTC/LPV/r was associated with the highest risk of giving birth to a VLBW infant. Conversely, placebo, AZT monotherapy and AZT/3TC/ABC HAART were the least efficacious ARTs and ranked in the bottom 3 places.

However, AZT monotherapy was associated with the lowest risk of LBW, VLBW and SGA, and AZT/3TC/ABC HAART was associated with the lowest risk for PTB, sPTB and VPTB. Surprisingly, LPV/r monotherapy, showed the highest efficacy for PMTCT and was not significantly associated with any adverse perinatal outcome.

HAART was comparatively the most efficacious antiretroviral regimen but it was associated with the highest risk of PTB, LBW and VLBW. Placebo followed by monotherapy were the least efficacious regimens; however, monotherapy was associated with the lowest risk of PTB, LBW and VLBW.

NNRTI and PI-based ART were the most efficacious class of antiretroviral; however, PI-based ART was associated with the highest risk of PTB, LBW and VLBW. Conversely, placebo and NRTI-based ART were the least efficacious class of antiretroviral; however, NRTI-based ART was associated with the lowest risk of PTB, LBW and VLBW.

4.5.2 Comparison of results to previous systematic review

One previous Cochrane review of RCTs has assessed the association between ART and adverse perinatal outcomes[267]. The present study is the first to use a network meta-analysis to provide evidence-based hierarchies for the safety (i.e. risk of adverse perinatal outcomes) and efficacy of commonly used antenatal ART drugs, antiretroviral regimens and classes of antiretroviral.

Brocklehurst et al. (2002)[296], conducted the first Cochrane review of RCTs assessing the efficacy of interventions to reduce MTCT, as well as their neonatal and maternal morbidity and mortality. This review was updated in 2007[305], and again in 2010[203] finally including 25 trials conducted between 1991 to 2008. Of the 25 trials included, 20 provided data on adverse perinatal outcomes including PTB (as defined by author) (9 trials), LBW (<2500g) (12 trials) and stillbirth (not defined) (19 trials).

Of the 20 trials assessed, 14 only initiated ART late in the third trimester; therefore, participants in both arms received ART for less than 30 days. Furthermore, 3 trials initiated the same antenatal ART and the only difference between the treatment groups was ART received during the intrapartum period. Both these comparisons essentially assess the effect of intrapartum treatment on adverse perinatal outcome; therefore, they were not included in the present systematic review. The remaining three trials (Mma Bana[204], PACTG 076[205], Kesho Bora[315]) were included. The authors showed that PI-based HAART significantly increased the risk of LBW, which supports the findings of the present review[203].

To the best of our knowledge, 9 previous systematic reviews of observational studies [281,294,298,299,301,304,320-322] – two of which also included RCTs[304, 299] – have assessed the association between ART and adverse perinatal outcome, with the most recent being published in 2016. These reviews are limited by the inherent biases associated with observational data, particularly because of the high probability of indication bias and the low quality of a large proportion of studies addressing this question. Some of these reviews concluded that ART significantly increases the risk of PTB, VPTB and LBW, albeit in the presence of a high degree of heterogeneity among the studies[294,299,304,321,322]. Furthermore, one review found that PI-based ART significantly increased the rate of PTB (13%; 0.11 – 0.16; I^2 = 51.7%) and LBW (16%; 95%CI 13% - 20%; I^2 = 67.4%)[299]. Others found no association between PTB and ART, even in sub-group analyses assessing the effect of PI-based ART[281]. The remaining reviews were inconclusive[298,301,320].

One factor that could not be addressed in the present review – as all women initiated treatment after the first trimester – was the association between the timing of ART initiation and perinatal outcomes. A systematic review and meta-analysis of 13 prospective cohort studies showed a significant increase in the risk of PTB in women initiating HAART before conception or during the first trimester (OR 1.71; 95%CI 1.09 – 2.67; I^2= not reported) compared to those initiating ART from the second trimester[281]. This was confirmed by a more recent review of 11 observational studies, which showed a significant increase in PTB (RR1.20; 95%CI 1.01 – 1.44; I^2 = 76.8%), VPTB (1.53; 1.22-

1.92; I^2 = 22.9%) and LBW (1.30; 1.04-1.62; I^2 = 15.6%) in women initiating HAART before conception[321].

4.5.3 Strengths of the review

This systematic review has multiple strengths. Firstly, it systematically synthesises all RCTs addressing this question, thus providing the most superior evidence on this topic to date. In order to minimise any bias in the conduct of the review, a rigorous, protocol driven approach was used, as recommended by the latest Cochrane guidelines[223]. The scientific literature search was extensive and rigorous, and was not restricted by language, study design or geographical location. The outcomes of interest were clearly defined *a priori*, and the stringent eligibility criteria, including those relating to the minimum duration of ART exposure during pregnancy, minimised the risk of misclassification bias and ensured that the effect estimates observed reflect the effect of ART.

All women included in this review initiated treatment during the antenatal period for PMTCT thus reducing the risk of indication bias. The use of similar dosages and routes of administration for the same drug across trials reduced heterogeneity and inconsistency. More than 80% of participants included in this review were from sub-Saharan Africa where 92% of HIV-positive pregnant women reside, 80% of whom received ART for PMTCT in 2015[130]. Therefore, the results of this review are directly applicable to those countries with the highest burden of maternal HIV-infection and the highest coverage of ART. Following the 2016 WHO recommendations that all HIV-positive pregnant and breastfeeding women should be initiated on lifelong HAART[12], there will be a significant increase in the use of both NNRTI and PI based HAART in pregnancy, which makes the results of this review critical and timely.

The most up to date and robust statistical analyses were used to pool the outcome data and the random effect model accounted for heterogeneity. The network meta-analyses made it possible to overcome previous limitations of an absence of comparative data from head-to-head trials. Furthermore, the

combination of direct and indirect evidence provided more accurate estimations of the effect size[178]. For the first time, the evidence generated provides information on both the comparative safety of ART in pregnancy and its comparative efficacy for PMTCT, which will greatly assist policymakers and clinicians in decisions regarding the use of ART in pregnancy. Lastly, the results of this review have been reported according to the PRISMA guidelines for network meta-analysis, thus eliminating reporting bias[323].

4.5.4 Implications of findings

The evidence in Chapter 3 showed that ART-naïve maternal HIV-infection increases the risk of PTB, LBW, SGA and stillbirth, compared to HIV-negative controls. The present study suggests that while the use of ART during pregnancy reduces the risk of MTCT, it also significantly increases the risk of PTB, sPTB, LBW and VLBW. This effect is most prominent when pregnant women initiate a HAART regimen containing a boosted PI drug and to a lesser extent an NNRTI drug, both of which have been established as the most effective ARTs to safeguard the health of the mother, prevent MTCT, ensure maximal viral suppression with minimal development of resistance, and prevent horizontal transmission[7,178,203]. Furthermore, as PI and NNRTI-based HAART were associated with the highest risk of adverse perinatal outcomes – even exceeding placebo – it suggests that the initiation of ART increases the burden of adverse perinatal outcomes in HIV-positive women, exceeding that observed in HIV-positive women not receiving any ART and HIV-negative women.

LPV/r monotherapy was found to be highly efficacious without any associated risk of adverse perinatal outcomes. However, it does not cross the placental barrier leaving the infant with no peri-exposure prophylaxis and PMTCT is entirely dependent on the viral suppressive properties of the ART in the mother[209,324]. Consequently, instances of virological failure – which has been described in African and European patients[325-327] – would lead to high rates of MTCT. The next logical assumption is that the intensification of treatment with a NRTI, e.g. AZT – which crosses the placental barrier - would improve efficacy, while still maintaining a low risk of adverse perinatal outcomes; however, the results show that AZT/LPV/r has a lower efficacy than PI-based HAART

or NNRTI-based HAART. Furthermore, recent studies have shown that the addition of 3TC to AZT/LPV/r significantly accelerates viral suppression in pregnant women, and it remains a crucial component of ART regimens for PMTCT[328].

No data were found on the association between integrase inhibitors and adverse perinatal outcomes; however, it has recently been shown to be more effective than NNRTI and PI-based HAART regimens with respect to viral suppression and rates of discontinuation[178]. While limited safety data prohibit the use of InSTIs in pregnancy in most developed and developing countries[12,324], Raltegravir is recommended for use in pregnancy in the USA[182]. Recent case reports suggest that INSTIs rapidly reduces the maternal viral load to undetectable levels – in one study the VL declined by 2.4 log in 9 days[329] - even when initiated in the third trimester of pregnancy[330,331]. Furthermore, it readily crosses the placental barrier, which significantly reduces the rate of MTCT, with no evidence of adverse perinatal outcomes[330,331]. The use of InSTIs in combination with NRTI could potentially provide the safest and most efficacious ART for PMTCT; however, this needs to be investigated in future RCTs.

In an effort to achieve the UNAIDS 90-90-90 target – 90% diagnosed, 90% on HAART, 90% virally suppressed – by 2020, countries have accelerated their response to HIV[332]. Developed[182,324] and developing countries[12] now recommend the initiation of lifelong boosted PI or NNRTI-based HAART for all HIV-positive women, including those who are pregnant or breastfeeding. The evidence presented in this review suggests that this will significantly increase the burden of PTB, spontaneous PTB, LBW and VLBW in these settings.

These outcomes all increase the risk of mortality and disability both in the short- and long-term[35]. Advances made in medical technology, strong health systems and improved access to universal health coverage ensure that the majority of these infants born in high-resource countries survive, albeit, with significant disability[49,54]. However, the majority of HIV-positive women reside in sub-Saharan Africa. The added burden of these outcomes in this setting, with its already high background rate of adverse perinatal outcomes, notoriously weak health systems and pervasive health inequity,

means that most of these infants will die during the first 4 weeks of life. Furthermore, the management of the short- and long-term morbidity associated with these outcomes in the surviving infants will have significant economic and social consequences at a family, community and country level.

4.5.4.1 Theories regarding the underlying mechanism of action

The mechanisms underlying the association between ART and adverse perinatal outcomes is not well understood; however, three main hypotheses have consistently been proposed. The initiation of HAART leads to immune reconstitution, which improves control of the HIV-infection but also skews the immune system towards a Th1 – pro-inflammatory – cytokine response, as oppose to the Th2 – anti-inflammatory – response normally seen in pregnancy[333,334]. A Th2 to Th1 shift has been described in HIV-negative women experiencing adverse perinatal outcomes, including sPTB and SGA[335], and Fiore et al (2006)[334] demonstrated an increase in IL-2 and decline in IL10 – which is consistent with a Th1 response – in HIV-positive pregnant women initiating HAART.

Others postulate that the immune reconstitution associated with HAART increases the risk of hypertension and pre-eclampsia in pregnancy, which leads to an increase in adverse perinatal outcomes[336].

Lastly, it is well established that progesterone is essential for the maintenance of a healthy pregnancy, and low levels of maternal progesterone increase the risk of PTB and SGA in HIV-negative women. Papp et al (2015)[337] recently described low levels of progesterone in HIV-positive pregnant women initiating a PI-based HAART, which could be the underlying mechanism for the increased risk of PTB observed in these women. This effect could be mediate by ritonavir's inhibition of the cytochrome P450 3A4 (CYP 3A4) enzymatic pathway[338], which is heavily involved in the production of progesterone from maternal cholesterol. Furthermore, it could also be mediated through a PI-mediated increased expression of placental 20-alpha-hydroxysteroid dehydrogenase enzyme, which renders progesterone inactive[339]. The ongoing ProSPAR pilot RCT, is the first trial

conducted in the process to determine whether progesterone supplementation of HIV-positive pregnant women on PI-based HAART reduces the risk of adverse perinatal outcome, including PTB[340].

4.5.5 Limitations of the review

This review did have a few limitations. The search of the literature only yielded 7 unique trials, reporting on 8 different treatments. Therefore, certain perinatal outcomes were not reported, a large proportion of ARTs currently used in clinical practice could not be assessed, and the limited power meant that only a limited number of analyses could be conducted. Furthermore, the threshold of 10 studies required to run a meaningful Peters test was not met[341], thus precluding the investigation of publication bias. All trials had a high risk of bias mostly because of a lack of blinding and inaccurate methods used to estimate gestational age, all of which could influence the effect estimate observed. Furthermore, a large proportion of participants were diagnosed with co-infections, some trials included multiple births and variations in the duration of ART received between trials were observed, which could also influence the effect estimate. Despite these limitations, the consistency of the magnitude and direction of the associations observed across these trials - which support a large proportion of the accumulating observational data - suggests that the findings of this review are valid.

4.6 Conclusion

The use of ART during pregnancy is essential both for the mother and infant, and clearly outweighs the risks of adverse perinatal outcomes. However, this study shows that the most effective ART regimens for PMTCT and to safeguard maternal health, are also associated with the highest risk of adverse perinatal outcomes. It suggests that recent WHO recommendations that all HIV-positive and breastfeeding women initiate lifelong HAART[12] could significantly increase the burden of PTB, spontaneous PTB, LBW and VLBW in developing countries, leading to a drastic rise in neonatal and under-5 mortality particularly in sub-Saharan Africa. Research efforts need to be directed towards understanding the biological mechanism driving these associations; whether InSTIs could

potentially be a safer and more efficacious option for pregnant women, and whether the initiation of ART before pregnancy further compounds the effect observed during pregnancy. Lastly, substantial efforts should be directed towards strengthening health systems within developing country settings, so as to mitigate mortalities associated with these outcomes and better manage the short- and long-term complications of these outcomes.

Section 3

Chapter 5: Soweto HIV and ART pregnancy study: The influence of HIV and Antiretroviral treatment on perinatal outcome in Soweto, South Africa (SHAPOSSA study)

5.1 Introduction

Almost the entire global burden of adverse perinatal outcomes – including PTB, LBW, SGA, stillbirth and neonatal deaths – occurs in sub-Saharan Africa, where 91% of HIV-positive pregnant women reside[6,35,38,63,130]. The majority [80% (95%CI 71-90%)] of these women receive ART, with 93% of those on treatment receiving lifelong HAART for PMTCT, to improve maternal health and prevent sexual transmission of the virus[2,130,147].

While the evidence presented in Chapters 3 and 4 confirms that HIV-infection and ART are associated with adverse perinatal outcomes, most of the perinatal outcomes assessed depend on a reliable estimate of GA. However, none of the studies included in the systematic reviews (Chapters 3 and 4) used an early ultrasound scan (<14 weeks) to estimate GA, despite this being the most accurate method of GA estimation[282]. Therefore, we still do not know the true magnitude of the association between maternal HIV/ART and perinatal outcomes, particularly in a "real-world" context in sub-Saharan Africa where the majority of maternal HIV-infections and adverse perinatal outcomes occur. South Africa, presented the perfect setting to explore this question using a prospective cohort study of HIV-positive and HI-negative pregnant women with pregnancies dated by early ultrasound (<14 weeks gestation).

5.1.1 HIV and ART in the South African context

South Africa has a large (54.96 million people) and young population (30.2% <15 years), with women constituting 51% of the population[342]. It currently has the largest HIV epidemic globally with an estimated 8.4 million (95% UI 7.9 – 8.8 million) HIV-positive people residing within its borders[2]. While the epidemic is generalised, women of reproductive age are most at risk of infection (HIV

prevalence: 23.2%): the highest HIV prevalence (31.6%) and incidence (4.5%) occurring in Black African women between the ages of 20 – 34 years (Figure 5.1)[343]. Between 2009 and 2015, South Africa contributed almost a third (26.7%) of all new infections in women of reproductive age (1.2 million; 95%CI 1.1 – 1.4 million)[130]. Adolescent girls and young women (15 – 24 years) are four times more likely to be HIV-positive than males in the same age group (incidence 2.5% vs. 0.6%) (Figure 5.1). In 2012, this group of women represented 24.1% of all new infections in South Africa[343].

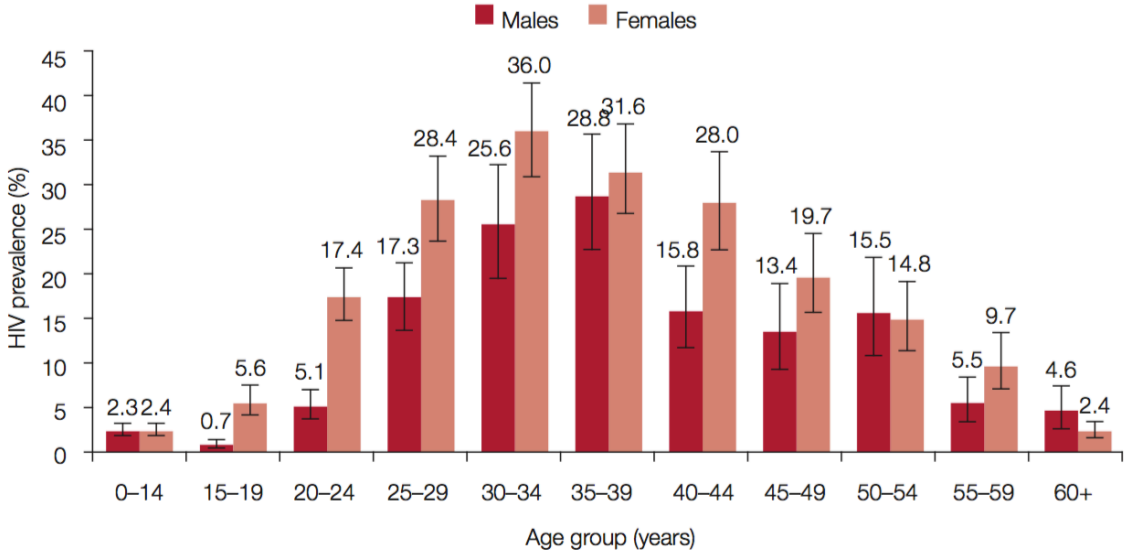

Figure 5.1: HIV prevalence by sex and age, South Africa 2012.
Source: Shisana, et al 2014[343]

The South African National ART programme – launched in 2009 – is currently the largest ART programme in the world with 51.0% (95%UI 48.5 – 53.6) of those infected receiving treatment (3.3 million HIV-positive individuals)[2,344]. On 1 September 2016, South Africa became the first African country to adopt the WHO's universal test-and-treat strategy, which will see all HIV-positive individuals initiated on lifelong ART at diagnosis, irrespective of their $CD4^+$ T-lymphocyte count[345]. This strategy was introduced in an effort to reach the UNAIDS 90-90-90 target by the year 2020[332], and it is estimated that the number of HIV-positive individuals residing in South Africa who receive lifelong ART will increase from 3.3 million in 2015 to an estimated 5 million by 2018-19[346].

HIV-infection remains the leading cause of death in the general population and women of reproductive age, particularly in the presence of co-infection with tuberculosis (Figure 5.2)[125].

A

2010 Rank	Disorder	Years of Life Lost in Thousands (% of total)
1	HIV/AIDS	11,201 (47%)
2	Diarrheal diseases	1,138 (4.9%)
3	Interpersonal violence	1,018 (4.4%)
4	Lower respiratory infections	873 (3.7%)
5	Tuberculosis	760 (3.7%)
6	Stroke	543 (2.3%)
7	Preterm birth difficulties	500 (2.1%)
8	Diabetes	489 (2.1%)
9	Mechanical forces	393 (1.7%)
10	Ischemic heart disease	383 (1.6%)
11	Neonatal encephalopathy	341 (1.5%)
12	Road injury	237 (1.0%)
13	Hypertensive heart disease	213 (0.9%)
14	Drug-use disorders	212 (0.9%)
15	Chronic kidney disease	211 (0.9%)
17	Congenital anomalies	
31	Syphilis	
39	Measles	
47	Protein-energy malnutrition	

B

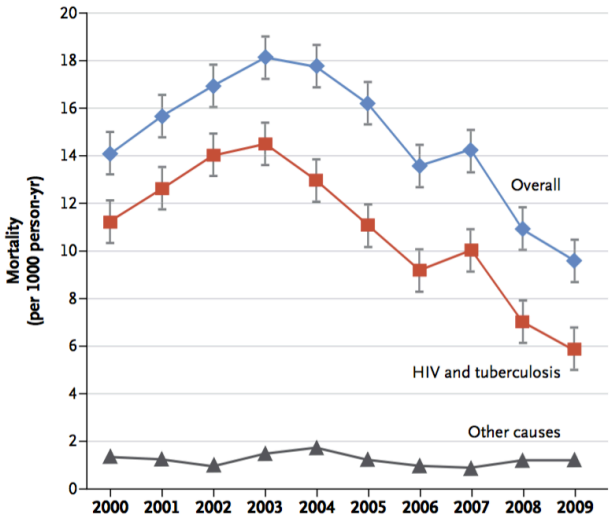

Figure 5.2: A. Cause of premature death in the general population in South Africa in 2010. B. All cause and cause-specific mortality in women of reproductive age (15 – 49 years) in South Africa, in 2000 – 2009. Source: Mayosi, et al.2014[125].

5.1.1.1 HIV-infection in South African pregnant women and PMTCT cascade

The South African government uses an "opt-out approach" for HIV counselling and testing in pregnancy[347]. All HIV-negative pregnant women who attend antenatal healthcare services within the South African public healthcare system receive HIV counselling and testing at the first antenatal visit, every 3 months thereafter and during the intrapartum period. Almost a third (national antenatal prevalence: 29.5%) of all pregnant women who attend antenatal care services in the South African public healthcare system are HIV-positive, and >95% of those infected receive HAART for PMTCT (Figure 5.3)[348,349]. In July 2013, when this study was initiated, the South African government implemented the WHO Option B PMTCT strategy, providing HAART [TDF (300mg po) & FTC

(200mg po) or 3TC (300mg po) & EFV (600mg po)] as a once daily dose – preferably as a FDC tablet – to all pregnant HIV-positive women, irrespective of their $CD4^+$ T-lymphocyte count or HIV-disease stage[349]. HAART was continued throughout pregnancy, during the intrapartum period and for 1 week after the end of breastfeeding. At this point, HIV-positive women who had a $CD4^+$ T-lymphocyte count ≤350cells/µL or advanced HIV-disease (WHO stage 3 or 4 – Appendix 5.1) continued HAART lifelong, while treatment was terminated in those with a $CD4^+$ T-lymphocyte count >350 cells/µL or early HIV-disease (WHO stage 1 or 2 – Appendix 5.1).

HIV-positive pregnant women who presented with active psychiatric or renal disease (creatinine levels > 85µmol/L) were initiated on AZT monotherapy (300mg po daily), and received a single dose of NVP (200mg po), TDF (300mg po) and FTC (200mg po) at the onset of labour, as well as AZT (300mg po) 3-hourly until delivery[349]. Furthermore, women requiring treatment for their own health with contra-indications to the standard treatment regimen were initiated on an alternative NNRTI or PI-based HAART regimen (Table 5.1). In January 2015, South Africa adopted Option B+ regimen and all HIV-positive pregnant women are now initiated on lifelong HAART[347].

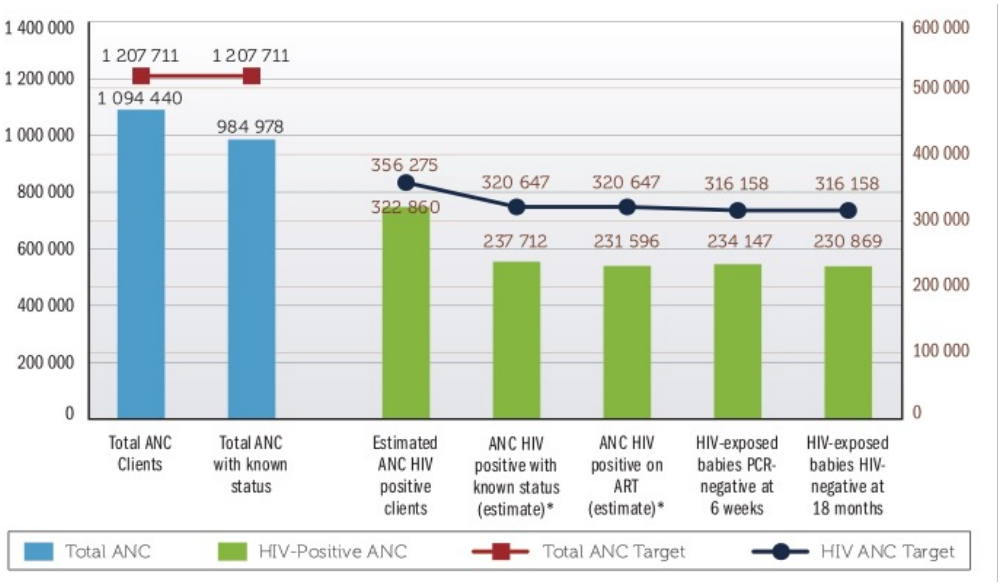

Figure 5.3: South African PMTCT cascade, 2014/2015.
Source: Health Systems Trust 2016[348]

Table 5.1: South African PMTCT program
Source: South African National Department of Health 2015[347]

First-line regimen	Pregnant women not on ART (any gestational age)	TDF + 3TC (or FTC) + EFV (Fixed-dose combination - FDC)
	Contraindication to TDF (renal insufficiency: creatinine > 85µmol/l)	AZT if Hb≥7g/dl
	Contraindication to EFV (active psychiatric illness)	TDF + 3TC + NVP or LPV/r
Second-line regimen	Failing on a TDF-based 1st line regimen	AZT + 3TC + LPV/r
		AZT + TDF + 3TC + LPV/r (4 drugs if HBV co-infected)
	Failing on a d4T or AZT-based 1st line regimen	AZT + 3TC (or FTC) + LPV/r
	Dyslipidaemia or diarrhoea associated with LPV/r	Switch LPV/r to ATV/r
Unbooked and presents in labour and tests HIV-positive	sdNVP + sdTruvada and AZT 3-hourly in labour	Woman qualifies for lifelong ART
Emergency caesarean section in an unbooked woman with no ART	sdNVP + sdTruvada for C/S	Woman qualifies for lifelong ART

Abbreviations: 3TC = lamivudine, ART = antiretroviral treatment, ATV/r = Atazanavir, AZT = zidovudine, d4T = stavudine, EFV = efavirenz, FDC = Fixed-dose combination, FTC = emtricitabine, Hb = haemoglobin, LPV/r = lopinavir/ritonavir, NVP = nevirapine, sd = single-dose, TDF = tenofovir disoproxil fumarate,

5.2 Research question

This chapter provides prospectively collected data of pregnant HIV-positive and negative women from South Africa, with accurately dated pregnancies (early ultrasound <14 weeks' gestation), to answer the question of whether HIV-positive women on treatment, in a sub-Saharan African setting, experience a higher incidence of adverse perinatal outcomes compared to their HIV-negative counterparts. The specific research question posed is as follows:

1. Do HIV-positive South African women, with accurately dated pregnancies have a higher incidence of PTB, VPTB, sPTB, sVPTB, LBW, VLBW, term-LBW, preterm-LBW, SGA, VSGA, miscarriage, and stillbirth, compared to HIV-negative women?

5.3 Methods

This study was conducted according to a predefined study protocol (Appendix 5.2).

5.3.1 Study setting

This study was conducted at the Chris Hani Baragwanath Academic Hospital (CHBAH) in Soweto, South Africa (Figure 5.4 – 5.5). Soweto is an urban informal settlement (i.e. township), found in the south-western part of the Johannesburg Metropolitan Municipality, in Gauteng Province[350] (Figure 5.4 – 5.5). Gauteng is the most populated province in South Africa, with 24% (13.2 million people) of the total country's population residing there[342]. The CHBAH serves a population of 3.5 million Soweto residents, as well as referrals from the surrounding primary healthcare clinics, community healthcare centres and regional hospitals in Gauteng and neighbouring provinces within South Africa[350,351]. The maternity unit at the CHBAH is the largest in South Africa, with an estimated 24,000 deliveries per year. Furthermore, it is the only public healthcare facility within the Soweto region with ultrasound facilities.

5.3.2 Study design

SHAPOSSA is a prospective cohort study, which was nested within the framework of the ongoing INTERBIO-21st[352] Fetal Study at the Soweto study site. INTERBIO-21st is a multi-centre, longitudinal study being conducted at 7 sites in six countries including; Oxford, United Kingdom; Pelotas, Brazil; Kilifi, Kenya; Nairobi, Kenya; Karachi, Pakistan; Mae Sot, Thailand, and Soweto, South Africa. It aims to use detailed phenotypic information based on fetal growth patterns, and maternal, fetal and placental biological samples to investigate the relationship between aetiological factors contributing to normal and abnormal (IUGR/SGA and PTB syndrome) pregnancy outcomes[352]. These data will be used to improve the characterisation of IUGR/SGA and PTB as phenotypes in an effort to identify targeted interventions and screening tools for pregnant mothers and infants[352].

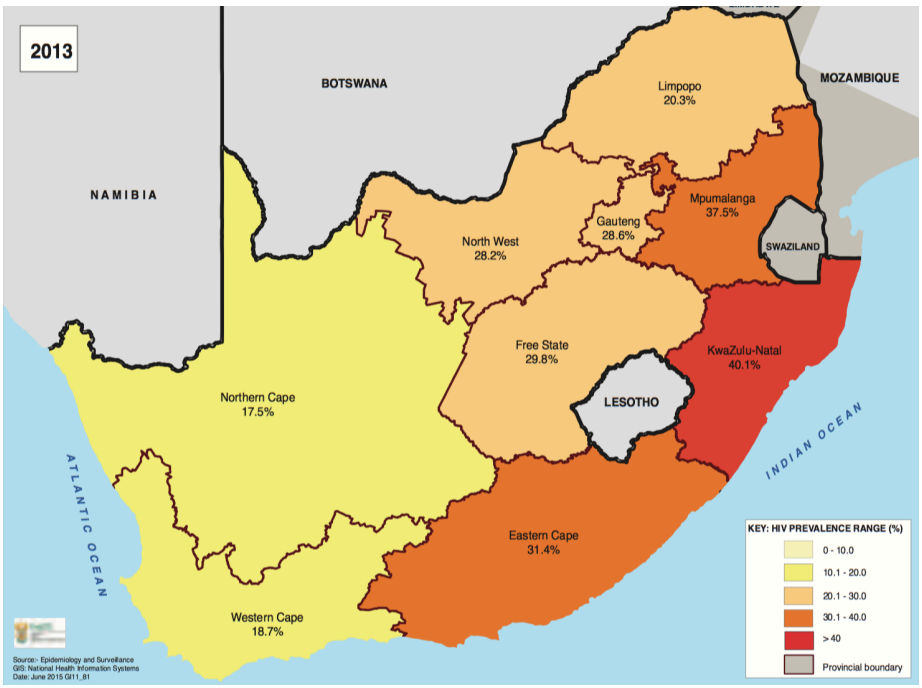

Figure 5.4: Map of South Africa illustrating its nine provinces, and HIV prevalence among pregnant women in 2013.

Source: South African National Department of Health 2015[353]

Figure 5.5: Map of South Africa illustrating its nine provinces; B. Map of Gauteng province illustrating the municipality of Johannesburg (purple) and Soweto Township.

South-African-tours-and travel.com[354]

5.3.3 Participants

HIV-positive and HIV-negative women who presented to the CHBAH Fetal Medicine Unit between 1 July 2013 and 31 December 2015 (31 months), with a singleton, intrauterine pregnancy of <14 weeks' gestation were identified by research assistants and assessed for eligibility (Table 5.2). This population included women with low-risk pregnancies and "unsure GA", who were referred to CHBAH for an ultrasound assessment, as well as women with moderate-to-high-risk pregnancies referred for further management. As the CHBAH is a referral hospital and only 10% of pregnant women present for an ultrasound scan in early pregnancy (<14 weeks' gestation), this cohort is not representative of the general South African population. Once a participant was deemed eligible for the study, she provided informed consent and was recruited at the booking visit (<14 weeks' gestation).

Table 5.2: INTERBIO-21st South African clinical site, eligibility criteria.

Parameter	**Eligible**	**Not eligible**
Maternal age	≥18 years old	<18 years old
Race	Black African	White, Indian, Coloured
Pregnancy	Singleton,	Multiple pregnancy (e.g. twins)
	naturally conceived	Fertility treatment
	No fetal abnormalities detected	Fetal abnormalities detected
Body-mass-index (BMI)(kg/m^2)	<35	>35
Place of residence	Soweto, Orange Farm	Lenasia, areas outside of Soweto
Medical conditions	-	Epilepsy, Type 1 diabetes Intellectually or physically disabled

5.3.4 Study procedures

5.3.4.1 Gestational age determination

At the booking visit, a dating scan was conducted by a trained ultrasonographer, and GA was determined using the fetal crown-rump length (CRL).

5.3.4.2 Sociodemographic data

Socioeconomic and demographic data was collected on standardised data collection forms by trained research nurses at the first study visit. Demographic information collected included the maternal age, marital status (whether married or cohabiting) and completed years of formal schooling (education), and household socio-economic status. The socioeconomic status (SES) of participants was assessed using a computed household SES score, derived from a validated, standardized questionnaire based on the Demographic and Health Survey for developing countries[355]. The questionnaire was administered to all participants who were asked whether they possessed 11 household assets: electricity, radio, television, refrigerator, cellular telephone, personal computer, farm animals, agricultural land, bicycle, motorcycle and a motor vehicle. The household SES score was generated by summing up the list of household assets owned. Participants could have a score between 0 (i.e. a low SES) to a maximum of 11 (i.e. a high SES). This measure of SES is more sensitive and robust than more traditional measures such as education and occupation, particularly in disadvantaged communities undergoing rapid economic and social transitions[355].

5.3.4.3 Clinical data

5.3.4.3.1 Maternal nutritional status

At the first study visit, trained nursing staff measured the maternal weight (SECA digital weighing scale, Hamburg, Germany) and height (SECA stadiometer, Hamburg, Germany) to the nearest 0.5cm and 0.1kg, to calculate the Body Mass Index (BMI) (Kg/m^2). A BMI ≥30 was classified as obese; 25.0 – 29.9 was overweight, 18.5 – 24.9 was normal weight and <18.5 was underweight[356].

5.3.4.3.2 Maternal comorbidities

At delivery, data were collected on whether the participant had experienced any of the following conditions during pregnancy:

- Hypertension, defined as a clinical diagnosis of pregnancy induced hypertension, pre-eclampsia, severe pre-eclampsia or HELLP syndrome;
- Anaemia, defined as a haemoglobin of <11g/dl at any time point during pregnancy;
- Medical comorbidities, defined as a clinical diagnosis of any of the following conditions: thyroid disease, epilepsy, respiratory disease (e.g. chronic asthma), Crohn's disease, coeliac disease, ulcerative colitis or any severe malabsorption condition, lupus erythematosus, sickle-cell anaemia, pyelonephritis or kidney disease, genital tract or sexually transmitted infection and cholestasis.

5.3.4.3.3 Obstetric factors

At delivery or as soon as possible thereafter participants provided information on the number of previous pregnancies (gravidity), births (parity) and any previous live born infant that weighed <2500g (LBW) or was born preterm (<37 weeks' gestation). Furthermore, information was also collected on the mode of delivery (vaginal delivery or Caesarean section).

5.3.4.3.4 HIV-status and ART factors

Participants provided information on their HIV-status at each of the 5 follow-up visits and at delivery through face-to-face interviews with a trained research nurse, which was confirmed by medical records. All participants received ART through the public healthcare system as part of the National PMTCT programme (Table 5.1). Data related to the timing of ART initiation, the use of ART in the current pregnancy and details related to the antiretroviral drugs, class and regimens used were collected through face-to-face interviews on a standardised SHAPOSSA data collection form at one time point during the second or third trimester of pregnancy (Appendix 5.3). Participants were encouraged to bring their ART drugs to the study visit where these data were collected, and any missing or additional ART-related information was abstracted from medical records. ART use in pregnancy was classified as monotherapy if only one antiretroviral drug was used during the antenatal period (e.g. AZT monotherapy) or HAART if three or more antiretroviral drugs were used. HAART regimens were

defined according to their unique third drug (e.g. PI-based HAART). If a participant's ART was changed during pregnancy, the treatment used for the longest duration during pregnancy was used in the analysis. Trained nursing staff determined each participant's HIV disease stage using the WHO clinical staging guideline.

5.3.4.3.5 $CD4^+$ T-lymphocyte count and viral load

The most recent $CD4^+$ T-lymphocyte count and VL results conducted during the current pregnancy by the National Health Laboratory Services (NHLS) were accessed for a sub-set of HIV-positive participants who provided informed consent. The $CD4^+$ T-lymphocyte count was determined using a FC-500 MPL flow cytometer and Beckman Coulter Flow-Care CD4 kit [Personal communication Dr. Sergio Carmona (specialist pathologist, NHLS)]. Furthermore, the VL was determined by DNA Polymerase Chain Reaction (PCR) (Roche CAPCTM HIV RNA version 2) (Personal communication Dr. Sergio Carmona (specialist pathologist, NHLS). In instances where a participant had more than one $CD4^+$ T-lymphocyte count or VL result, the lowest $CD4^+$ T-lymphocyte count and the highest VL during the pregnancy were used.

5.3.4.4 Behavioural data

During each trimester of pregnancy, and at delivery, data were collected on whether the participant smoked or consumed alcohol during pregnancy.

5.3.5 Perinatal outcomes

When a study participant delivered at the CHBAH, a trained research nurse or assistant obtained anthropometric measurements of the newborn within 12 hours of delivery using an electronic scale (Seca, Hangzhou, China) for the birthweight and a Harpenden infantometer for the length (Chasmors, London)[226,357]. The scale and infantometer were calibrated on a 2-weekly basis, and rigorous training of staff and quality control ensured that the coefficient of variation of anthropometric measurements between research staff was <1% (Personal communication: Professor Shane Norris). When study

participants did not deliver at the CHBAH, or the infant was not measured by the study team within 12 hours of delivery, the values were taken from routinely collected hospital records

5.3.5.1.1 Newborn z-scores

The INTERGROWTH-21st Newborn Size for GA and sex application tool[358] was used to compute the BW and birth length z-scores (standard normal deviation from the population mean)[359].

5.3.5.1.2 Perinatal outcomes of interest

PTB (≥16 weeks 0 days', <37 weeks 0 days' gestation)[360]; VPTB (≥16 weeks 0 days', <32 weeks 0 days' gestation); sPTB (PTB after spontaneous initiation of labour); sVPTB (VPTB after spontaneous initiation of labour); LBW (<2500g); VLBW (<1500g); term-LBW (≥37 weeks 0 days' gestation and BW<2500g); preterm-LBW (≥16 weeks 0 days', <37 weeks 0 days' gestation and BW <2500g); SGA (BW for GA<10th centile based on INTERGROWTH-21st standards[226]); VSGA (BW for GA<3rd centile based on INTERGROWTH-21st standards[222]); miscarriage (spontaneous expulsion of fetus <24 weeks 0 days' gestation), and stillbirth (spontaneous expulsion of fetus ≥ 24 weeks 0 days' gestation).

5.3.6 Statistical analysis

Using HIV-status, the study cohort was divided into two groups (HIV-positive and HIV-negative women) for descriptive statistics to report on the participants' characteristics. These included the mean and standard deviation (SD) or the median and lower (25% centile) and upper (75% centile) quantiles (Q1-Q3) for continuous variables, and proportions for categorical variables. The Shapiro-Wilk test (function "swilk" in Stata), coupled with the frequency distribution, was used to assess whether continuous variables met the assumption for normality[361,362]. Normally distributed variables were reported as a mean (SD) and variables found to have a skewed distribution were reported as a median (IQR).

Inferential statistics, including chi-square tests and the Student t-test, were used to assess differences in demographic characteristics and perinatal outcomes between HIV-positive and HIV-negative

participants: the chi-square test to assess categorical variables, and the Student t-test to assess normally distributed continuous variables if there was equal variance in the two groups. The Folded F-test (function "sdtest" in Stata) was used to assess whether continuous variables violate the assumption of homoscedasticity (equal variance)[361,363]. In instances where there was a statistically significant difference in the variance (unequal variance or heteroscedasticity) ($p<0.05$), it was not appropriate to perform a standard t-test due to alterations in the Type 1 error rates[364]. Therefore, the Satterthwaite approximation t-test statistic was used instead (function "unequal" in Stata), which does not assume homoscedasticity but still controls the Type 1 error rate[363,365]. The Wilcoxon rank sum (Mann-Whitney) test was used to assess differences between continuous variables with a skewed distribution. A two-tailed p-value of <0.05 was considered as being statistically significant. All statistical analyses were conducted using Stata (Stata Corporation, College Station, Texas, USA; version 13).

5.3.7 Ethical considerations

Ethical approval for the SHAPOSSA study was received from the Oxford Tropical Research Ethics Committee (OXTREC) (Appendix 5.4) at the University of Oxford, and from the Human Research Ethics Committee (Medical) of the University of the Witwatersrand, South Africa (certificate number: M130134) (Appendix 5.5). All participants signed written consent for inclusion in the study.

5.4 Results

5.4.1 Maternal characteristics

Between 1 July 2013 and 31 December 2015 (31 months), 680 pregnant women were recruited into the study. Of those recruited, 632 women (92.9%) were followed to the end of their pregnancy, of whom 403 (63.7%) were HIV-negative and 229 (36.2%) HIV-positive (Figure 5.6). Of the 48 women who were not followed to delivery, 36 (75%) were lost to follow-up and 6 (12.5%) withdrew consent mostly because of work-related demands that prohibited them from attending follow-up visits. The remaining 6 (12.5%) women withdrew because they lost interest in the study, did not feel comfortable

with the blood drawing components of the study, medical challenges prohibited them from attending the follow-up visits and one woman relocated to another province.

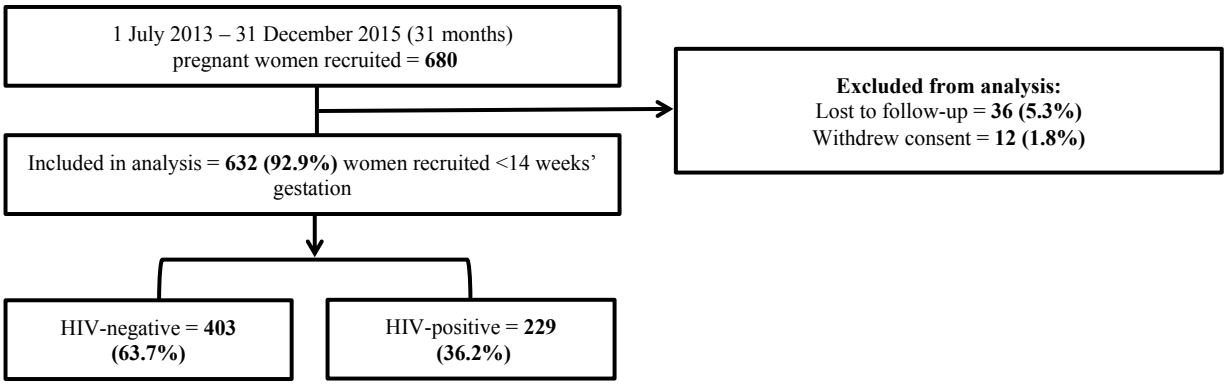

Figure 5.6 Flowchart illustrating the study population included in the analysis.

The demographic characteristics stratified according to maternal HIV-status are presented in Table 5.3. The median maternal age at enrolment was 30 years (IQR 26 - 35); HIV-positive women were significantly older than HIV-negative women [32 (28 – 36) years vs. 29 (25 – 34) years; P<0.001), with more HIV-positive women at an advanced maternal age (≥35 years) (33% vs. 23.3%; P=0.018) (Table 5.3). While most women had completed secondary school education, HIV-positive women had completed less years of formal education [median (IQR): 12 (12 – 12) versus 12 (11 – 12); P<0.001] and possessed a significantly lower average number of household assets [median (IQR): 6 (5 – 6) vs. 5 (5 – 6); P=0.022] (Table 5.3). Over a third of women (38.8%) were married or cohabiting (Table 5.3).

The majority (63%) of women were either overweight or obese [BMI: 26.7 (IQR 23.2 – 30.1)]; with no significant difference between HIV-positive and HIV-negative women. Almost a third of participants (29.5%) of women had delivered a LBW or PTB infant in a previous pregnancy, with a higher rate observed in HIV-negative than HIV-positive women (31.8% vs. 26.0%; P=0.174) (Table 5.3). Furthermore, despite having a similar gravidity to HIV-negative women, HIV-positive women displayed a significantly higher parity than HIV-negative women (P=0.007) owing to the high

frequency of previous miscarriages in HIV-negative women [median (IQR) =1 (0 – 2) versus 0 (0 – 1); P=0.0004].

Only a small proportion of women had a medical comorbidity (8.9%) or genital tract infection (1.4%) during this pregnancy with no difference observed between HIV-positive and HIV-negative women. However, 54.1% of the cohort were anaemic [haemoglobin (Hb)<11g/dl] [mean(sd) Hb: 10.8 (1.5)]. HIV-positive women had a significantly lower mean Hb than HIV-negative women [11.1 (1.5) vs 10.8 (1.4); P=0.007], with substantially more HIV-positive women being diagnosed with anaemia during pregnancy (63.0% vs. 49.1%; P=0.001).

A significantly higher proportion of HIV-positive women smoked during pregnancy (15.3% vs. 8.9%, P=0.015); however, no difference was observed between HIV-positive and HIV-negative women in the use of alcohol (10.0% vs 7.2%; P=0.210) (Table 5.3). Only three women (0.5%) used illicit drugs (Table 5.3).

Table 5.3: Maternal characteristics, stratified according to HIV-status.

Variable	Total (N = 632)	HIV-negative (N = 403)	HIV-positive (N = 229)	P-Value
Demographic factors (socio-economic status)				
Maternal age (years), median (IQR)	30 (26 – 35)	29 (25 – 34)	32 (28 – 36)	<0.001*** [a]
<25 years	110 (17.4%)	69 (20.9%)	24 (13.2%)	0.018*
25 - 35 years	356 (56.3%)	185 (55.9%)	98 (53.9%)	
≥35 years	166 (26.3%)	77 (23.3%)	60 (33.0%)	
Married or cohabiting				0.097
Yes, n (%)	245 (38.8%)	166 (41.2%)	79 (34.5%)	
No, n (%)	387 (61.2%)	237 (58.8%)	150 (65.5%)	
Years of formal education, median (IQR)	12 (11 – 12)	12 (12 – 12)	12 (11 – 12)	<0.001*** [a]
Household socio-economic status, median (IQR)	5 (5 – 6)	6 (5 – 6)	5 (5 – 6)	0.022* [a]
Maternal factors				
Maternal body-mass index (kg/m^2), median (IQR)	26.7 (23.2 – 30.1)	26.8 (23.3 – 30.4)	27.3 (23.4 – 30.2)	0.817 [a]
Underweight (<18.5)	10 (1.6%)	8 (2.0%)	2 (0.9%)	0.573
Normal weight (18.5 - 24.9)	224 (35.4%)	138 (34.2%)	86 (37.6%)	
Overweight (25.0 - 29.9)	233 (36.9%)	153 (38.0%)	80 (34.9%)	
Obese (≥30.0)	165 (26.1%)	104 (25.8%)	61 (26.6%)	
Maternal gravidity				0.209
1	39 (6.2%)	29 (7.2%))	10 (4.4%)	
2	175 (27.7%)	116 (28.8%)	59 (25.8%)	
≤3	418 (66.1%)	258 (64.0%)	160 (69.9%)	
Maternal parity				0.007**
0	102 (17.2%)	75 (20.1%)	27 (12.3%)	
1	278 (46.9%)	189 (48.1%)	98 (44.8%)	
≤2	213 (35.9%)	119 (31.8%)	94 (42.9%)	
Previous adverse perinatal outcome [b]				0.174
Yes, n (%)	145 (29.5%)	95 (31.8%)	50 (26.0%)	
No, n (%)	346 (70.5%)	204 (68.2%)	142 (74.0%)	
Hypertensive in pregnancy [c]				0.662
Yes, n (%)	45 (7.1%)	27 (8.2%)	10 (5.5%)	
No, n (%)	585 (92.9%)	302 (91.8%)	173 (94.5%)	
Lowest haemoglobin in pregnancy, mean (sd)	10.8 (1.5)	11.1 (1.5)	10.8 (1.4)	0.007**
Anaemia in pregnancy (<11g/dl), n (%)[d]				
Yes, n (%)	340 (54.1%)	197 (49.1%)	143 (63.0%)	0.001**
No, n (%)	288 (45.9%)	204 (50.9%)	84 (37.0%)	
Maternal comorbidities [e]				
Yes, n (%)	56 (8.9%)	34 (8.4%)	22 (9.6%)	0.619
No, n (%)	576 (91.1%)	369 (91.6%)	207 (90.4%)	
Smoking				0.015*
Yes, n (%)	71 (11.2%)	36 (8.9%)	35 (15.3%)	
No, n (%)	561 (88.8%)	367 (91.1%)	194 (84.7%)	
Alcohol				
Yes, n (%)	52 (8.2%)	29 (7.2%)	23 (10.0%)	0.210
No, n (%)	580 (91.8%)	374 (92.8%)	206 (90.0%)	
Recreational drug use [f]				
Yes, n (%)	3 (0.5%)	1 (0.2%)	2 (0.9%)	0.272
No, n (%)	629 (99.5%)	402 (99.8%)	227 (99.1)	
Genital tract infections				
Yes, n (%)	9 (1.4%)	4 (1.0%)	5 (2.2%)	0.226
No, n (%)	622 (98.6%)	398 (99.0%)	224 (97.8)	

a = Wilcoxon rank-sum (Mann-Whitney) test; b = Previous adverse perinatal outcome defined history of a low birthweight (<2500g) or preterm birth (<37 weeks 0 days) infant; c = Hypertension in pregnancy includes women diagnosed with pregnancy induced hypertension, pre-eclampsia, severe pre-eclampsia, eclampsia or HELLP syndrome during current pregnancy, data only available for 630 women; d = Anaemia defined as a haemoglobin of <11g/dl at any point during pregnancy: sample size = 510; e = Maternal comorbidities include women diagnosed with any of the following conditions: thyroid disease, epilepsy, respiratory disease (e.g. chronic asthma), Crohn's disease, coeliac disease, ulcerative colitis or any severe malabsorption condition, lupus erythematosus, sickle-cell anaemia, pyelonephritis or kidney disease, genital tract or sexually transmitted infection, cholestasis, any other medical/surgical condition requiring treatment or referral; f = Recreational drug use during pregnancy includes any of the following cannabis, benzodiazepines, heroine, methadone, crack cocaine, amphetamines, hallucinogens, inhalant solvents and any other drugs, * = statistically significant, *P < 0.05; ** P<0.01; ***P<0.001

5.4.1.1 HIV-specific factors

5.4.1.1.1 Antiretroviral treatment

While almost all HIV-positive participants in this cohort - barring one woman (0.4%) - received ART during pregnancy (personal communication: Professor Shane Norris), detailed ART data were collected on 124 (54.2%) women (Figure 5.7). The majority (97.6%) received HAART; two women were initiated on AZT monotherapy because of renal impairment prohibiting the initiation of HAART, and one woman did not receive any ART during pregnancy. Of those who were on HAART, 50.4% initiated treatment before conception ("preconception") for maternal health reasons (Figure 5.4); 48.8% of women were diagnosed as being HIV-positive during pregnancy and initiated HAART during the antenatal period for PMTCT ("antenatal"). The majority (97.5%) of women on HAART received first-line NNRTI-based regimens as per the South African guidelines (Table 5.4); EFV was the most common NNRTI used contributing to 94.4% of the treatment regimens received (Table 5.4). Three women (2.5%) received a boosted PI-based HAART regimen, which had been initiated before pregnancy (preconception HAART). One participant who initiated treatment before conception was switched from a boosted PI-based HAART regimen to a NNRTI-based HAART (TDF + FTC + EFV) regimen during pregnancy.

5.4.1.1.2 $CD4^+$ T-lymphocyte count, viral load and WHO stage of disease

The antenatal $CD4^+$ T-lymphocyte count was available for 107 (46.7%) HIV-positive women, and the median $CD4^+$ T-lymphocyte count was 447 (320 – 582) cells/µl. Only 9.4% of participants had a $CD4^+$ T-lymphocyte count <200 cells/µl (Table 5.5). A large proportion of women who initiated HAART before conception had a significantly higher $CD4^+$ T-lymphocyte count than those initiating HAART

during the antenatal period (P=0.019) (Table 5.5). Almost all (93.6%) HIV-positive women were asymptomatic during pregnancy (WHO stage 1), and none of the women were diagnosed with WHO stage 4 disease (Table 5.5).

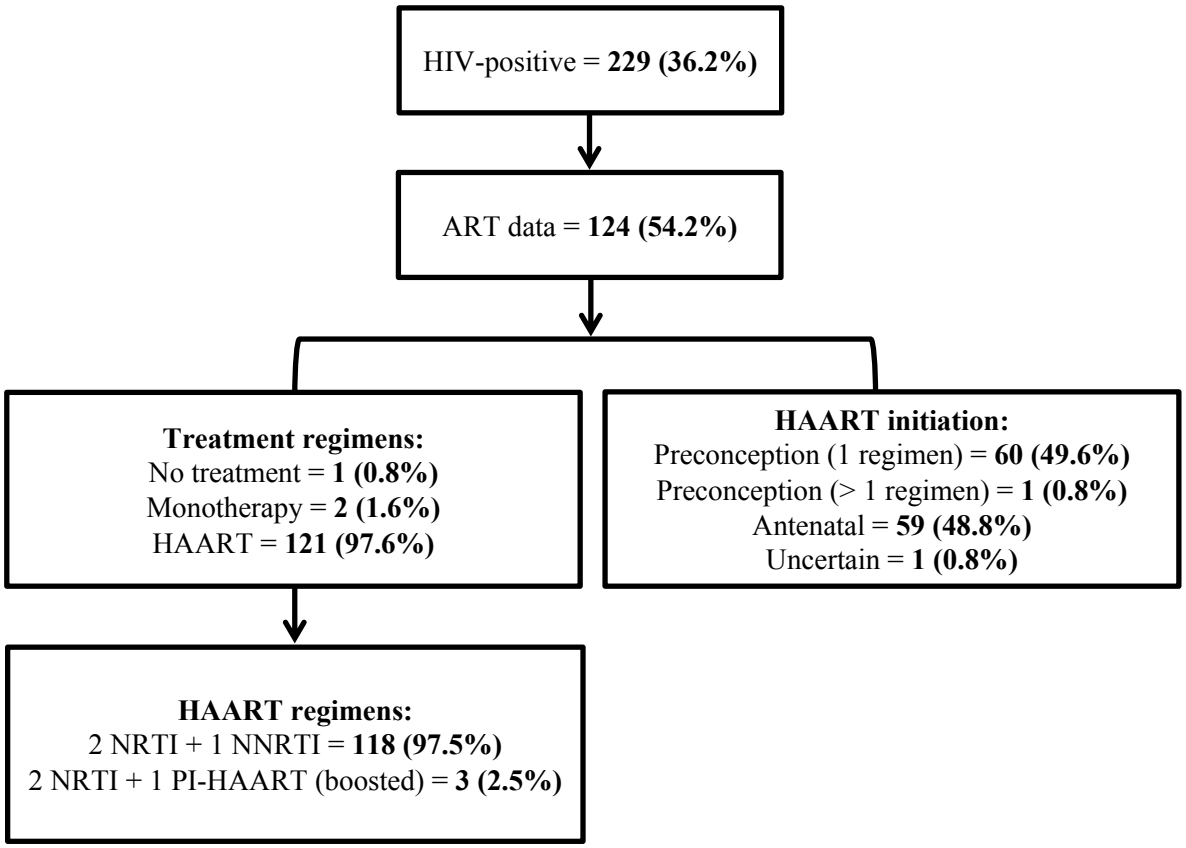

Figure 5.7. Flowchart illustrating the ART regimens received by HIV-positive participants. All participants on monotherapy received zidovudine. HAART was defined as ≥3 ART drugs. Participants who initiated treatment before conception according to first trimester ultrasound were classified as preconception initiation. Preconception (>1 regimen) includes participants whose HAART regimen was changed to another HAART regimen during pregnancy. Abbreviations: ART = antiretroviral treatment, NRTI = Nucleoside/nucleotide reverse transcriptase inhibitor, NNRTI = Non-nucleoside reverse transcriptase inhibitor, PI = protease inhibitor, HAART = Highly Active Antiretroviral Treatment.

Table 5.4. ART received in HIV-positive women.

ART regimen	Antiretroviral drug	HIV-positive women (N = 123)
		N (%)
Monotherapy	AZT	2 (1.6%)
NNRTI-based HAART	TDF + FTC + EFV	105 (85.4%)
	TDF + 3TC + EFV	6 (4.9%)
	AZT + 3TC + EFV	4 (3.3%)
	TDF + FTC + NVP	2 (1.6%)
	D4T + 3TC + EFV	1 (0.8%)
Boosted PI-based HAART	TDF + 3TC + LPV/r	3 (2.4%)

Abbreviations: 3TC = lamivudine, AZT = zidovudine, D4T = stavudine, EFV = efavirenz, FTC = emtricitabine, HAART = Highly Active Antiretroviral Therapy, LPV/r = lopinavir/ritonavir, NRTI = Nucleoside Reverse Transcriptase Inhibitors, NNRTI = Non-nucleoside Reverse Transcriptase Inhibitor, NVP = nevirapine, PI = Protease Inhibitor, TDF = tenofovir disoproxil fumarate.

Table 5.5: Antenatal $CD4^+$ T-lymphocyte count, viral load and WHO stage of disease

	Total	All HAART	HAART initiation		
			Preconception	Antenatal	Preconception versus antenatal HAART p-value
	124	(N = 121)	(N = 61)	(N = 59)	
Antenatal $CD4^+$ T-lymphocyte count (cells/µL)					
Median (IQR) (n=109)	447 (320 – 582)	447 (320 – 573)	456 (369 – 501)	416.5 (270.5 – 641)	0.657 [a]
<200, n (%)	10 (9.4%)	10 (9.5%)	2 (4.2%)	7 (12.5%)	
200-349, n (%)	20 (18.7%)	20 (19.1%)	7 (14.6%)	13 (23.2%)	
350-499, n (%)	41 (38.3%)	40 (38.1%)	26 (54.2%)	14 (25.0%)	0.019*
≥500, n (%)	36 (33.6%)	35 (33.3%)	13 (27.1%)	22 (39.3%)	
Antenatal viral load					
LDL, n (%)	28 (57.1%)	28 (58.3%)	19 (54.3%)	9 (75.0%)	
>LDL, n (%)	21 (42.9%)	20 (41.7%)	16 (45.7%)	3 (25.0%)	0.207
WHO stage of disease					
Clinical stage 1, n (%)	116 (93.6%)	113 (93.4%)	57 (93.4%)	55 (93.2%)	
Clinical stage 2, n (%)	2 (1.6%)	2 (1.7%)	0 (0.0%)	2 (3.4%)	
Clinical stage 3, (%)	2 (1.6%)	2 (1.7%)	2 (3.3%)	0 (0.0%)	0.261
Clinical stage 4, (%)	0 (0.0%)	0.(0.0%)	0 (0.0%)	0 (0.0%)	
Not available	4 (3.2%)	4 (3.3%)	2 (3.3%)	2 (3.4%)	

a = Wilcoxon rank-sum (Mann-Whitney) test.
Antenatal $CD4^+$ T-lymphocyte count: n = 107, antenatal VL: n = 49, WHO stage of disease: n = 124
Abbreviations: HAART = Highly Active Antiretroviral Treatment, IQR = Interquartile range, LDL = lower than detectable level, * = statistically significant, $P < 0.05$; ** $P<0.01$; ***$P<0.001$

5.4.2 Obstetric factors and perinatal outcomes

The majority of women delivered a live born infant (n=577; 91.3%); 5.9% (n=37) of women had a miscarriage and 2.9% (n=18) delivered a stillborn infant (Table 5.6). While more HIV-negative than HIV-positive women experienced pregnancy loss (9.9% vs 6.6%; P=0.148), this did not reach significance (Table 5.6). The median gestational age at delivery was 38 weeks (IQR: 37 – 39) and the majority of women delivered by caesarean section (53.6%).

The median birthweight was 2900g (IQR: 2595 – 3260), with no difference observed in the birthweight or birth length z-scores between HIV-exposed and unexposed newborns (Table 5.6). Almost a quarter (20.4%) of babies were born LBW, and 19.1% of these had a birthweight <1500g (Table 5.6). The majority of LBW newborns were also preterm (67.0%). While there no statistically significant difference in the rate of LBW, VLBW, term-LBW or preterm-LBW between HIV-exposed and unexposed newborns, a larger proportion of HIV-exposed newborns were LBW (22.9% vs. 18.6%; P=0.223) (Table 5.6), and term-LBW (7.1% vs 6.4%; P=0.728) and preterm-LBW (15.7% vs. 12.2%; P=0.239) (Table 5.6).

5.6: Obstetric factors and perinatal outcomes, stratified according to HIV-status.

Variable	Total (N = 632)	HIV-negative (N = 403)	HIV-positive (N = 229)	P-Value
Obstetric factors				
Onset of labour				
Spontaneous, n (%)	386 (61.2%)	252 (62.7%)	134 (58.5%)	
Induced, n (%)	65 (10.3%)	46 (11.4%)	19 (8.3%)	0.103
No labour, n (%)	180 (28.5%)	104 (25.9%)	76 (33.2%)	
Mode of delivery				0.719
Vaginal, n (%)	293 (46.4%)	189 (46.9%)	104 (45.4%)	
Caesarean section, n (%)	339 (53.6%)	214 (53.1%)	125 (54.6%)	
Perinatal outcomes				
Live births, n (%)	577 (91.3%)	363 (90.1%)	214 (93.5%)	0.148
Pregnancy loss, n (%)	55 (8.7%)	40 (9.9%)	15 (6.6%)	
Miscarriage, n (%)				0.120
Yes, n (%)	37 (5.9%)	28 (7.0%)	9 (3.9%)	
No, n (%)	595 (94.2%)	375 (93.1%)	220 (96.1%)	
Stillbirth, n (%)				0.795
Yes, n (%)	18 (2.9%)	12 (3.0%)	6 (2.6%)	
No, n (%)	614 (97.2%)	391 (97.0%)	223 (97.4%)	
Sex				0.547
Male	324 (52.6%)	201 (51.7%)	123 (54.2%)	
Female	292 (47.4%)	188 (48.3%)	104 (45.8%)	
Gestational age at delivery (weeks), median (IQR)[a]	38 (37 – 39)	38 (37 – 39)	38 (37 – 39)	0.740
Birthweight (g), median (IQR)[a]	2990 (2595 – 3260)	2995 (2653 – 3265)	2953 (2540 – 3250)	0.190
Sex-specific birthweight for gestational age (z-score), median (IQR)[a]	- 0.4 (-1.1 – 0.3)	- 0.4 (-1.0 – 0.3)	- 0.5 (-1.2 – 0.3)	0.244
Sex-specific birth length for gestational age (z-score), median (IQR)[a]	- 0.3 (-1.2 – 0.7)	- 0.3 (-1.1 – 0.7)	- 0.3 (-1.4 – 0.7)	0.173
Low birthweight, n (%)[b]				
Yes, n (%)	115 (20.2%)	67 (18.6%)	48 (22.9%)	0.223
No, n (%)	455 (79.8%)	293 (81.4%)	162(77.1%)	
Very low birthweight, n (%)	22 (19.1%)	14 (20.9%)	8 (16.7%)	0.570
Term low birthweight, n (%)	38 (33.0%)	23 (34.3%)	15 (31.3%)	0.729
Preterm low birthweight, n (%)	77 (67.0%)	44 (65.7%)	33 (68.8%)	0.729
Preterm birth, n (%)[c]				0.657
Yes, n (%)	151 (23.9%)	94 (23.3%)	57 (24.9%)	
No, n (%)	481 (76.1%)	309 (76.7%)	172 (75.1%)	
Spontaneous preterm birth, n (%)	91 (60.7%)	56 (60.2%)	35 (61.4%)	0.885
Very preterm birth, n (%)	66 (45.8%)	44 (49.4%)	22(40.0%)	0.269
Spontaneous very preterm birth, n (%)	42 (29.4%)	26 (29.6%)	16 (29.1%)	0.954
Small for gestational age, n (%)[d]				
Yes, n (%)	80 (14.1%)	42 (11.7%)	38 (18.2%)	0.032*
No, n (%)	488 (85.9%)	317(88.3%)	171 (81.8%)	
Very small for gestational age, n (%)	24 (30.0%)	13 (12.6%)	11 (29.0%)	0.845
Adverse perinatal outcome [e]	227 (36.3%)	136 (34.3%)	91 (39.9%)	0.157
Severe adverse perinatal outcome [f]	90 (14.7%)	57 (14.7%)	33 (14.7%)	0.157

a = Wilcoxon rank-sum (Mann-Whitney) test, b = Subsets of LBW outcome (e.g. VLBW) reported as a percentage of LBW infants; c = Subsets of PTB outcome (e.g. VPTB) reported as a percentage of PTB infants; d = Subsets of SGA outcome (e.g. VPTB) reported as a percentage of SGA infants; e = Adverse perinatal outcome was defined as any infant born either preterm, LBW or SGA; f = Severe adverse perinatal outcome was defined as any infant born either VPTB, VLBW or VSGA; Sex: n = 616, miscarriage: n = 632, stillbirth = 632, preterm birth: n = 632, low birthweight: n = 570, small for gestational age: n = 568; Very small for gestational age: n = 568; Abbreviations: IQR = Interquartile range. * = statistically significant, P < 0.05; ** P<0.01; ***P<0.001

Almost a quarter of participants delivered preterm (23.9%), with the majority of PTBs occurring after spontaneous onset of labour (60.7%). Almost half (45.8%) of the preterm babies were born <32 weeks' gestation (VPTB), and 29.4% of PTBs occurred <32 weeks' gestation in women who laboured spontaneously. Similar to LBW, there was no statistically significant difference between HIV-positive and HIV-negative women; however, HIV-positive women experienced a higher rate of PTBs (24.9% vs 23.3%; P=0.657) (Table 5.6), spontaneous PTBs (15.3% vs 13.9%; P=0.642) and spontaneous VPTBs (7.1% vs. 6.6%; P=0.811) compared to HIV-negative women. Interestingly, HIV-negative participants had a higher frequency of VPTB (11.1% vs. 9.7%) (Table 5.6).

Fourteen percent (14.1%) of participants delivered an SGA newborn, and a third (30.0%) of these had a birthweight-for-gestational age below the 3rd percentile (Table 5.6). A significantly larger proportion of HIV-exposed neonates were SGA (18.2% vs. 11.7%; P=0.032) (Table 5.6), and while a larger proportion of HIV-exposed infants were also VSGA (5.3% vs 3.6%), this difference was not statistically significant (Appendix 5.6).

Overall, a third (36.3%) of women in this cohort - including 39.9% of HIV-positive women and 34.3% of HIV-negative women - gave birth to a LBW, PTB or SGA baby, and many of these had a combination of these outcomes (Table 5.6). Furthermore, 14.7% of these women gave birth to VLBW, VPTB or VSGA babies.

5.5 Discussion

The aim of this study was to determine whether there is an increased risk of adverse perinatal outcomes in treated HIV-positive women compared to HIV-negative women, if the pregnancies are accurately dated. The principal findings were that treated HIV-positive women and HIV-negative women have similar rates of PTB, VPTB, spontaneous PTB, spontaneous VPTB, LBW, VLBW, term-LBW, preterm-LBW, VSGA, miscarriage and stillbirth. Only maternal HIV/ART was associated with SGA.

To the best of my knowledge, this is the only sub-Saharan Africa study that has ever compared perinatal outcomes in HIV-positive and HIV-negative women with pregnancies dated by early ultrasound (<14 weeks' gestation). Furthermore, unlike previous studies addressing this question, detailed information was collected on potential confounding factors and ART received during the antenatal period, and all perinatal outcomes, which were measured objectively using standardised and validated measurement methods, were clearly defined *a priori*.

Only two other sub-Saharan African cohort studies have compared perinatal outcomes in HIV-positive women on ART and HIV-negative women, both of which also confirmed an association between maternal HIV-infection and SGA[280,366]. However, contrary to the findings of the present study, Chen and colleagues (2012)[280], who conducted the largest retrospective study to date assessing this question (N = 33 148 women), also showed a significant association with stillbirth and PTB. One explanation for their findings could be their use of multiple inaccurate methods to assess GA (LNMP, symphysis-fundal height and late ultrasound), or because 13% of their cohort – as opposed to 0.4% of the cohort in the present study – were not taking any ART during pregnancy, a factor shown to be associated with PTB and stillbirth (Chapter 3)[3,26]. Furthermore, a larger proportion of their cohort received a PI-based HAART regimen during pregnancy (9% vs. 2.4%), and were diagnosed with hypertension in pregnancy (17.6% vs. 7.1%), both of which are associated with PTB[9] and stillbirth[367]. Similarly, Olagbuji and colleagues (2010)[366], showed an association between

maternal HIV-infection and LBW; however, the limited demographic and ART related information reported in the study, including the lack of specification on the method used to determine GA, prohibits one from identifying factors that may have contributed to these differences observed.

Similar studies conducted in Europe[283,368] and the USA[369] used ultrasound estimates (early or late) in conjunction with LNMP to determine GA. While all found an association between maternal HIV-infection and PTB, these findings should be interpreted with caution as the majority of participants were on a PI-based HAART regimen [Boer, et al. (2007) = 83%[283], Haeri, et al. (2009) = 74%[369], Lopez, et al (2012) = 59.7%[368]). Haeri, et al (2009)[369] also showed an association between maternal HIV-infection and SGA; however, this was attributed to an increased use of tobacco and illicit drugs in the HIV-positive women[369]. While the use of illicit drugs was negligible in the present study (0.5% of cohort), significantly more HIV-positive women smoked during pregnancy (15.3% vs. 8.9%, p = 0.015), which could have contributed to the increased rates of SGA observed in HIV-positive women (a topic investigated further through regression analysis in Chapter 6). If smoking is the underlying cause of the increased SGA rate observed in HIV-positive women in this cohort, it suggests that HIV-negative women and HIV-positive women on ART experience similar rates of adverse perinatal outcomes.

Almost all women in this cohort were asymptomatic and received ART during pregnancy, with the majority on a NNRTI-based HAART regimen. In Chapters 3 and 4, untreated HIV-positive women and those treated with a PI-based HAART regimen were shown to experience a significantly higher rate of adverse perinatal outcomes. Furthermore, in Chapter 4, RCT data showed that NNRTI-based HAART significantly increases the risk of giving birth to a LBW infant. In the present study, a non-significant increase in the risk of giving birth to a LBW infant was observed in HIV-positive women, compared to HIV-negative women (22.9% vs. 18.6%; p=0.223). The failure to achieve statistical significance in the present study could be because of the limited sample size, underlying confounding or because of the high background rate of LBW in HIV-negative women. However, it could also be that in the "real-world" context, the use of NNRTI-based HAART during pregnancy attenuates the

effect of HIV-infection on perinatal outcomes, thus reducing the rate of adverse perinatal outcomes to that observed in the HIV-negative population. This effect may be mediated by the ability of HAART to improve maternal health status and prevent AIDS[145] or by preventing MTCT as neonates infected in-utero are at a greater risk of PTB, LBW and stillbirth[277,370]. Furthermore, the heightened state of inflammation and activation found in untreated HIV infection far exceeds the controlled and mild inflammatory activity observed in normal pregnancy, and may have detrimental effects on pregnancy outcomes[274]. HAART reduces the persistent activation and inflammation associated with HIV-infection[371], which possibly improves maternal-fetal tolerance and creates a more conducive intrauterine environment for fetal development and growth.

Surprisingly, a third of HIV-exposed (39.9%) and unexposed (34.3%) neonates were either preterm, LBW or SGA; outcomes which are associated with a 3 – 18-fold increased risk of death in the neonatal period and during infancy[35]. Furthermore, the majority of LBW neonates were also preterm (67%), and 14.7% of neonates were either VLBW, VSGA or VPTB, all conditions associated with a substantially higher risk of mortality in the first week of life[35]. The effects of these outcomes on neonatal mortality have already been observed at a population level; Allanson, et al. (2015)[372] conducted a population based study in South Africa and found that a large proportion of newborns who died during the neonatal period were either SGA (25.8%), LBW (64.9%) or preterm (48.7%). Furthermore, the majority (74.2%) of neonatal deaths occurred in healthy HIV-negative mothers[372], which confirms the finding in the present study that both HIV-positive and "healthy" HIV-negative South African women are at increased risk of adverse perinatal outcome.

Other maternal factors that may have contributed to the elevated rates of adverse perinatal outcomes include the fact that 54% of women in this cohort were anaemic - a condition associated with PTB and LBW[373,374]. In this setting, the most probable underlying cause in "healthy" HIV-negative women is nutritional (iron deficiency), while normochromic normocytic anaemia of chronic disease is most common in HIV-positive pregnant women[375]. The high rates of anaemia in the present study confirm findings from other South African cohorts of pregnant HIV-positive and HIV-negative

women where anaemia occurred more frequently in HIV-positive women[375,376]. Secondly, 63% of women were overweight or obese, which is associated with high rates of adverse maternal and perinatal outcomes. Both these factors (anaemia and increased BMI) are a reflection of the double burden of poor nutrition associated with the rapid socio-economic transition occurring in developing countries like South Africa, which re-affirms the need for a continuum of care approach to nutritional intervention in women of reproductive age[19,21].

One cause for concern is the fact that 53.6% of participants delivered by Caesarean section, which exceeds the national Caesarean section rate (24.7%)[24] and the WHO Caesarean section threshold (15%)[377]. The rate observed in this cohort is higher than that in countries with the highest caesarean section rates in the world, including Mexico (46.9%), Turkey (48.0%), and Egypt (51.8%), with only Brazil (58.9) and the Dominican Republic (58.9%) having higher estimates[24]. Similar to other low-to-middle income countries, South Africa is experiencing an obstetric transition from a high maternal mortality and morbidity predominantly from direct causes (e.g. haemorrhage) to a lower incidence predominantly due to indirect causes[24]. This transition is characterised by an excessive amount of unnecessary or inappropriate use of obstetric interventions, including Caesarean sections[24], which significantly increase the incidence of maternal and perinatal morbidity and mortality[378]. This factor in itself may be a cause for the increased rate of PTBs observed in this cohort, as similar trends have been observed in Brazil, where increased rates of Caesarean deliveries (from 6.3% to 16.2%), resulted in an almost three-fold increase in the rates of PTBs (6.3 to 16.2%)[24].

In order to optimise the use of Caesarean sections in this setting and reduce the burden of adverse maternal and perinatal outcomes attributed to the excessive and inappropriate use of this intervention, more efforts should be directed towards monitoring trends in the Caesarean section rate using a validated monitoring tool, e.g. the Robson classification for Caesarean section[379]. This information could then be used to guide policies, and clinical practice in an effort to reduce the rate of Caesarean sections to a level that ensures maximal health benefits, with minimal harm.

The present study did have a few limitations. As the SHAPOSSA Study was initiated after the Fetal Study had already commenced, it was not possible to collect detailed ART data on a larger proportion of HIV-positive women. Furthermore, as data related to the level of compliance to ART was not collected, adherence to treatment could not be assessed. Maternal health status during pregnancy has a direct impact on perinatal outcome, and advanced maternal HIV-infection significantly increases the risk of adverse perinatal outcome, including PTB, LBW, SGA and stillbirth. Successful HIV treatment, which improves maternal health, largely depends on patient compliance to ART[380]. Sub-optimal compliance predicts virological failure, the development of drug resistance, HIV-disease progression and death[380]. While no gold standard has been established for monitoring ART adherence, treatment compliance can be assessed using data obtained from patients, providers, pill bottles, pharmacy records, electronic devises, biochemical assays or a combination of these sources[381,382]. The most inexpensive and practical methods are (i) patient self-reported adherence, which can be affected by recall or social desirability bias, and tends to overestimate treatment adherence, and (ii) prescription or pill-based adherence measures, which are objective and can be derived from routinely collected data from medical and pharmacy records[380]. These methods assume rather than prove the patient's actual medication intake[383]. A combination of pill count, patient self-report in combination with $CD4^+$ T-lymphocyte counts and viral load estimation provide a good estimate of patient adherence to treatment[384].

Objective measures of adherence through biochemical (detection of drug or metabolites level in hair or body fluids) and electronic drug monitoring (e.g. Medication Event Monitoring System) are sensitive and routinely used in research settings; however, their high cost, complexity and intrusiveness make them impractical for use in routine clinical care settings[385]. Future studies should include a rigorous assessment of ART compliance, and the impact on perinatal outcomes.

Some participants delivered at other hospitals, which necessitated using routinely collected birthweight and birth length estimates for the analyses. Although the Caesarean section rate was high, insufficient information was collected to determine whether these were elective or emergency sections. No analyses could be conducted on the association between ART dose and rate of adverse perinatal outcomes, as HIV-positive patients were managed according to the Government of South

Africa's standardised ART protocols with standardised dosing regimens. A limited number of women provided data on the timing of ART initiation (n = 120), so questions related to the influence of timing of treatment initiation on perinatal outcomes could not be explored. However, preliminary data suggests that women in the SHAPOSSA cohort who initiated HAART before conception had higher rates of PTB (22.9% vs. 15.5%; p=0.305), LBW (23.3% vs. 13.2%; p=0.167) and SGA (18.3% vs. 17.0%) than those initiating treatment during the antenatal period. These findings are consistent with those from a recent meta-analysis of 11 studies (PTB = 1.20; 95%CI 1.01-1.44, LBW = 1.30, 95%CI 1.04-1.62)[321], as well as a large (n = 47, 124 births) surveillance study from Botswana (SGA: ARR 1.25; 95%CI 1.15-1.36)[386]. A larger sample size, adjustments for indication bias and correction for confounding factors are needed to confirm these findings. Lastly, a self-reported, interviewer administered questionnaire was used to collect data on the prevalence of recreational drug use. This may have under-represented the prevalence of drug use in the cohort and introduced bias as some participants may have been reluctant to report drug use to health professionals[387].

While the study is not representative of the general population, the reported incidence of these outcomes in the general South African population (LBW = 14.8%[388], PTB = 14%[389]) is still substantially higher than that observed in healthy women in other parts of the world (INTERGROWTH-21st: LBW = 5.5%, PTB = 5.5%)[226]. Furthermore, as a large proportion of babies in this setting are not weighed at birth, and ultrasound dating of pregnancies is extremely limited, and almost non-existent in the first trimester of pregnancy, the national estimates may significantly underestimate the real burden. The outcomes in this setting could explain South Africa's stubbornly high neonatal mortality rate, and its inability to meet the child-related MDG. If South Africa is to meet the child related Sustainable Development Goals of reducing neonatal mortality to 12 per 1000 live births and child mortality to ≤25 per 1000 live births[62], it will need to invest in strategies that ensure a continuum of care approach to reproductive and perinatal health[20] increase efforts to identify these outcomes when they do occur, and invest in strengthening health systems, including antenatal (steroids) and neonatal interventions (neonatal ICU and KMC) to manage these outcomes in a way that reduces mortality.

Chapter 6: Association between HIV, ART and adverse perinatal outcomes: logistic regression analysis

6.1 Background

Of the estimated 32 million livebirths that occur in sub-Saharan Africa every year, more than 10% are preterm (12.3%), LBW (14.2%), or SGA (25.5%)[38]. The debate on the overlapping nature of these outcomes is ongoing and there are still challenges related to their definition, measurement, diagnosis and heterogeneity. However, PTB, LBW and SGA have clearly been established as predictors of neonatal and infant mortality, and disability[31,35,41,42,54].

PTB accounts for almost a fifth of neonatal (15.9%) and under-5 deaths (17.8%) globally[6]. LBW infants are 40 times more likely to die within the first 4 weeks of life, and account for 60-80% of neonatal deaths[33,39], and infants born SGA are 3-times more likely to die within the first 4 weeks of life compared to infants born with an appropriate weight for GA[35]. A large proportion of those who survive the neonatal period suffer from long-term neurodevelopmental, auditory and visual impairments, and they are predisposed to developing infectious and non-communicable diseases in adolescence and adulthood[41,42,54,55].

An accumulating body of predominantly observational evidence has suggested that maternal HIV-infection is associated with PTB[285], LBW[10,366] and SGA[390], even in the presence of effective ART. Most of these studies have been conducted in Europe and the USA, where boosted PI-based ART is recommended as first-line treatment, and is the most common ART provided in pregnancy[324,391]. In this thesis, PI-based ART has been shown to be associated with PTB and LBW (Chapter 4), which explains the findings observed in the European and American settings.

Within sub-Saharan Africa - where the majority of HIV-positive pregnant women reside - PI-based ART is rarely used in pregnancy: NNRTI-based HAART is the recommended first-line regimen for

PMTCT[12]. In Chapter 5, South African HIV-positive women on a predominantly NNRTI-based HAART regimen were shown to have similarly high rates of PTB and LBW as HIV-negative pregnant women. The fact that both HIV-positive and HIV-negative women have high rates of adverse perinatal outcomes in this setting suggests that other proximal and distal risk factors may also be contributing to the development of these outcomes.

In this chapter, the association between maternal HIV/ART and LBW, PTB and SGA is explored independently of known sociodemographic, clinical and behavioural confounding factors. Secondly, the underlying sociodemographic, clinical and behavioural determinants for PTB, LBW and SGA in a South African cohort are determined.

6.2 Research questions

1. Is HIV/ART independently associated with PTB, LBW or SGA in South African with pregnancies dated by early ultrasound (<14 weeks' gestation, following adjustment for known confounding factors?
2. Which sociodemographic, clinical and behavioural risk factors are independently associated with PTB, LBW and SGA in South African women)?

6.3 Methods

6.3.1 Study design

SHAPOSSA was a prospective cohort study conducted at the Chris Hani Baragwanath Academic Hospital (CHBAH), in Soweto, South Africa, between 1 July 2013 and 31 December 2015 (31 months).

6.3.2 Study population

HIV-positive and HIV-negative women with a singleton pregnancy were recruited before 14 weeks' gestation, and followed until delivery.

6.3.3 Study procedure

A detailed description of the study procedure is provided in Chapter 5.

6.3.3.1 Gestational age determination

The gestational age of the pregnancy was determined by an early ultrasound scan (<14 weeks' gestation) performed by a trained ultrasonographer at the first study visit.

6.3.3.2 Sociodemographic factors

Data related to maternal sociodemographic status was collected at the first study visit and included maternal age, marital status (whether married or cohabiting), completed years of formal schooling (education), and household socio-economic status (SES).

6.3.3.3 Clinical factors

At the first study visit, the maternal weight and height were measured, and used to compute the BMI. Participants were categorised into three BMI groups: underweight or normal weight (<18.5kg/m^2–24.9kg/m^2), overweight (≥25 – 29.9kg/m^2) or obese (≥30kg/$m^{2)}$). Participants provided information on the number of previous pregnancies (gravidity), births (parity) and any previous live born infant that

weighed <2500g (LBW) or was born preterm (<37 weeks' gestation). Participants gave their HIV-status (HIV-positive or HIV-negative) at each study visit, and at delivery. Specific information was collected on the ART regimen received during pregnancy at one time point during the second or third trimester of pregnancy. All information relating to HIV was confirmed by examining the medical records.

At delivery, data were collected on whether the participant had experienced any of the following conditions during pregnancy:

- Hypertension, defined as a clinical diagnosis of pregnancy induced hypertension, pre-eclampsia, severe pre-eclampsia or HELLP syndrome;
- Anaemia, defined as a haemoglobin of <11g/dl at any time point during pregnancy;
- Medical comorbidities, defined as a clinical diagnosis of any of the following conditions: thyroid disease, epilepsy, respiratory disease (e.g. chronic asthma), Crohn's disease, coeliac disease, ulcerative colitis or any severe malabsorption condition, lupus erythematosus, sickle-cell anaemia, pyelonephritis or kidney disease, genital tract or sexually transmitted infection and cholestasis.

6.3.3.4 Behavioural factors

During each trimester of pregnancy, and at delivery, data were collected on whether the participant smoked or consumed alcohol during pregnancy.

6.3.3.5 Obstetric factors

At delivery, information were collected on the mode of delivery (vaginal delivery or Caesarean section).

6.3.4 Outcome variables

The three binary outcomes of interest assessed were PTB (≥16 weeks 0 days', <37 weeks 0 days' gestation)[360], LBW (BW <2500g), and SGA (BW for GA <10th centile according to INTERGROWTH-21st standards[226]).

6.1.1. Statistical analysis

Descriptive statistics for the baseline characteristics of the HIV-positive and HIV-negative women included in this analysis have already been reported in Chapter 5 (Table 5.3).

Univariate logistic regression models were used to test the unadjusted association between binary perinatal outcomes (PTB, LBW and SGA) and exposures, which included HIV/ART, sociodemographic factors (maternal age, marital status, education, household SES), clinical factors (BMI, hypertension, anaemia, parity, previous PTB or LBW infant, maternal comorbidities), behavioural factors (smoking and alcohol) and obstetric factors (mode of delivery). Effect estimates were reported as odds ratios (OR) and 95% confidence interval (CI), and a p-value of <0.05 was considered statistically significant.

Multivariate logistic regression models were used to quantify the strength of the association between maternal HIV/ART and each of the perinatal outcomes, independent of potential confounding factors. The confounders included in the model were identified *a priori* and included variables that were consistently reported to be associated with PTB, LBW and SGA[31,37,280,392-394]. Three multivariate regression models were used to assess the association between maternal HIV/ART and each perinatal outcome, the first model adjusted for sociodemographic factors (maternal age, marital status, education, household SES); the second model adjusted for sociodemographic and clinical factors (BMI, hypertension, anaemia, parity, previous PTB or LBW infant, maternal comorbidities), and the third model adjusted for sociodemographic, clinical and behavioural factors (smoking and alcohol). Collinearity between exposure variables was resolved by only including the variable with the larger effect estimate. Effect estimates were reported as adjusted odds ratios (AOR) with 95%CI, and a p-value of <0.05 was considered statistically significant.

All statistical analyses were conducted using Stata (Stata Corporation, College Station, Texas, USA; version 13).

6.4 Results

This analysis includes 632 women [403 (63.8%) HIV-negative and 229 (36.2%) HIV-positive] with singleton pregnancies who were followed to delivery, and provided data on perinatal outcomes. The baseline characteristics of these two groups are reported in detail in Chapter 5. Almost all HIV-positive women received ART during pregnancy (>99%), and the majority were initiated on a NNRTI-based HAART regimen (97.5%).

6.4.1 Preterm birth

Data on the duration of pregnancy was available for all participants included in this analysis (n = 632): 151 (23.9%) women delivered preterm, of whom 94 (62.3%) were HIV-negative and 57 (37.7%) HIV-positive (HIV/ART group) (Chapter 5).

6.4.1.1 Univariate analysis

In the univariate analysis, which included all 632 participants, no association was observed between PTB and HIV/ART (OR1.0; 95%CI 0.7-1.5) (Table 6.1). The only maternal risk factors that significantly increased the risk of PTB were maternal age between 25 – 35 years (OR1.7;95%CI 1.0-3.0, p=0.04), parity = 1 (OR0.4; 95%CI 0.3-0.7, p=0.001), parity ≤2 (OR0.5;95%CI 0.3-0.8, p=0.002), and having previously delivered a LBW or PTB infant (OR1.6; 1.0-2.5, p=0.04) (Table 6.1). The remaining factors were not significantly associated with PTB.

Table 6.1. Univariate logistic regression analysis of the association between PTB, HIV-infection, and demographic, behavioural and obstetric risk factors, reported as OR and 95%CI (n = 632).

Risk factor	PTB rate (%)	PTB (n/N)	OR (95%CI)	p-value
Exposure of interest				
HIV status				
HIV-negative	23.2%	94/403	Reference	
HIV-positive	24.9%	57/229	1.0 (0.7 – 1.5)	0.65
Sociodemographic factors				
Maternal age (years)				
<25 years	17.3%	19/110	Reference	
25 - 35 years	27.0%	96/356	1.7 (1.0 – 3.0)	0.04*
≥ 35 years	21.7%	36/166	1.3 (0.7 – 2.4)	0.37
Married or cohabiting				
No	25.6%	99/387	Reference	
Yes	21.2%	52/245	0.7 (0.5 – 1.1)	0.21
Years of formal education	-	-	0.9 (0.8 – 1.0)	0.66
Household SES	-	-	0.9 (0.8 – 1.0)	0.35
Clinical factors				
Maternal BMI (kg/m^2)	-	-	1.0 (0.9 – 1.0)	0.96
Underweight or normal weight (<24.9)	24.4%	57/234	Reference	
Overweight (25.0 - 29.9)	23.2%	54/233	0.9 (0.6 – 1.4)	0.76
Obese (≥30.0)	24.2%	40/165	0.9 (0.6 – 1.6)	0.98
Maternal parity				
0	39.2%	40/102	Reference	
1	21.9%	61/278	0.4 (0.3 – 0.7)	0.001**
≤2	22.5%	48/213	0.5 (0.3 – 0.8)	0.002**
Previous adverse perinatal outcome [a]				
No	19.7%	68/346	Reference	
Yes	28.3%	41/145	1.6 (1.0 – 2.5)	0.04*
Hypertensive in pregnancy [b]				
No	23.1%	135/585	Reference	
Yes	31.1%	14/45	1.3 (0.7 – 2.4)	0.28
Anaemia in pregnancy (<11g/dl) [c]				
No	25.7%	74/288	Reference	
Yes	21.5%	73/340	0.7 (0.5 – 1.1)	0.213
Maternal comorbidities [d]				
No	24.0%	138/576	Reference	
Yes	23.2%	13/56	0.9 (0.5 - 1.8)	0.901
Behaviour factors				
Smoking				
No	23.2%	130/561	Reference	
Yes	30.0%	21/71	1.3 (0.8 – 2.4)	0.23
Alcohol				
No	23.6%	137/580	Reference	
Yes	26.9%	14/52	1.1 (0.6 – 2.2)	0.59
Obstetric factors				
Mode of delivery				
Vaginal	25.9%	76/293	Reference	
Caesarean section	22.1%	75/339	0.8 (0.5 – 1.1)	0.26

a = Previous adverse perinatal outcome, defined as a history of giving birth to a LBW (<2500g) or PTB (<37 weeks 0 days) infant; b = Hypertension in pregnancy includes women diagnosed with pregnancy induced hypertension, pre-eclampsia, severe pre-eclampsia, eclampsia or HELLP syndrome during current pregnancy; c = Anaemia defined as a haemoglobin of <11g/dl at any point during pregnancy; d = Maternal comorbidities include women diagnosed with any of the following conditions: thyroid disease, epilepsy, respiratory disease (e.g. chronic asthma), Crohn's disease, coeliac disease, ulcerative colitis or any severe malabsorption condition, lupus erythematosus, sickle-cell anaemia, pyelonephritis or kidney disease, genital tract or sexually transmitted infection, cholestasis, any other medical/surgical condition requiring

treatment or referral; Abbreviations: BMI = body mass index, OR = odds ratio, PTB = preterm birth, SES = socio-economic status; * = statistically significant, p<0.05 *; p<0.01 **; p<0.001***

6.4.1.2 Multivariate analysis

6.4.1.2.1 Model 1: association between HIV/ART and PTB, adjusting for sociodemographic factors.

The first multivariate model assessed the association between maternal HIV/ART and PTB, while adjusting for maternal sociodemographic factors, including maternal age, marital status (married or cohabiting), education and household SES (Figure 6.1; Table 6.2). HIV/ART was not significantly associated with PTB (AOR1.0; 95%CI 0.7-1.5, p=0.86); however, women between the age of 25 - 35 years were almost twice as likely to deliver preterm than women <25 years (AOR1.8; 95%CI 1.0-3.0, p=0.03) (Figure 6.1. Table 6.2).

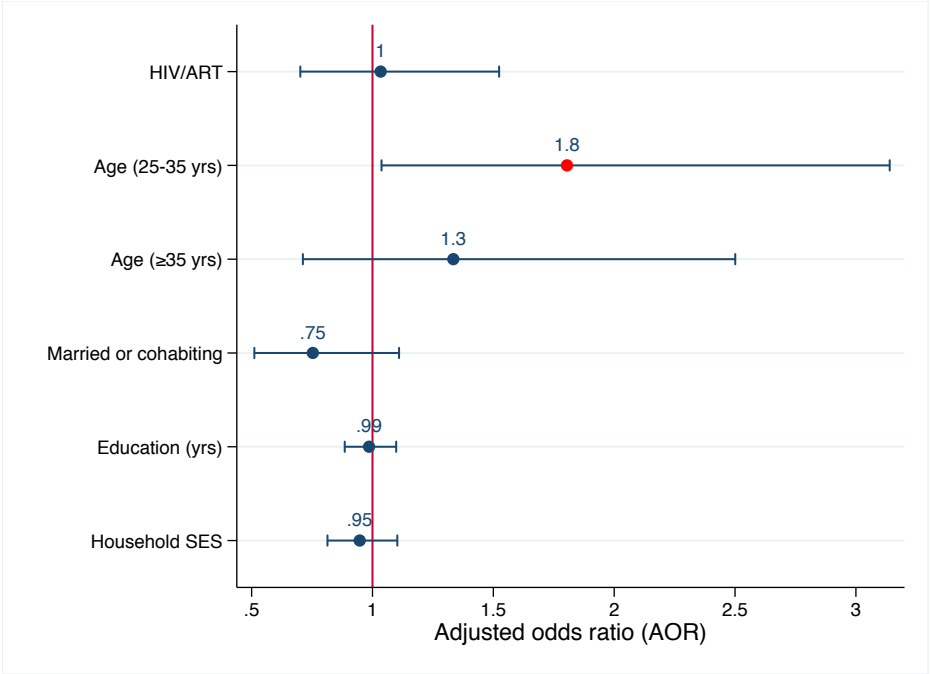

Figure 6.1: Multivariate logistic regression model for the association between HIV/ART and PTB, adjusting for sociodemographic factors. Reference group for HIV-positive women on HAART (HIV/ART) is HIV-negative women. Sociodemographic factors included are maternal age (reference <25years), whether participant is married or cohabiting (reference = no), years of formal education (continuous variable), and household SES (continuous variable). Effect estimates are reported as an adjusted OR (AOR) and 95%CI, and considered as statistically significant if the CI does not include 1 (red). Maternal age between 25-35 years significantly increases the odds of PTB.

6.4.1.2.2 Model 2: association between HIV/ART and PTB, adjusting for sociodemographic factors, and clinical factors.

Model 2 included sociodemographic factors and clinical factors including BMI, hypertension, anaemia, parity, previous LBW or PTB, maternal comorbidities. HIV-infection was associated with a non-significant 1.2-fold increase in PTB (AOR1.2; 95%CI 0.7-1.8, p=0.47) (Figure 6.2; Table 6.2). Having previously delivered a LBW or PTB infant was significantly associated with PTB (AOR 1.7; 95%CI 1.1-2.7, p=0.02). Adjusting for clinical factors led to a reduction in the effect estimate of maternal age between 25-35years, from AOR1.8 to AOR 1.3, which was no longer statistically significant (AOR1.3; 95%CI 0.6-3.0; p=44) (Figure 6.2; Table 6.2).

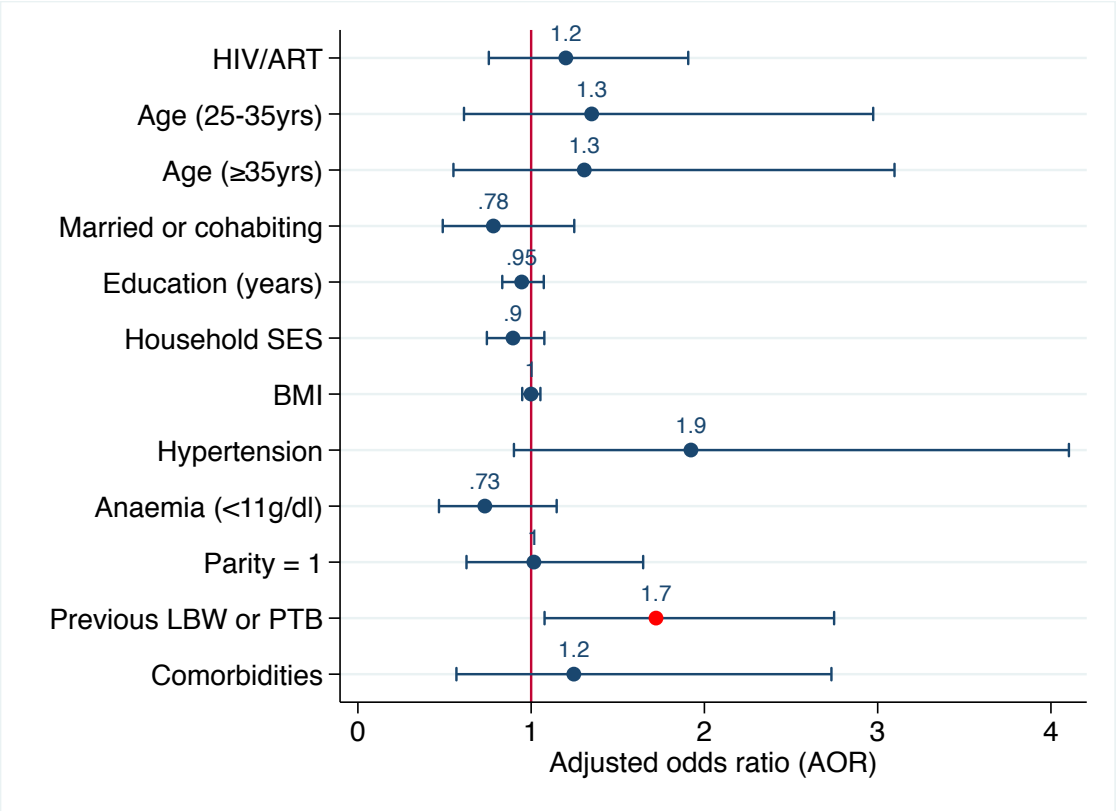

Figure 6.2: Multivariate logistic regression model for the association between HIV/ART and PTB, adjusting for sociodemographic factors and clinical factors. Reference group for HIV-positive women on HAART (HIV/ART) is HIV-negative women. Sociodemographic factors included are maternal age (reference <25years), whether participant is married or cohabiting (reference = no), years of formal education (continuous variable), and household socio-economic status (continuous variable). Clinical factors include BMI, hypertension in pregnancy (reference = no), anaemia in pregnancy (reference = no), parity (reference = 0), previous history of LBW or PTB (reference = no), any comorbidities during pregnancy (reference = no). Effect estimates are reported as an adjust OR (AOR) and 95%CI, and considered as statistically significant if the CI does not include 1 (red). Previous history of LBW or PTB significantly increased the odds of PTB.

6.4.1.2.3 Model 3: Association between HIV/ART and PTB, adjusting for sociodemographic factors,

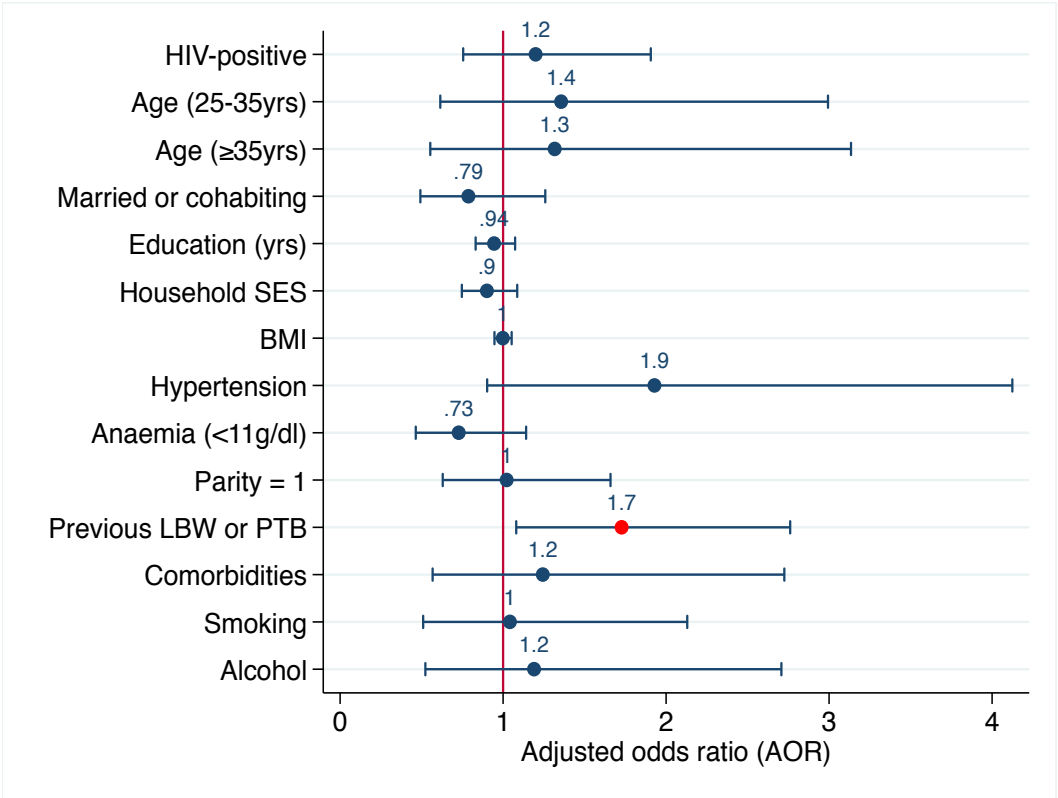

Figure 6.3: Multivariate logistic regression model for the association between HIV/ART and PTB, adjusting for sociodemographic factors, clinical factors and behavioural factors. Reference group for HIV-positive women on HAART (HIV/ART) is HIV-negative women. Sociodemographic factors included are maternal age (reference <25years), whether participant is married or cohabiting (reference = no), years of formal education (continuous variable), and household socio-economic status (continuous variable). Clinical factors include BMI, hypertension in pregnancy (reference = no), anaemia in pregnancy (reference = no), parity (reference = 0), previous history of LBW or PTB (reference = no), any comorbidities during pregnancy (reference = no). Behavioural factors include smoking during pregnancy (reference = no), or use of alcohol during pregnancy (reference = no). Effect estimates are reported as an adjusted OR and 95%CI, and considered as statistically significant if the CI does not include 1 (red). Previous history of LBW or PTB significantly increased the odds of PTB.

clinical factors and behavioural factors.

The third multivariate model assessed the association between maternal HIV/ART and PTB, after adjusting for sociodemographic factors, maternal health factors, and behavioural factors including smoking and the use of alcohol during pregnancy (Figure 6.3, Table 6.2). Maternal HIV-infection remained non-significantly associated with PTB (AOR 1.2; 95%CI 0.8-1.9; p=0.44); however, a previous history of PTB or LBW was significantly associated with PTB, and almost doubled the odds of delivering a preterm infant (AOR 1.7; 95%CI 1.0-2.8 (Figure 6.3, Table 6.2).

Table 6.2: Multivariate logistic regression analysis showing the association between exposures and preterm birth

Variables	Multivariate (AOR (95%CI)					
	Model 1 (n = 632)	**p-value**	**Model 2 (n = 486)**	**p-value**	**Model 3 (n = 486)**	**p-value**
Exposure of interest						
HIV status						
HIV-negative	Reference		Reference		Reference	
HIV-positive	1.0 (0.7 – 1.5)	0.86	1.2 (0.8 – 1.9)	0.44	1.2 (0.8 – 1.9)	0.44
Demographic factors						
Maternal age (years)						
<25 years	Reference		Reference		Reference	
25 - 35 years	1.8 (1.0 – 3.1)	0.03*	1.3 (0.6 – 3.0)	0.46	1.4 (0.6 – 3.0)	0.45
≥35 years	1.3 (0.7 – 2.5)	0.36	1.3 (0.6 – 3.1)	0.54	1.3 (0.6 – 3.1)	0.53
Married or cohabiting						
No	Reference		Reference		Reference	
Yes	0.7 (0.5 – 1.1)	0.15	0.8 (0.5 – 1.2)	0.30	0.8 (0.5 – 1.3)	0.32
Years of formal education	0.9 (0.8 – 1.0)	0.79	0.9 (0.8 – 1.1)	0.39	0.9 (0.8 – 1.1)	0.38
Household SES	0.9 (0.8 – 1.1)	0.48	0.9 (0.7 – 1.1)	0.24	0.9 (0.7 – 1.1)	0.27
Clinical factors						
Maternal BMI (kg/m^2)			1.0 (0.9 – 1.1)	1.0	1.0 (0.9 – 1.1)	0.95
Maternal parity						
0			Reference			
1			1.0 (0.6 – 1.6)	0.95	1.0 (0.6 – 1.7)	0.93
≤2			Omitted		Omitted	
Previous adverse perinatal outcome [a]						
No			Reference		Reference	
Yes			1.7 (1.1 – 2.7)	0.02	1.7 (1.0 – 2.8)	0.02*
Hypertensive in pregnancy [b]						
No			Reference		Reference	
Yes			1.9 (0.9 – 4.1)	0.09	1.9 (0.9 – 4.1)	0.09
Anaemia in pregnancy (<11g/dl) [c]						
No			Reference		Reference	
Yes			0.7 (0.5 – 1.1)	0.17	0.7 (0.4 – 1.1)	0.17
Maternal comorbidities [d]						
No			Reference		Reference	
Yes			1.2 (0.6 – 2.7)	0.58	1.2 (0.6 – 2.7)	0.59
Behavioural factors						
Smoking						
No					Reference	
Yes					1.0 (0.5 - 2.1)	0.91
Alcohol						
No					Reference	
Yes					1.2 (0.5 - 2.7)	0.68

a = Previous adverse perinatal outcome, defined as a history of giving birth to a LBW (<2500g) or PTB (<37 weeks 0 days) infant; b = Hypertension in pregnancy includes women diagnosed with pregnancy induced hypertension, pre-eclampsia, severe pre-eclampsia, eclampsia or HELLP syndrome during current pregnancy; c = Anaemia defined as a haemoglobin of <11g/dl at any point during pregnancy; d = Maternal comorbidities include women diagnosed with any of the following conditions: thyroid disease, epilepsy, respiratory disease (e.g. chronic asthma), Crohn's disease, coeliac disease, ulcerative colitis or any severe malabsorption condition, lupus erythematosus, sickle-cell anaemia, pyelonephritis or kidney disease, genital tract or sexually transmitted infection, cholestasis, any other medical/surgical condition requiring treatment or referral; Abbreviations: BMI = body mass index, OR = odds ratio, PTB = preterm birth, SES = socio-economic status * = statistically significant, p<0.05 *; p<0.01 **; p<0.001***

6.4.2 Low birthweight

Of the 632 women with perinatal outcome data, the birthweight was available for 570 (90.2%) babies. The birthweight data was not available for 62 women for the following reason; the pregnancy ended as a stillbirth or miscarriage and the baby was not weighed (n=54, 87.1%); the mother delivered at another hospital and the birthweight could not be retrieved (n=3, 4.8%), the baby required resuscitation at delivery and the birthweight could not be conducted (n=4, 6.5%) the delivery was missed at the CHBAH (n=1, 1.6%). Of the 570 women who provided birthweight data, 360 (63.2%) were HIV-positive and 210 (36.8%) HIV-negative.

6.4.2.1 Univariate analysis

The univariate analysis showed a non-significant 1.2-fold increase in LBW in HIV-positive compared to HIV-negative women (OR 1.2; 95%CI 0.8-1.9, p=0.22) (Table 6.3). Maternal age was significantly associated with LBW; participants aged 25-35 years (OR2.6; 95%CI 1.2-5.2, 0.008), and those ≥ 35 years (OR2.6; 95%CI 1.2 -5.6, p=0.01) were almost 3 times more likely to deliver a LBW infant than participants <25years (Table 6.3).

Maternal BMI was significantly associated with LBW, and each unit increase in the BMI reduced the odds of LBW by 0.9-fold (OR0.9; 95%CI 0.9-0.9, p=0.02) (Table 6.3). Furthermore, parity = 1 was associated with a 0.5-fold reduction in the rate of LBW (OR 0.5; 95%CI 0.3-0.9, p=0.02). The remaining factors were not significantly associated with LBW.

Table 6.3. Univariate logistic regression analysis of the association between LBW, HIV-infection, and demographic, behavioural and obstetric risk factors, reported as OR and 95%CI (n = 570).

Risk factor	LBW rate (%)	LBW (n/N)	OR (95%CI)	p-value
Exposure of interest				
HIV status				
HIV-negative	18.6%	67/360	Reference	
HIV-positive	22.9%	48/210	1.2 (0.8 – 1.9)	0.22
Demographic factors				
Maternal age (years)				
<25 years	9.9%	10/101	Reference	
25 - 35 years	22.2%	70/315	2.6 (1.2 – 5.2)	0.008**
≥35 years	22.7%	35/154	2.6 (1.2 – 5.6)	0.01*
Married or cohabiting				
No	22.5%	78/347	Reference	
Yes	16.6%	37/223	0.6 (0.4 – 1.0)	0.08
Years of formal education	-	-	0.9 (0.8 – 1.0)	0.69
Household SES	-	-	0.9 (0.8 – 1.1)	0.60
Clinical factors				
Maternal BMI (kg/m^2)	-	-	0.9 (0.9 – 0.9)	0.02*
Underweight or normal weight (<24.9)	22.8%	48/211	Reference	
Overweight (25.0 - 29.9)	20.5%	44/215	0.8 (0.5 – 1.3)	0.56
Obese (≥30.0)	16.0%	23/144	0.6 (0.3 – 1.1	0.11
Maternal parity				
0	29.8%	25/84	Reference	
1	18.4%	46/250	0.5 (0.3 – 0.9)	0.02*
≤2	20.3%	40/197	0.6 (0.3 – 1.0)	0.08
Previous adverse perinatal outcome [a]				
No	17.1%	54/316	Reference	
Yes	24.4%	32/131	1.6 (0.9 – 2.6)	0.08
Hypertensive in pregnancy [b]				
No	19.5%	103/528	Reference	
Yes	28.6%	12/42	1.6 (0.8 – 3.3)	0.16
Anaemia in pregnancy (<11g/dl) [c]				
No	21.2%	53/250	Reference	
Yes	19.1%	61/319	0.8 (0.5 – 1.3)	0.53
Maternal comorbidities [d]				
No	20.1%	104/518	Reference	
Yes	21.2%	11/52	1.0 (0.5 – 2.1)	0.85
Maternal behaviour				
Smoking				
No	19.1%	96/504	Reference	
Yes	28.8%	19/66	1.7 (0.9 – 3.0)	0.06
Alcohol				
No	20.1%	105/523	Reference	
Yes	21.3%	10/47	1.07 (0.5 – 2.2)	0.84
Obstetric factors				
Mode of delivery				
Vaginal	16.7%	40/240	Reference	
Caesarean section	22.7%	75/330	1.4 (0.9 – 2.2)	0.07

a = Previous adverse perinatal outcome, defined as a history of giving birth to a LBW (<2500g) or PTB (<37 weeks 0 days) infant; b = Hypertension in pregnancy includes women diagnosed with pregnancy induced hypertension, pre-eclampsia, severe pre-eclampsia, eclampsia or HELLP syndrome during current pregnancy; c = Anaemia defined as a haemoglobin of <11g/dl at any point during pregnancy; d = Maternal comorbidities include women diagnosed with any of the following conditions: thyroid disease, epilepsy, respiratory disease (e.g. chronic asthma), Crohn's disease, coeliac disease, ulcerative colitis or any severe malabsorption condition, lupus erythematosus, sickle-cell anaemia, pyelonephritis or kidney disease, genital tract or sexually transmitted infection, cholestasis, any other medical/surgical condition requiring treatment or referral.
Abbreviations: BMI = body mass index, OR = odds ratio, PTB = preterm birth, SES = socio-economic status; * = statistically significant, p< 0.05 *; p<0.01 **; p<0.001***

6.4.2.2 Multivariate analysis

6.4.2.2.1 Model 1: Association between HIV/ART and LBW, adjusting for sociodemographic factors.

The first multivariate model, which assessed the association between maternal HIV/ART and LBW, adjusting for sociodemographic factors, showed a non-significant increase in the rate of LBW, in HIV-positive compared to HIV-negative women (AOR 1.1; 95%CI 0.7-1.8, p=0.53) (Figure 6.4, Table 6.4). Maternal age remained significantly associated with LBW after adjusting for sociodemographic risk factors; in comparison to women <25 years, those aged 25-35 years (AOR2.7; 95%CI 1.3-5.4, p=0.007), and those ≥35 years (AOR 2.7; 95%CI 1.2-5.8, p=0.011), were almost 3 times more likely to deliver a LBW infant (Figure 6.4, Table 6.4).

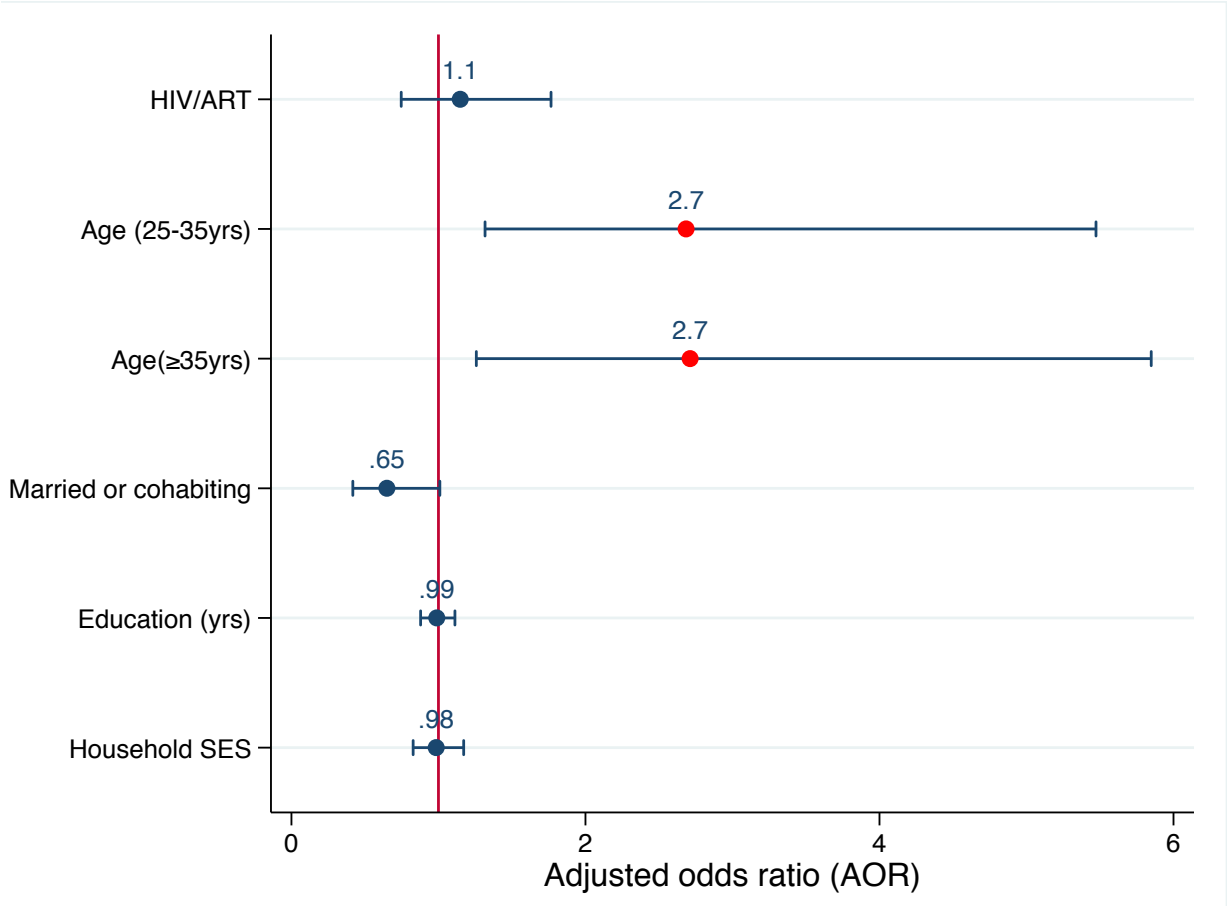

Figure 6.4: Multivariate logistic regression model for the association between HIV/ART and LBW, adjusting for sociodemographic factors. Reference group for HIV-positive women on HAART (HIV/ART) is HIV-negative women. Sociodemographic factors included are maternal age (reference <25years), whether participant is married or cohabiting (reference = no), years of formal education (continuous variable), and household socio-economic status (continuous variable). Effect estimates are reported as an adjusted OR (AOR) and 95%CI, and considered as statistically significant if the CI does not include 1 (red). Maternal age between 25-35 years and ≥ 35 years significantly increases the odds of PTB.

6.4.2.2.2 Model 2: Association between HIV/ART and LBW, adjusting for sociodemographic factors and clinical factors.

The second model, which adjusted for sociodemographic factors and clinical factors resulted in an increase in the effect of maternal HIV-infection on LBW from AOR1.1 to AOR1.6; however, the association remained non-significant (Figure 6.5, Table 6.4). Maternal age between 25-35 years was no longer statistically significant, possibly because of the reduced sample size (Table 6.4). Advanced maternal age (≥35 years) (AOR3.3; 95%CI 1.1-10.5, p=0.04), maternal BMI (AOR 0.9; 95%CI 0.9-0.9, p=0.04) and hypertension in pregnancy (AOR 2.8; 95%CI 1.3-6.1, p=0.01) were all strongly associated with LBW (Figure 6.5, Table 6.4).

6.4.2.2.3 Model 3: Association between HIV/ART and LBW, adjusting for sociodemographic factors, clinical factors and behavioural factors.

In the third model, that adjusted for demographic factors, clinical factors and behavioural factors (smoking and alcohol), maternal HIV-infection increased the odds of LBW by 60%; however, the association was not statistically significantly (Figure 6.6, Table 6.4). Advanced maternal age (≥35 years) remained the most significant risk factor for LBW (AOR 3.3; 95%CI 1.0-10.6, p=0.04), followed by hypertension in pregnancy (AOR 2.8; 95%CI1.3-6.1, p=0.01) (Figure 6.6, Table 6.4). Furthermore, every unit increase in maternal BMI was associated with a 9% reduction in the odds of delivering a LBW infant (AOR 0.9; 95%CI 0.8-0.9, p=0.03) (Figure 6.6, Table 6.4).

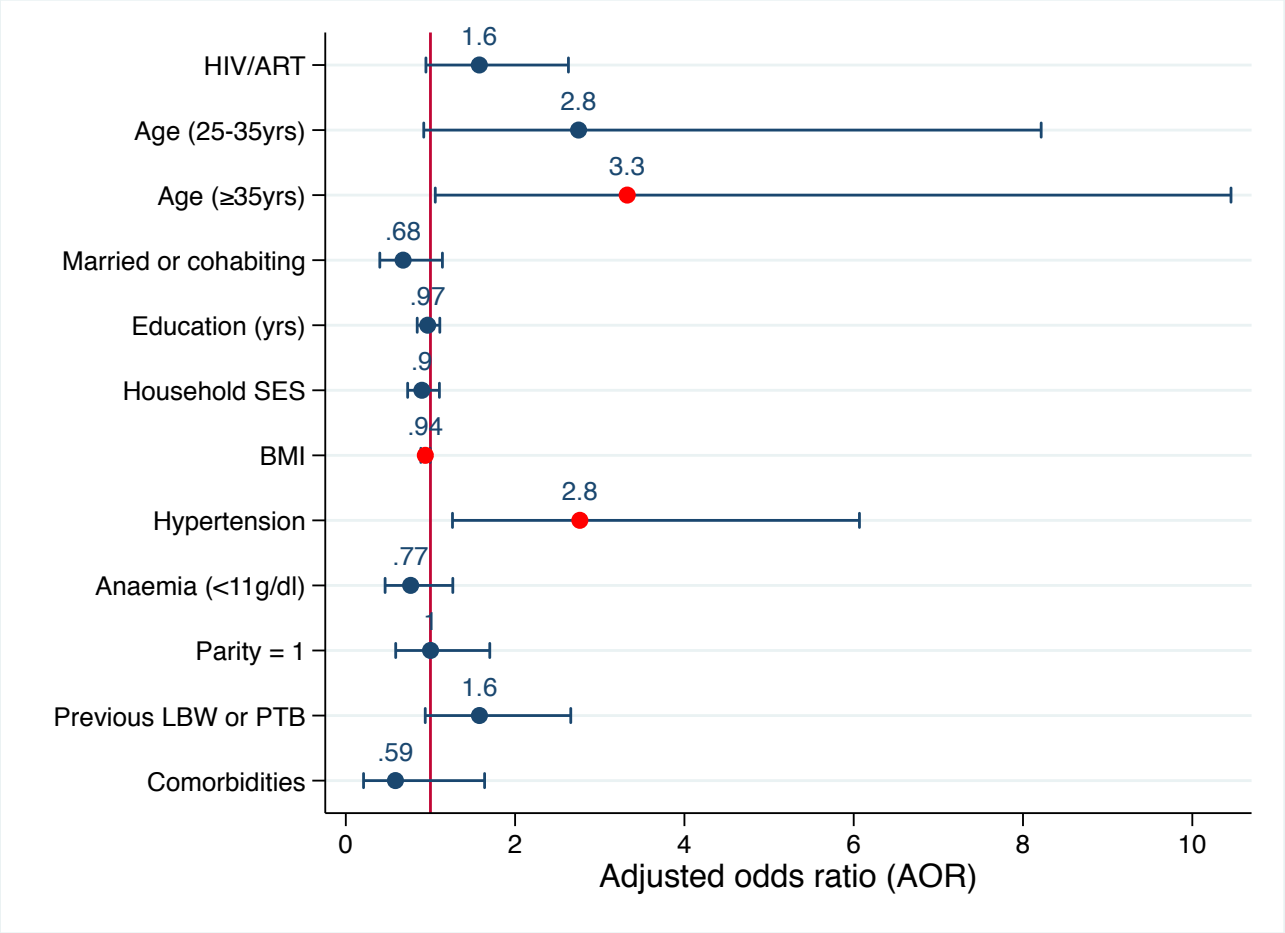

Figure 6.5: Multivariate logistic regression model for the association between HIV/ART and LBW, adjusting for sociodemographic factors and clinical factors. Reference group for HIV-positive women on HAART (HIV/ART) is HIV-negative women. Sociodemographic factors included are maternal age (reference <25years), whether participant is married or cohabiting (reference = no), years of formal education (continuous variable), and household socio-economic status (continuous variable). Clinical factors include BMI, hypertension in pregnancy (reference = no), anaemia in pregnancy (reference = no), parity (reference = 0), previous history of LBW or PTB (reference = no), any comorbidities during pregnancy (reference = no). Effect estimates are reported as an adjust OR (AOR) and 95%CI, and considered as statistically significant if the CI does not include 1 (red). Advanced maternal age (≥35 years), maternal BMI and hypertension in pregnancy were significantly associated with LBW.

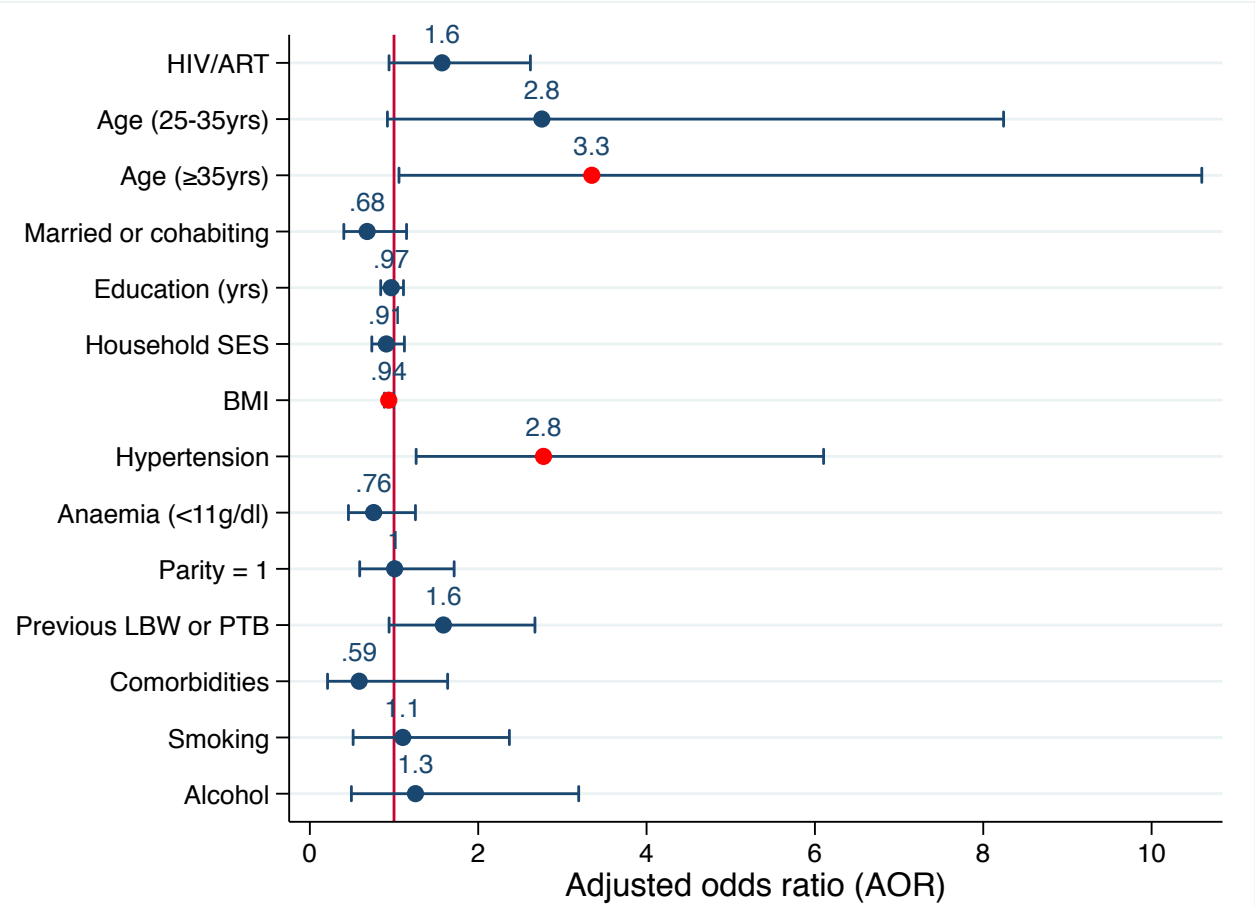

Figure 6.6: Multivariate logistic regression model for the association between HIV/ART and LBW, adjusting for sociodemographic factors, clinical factors and behavioural factors. Reference group for HIV-positive women on HAART (HIV/ART) is HIV-negative women. Sociodemographic factors included are maternal age (reference <25years), whether participant is married or cohabiting (reference = no), years of formal education (continuous variable), and household socio-economic status (continuous variable). Clinical factors include BMI, hypertension in pregnancy (reference = no), anaemia in pregnancy (reference = no), parity (reference = 0), previous history of LBW or PTB (reference = no), any comorbidities during pregnancy (reference = no). Behavioural factors include smoking during pregnancy (reference = no), or use of alcohol during pregnancy (reference = no). Effect estimates are reported as an adjusted OR and 95%CI, and considered as statistically significant if the CI does not include 1 (red). Advanced maternal age (≥35years), maternal BMI, and hypertension in pregnancy were significantly associated with LBW.

Table 6.4: Multivariate logistic regression analysis showing the association between exposures and low birthweight

Variables	Multivariate (AOR (95%CI)					
	Model 1 (n = 570)	**p-value**	**Model 2 (n = 446)**	**p-value**	**Model 3 (n = 446)**	**p-value**
Exposure of interest						
HIV status						
HIV-negative	Reference		Reference		Reference	
HIV-positive	1.1 (0.7 – 1.8)	0.53	1.6 (0.9 – 2.6)	0.08	1.6 (0.9 – 2.6)	0.08
Demographic factors						
Maternal age (years)						
<25 years	Reference		Reference		Reference	
25 - 35 years	2.7 (1.3 – 5.5)	0.007**	2.8 (0.9 – 8.2)	0.07	2.8 (0.9 – 8.2)	0.07
≥35 years	2.7 (1.3 – 5.8)	0.01*	3.3 (1.1 – 10.5)	0.04*	3.3 (1.1 – 10.6)	0.04*
Married or cohabiting						
No	Reference		Reference		Reference	
Yes	0.6 (0.4 – 1.0)	0.05	0.7 (0.4 – 1.1)	0.14	0.7 (0.4 – 1.1)	0.15
Years of formal education	1.0 (0.9 – 1.1)	0.84	1.0 (0.8 – 1.1)	0.64	1.0 (0.8 – 1.1)	0.64
Household SES	1.0 (0.8 – 1.2)	0.86	0.9 (0.7 – 1.1)	0.31	0.9 (0.7 – 1.1)	0.40
Clinical						
Maternal BMI (kg/m^2)			0.9 (0.9 – 0.9)	0.04*	0.9 (0.8 – 0.9)	0.03*
Maternal parity						
0			Reference			
1			1.0 (0.6 – 1.7)	1.0	1.0 (0.6 – 1.7)	0.98
≤2			Omitted		Omitted	
Previous adverse perinatal outcome [a]						
No			Reference		Reference	
Yes			1.6 (0.9 – 2.7)	0.09	1.6 (0.9 – 2.7)	0.08
Hypertensive in pregnancy [b]						
No			Reference		Reference	
Yes			2.8 (1.3 – 6.1)	0.01*	2.8 (1.3 – 6.1)	0.01*
Anaemia in pregnancy (<11g/dl) [c]						
No			Reference		Reference	
Yes			0.8 (0.5 – 1.2)	0.30	0.8 (0.4 – 1.3)	0.28
Maternal comorbidities [d]						
No			Reference		Reference	
Yes			0.6 (0.2 – 1.6)	0.31	0.6 (0.2 – 1.6)	0.31
Maternal behaviour						
Smoking						
No					Reference	
Yes					1.1 (0.5 - 2.4)	0.80
Alcohol						
No					Reference	
Yes					1.3 (0.5 - 3.2)	0.63

a = Previous adverse perinatal outcome, defined as a history of giving birth to a LBW (<2500g) or PTB (<37 weeks 0 days) infant; b = Hypertension in pregnancy includes women diagnosed with pregnancy induced hypertension, pre-eclampsia, severe pre-eclampsia, eclampsia or HELLP syndrome during current pregnancy; c = Anaemia defined as a haemoglobin of <11g/dl at any point during pregnancy; d = Maternal comorbidities include women diagnosed with any of the following conditions: thyroid disease, epilepsy, respiratory disease (e.g. chronic asthma), Crohn's disease, coeliac disease, ulcerative colitis or any severe malabsorption condition, lupus erythematosus, sickle-cell anaemia, pyelonephritis or kidney disease, genital tract or sexually transmitted infection, cholestasis, any other medical/surgical condition requiring treatment or referral; Abbreviations: BMI = body mass index, OR = odds ratio, PTB = preterm birth, SES = socio-economic status; * = statistically significant, p< 0.05 *; p<0.01 **; p<0.001***

6.4.3 Small for gestational age

A total of 568 women, of whom 359 (63.2%) were HIV-negative and 209 (36.8%) HIV-positive, provided data for the SGA outcome.

6.4.3.1 Univariate analysis

Maternal HIV/ART was significantly associated with SGA, and increased the odds of delivering a SGA infant by 60% (OR 1.6; 95%CI 1.0-2.7) (Table 6.5). Advanced maternal age was also significantly associated with SGA, and women in this group were 3 times more likely to deliver an SGA infant, than those <25 years (OR 3.0; 95%CI 1.3-7.3, p = 0.01) (Table 6.5). The remaining factors were not significantly associated with SGA.

6.4.3.2 Multivariate analysis

6.4.3.2.1 Model 1: Association between HIV/ART and SGA, adjusting for sociodemographic factors.

The adjustment for sociodemographic factors reduced the association between HIV/ART and SGA by 10%, from OR1.6 to AOR1.5, and was no longer statistically significant (AOR 1.5; 95%CI 0.9-2.4, p=0.12) (Figure 6.7, Table 6.6). However, advanced maternal age (≥35 years) was strongly associated with SGA, and women in this group were 3 times more likely to deliver an SGA infant than women <25 years (AOR 2.8; 95%CI 1.2-6.9, p=0.02) (Figure 6.7, Table 6.6). Maternal age 25-35 years was associated with a non-significant 2.1-fold increase in SGA (Figure 6.7, Table 6.6).

Table 6.5. Univariate logistic regression analysis of the association between SGA, HIV-infection, and demographic, behavioural and obstetric risk factors, reported as OR and 95%CI (n = 568).

Risk factor	SGA rate (%)	SGA (n/N)	OR (95%CI)	p-value
Exposure of interest				
HIV status				
HIV-negative	11.7%	42/359	Reference	
HIV-positive	18.2%	38/209	1.6 (1.0 – 2.7)	0.03*
Demographic factors				
Maternal age (years)				
<25 years	7.0%	7/100	Reference	
25 - 35 years	14.0%	44/314	2.2 (0.9 – 5.0)	0.07
≥ 35 years	18.8%	29/154	3.0 (1.3 – 7.3)	0.01*
Married or cohabiting				
No	15.1%	52/345	Reference	
Yes	12.6%	28/223	0.8 (0.4 – 1.3)	0.40
Years of formal education	-	-	0.9 (0.8 – 1.0)	0.40
Household SES	-	-	0.9 (0.7 – 1.1)	0.69
Clinical factors				
Maternal BMI (kg/m^2)			0.9 (0.9 – 1.0)	0.14
Underweight or normal weight (<24.9)	15.7%	33/210	Reference	
Overweight (25.0 - 29.9)	14.9%	32/215	0.9 (0.5 – 1.5)	0.81
Obese (≥30.0)	10.5%	15/143	0.6 (0.3 – 1.2	0.16
Maternal parity				
0	16.7%	14/84	Reference	
1	14.9%	37/249	0.8 (0.4 – 1.7)	0.69
≤2	11.7%	23/196	0.6 (0.3 – 1.3)	0.26
Previous adverse perinatal outcome [a]				
No	12.7%	40/316	Reference	
Yes	15.5%	20/129	1.3 (0.7 – 2.3)	0.43
Hypertensive in pregnancy [b]				
No	19.5%	103/528	Reference	
Yes	28.6%	12/42	1.4 (0.6 – 3.3)	0.33
Anaemia in pregnancy (<11g/dl) [c]				
No	21.1%	53/250	Reference	
Yes	19.1%	61/319	1.0 (0.6 – 1.7)	0.70
Maternal comorbidities [d]				
No	20.1%	104/518	Reference	
Yes	21.2%	11/52	0.9 (0.4 – 2.2)	0.93
Maternal behaviour				
Smoking				
No	19.1%	96/504	Reference	
Yes	28.8%	19/66	1.5 (0.8 – 3.0)	0.16
Alcohol				
No	20.1%	105/523	Reference	
Yes	21.3%	10/47	1.0 (0.4 – 2.4)	0.86
Obstetric factors				
Mode of delivery				
Vaginal	16.7%	40/240	Reference	
Caesarean section	22.7%	75/330	1.3 (0.8 – 2.1)	0.256

a = Previous adverse perinatal outcome, defined as a history of giving birth to a LBW (<2500g) or PTB (<37 weeks 0 days) infant; b = Hypertension in pregnancy includes women diagnosed with pregnancy induced hypertension, pre-eclampsia, severe pre-eclampsia, eclampsia or HELLP syndrome during current pregnancy; c = Anaemia defined as a haemoglobin of <11g/dl at any point during pregnancy; d = Maternal comorbidities include women diagnosed with any of the following conditions: thyroid disease, epilepsy, respiratory disease (e.g. chronic asthma), Crohn's disease, coeliac disease, ulcerative colitis or any severe malabsorption condition, lupus erythematosus, sickle-cell anaemia, pyelonephritis or kidney disease, genital

tract or sexually transmitted infection, cholestasis, any other medical/surgical condition requiring treatment or referral; Abbreviations: BMI = body mass index, OR = odds ratio, PTB = preterm birth, SES = socio-economic status; * = statistically significant, p< 0.05 *; p<0.01 **; p<0.001***

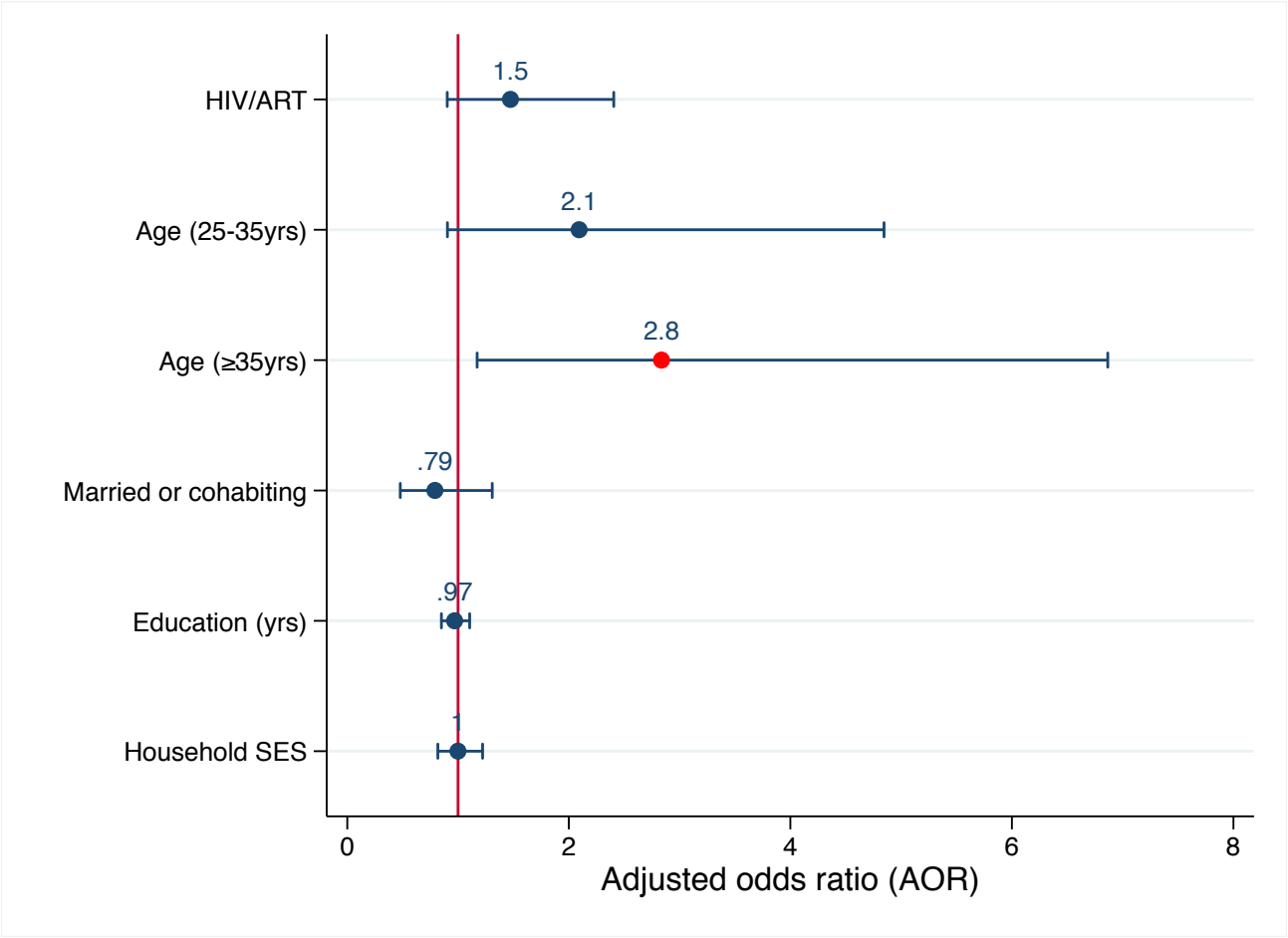

Figure 6.7: Multivariate logistic regression model for the association between HIV/ART and SGA, adjusting for sociodemographic factors. Reference group for HIV-positive women on HAART (HIV/ART) is HIV-negative women. Sociodemographic factors included are maternal age (reference <25years), whether participant is married or cohabiting (reference = no), years of formal education (continuous variable), and household socio-economic status (continuous variable). Effect estimates are reported as an adjusted OR (AOR) and 95%CI, and considered as statistically significant if the CI does not include 1 (red). Advance maternal age (≥ 35 years) significantly increases the odds of SGA.

6.4.3.2.2 Model 2: Association between HIV/ART and SGA, adjusting for sociodemographic factors and clinical factors.

In model two, which included sociodemographic and clinical factors, maternal HIV/ART was associated with a 60% increased odds of SGA; however, this association was not statistically significant (Figure 6.8, Table 6.6), nor were the remaining factors statistically significant (Figure 6.8, Table 6.6).

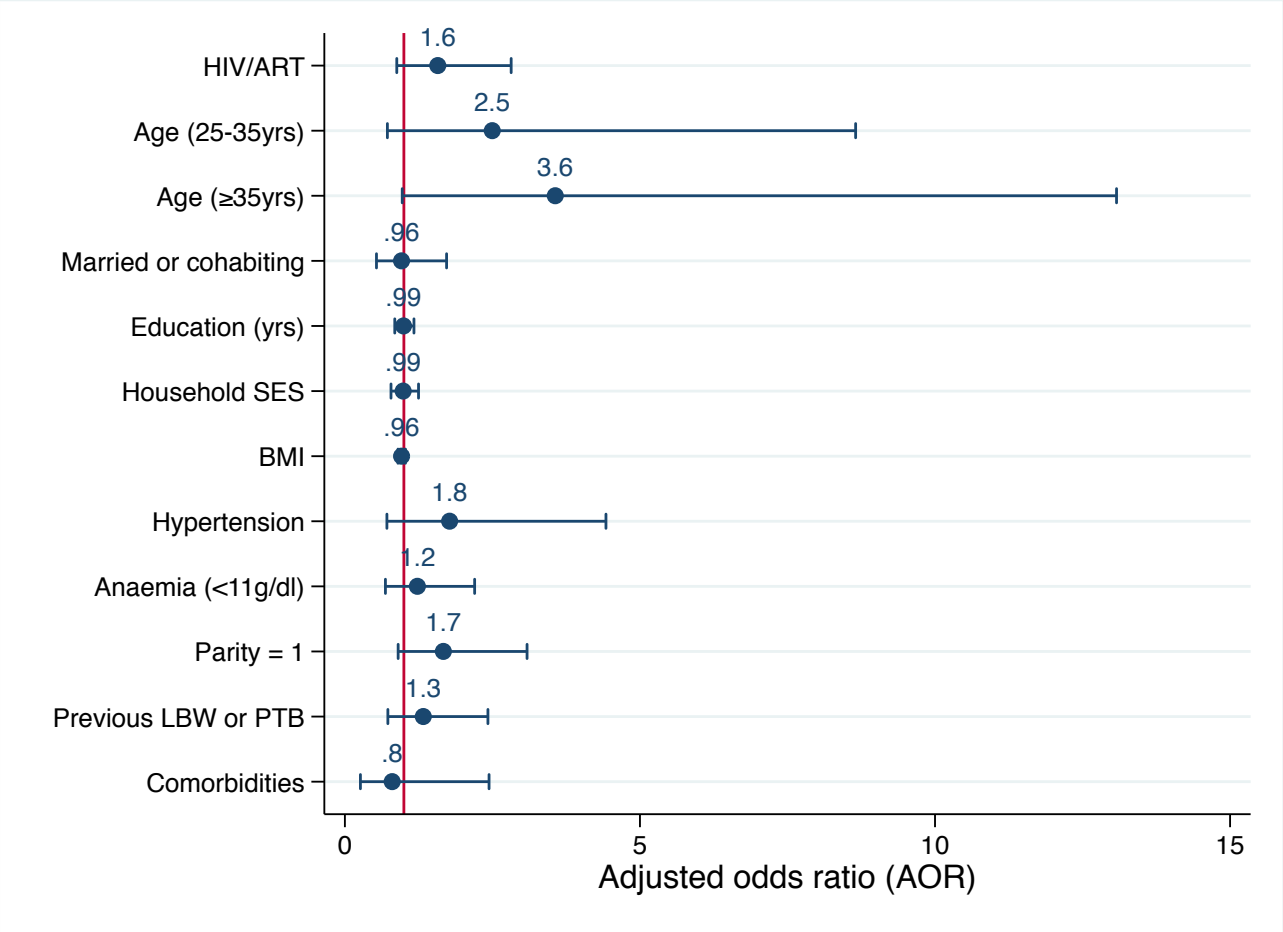

Figure 6.8: Multivariate logistic regression model for the association between HIV/ART and SGA, adjusting for sociodemographic factors and clinical factors. Reference group for HIV-positive women on HAART (HIV/ART) is HIV-negative women. Sociodemographic factors included are maternal age (reference <25years), whether participant is married or cohabiting (reference = no), years of formal education (continuous variable), and household socio-economic status (continuous variable). Clinical factors include BMI, hypertension in pregnancy (reference = no), anaemia in pregnancy (reference = no), parity (reference = 0), previous history of LBW or PTB (reference = no), any comorbidities during pregnancy (reference = no). Effect estimates are reported as an adjust OR (AOR) and 95%CI, and considered as statistically significant if the CI does not include 1 (red). None of the variables were independently associated with SGA.

6.4.3.2.3 Model 3: Association between HIV/ART and SGA, adjusting for sociodemographic factors, clinical and behavioural factors.

In model three, which included sociodemographic, clinical and behavioural factors, maternal HIV/ART was associated with a 50% increased odds of SGA; however, this association was not statistically significant (Figure 6.9, Table 6.6), nor were the remaining factors statistically significant (Figure 6.9, Table 6.6).

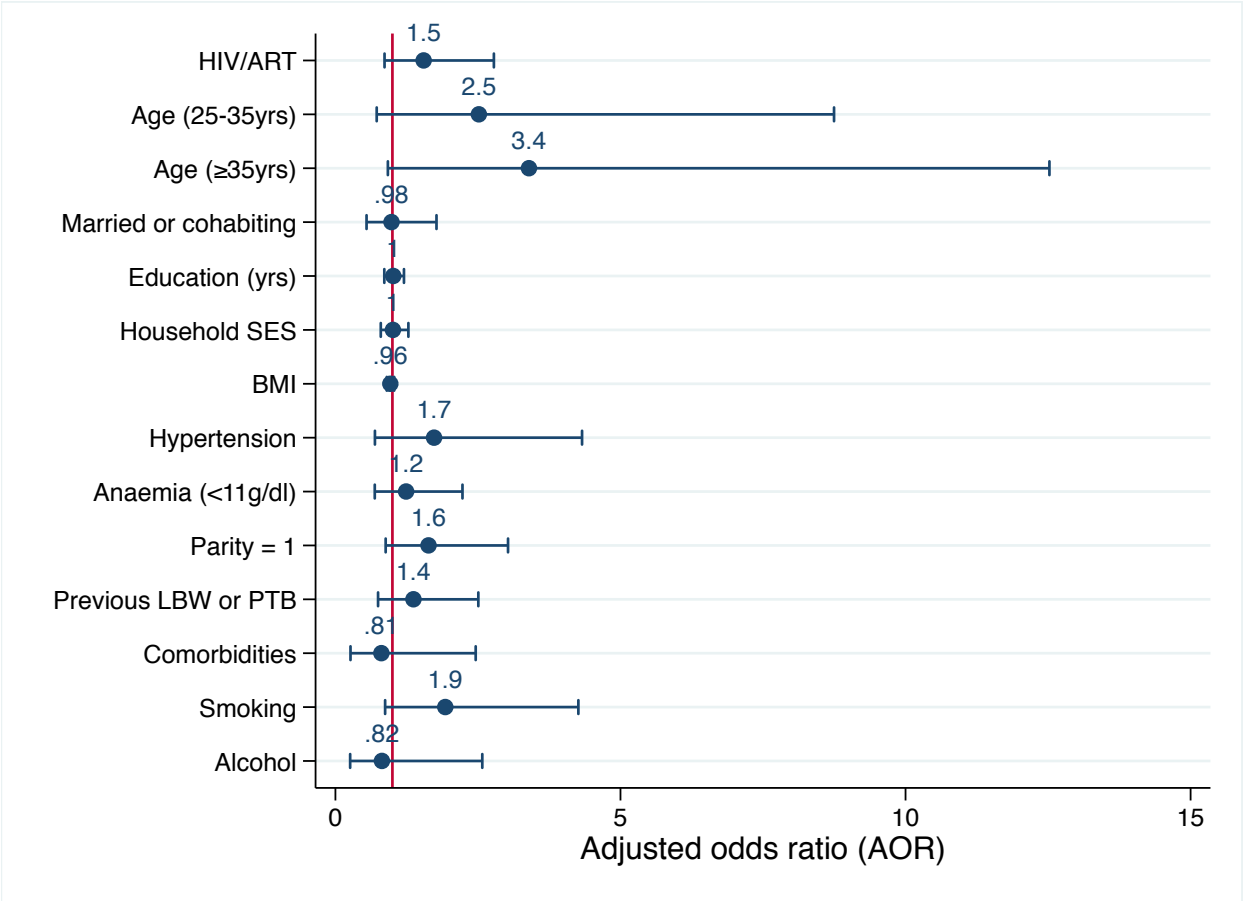

Figure 6.9: Multivariate logistic regression model for the association between HIV/ART and SGA, adjusting for sociodemographic factors, clinical factors and behavioural factors. Reference group for HIV-positive women on HAART (HIV/ART) is HIV-negative women. Sociodemographic factors included are maternal age (reference <25years), whether participant is married or cohabiting (reference = no), years of formal education (continuous variable), and household socio-economic status (continuous variable). Clinical factors include BMI, hypertension in pregnancy (reference = no), anaemia in pregnancy (reference = no), parity (reference = 0), previous history of LBW or PTB (reference = no), any comorbidities during pregnancy (reference = no). Behavioural factors include smoking during pregnancy (reference = no), or use of alcohol during pregnancy (reference = no). Effect estimates are reported as an adjusted OR and 95%CI, and considered as statistically significant if the CI does not include 1 (red).
None of the variables were independently associated with SGA.

Table 6.6: Multivariate logistic regression analysis showing the association between exposures and small for gestational age

Variables	Multivariate (AOR (95%CI)					
	Model 1 (n = 568)	**p-value**	**Model 2 (n = 444)**	**p-value**	**Model 3 (n = 444)**	**p-value**
Exposure of interest						
HIV status						
HIV-negative	Reference		Reference		Reference	
HIV-positive	1.5 (0.9 – 2.4)	0.12	1.6 (0.9 – 2.8)	0.13	1.5 (0.9 – 2.8)	0.14
Demographic factors						
Maternal age (years)						
<25 years	Reference		Reference		Reference	
25 - 35 years	2.1 (0.9 – 4.8)	0.08	2.5 (0.7 – 8.7)	0.146	2.5 (0.7 – 8.7)	0.15
≥35 years	2.8 (1.2 – 6.9)	0.02*	3.6 (0.9 – 13.1)	0.06	3.4 (0.9 – 12.5)	0.07
Married or cohabiting						
No	Reference		Reference		Reference	
Yes	0.8 (0.5 – 1.3)	0.36	1.0 (0.5 – 1.7)	0.89	1.0 (0.5 – 1.8)	0.96
Years of formal education	1.0 (0.9 – 1.1)	0.63	1.0 (0.8 – 1.1)	0.95	1.0 (0.9 – 1.2)	0.86
Household SES	1.0 (0.8 – 1.2)	1.00	1.0 (0.8 – 1.2)	0.91	1.0 (0.8 – 1.3)	0.86
Clinical factors						
Maternal BMI (kg/m^2)			1.0 (0.9 – 1.0)	0.24	1.0 (0.9 – 1.0)	0.28
Maternal parity						
0			Reference			
1			1.7 (0.9 – 3.1)	0.10	1.6 (0.9 – 3.0)	0.12
≤2			Omitted		Omitted	
Previous adverse perinatal outcome [a]						
No			Reference		Reference	
Yes			1.3 (0.7 – 2.4)	0.35	1.4 (0.7 – 2.5)	0.31
Hypertensive in pregnancy [b]						
No			Reference		Reference	
Yes			1.8 (0.7 – 4.4)	0.28	1.7 (0.7 – 4.3)	0.24
Anaemia in pregnancy (<11g/dl) [d]						
No			Reference		Reference	
Yes			1.2 (0.7 – 2.2)	0.49	1.2 (0.7 – 2.2)	0.47
Maternal comorbidities [c]						
No			Reference		Reference	
Yes			0.8 (0.3 – 2.4)	0.70	0.8 (0.3 – 2.5)	0.71
Maternal behaviour						
Smoking						
No					Reference	
Yes					1.9 (0.9 – 4.3)	0.11
Alcohol						
No					Reference	
Yes					0.8 (0.3 – 2.6)	0.73

a = Previous adverse perinatal outcome, defined as a history of giving birth to a LBW (<2500g) or PTB (<37 weeks 0 days) infant; b = Hypertension in pregnancy includes women diagnosed with pregnancy induced hypertension, pre-eclampsia, severe pre-eclampsia, eclampsia or HELLP syndrome during current pregnancy; c = Anaemia defined as a haemoglobin of <11g/dl at any point during pregnancy; d = Maternal comorbidities include women diagnosed with any of the following conditions: thyroid disease, epilepsy, respiratory disease (e.g. chronic asthma), Crohn's disease, coeliac disease, ulcerative colitis or any severe malabsorption condition, lupus erythematosus, sickle-cell anaemia, pyelonephritis or kidney disease, genital tract or sexually transmitted infection, cholestasis, any other medical/surgical condition requiring treatment or referral; abbreviations: BMI = body mass index, OR = odds ratio, PTB = preterm birth, SES = socio-economic status; * = statistically significant, p< 0.05; ** p<0.01; *** p<0.001.

6.5 Discussion

The aim of this study was to determine whether treated maternal HIV-infection increases the risk of PTB, LBW or SGA independently of sociodemographic, clinical and behavioural risk factors, in sub-Saharan African women with pregnancies dated accurately by early ultrasound (<14 weeks' gestation). The principal finding was that treated maternal HIV-infection is not independently associated with PTB, LBW or SGA within this context.

A previous history of delivering a preterm or LBW infant was significantly and positively associated with PTB. Advanced maternal age (≥35 years), hypertension and BMI were independently associated with LBW; advanced maternal age increased the odds of giving birth to a LBW infant 3-fold, while hypertension in pregnancy was associated with a 2.8-fold increase in LBW. Similar to what has previously been reported, an increase in BMI was associated with a decline in LBW[395]. None of the variables assessed were independently associated with SGA, possibly due to the limited sample size.

To the best of my knowledge, this is the first sub-Saharan African study to identify the determinants of PTB, LBW and SGA in black African women with pregnancies dated by early ultrasound. Furthermore, sufficient information was collected on a broad range of well-established risk factors for PTB, LBW and SGA and used in robust statistical analyses to assess both the strength and direction of the associations between maternal risk factors and perinatal outcomes.

Healthy HIV-positive women, who are well controlled on a predominantly NNRTI-based HAART regimen, do not have a significantly increased risk of PTB, LBW and SGA compared to HIV-negative women. While the observed results are most likely because the study was under powered (power calculation: PTB = 7.9%, LBW = 23.9%, SGA = 57.1%), one would need to assess at least 26,580 pregnant women (HIV-negative = 19935, HIV-positive 6645) in order to have sufficient power (80% power) to reject the null hypothesis for PTB, the most severe adverse perinatal outcome.

This would not be feasible or cost-effective, and suggests that the association between HIV/ART within this context is sufficiently small that the benefits of NNRTI-based HAART in pregnancy for the mother, baby and community, outweigh the risks of adverse perinatal outcome.

The sociodemographic and clinical determinants found to be independently associated with PTB (previous history of PTB or LBW), and LBW (advanced maternal age, hypertension and BMI), confirm findings from other studies conducted in sub-Saharan Africa[280,394]. A previous history of PTB or LBW was associated with a 1.7-fold increase in the odds of delivering preterm. This is slightly lower than estimates reported by Mercer et al (1999)[396] who observed a 2.5-fold increase in the risk of PTB in women reporting a previous PTB. The fact that this was the only risk factor independently associated with PTB may be because this study only included black African women from a low-income community, who are inherently at risk for PTB[392].

The incidence of PTB in Black women in the USA and UK ranges from 16-18% - which is similar to estimates in HIV-negative women in this cohort (18.6%) - versus 5-9% in white women in these communities[392]. While this may be related to biological – most probably infectious – factors not investigated in this study, it may also be a symptom of the pervasive structural inequalities experienced by most black South Africans[125], including pregnant women in this setting. Such socio-economic challenges could substantially increase the level of stress experienced by these women, and stress has been associated with a 2-fold increase in PTB[392,397]. The fact that in this cohort, spontaneous PTB was independently associated with household SES (AOR 0.7; 95%CI 0.6-0.9, p=0.01) after adjusting for sociodemographic, clinical and behavioural factors, suggests that this is a hypothesis that needs to be explored in future studies. Future population-based studies within the sub-Saharan African context, should include women of different ethnicities and socio-economic backgrounds in order to assess whether the high rates of PTB observed in this setting are specific to Black women of a specific socio-economic status or whether the effect is mediated by alternative biological or social factors.

Chapter 7: Final discussion and policy implication

This thesis explored the impact of maternal HIV-infection and ART on perinatal outcomes using three different, but robust methodologies. Each chapter contains a detailed discussion on the results of each study and the implications of these finding. In short, in Chapter 3 a systematic review and pairwise meta-analysis of observational data was used to answer the question of whether ART-naïve maternal HIV-infection is associated with adverse perinatal outcomes. It showed that ART-naïve maternal HIV-infection significantly increases the risk of PTB, LBW, SGA and stillbirth. This effect was most prominent within the sub-Saharan African context, it persisted even after the adjustment for confounders and was directly correlated with the clinical stage of HIV.

In Chapter 4, a systematic review, pairwise, and network meta-analysis of RCTs was used to assess (1) whether ART is associated with adverse perinatal outcomes, and (2) to determine which ART is associated with the lowest risk of adverse perinatal outcome, and still has a high efficacy for PMTCT. The evidence in this chapter showed unequivocally that antenatal ART is associated with adverse perinatal outcomes, and significantly increases the risk of PTB, sPTB, LBW and VLBW. Furthermore, it showed that the effect of ART on perinatal outcomes varied depending on the class and regimen initiated, with PI-based ARTs and HAART regimens associated with the highest risks of adverse perinatal outcomes. LPV/r monotherapy was found to be the most efficacious ART, with no significant risk of adverse perinatal outcome; however, as it does not cross the placental barrier – thus cannot provide peri-exposure prophylaxis for the infant – it cannot be recommended for PMTCT[209,324]. PI and NNRTI-based HAART regimens were found to be highly efficacious for PMTCT, but they were also associated with the highest risk of adverse perinatal outcome. PI-based HAART in particular significantly increased the risk of PTB, sPTB, LBW and VLBW.

Lastly, Chapter 5 and 6 sought to determine whether maternal HIV-infection/NNRTI-HAART increased the risk of adverse perinatal outcomes in a "real-world" context, independently of known

confounding. The SHAPOSSA study, showed a significant association between maternal HIV/HAART and SGA. However, this association disappeared following adjustment for confounding factors. While maternal HIV/NNRTI-HAART was not independently associated with PTB, LBW or SGA within this cohort; LBW was independently associated with advanced maternal age (≥35 years), hypertension and BMI, and a previous history of giving birth to a preterm or LBW infant was the only risk factor independently associated with PTB. The SHAPOSSA study also confirmed that HIV-negative pregnant women in this region have a high background incidence of all adverse perinatal outcomes, including PTB, LBW and SGA. The combination of weak health systems, pervasive social inequity and the rapid socioeconomic and epidemiological transition in the South African context[124], meant that underlying clinical and social risk factors contributed to the high risk of adverse perinatal outcomes observed, particularly in HIV-negative women. However, the findings of this thesis suggest that HAART could also be driving the effect in HIV-positive women, and the limited power of the study precluded the detection of this association in the cohort study.

7.1 Overall policy implications and recommendations

The policy implications, recommendations and research gaps that should be explored have already been discussed in individual chapters. However, it is important to point out the implications of the overall findings of this thesis, which are centred around 3 key policies (1) Acceleration of ART coverage for PMTCT, (2) specific ART initiated during pregnancy and, (3) need for accelerated health system strengthening to improve maternal and child health in developing countries.

7.1.1 Acceleration of ART coverage for PMTCT

While the linkage of HIV-services and PMTCT has significantly improved on the coverage of PMTCT programs, 7 of the 20 priority countries have achieved a PMTCT coverage of less than 80%, with Nigeria (30%), Angola (40%) and Chad (46%) achieving a PMTCT coverage of <50%[130]. The evidence in this thesis suggests that in addition to a high rate of MTCT, and maternal morbidity and

mortality, the reduced PMTCT coverage in these countries will significantly increase the burden of stillbirths and babies born preterm, LBW and SGA, particularly in women with advanced disease. The evidence presented in this thesis supports recommendations to initiate all HIV-positive women of reproductive age on ART[12,181,324]. While this may not directly reduce the rate of adverse perinatal outcome, it will improve maternal health before conception and during pregnancy, and will significantly reduce MTCT, both of which would indirectly improve perinatal outcomes in these setting.

7.1.2 Specific ART initiated during pregnancy

While the benefits of ART clearly still outweigh the risks, this thesis has shown that the unintended negative consequences of certain ART are both substantial and severe. Previous inconsistencies in the literature meant that there was uncertainty as to the safest and most efficacious ART to initiated during pregnancy[324]. This is the first document to provide conclusive evidence on the ranking of ART for PMTCT according to its safety and efficacy profile. PI-based HAART is recommended in pregnancy in Europe[324], USA[181] and in developing countries[12]. The evidence suggests that PI-based HAART is associated with the highest risk of adverse perinatal outcomes (PTB, sPTB, LBW, VLBW) and should be used with caution in pregnancy. In instances where both PI-based HAART and NNRTI-based HAART are available e.g. UK and US, clinicians should preferably initiate a NNRTI-based HAART regimen, as it was shown to be more efficacious than PI-based HAART, and was only associated with LBW.

InSTI-based HAART has not been recommended as first line treatment in pregnancy by WHO[12] or in Europe[324] because of insufficient safety data. It is comparatively superior with regards to virological suppression than both PI and NNRTI-based HAART[178], particularly in women[189-191], and to date, no associated risk of adverse perinatal outcome has been reported, which is probably why it is the recommended first line treatment for pregnant women in the USA[181]. In addition to a lack of safety data, a second challenge to the recommendation of InSTIs for developing countries is its

enormous cost. Governments pay an estimated $32,000 per person/year for the patented co-formulated DTG-based HAART regimen, as oppose to the estimated $110 to $184 per person/year paid for the first-line, co-formulated, EFV-based HAART regimen[12,398,399]. The potential of InSTIs as possibly the safest option for PMTCT is significant, and requires urgent attention. Research effort should be directed towards the generation of RCT data on the effects of InSTIs in pregnancy. Furthermore, cost-effectiveness analyses should be conducted on PI, NNRTI and InSTIs taking into account short- and long-term cost-saving associated with ARTs that avert the development of PTB, LBW and SGA.

7.1.3 Need for accelerated health system strengthening to improve maternal and child health in developing countries.

The largest burden of maternal HIV-infections occurs in sub-Saharan Africa, and the WHO's recommendation that all women initiate NNRTI-based HAART (first-line) or PI-based HAART (second line) in pregnancy will significantly increase the burden of PTB, sPTB, LBW and VLBW in these settings[12]. These infants will be managed by already overburdened and under-resourced health systems, leading to high neonatal and under-5 mortality. Resilient and dynamic health systems that address both the supply (health system) and demand side (patient) challenges are crucial to addressing poor maternal health and the large burden of adverse perinatal outcomes that occur within these settings[14].

In light of the findings of this thesis, policy makers need to re-prioritise the response to HIV-infection so that the focus is not purely on the prevention of new HIV-infections in women of reproductive age, and PMTCT. Policy makers should galvanise efforts to better understand the implications of HIV-infection and ART on perinatal outcomes, how best to reduce the incidence of these outcomes in HIV endemic countries and identify better strategies of managing infants born with these conditions to achieve the best outcomes. As we move towards an era where all women will be initiated on HAART before conception through the wide adoption of the Option B+ policy[12],

mechanisms to monitor trends in the burden of adverse perinatal outcomes need to be escalated. This can be achieved by the strengthening of civil registration and vital statistic processes in developing countries. These efforts will generate robust data that can be used by countries to assess the extent to which HAART contributes to the burden of adverse perinatal outcomes within these contexts, and can guide policy decisions.

The high burden of poor maternal health and adverse perinatal outcomes observed in both HIV-positive and HIV-negative women in the SHAPOSSA cohort, supports the need for policy makers to continuously re-evaluate and re-assess health system weaknesses at the three-main maternal and child health delivery platforms i.e. community level, primary healthcare and referral hospitals[19]. Structural weaknesses at these delivery platforms – e.g. barriers to healthcare access, poor quality healthcare services, dilapidated infrastructure, shortages of trained healthcare providers, insufficient quantity or poor quality of medication and equipment etc. – pose significant barriers to the improvement of maternal and perinatal health outcomes in these settings[19,24]. A host of feasible, high quality evidence based interventions have been identified, and shown to significantly improve maternal health and perinatal outcomes[19]. However, these interventions need to be implemented at the three service delivery platforms, using a continuum of care approach that will ensure that HIV-positive and HIV-negative women within these settings gain access to high quality maternal and reproductive health services along the life course[19,20].

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Appendix 1: Publication, conference abstract and presentations arising from this research

	Relevant Thesis Chapter
Original manuscripts	
Wedi, COO.; Kirtley, S.; Hopewell, S.; Corrigan, R.; Kennedy, SH.; Hemelaar, J. Perinatal outcomes associated with maternal HIV infection: a systematic review and meta-analysis. Lancet HIV 2016; 3(1): e33-48	3
Conference abstracts and presentations	
Wedi, COO.; Norris, S.; Kennedy, SH.; Hemelaar, J. Soweto HIV and ART pregnancy study: the influence of HIV and antiretroviral treatment on perinatal outcomes in Soweto, South Africa (SHAPOSSA study). 21st International AIDS Conference, Durban, South Africa, 18-22 July 2016. Abstract TUPEB039 (Poster presentation).	5
Wedi, COO.; Kirtley, S.; Hopewell, S.; Corrigan, R.; Kennedy, SH.; Hemelaar, J. Perinatal outcomes associated with Maternal HIV infection: A systematic Review and Meta-analysis. SA HIV Clinicians Society Conference, Johannesburg, South Africa, 13-16 April 2016. Abstract TUPEB187 (Oral presentation).	3
Wedi, COO.; Norris, S.; Kennedy, SH.; Hemelaar, J. The influence of maternal HIV-infection and antiretroviral therapy on perinatal outcomes: Implications for the sub-Saharan African context. 9th DOHad World Congress, Satellite Workshop, Cape Town, South Africa, 8-11 November 2015 (Oral presentation).	3 & 5

Perinatal outcomes associated with maternal HIV infection: a systematic review and meta-analysis

Chrystelle O O Wedi, Shona Kirtley, Sally Hopewell, Ruth Corrigan, Stephen H Kennedy, Joris Hemelaar

Summary

Background The HIV pandemic affects 36·9 million people worldwide, of whom 1·5 million are pregnant women. 91% of HIV-positive pregnant women reside in sub-Saharan Africa, a region that also has very poor perinatal outcomes. We aimed to establish whether untreated maternal HIV infection is associated with specific perinatal outcomes.

Methods We did a systematic review and meta-analysis of the scientific literature by searching PubMed, CINAHL (Ebscohost), Global Health (Ovid), EMBASE (Ovid), and the Cochrane Central Register of Controlled Trials and four clinical trial databases (WHO International Clinical Trials Registry Platform, the Pan African Clinical Trials Registry, the ClinicalTrials.gov database, and the ISRCTN Registry) for studies published from Jan 1, 1980, to Dec 7, 2014. Two authors independently reviewed the studies retrieved by the scientific literature search, identified relevant studies, and extracted the data. We investigated the associations between maternal HIV infection in women naive to antiretroviral therapy and 11 perinatal outcomes: preterm birth, very preterm birth, low birthweight, very low birthweight, term low birthweight, preterm low birthweight, small for gestational age, very small for gestational age, miscarriage, stillbirth, and neonatal death. We included prospective and retrospective cohort studies and case-control studies reporting perinatal outcomes in HIV-positive women naive to antiretroviral therapy and HIV-negative controls. We used a random-effects model for the meta-analyses of specific perinatal outcomes. We did subgroup and sensitivity analyses and assessed the effect of adjustment for confounders. This systematic review and meta-analysis is registered with PROSPERO, number CRD42013005638.

Findings Of 60 750 studies identified, we obtained data from 35 studies (20 prospective cohort studies, 12 retrospective cohort studies, and three case-control studies) including 53 623 women. Our meta-analyses of prospective cohort studies show that maternal HIV infection is associated with an increased risk of preterm birth (relative risk 1·50, 95% CI 1·24–1·82), low birthweight (1·62, 1·41–1·86), small for gestational age (1·31, 1·14–1·51), and stillbirth (1·67, 1·05–2·66). Retrospective cohort studies also suggest an increased risk of term low birthweight (2·62, 1·15–5·93) and preterm low birthweight (3·25, 2·12–4·99). The strongest and most consistent evidence for these associations is identified in sub-Saharan Africa. No association was identified between maternal HIV infection and very preterm birth, very small for gestational age, very low birthweight, miscarriage, or neonatal death, although few data were available for these outcomes. Correction for confounders did not affect the significance of these findings.

Interpretation Maternal HIV infection in women who have not received antiretroviral therapy is associated with preterm birth, low birthweight, small for gestational age, and stillbirth, especially in sub-Saharan Africa. Research is needed to assess how antiretroviral therapy regimens affect these perinatal outcomes.

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Introduction

Maternal HIV infection, with its associated maternal morbidity and mortality and risk of mother-to-child transmission of HIV, affected the outcomes of three millennium development goals (MDGs): the reduction of child mortality (MDG 4), the improvement of maternal health (MDG 5), and the combating of HIV/AIDS and other diseases (MDG 6).[1] The disease burden caused by HIV infection is reduced by antiretroviral therapy, but coverage is incomplete.[2] In part because of incomplete antiretroviral therapy coverage, MDG targets were not met, and high rates of maternal and child mortality and morbidity persist, particularly in sub-Saharan Africa where 91% of HIV-positive pregnant women live.[1–3]

Whether maternal HIV infection affects perinatal outcomes, which are major contributors to poor health worldwide, is unknown. An estimated 2·6 million stillbirths,[4] 2·8 million neonatal deaths,[5] 14·9 million preterm births,[6] 32·4 million small-for-gestational-age births,[7] and 18 million low birthweight babies[7] occur every year. These outcomes are inter-related, with preterm birth being the leading cause of neonatal and child mortality,[5,8] fetal growth restriction being associated with stillbirths,[9] and 41% of low birthweight infants being preterm.[7] Sub-Saharan Africa has the highest rates of stillbirths and neonatal deaths worldwide,[4,10] and nine of the 11 countries with the highest preterm birth rates are also in sub-Saharan Africa,[6] which has the second highest small for

Research in context

Evidence before this study

A systematic review and meta-analysis on perinatal outcomes associated with maternal HIV infection was published in 1998. Only publications up to 1996 were included, many of which were abstracts. The perinatal outcomes were poorly or not defined, gestational age estimation methods were not assessed, and a fixed-effects model was used to pool the data despite substantial between-study heterogeneity.

Added value of this study

We have done a systematic review and meta-analysis including studies published up to 2014, at a stage in the epidemic when an increasing number of pregnant women worldwide are receiving antiretroviral therapy, making the likelihood of new evidence emerging in the future low. Perinatal outcomes were clearly defined a priori, inclusion and exclusion criteria were strictly applied to reduce bias, and a random-effects model was used to pool the data. Our study shows that antiretroviral therapy-naive maternal HIV infection is strongly associated with increased risks of preterm birth, low birthweight, small for gestational age, and stillbirth, and weakly associated with term low birthweight and preterm low birthweight. The evidence did not support an effect on very preterm birth, very low birthweight, very small for gestational age, miscarriage, or neonatal death.

Implications of all the available evidence

The evidence for an association of maternal HIV infection with adverse perinatal outcomes is strongest and most consistent in sub-Saharan Africa, the region with the highest burden of maternal HIV infection. Antiretroviral therapy reduces maternal morbidity and mortality and greatly reduces mother-to-child transmission of HIV, but the effect of different antiretroviral therapy regimens on perinatal outcomes is unknown. WHO recommends triple-drug antiretroviral therapy regimens during pregnancy for all HIV-positive women and lifelong treatment thereafter. With continued expansion of treatment programmes, especially in sub-Saharan Africa, an urgent need exists to assess the effect of antenatal antiretroviral therapy regimens on perinatal outcomes.

gestational age and low birthweight rates after south Asia.[7] Therefore, sub-Saharan Africa carries very high rates of adverse perinatal outcomes and the highest burden of maternal HIV infection. Because an increasing number of pregnant women worldwide are receiving antiretroviral therapy, the likelihood of new data emerging on the associations between treatment naive HIV and perinatal outcomes is low.

We did a systematic review and meta-analysis with the aim of establishing whether antiretroviral therapy-naive maternal HIV infection is associated with 11 specific perinatal outcomes.

Methods

Search strategy and selection criteria

We did this systematic review and meta-analysis according to a protocol developed with the Cochrane guidelines.[11] We searched five electronic scientific literature databases (PubMed, CINAHL (Ebscohost), Global Health (Ovid), EMBASE (Ovid), and the Cochrane Central Register of Controlled Trials) and four trial databases (WHO International Clinical Trials Registry Platform, the Pan African Clinical Trials Registry, ClinicalTrials.gov database, and the ISRCTN Registry) to identify studies published between Jan 1, 1980, and Dec 7, 2014. In an effort to ensure that we identified all relevant studies, we developed two search strategies and adapted these for each database (appendix, pp 1–12). We used the search terms "HIV", "antiretroviral therapy", "pregnancy outcome", "preterm birth", "intrauterine growth restriction", "low birthweight", and "stillbirth". No method or language restrictions were applied and studies from all countries were eligible. We included full text articles and abstracts. The references of studies included in this analysis, and a review published in 1998,[12] were assessed for additional citations. Study investigators were contacted when the full text manuscript contained insufficient information.[13] Inclusion criteria for studies were randomised controlled trials, prospective and retrospective cohorts, case-control, and cross-sectional studies. COOW obtained and screened full text manuscripts meeting the eligibility criteria: population (pregnant women with known perinatal outcomes); exposure (HIV-infection, contracted before or during pregnancy, irrespective of the route of transmission; women should not have been exposed to antiretroviral therapy during pregnancy, with the exception of a single dose at delivery); and comparison (HIV-negative pregnant women from the same community as the HIV-positive participants).

Studies were excluded if they reported ambiguous eligibility criteria, no HIV-negative pregnant participants as the control group, or HIV-positive women included received more than an intrapartum dose of antiretroviral therapy during the pregnancy. Furthermore, studies were excluded if the outcomes of interest were not defined or the study definition differed to that specified in our protocol. Ambiguities about study eligibility were resolved by discussion (a list of excluded studies is available on request). COOW and JH independently assessed the uniqueness of each study population. If the same cohort was reported more than once, the manuscript with the most recent and complete data was retained; however, manuscripts reporting different outcomes for the same cohort were both included.[14,15] The protocol is available online.

See Online for appendix

For the **protocol** see http://www.crd.york.ac.uk/PROSPERO/display_record.asp?ID=CRD42013005638

	Country	Study design	Recruitment time period	Number of pregnant women included in analysis		Perinatal outcomes	Population characteristics	HIV disease stage and reason why antenatal antiretroviral therapy not provided	Methods to correct for confounding factors†	Gestational age estimation method	Quality assessment
				HIV-positive	HIV-negative						
Sub-Saharan Africa											
Adjorlolo et al (1991)[16]	Ivory Coast	Retrospective cohort	Not reported	290	2768	Low birthweight	Low socioeconomic status, largest urban area in the country	4–15% of women met criteria for AIDS; antiretroviral therapy not yet shown to be effective in pregnancy	None	Unspecified	Poor
Ayisi et al (2003)[17]	Kenya	Prospective cohort	June, 1996, to March, 1999	420	1503	Preterm birth, low birthweight, small for gestational age	Uncomplicated singleton pregnancies without comorbidities; urban setting; all women delivered in hospital	No description of HIV disease stage or why antiretroviral therapy not provided	Regression analysis	Neonatal assessment (Dubowitz)	Average
Bergstrom et al (1995)[18]	Mozambique	Case–control	Not reported	51 cases	51 controls	Low birthweight	No description	No description of HIV disease stage; antiretroviral therapy not yet shown to be effective in pregnancy	Matching	Unspecified	Average
Braddick et al (1990)[19]*	Kenya	Prospective cohort	January, 1986, to March, 1989	177	326	Preterm birth, low birthweight	Low socioeconomic status; urban setting; no history of intravenous drug use; all women delivered in hospital	55% of women were asymptomatic, 45% were symptomatic, and one woman had AIDS; antiretroviral therapy not yet shown to be effective in pregnancy	Risk factor analysis	Neonatal assessment (Ballard) and last normal menstrual period	Average
Bulterys et al (1994)[20]	Rwanda	Prospective cohort	October, 1989, to February, 1992	308	299	Preterm birth, low birthweight, small for gestational age, stillbirth, neonatal death	Semirural setting where 75% of deliveries occurred at home	Majority of HIV-positive women were asymptomatic and none met the criteria for AIDS; antiretroviral therapy not yet shown to be effective in pregnancy	Risk factor analysis, regression analysis	Neonatal assessment (Ballard) and late ultrasound	Average
Coley et al (2001)[21]	Tanzania	Prospective cohort	April, 1995, to July, 1997	522	485	Preterm birth, low birthweight, term low birthweight, preterm low birthweight	Cohort representative of the general population; 93·5% of deliveries occurred at medical facility, 3·6% at home, and 2·9% at non–medical facilities	83% of women were asymptomatic (WHO stage 1), 17% were symptomatic (WHO ≥2); no description of why antiretroviral therapy not provided	Risk factor analysis, regression analysis	Unspecified	Average
Ezeaka et al (2009)[22]	Nigeria	Prospective cohort	August, 2002, to August, 2005	220	218	Preterm birth, low birthweight	Participants recruited in a tertiary hospital in urban setting; none of the women reported use of intravenous drugs, alcohol, or tobacco; all women delivered in hospital	85·5% of women were asymptomatic and 14·5% met WHO criteria for AIDS; no description on why antiretroviral therapy not provided	Matching, risk factor analysis	Last normal menstrual period and late ultrasound	Average
Friis et al (2004)[23]	Zimbabwe	Prospective cohort	1996–97	360	725	Preterm birth, low birthweight, term low birthweight	Low socioeconomic status, urban setting.	No description of HIV disease stage; antiretroviral therapy recommended nationally, but not available	Regression analysis	Last normal menstrual period and symphysio-fundal height	Poor

(Table continues on next page)

	Country	Study design	Recruitment time period	Number of pregnant women included in analysis		Perinatal outcomes	Population characteristics	HIV disease stage and reason why antenatal antiretroviral therapy not provided	Methods to correct for confounding factors†	Gestational age estimation method	Quality assessment
				HIV-positive	HIV-negative						
(Continued from previous page)											
Habib et al (2008)[24]	Tanzania	Retrospective cohort	1999–2006	137	5436	Preterm birth, small for gestational age	Urban and rural settings in six districts in Tanzania; study done at a referral hospital and data not representative of the population; all women delivered in hospital	No description on HIV disease stage or why antiretroviral therapy not provided	Regression analysis	Last normal menstrual period	Poor
Ladner et al (1998)[25]	Rwanda	Prospective cohort	July, 1992, to August, 1993	275	286	Stillbirth	Urban setting; all women delivered in hospital	No description of HIV disease stage; antiretroviral therapy not yet shown to be effective in pregnancy	None	Late ultrasound	Poor
Lallemant et al (1989)[26]	Republic of the Congo	Prospective cohort	May, 1987, to March, 1988	61	130	Low birthweight	Urban setting; all women delivered in hospital	One woman met the WHO criteria for AIDS; antiretroviral therapy not yet shown to be effective in pregnancy	Matching, risk factor analysis	Unspecified	Average
Lepage et al (1991)[27]	Rwanda	Retrospective cohort	November, 1988, to June, 1989	215	216	Low birthweight, neonatal death	Urban setting; all women delivered in hospital	81% were asymptomatic and none met the WHO criteria for AIDS; antiretroviral therapy not yet shown to be effective in pregnancy	Matching, risk factor analysis, regression analysis	Neonatal assessment (Finnström)	Average
Leroy et al (1998)[28]	Rwanda	Prospective cohort	July, 1992, to August, 1993	364	365	Preterm birth, low birthweight, term low birthweight, preterm low birthweight	Urban setting; study sample representative of the population; 12·3% of deliveries occurred at home and 87·7% at a hospital	92% of women were asymptomatic and one fulfilled the WHO criteria for AIDS; 7·5% of women had a CD4 count <200 cells per μL; antiretroviral therapy not yet shown to be effective in pregnancy	Matching, risk factor analysis, regression analysis, Mantel-Haenzel analysis	Neonatal assessment (Finnström) and late ultrasound	Average
Mmiro et al (1993)[29]	Uganda	Prospective cohort	December, 1988, to November, 1990	557	697	Preterm birth	Urban setting. No evidence of illicit drug use; 50% of deliveries occurred at home and 50% at a hospital	46·8% of women were symptomatic; antiretroviral therapy not yet shown to be effective in pregnancy	Risk factor analysis	Unspecified	Poor
Musana et al (2009)[30]	Kenya	Prospective cohort	September, 2004, to April, 2005	68	68	Preterm birth, low birthweight	No description	All women met the criteria for advanced stage of disease (WHO stage 3 and 4); no description on why antiretroviral therapy not provided	Matching, risk factor analysis	Last normal menstrual period	Average
Mwanyumba et al (2001)[31]	Kenya	Retrospective cohort	May, 1996, to April, 1999	1169	7188	Low birthweight	Singleton pregnancies, no obstetric complications, illicit drug use or smoking; all women delivered in hospital	No description on HIV disease stage or why antiretroviral therapy not provided	Regression analysis	Unspecified	Poor

(Table continues on next page)

	Country	Study design	Recruitment time period	Number of pregnant women included in analysis		Perinatal outcomes	Population characteristics	HIV disease stage and reason why antenatal antiretroviral therapy not provided	Methods to correct for confounding factors†	Gestational age estimation method	Quality assessment
				HIV-positive	HIV-negative						
(Continued from previous page)											
Ndirangu et al (2012)[14]	South Africa	Prospective cohort	August, 2001, to September, 2004	1189	1179	Preterm birth, very preterm birth, small for gestational age, very small for gestational age	Area with a high HIV–prevalence (39·5%); rural setting, low socioeconomic status; 11·8% of deliveries occurred at home or on the way to a health-care facility and 88·2% of deliveries in hospital	5·5% of HIV–positive women had a CD4 count <200 cells per μL; antiretroviral therapy recommended nationally, but not available	Risk factor analysis, regression analysis	Neonatal assessment (Ballard) and last normal menstrual period	Poor
Noble et al (2005)[32]	Zimbabwe	Case–control	July, 1998, to March, 1999	53 cases	444 controls	Preterm birth	Urban setting. Convenience sample of pregnant women who delivered term and preterm infants at a university affiliated hospital all women delivered in hospital	No description on HIV disease stage HIV–diagnoses made after delivery, hence no antenatal antiretroviral therapy	Risk factor analysis, regression analysis	Neonatal assessment (Dubowitz), last normal menstrual period and symphysio-fundal height	Average
Rollins et al (2007)[15]	South Africa	Prospective cohort	September, 2001, to September, 2004	1449	1401	Low birthweight	Low socioeconomic status, rural, semirural, and urban antenatal clinics in an area with a high HIV prevalence (39·5%); 12·4% of deliveries occurred at home, 28% at a clinic, 52·9% in hospital, and 6·5% in an unknown location	11% of women had a CD4 count <200 cells per μL; participants received intrapartum antiretroviral therapy only	Regression analysis	Neonatal assessment (Ballard) and last normal menstrual period	Average
Ryder et al (1989)[33]	Democratic Republic of the Congo	Prospective cohort	April 30, 1986, to April 30, 1987	466	606	Low birthweight, neonatal death	Urban setting; recruited from 2 hospitals: hospital A (women of low socioeconomic status and hospital B (women of high socioeconomic status); no illicit drug use	18% of women met criteria for AIDS; antiretroviral therapy not yet shown to be effective in pregnancy	Matching	Neonatal assessment (Dubowitz)	Average
Sutton et al (1999)[34]*	Democratic Republic of the Congo	Retrospective cohort	October, 1989, to April, 1990	215	206	Low birthweight	Urban setting; singleton pregnancies; all women delivered in hospital	No description on HIV disease stage; antiretroviral therapy not yet shown to be effective in pregnancy	Regression analysis	Neonatal assessment (Ballard) and last normal menstrual period	Average
Taha et al (1995)[35]	Malawi	Retrospective cohort	October, 1989, to October, 1990	679	687	Preterm birth, low birthweight, term low birthweight, preterm low birthweight	Urban setting; all women delivered in hospital	No description of HIV disease stage; antiretroviral therapy not yet shown to be effective in pregnancy	None	Neonatal assessment (Ballard) and last normal menstrual period	Poor

(Table continues on next page)

	Country	Study design	Recruitment time period	Number of pregnant women included in analysis		Perinatal outcomes	Population characteristics	HIV disease stage and reason why antenatal antiretroviral therapy not provided	Methods to correct for confounding factors†	Gestational age estimation method	Quality assessment
				HIV-positive	HIV-negative						
(Continued from previous page)											
Temmerman et al (1994)[13]	Kenya	Prospective cohort	January, 1989, to December, 1991	315	311	Preterm birth, low birthweight, term low birthweight, neonatal death	Low socioeconomic status, urban setting, no illicit drug use; 20% of deliveries occurred at home or at another maternity unit	52·2% of women were asymptomatic, 43·6% were symptomatic and 4·2% met the criteria for AIDS; antiretroviral therapy not yet shown to be effective in pregnancy	Matching, risk factor analysis	Late ultrasound	Poor
Ticconi et al (2003)[36]	Zimbabwe	Retrospective cohort	Jan 1, to June 30, 2000, and Jan 1, to June 30, 2001	52	789	Preterm birth, small-for-gestational-age	Referral hospital in a malaria endemic area; recruitment only during peak malaria season	No description on HIV disease stage; participants received intrapartum antiretroviral therapy only	Regression analysis	Last normal menstrual period	Poor
Van den Broek et al (2014)[37]	Malawi	Case–control	February, 2004, to September, 2005	351 cases	1729 controls	Preterm birth	Rural setting, high HIV prevalence (26·2%), singleton pregnancies	No description on HIV disease stage; HIV diagnoses made after delivery, hence no antenatal antiretroviral therapy	Risk factor analysis, regression analysis	Late ultrasound	Average
Europe											
Bucceri et al (1997)[38]	Italy	Prospective cohort	1985–93	151	164	Preterm birth, low birthweight	Urban setting; intravenous drug users; all women delivered in hospital	93% of women were asymptomatic (Centers for Disease Control and Prevention stage A) and 5% had a CD4 <200 cells per μL; antiretroviral therapy not yet shown to be effective in pregnancy	Regression analysis	Neonatal assessment (not specified), last normal menstrual period and late ultrasound	Average
Johnstone et al (1996)[39]	UK	Retrospective cohort	1983–92	82	302	Preterm birth	Intravenous drug users or sexual partners of intravenous drug user; singleton pregnancies	No description on HIV disease stage; antiretroviral therapy not yet shown to be effective in pregnancy	Matching, regression analysis	Late ultrasound and last normal menstrual period	Poor
The Americas											
Alger et al (1993)[40]	USA	Prospective cohort	July, 1987, to July, 1991	101	97	Low birthweight	Low socioeconomic status, inner city; current or former intravenous drug users or sexual partners of intravenous drug users	One woman with an AIDS-defining opportunistic infection and 15·8% of women had a CD4 count <200 cells per μL; antiretroviral therapy not yet shown to be effective in pregnancy	Risk factor analysis	Unspecified	Average
Ellis et al (2002)[41]	USA	Retrospective cohort	January, 1988, to December, 1995	563	2252	Preterm birth, low birthweight, small-for-gestational-age	Low socioeconomic status , inner city; referral centre for high–risk obstetric patients; all women delivered in hospital	No description on HIV disease stage; antiretroviral therapy not yet recommended in pregnancy in national guideline	Regression analysis	Unspecified	Poor

(Table continues on next page)

	Country	Study design	Recruitment time period	Number of pregnant women included in analysis		Perinatal outcomes	Population characteristics	HIV disease stage and reason why antenatal antiretroviral therapy not provided	Methods to correct for confounding factors†	Gestational age estimation method	Quality assessment
				HIV-positive	HIV-negative						
(Continued from previous page)											
Halsey et al (1990)[42]	Haiti	Prospective cohort	August, 1986, to August, 1988	199	1994	Preterm birth, low birthweight	Low socioeconomic status, with no history of intravenous drug use or cocaine; sample was representative of the community	All women were asymptomatic; antiretroviral therapy not yet shown to be effective in pregnancy	None	Neonatal assessment (Dubowitz)	Average
Markson et al (1996)[43]	USA	Retrospective cohort	1989–1990	772	2377	Preterm birth, low birthweight, term low birthweight	Urban setting, singleton pregnancy; data drawn from New York city Medicaid claims database	No description on HIV disease stage; antiretroviral therapy not yet shown to be effective in pregnancy	Matching, risk factor analysis, regression analysis	Last normal menstrual period	Poor
Maynard et al (1990)[44]	USA	Retrospective cohort	March, 1985, to February, 1988	17	41	Preterm birth, low birthweight, very low birthweight	Intravenous drug users; all women delivered in hospital	82·4% of women were asymptomatic and 17·6% were symptomatic; antiretroviral therapy not yet shown to be effective in pregnancy	None	Unspecified	Average
Selwyn et al (1989)[45]	USA	Prospective cohort	June, 1985, to 1988	39	58	Term low birthweight, miscarriage	Inner city intravenous drug users; all women delivered in hospital	54% of women were asymptomatic (Centers for Disease Control and Prevention stage II), 42% had persistent generalised lymphadenopathy (Centers for Disease Control and Prevention stage III), 4% had advanced disease (Centers for Disease Control and Prevention stage IV C.2) and none of the women met the criteria for AIDS; antiretroviral therapy not yet shown to be effective in pregnancy	None	Unspecified	Poor

(Table continues on next page)

Data extraction

COOW and RC independently reviewed the citations retrieved by the scientific literature search and identified relevant citations. COOW and JH extracted perinatal outcome data (table), plus reported unadjusted and adjusted relative risks (RR) and odds ratios (OR) and 95% CI to assess the effect of adjusting for confounders. Any disagreements were resolved by discussion between the authors.

Outcomes

We assessed perinatal outcomes in four categories: gestational age at delivery; birthweight; gestational age and birthweight combined; and fetal and neonatal mortality. We had 11 birth outcomes of interest: preterm birth (<37 weeks 0 days gestation),[6] very preterm birth (<32 weeks 0 days gestation[6]); low birthweight (<2500 g)[7] or very low birthweight (<1500 g);[7] term birth with low birthweight (≥37 weeks 0 days gestation and <2500 g),[7] preterm low birthweight (<37 weeks 0 days and <2500 g), small for gestational age (birthweight <10th centile)[46] or very small for gestational age (birthweight <3rd centile)[46] according to the reference chart used at the study site; and miscarriage (spontaneous expulsion of fetus <24 weeks 0 days gestation),[47] stillbirth (any third trimester delivery of stillborn infant with birthweight ≥1000 g[9] or ≥24 weeks

	Country	Study design	Recruitment time period	Number of pregnant women included in analysis		Perinatal outcomes	Population characteristics	HIV disease stage and reason why antenatal antiretroviral therapy not provided	Methods to correct for confounding factors†	Gestational age estimation method	Quality assessment
				HIV-positive	HIV-negative						
(Continued from previous page)											
Asia											
Kumar et al (1995)[53]	India	Prospective cohort	January, 1992, to January, 1993	150	152	Preterm birth, low birthweight, small for gestational age	Low socioeconomic status, no illicit drug use, referral hospital; all women delivered in hospital	53% of women were asymptomatic (Centers for Disease Control and Prevention stage I/II), 38% were symptomatic (Centers for Disease Control and Prevention stage III) and 9% met the criteria for AIDS (Centers for Disease Control and Prevention stage IV); antiretroviral therapy not yet shown to be effective in pregnancy	Matching, risk factor analysis	Neonatal assessment (Dubowitz)	Average
Mitgitti et al (2008)[54]	Thailand	Retrospective cohort	January, 1997, to December, 2002	266	5872	Preterm birth, low birthweight	Singleton pregnancies; all women delivered in hospital	No description on HIV disease stage or why antiretroviral therapy not provided	Risk factor analysis, regression	Unspecified	Poor

*Studies designated as a case-control studies by authors, but identified to be cohort studies and analysed as cohort studies in this systematic review. †Confounding factors corrected for in each study are detailed elsewhere (appendix, p16).

Table: **Characteristics of studies included in the systematic review**

0 days gestation[48] or ≥35 cm body length),[9] or neonatal death (death within first 28 days of life).[49] Outcomes were not extracted if they were not defined or if the definition in the study differed to that in our protocol. Data for the preterm prelabour rupture of membranes were also sought, but no studies reporting this outcome were identified.

Quality analysis

COOW and JH independently assessed the methodological quality of each study with the modified Newcastle-Ottawa Scale.[50] Cohort studies were assessed according to nine criteria in three groups (appendix, p 13): selection of HIV-positive and HIV-negative participants (maximum 4 points), comparability of exposed and unexposed participants, and whether corrections were made for confounding factors (maximum 2 points), and methods to assess the outcome of interest, including methods used to estimate gestational age at delivery (maximum 3 points). A similar grading system was used for case-control studies with minor changes to accommodate the study design (appendix, p 14). Studies were defined as good, average, and poor quality according to our preset definitions (appendix, p 15).

Statistical analysis

Dichotomous outcome data extracted from individual studies were used to generate RR (cohort studies) or OR (case-control studies), irrespective of study quality. A meta-analysis was done when two or more studies with the same study design reported the same outcome. A random-effects model was used to calculate a weighted summary effect estimate and 95% CI for the association between maternal HIV infection and individual perinatal outcomes.[11] Thereafter, the summary effect estimates and 95% CI for individual outcomes, according to study design, were represented with a forest plot. Within each meta-analysis, the I^2 statistic was used to establish the proportion of heterogeneity in the effect estimate attributable to clinical or methodological variability, as opposed to sampling error.[11] The degree of heterogeneity was interpreted as none (<25%), low (25–49%), moderate (50–74%), or high (≥75%).[11]

Prespecified subgroup analyses were done to explore the effect of covariates on the association between maternal HIV infection and outcomes,[11] including geographical location of the study and the method used to establish gestational age.

Sensitivity analyses were done to explore whether methodological quality and the adjustment for confounding factors affected the reported associations between maternal HIV infection and outcomes.[11] The Peters' test was used to assess publication bias in meta-analyses containing a minimum of ten studies.[51] All statistical analyses were done with Stata version 13 and the systematic review is reported according to the

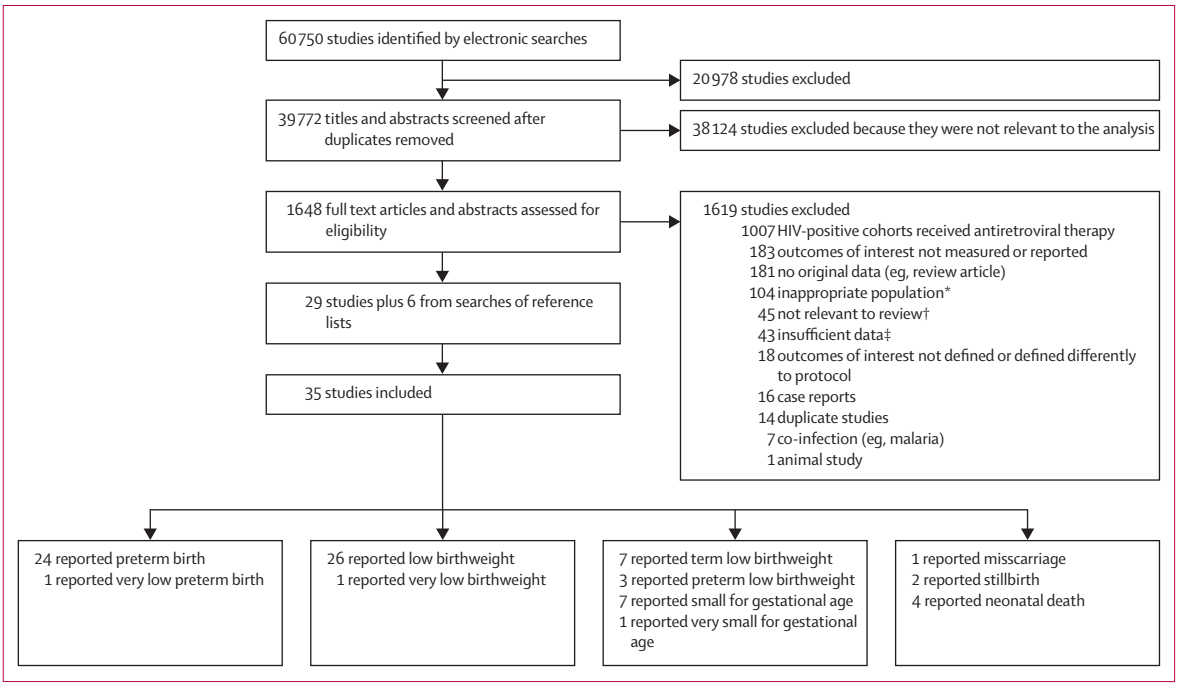

Figure 1: **Study selection**
*For example, HIV-positive women who are not pregnant. †For example, ART refers to assisted reproductive technology rather than antiretroviral therapy. ‡For example, perinatal outcomes not reported according to HIV status of women.

Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.[52] This analysis is registered online with PROSPERO, number CRD42013005638.

Role of the funding source

The study received no funding. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Our search yielded 60750 citations, 35 of which were included in this meta-analysis (figure 1). These studies included data for 53623 women in 18 countries in 20 prospective and 12 retrospective cohort studies, and three case-control studies.[13,14,15,16–45,53,54] No randomised controlled trials were identified. The study and population characteristics, methods used to correct for confounders, gestational age estimation methods, and quality assessment ratings are displayed (table).

Of these 35 studies, 25 (71%) were done in sub-Saharan Africa (table). The most commonly reported perinatal outcomes were low birthweight (26 studies), preterm birth (24), small for gestational age (seven), and term low birthweight (seven). The remaining outcomes were each reported in fewer than five studies (figure 1, table).

24 (69%) of the included studies reported methods for gestational age estimation (table). Half of these used only one method: late ultrasound (≥14 weeks' gestation; three studies), last normal menstrual period (four studies), or neonatal assessment (five studies); the other half used mixed methods (appendix, p 18). No study used a first trimester ultrasound scan, the most accurate method to establish gestational age. As a result, no studies were classified as good quality (table; appendix, p 19, 20).

In 21 (60%) of the studies, antenatal antiretroviral therapy was not provided because the studies were done before 1994 when antiretroviral treatment for the prevention of mother-to-child transmission of HIV was not in clinical practice (table).[55] In other studies, antiretroviral therapy was not recommended in national treatment guidelines (one study),[41] recommended but not available (two studies),[14,23] only provided intrapartum (two studies)[15,36] and precluded because HIV was diagnosed post partum (two case-control studies).[32,37] In the remaining seven studies, no reason was specified. No association was found between the place of delivery and perinatal outcomes.

34337 women were included in the assessment of the association between maternal HIV infection and preterm birth. Maternal HIV infection was significantly associated with preterm birth in the meta-analysis of 14 prospective (RR 1·50, 95% CI 1·24–1·82) and eight retrospective (1·82, 1·41–2·34) cohort studies (figure 2). The association was significant in both sets of studies in sub-Saharan Africa and the Americas (figure 3). In prospective cohort studies where method of gestational age assessment was specified, the type of method of gestational age assessment did not change the significant association between maternal HIV infection and preterm birth (appendix, p 22). Sensitivity

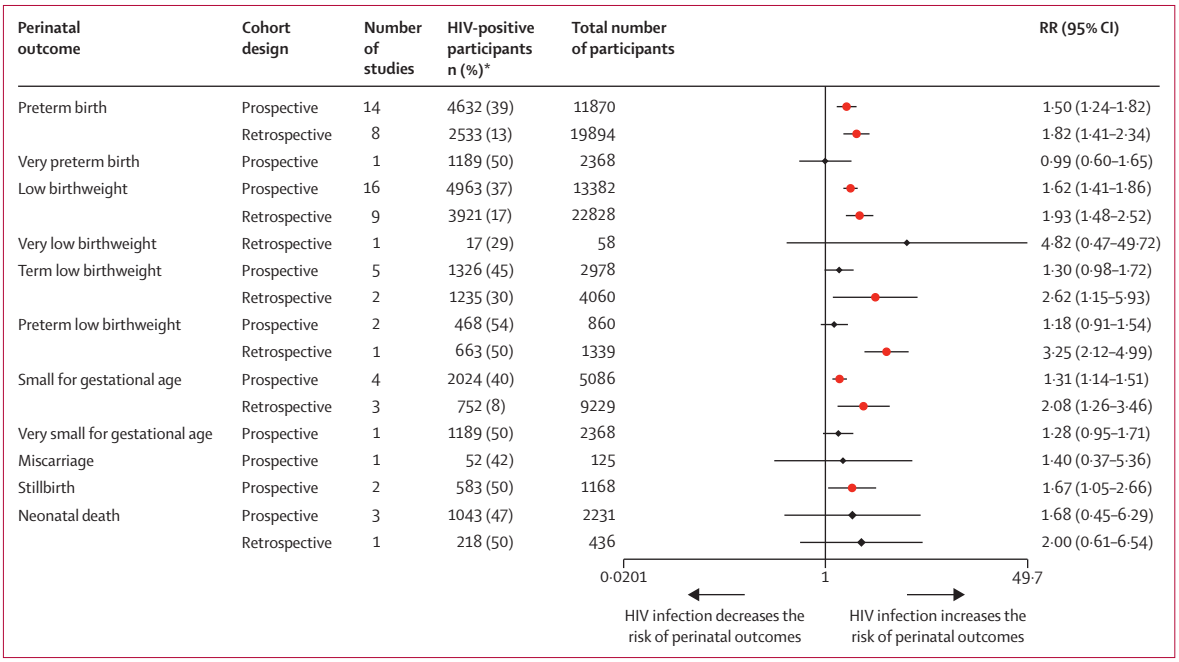

Figure 2: **Perinatal outcomes associated with maternal HIV infection in women naive to antiretroviral therapy by study design**
Random-effects meta-analysis results of the association between maternal HIV infection in women naive to antiretroviral therapy and perinatal outcomes in prospective and retrospective cohort studies. Statistically significant effects are presented with red dots and black dots show non-significant effects. RR=relative risk. *Percentage represents the proportion of the total number of participants in the included studies (for each outcome and study design).

analysis according to methodological quality showed a significant association in both average (RR 1·64, 1·23–2·20) and poor quality prospective studies (1·34, 1·06–1·71; appendix, p 23). Retrospective studies were heterogeneous (I^2 81·0%; appendix, p 25). Seven studies reported both unadjusted and adjusted effect estimates for preterm birth (figure 4). In all cases, the adjustment for covariates had no effect on the direction or significance level of the effect estimates. The Peters' test showed no evidence of publication bias (p=0·7). Two prospective cohort studies reported an increased risk of preterm birth with more advanced HIV disease (appendix, p 24).[13,21] Two case-control studies showed no significant association with preterm birth (appendix, p 21).[32,37]

We included 2368 women in the analysis for very preterm birth. One prospective cohort study[14] reported no association between maternal HIV infection and very preterm birth (RR 0·99, 95% CI 0·60–1·65; figure 2).

We included 36 312 women in the assessment of the association between maternal HIV and low birthweight. Maternal HIV infection was significantly associated with low birthweight in the meta-analysis of 16 prospective (RR 1·62, 95% CI 1·41–1·86) and nine retrospective (1·93, 1·48–2·52) cohort studies (figure 2). The association was significant in both sets of studies in sub-Saharan Africa and Asia (figure 3), irrespective of study quality (appendix, p 23). Heterogeneity was present among retrospective studies (I^2 87·2%; appendix, p 36). Adjustment for covariates had no effect on the direction or significance level of the effect estimates (figure 4). Two prospective cohort studies, reported an increasing risk of low birthweight with more advanced HIV disease (appendix, p 24). A small case-control study showed no association with low birthweight (appendix, p 21).[18]

58 women were included in the assessment for an association between very low birthweight and maternal HIV infection. One small retrospective cohort study[44] reported a positive, but non-significant, association between maternal HIV infection and very low birthweight (RR 4·82, 95% CI 0·47–49·72; figure 2).

We included 7038 women in the assessment for the association between maternal HIV infection and term low birthweight. Maternal HIV infection was significantly associated with term low birthweight in the meta-analysis of two retrospective cohort studies (RR 2·62, 95% CI 1·15–5·93; figure 2),[21,28] irrespective of geographical region, gestational age assessment method, or study quality (figure 3; appendix, p 22, 23), and after adjustment for confounders in the study[43] which assessed this (figure 4). This finding, however, was in the presence of a high degree of between-study heterogeneity (I^2 89·8%; appendix, p 47). The association in the meta-analysis of five prospective cohort studies did not reach significance (1·30, 0·98–1·72). No effect of HIV disease stage on the rate of term low birthweight was seen in the one study[21] which examined this (appendix, p 24).

2199 women were assessed for an association between maternal HIV infection and preterm low birthweight. Maternal HIV infection was significantly associated with preterm low birthweight in one retrospective cohort study

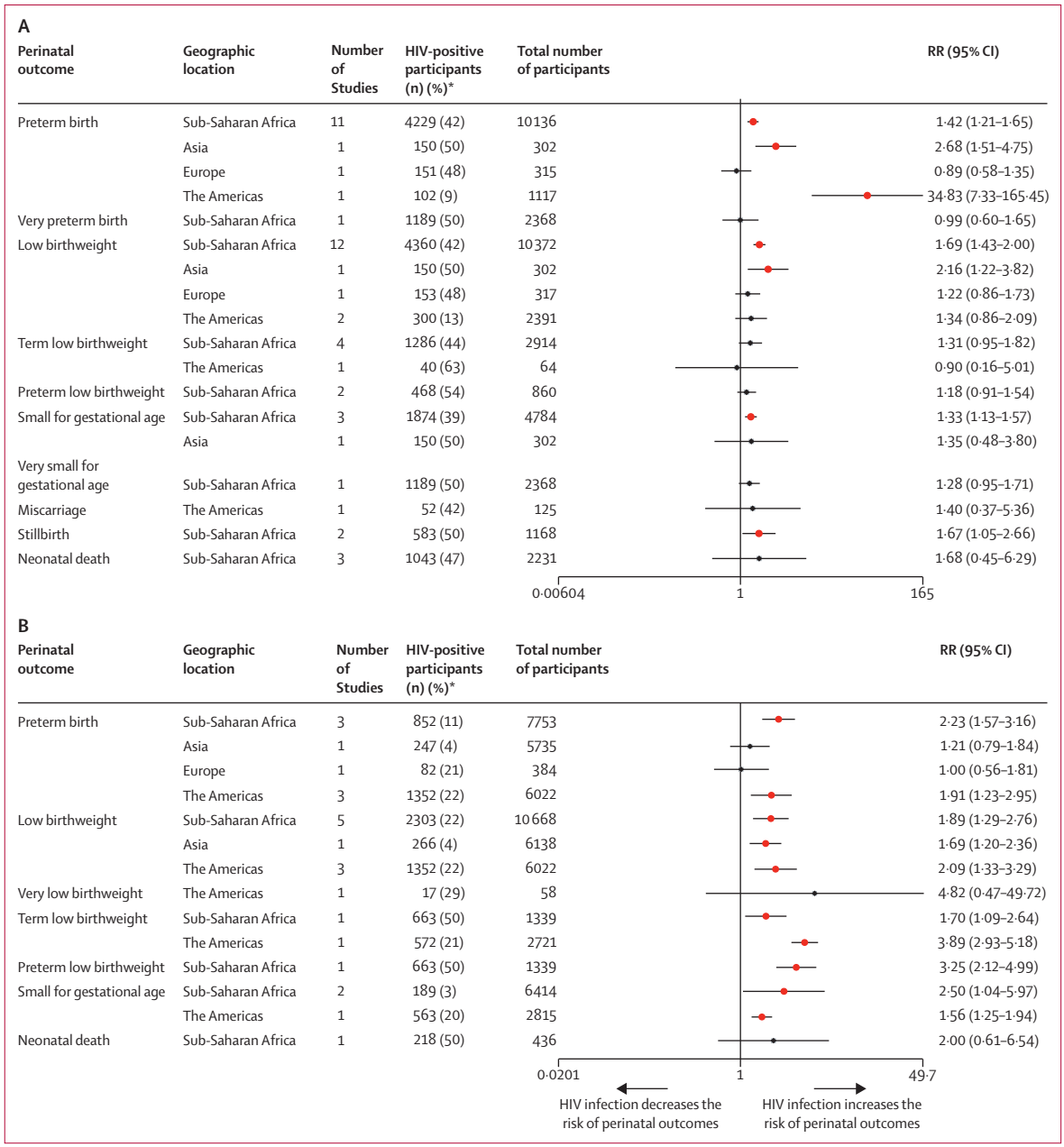

Figure 3: **Perinatal outcomes associated with maternal HIV infection in women naive to antiretroviral therapy by geographic region**
Prospective cohort studies (A) and retrospective cohort studies (B). Random-effects meta-analysis results of the association between maternal HIV infection in women naive to antiretroviral therapy and perinatal outcomes, stratified according to the geographic region where the studies were done. Statistically significant effects are presented with red dots and black dots show non-significant effects. RR=relative risk. *Percentage represents the proportion of the total number of participants in the included studies for each outcome and study design.

(RR 3·25, 95% CI 2·12–4·99; figure 2),[35] which was done in sub-Saharan Africa, used mixed methods for gestational age assessment, and was of low quality (figure 3; appendix, pp 22,23). However, the association in the meta-analysis of two prospective cohort studies[21,28] was not significant (1·18, 0·91–1·54). One prospective cohort study reported an increased risk of preterm low birthweight with more advanced HIV disease (appendix, p 24).[21]

In the 14 315 women assessed, maternal HIV infection was significantly associated with small-for-gestational-age births in the meta-analysis of four prospective (RR 1·31, 95% CI 1·14–1·51) and three retrospective (2·08, 1·26–3·46) cohort studies (figure 2). The association was significant in both sets of studies in sub-Saharan Africa (figure 3), irrespective of gestational age assessment method and study quality, apart from

one poor quality prospective study (appendix, p 22,23).[14] Heterogeneity was reported in the meta-analysis of retrospective studies (I^2 85·2%; appendix, p 61). In the studies that assessed confounding, the effect estimates remained significant after adjustment for covariates in three studies (figure 4); in one study,[14] the multivariate

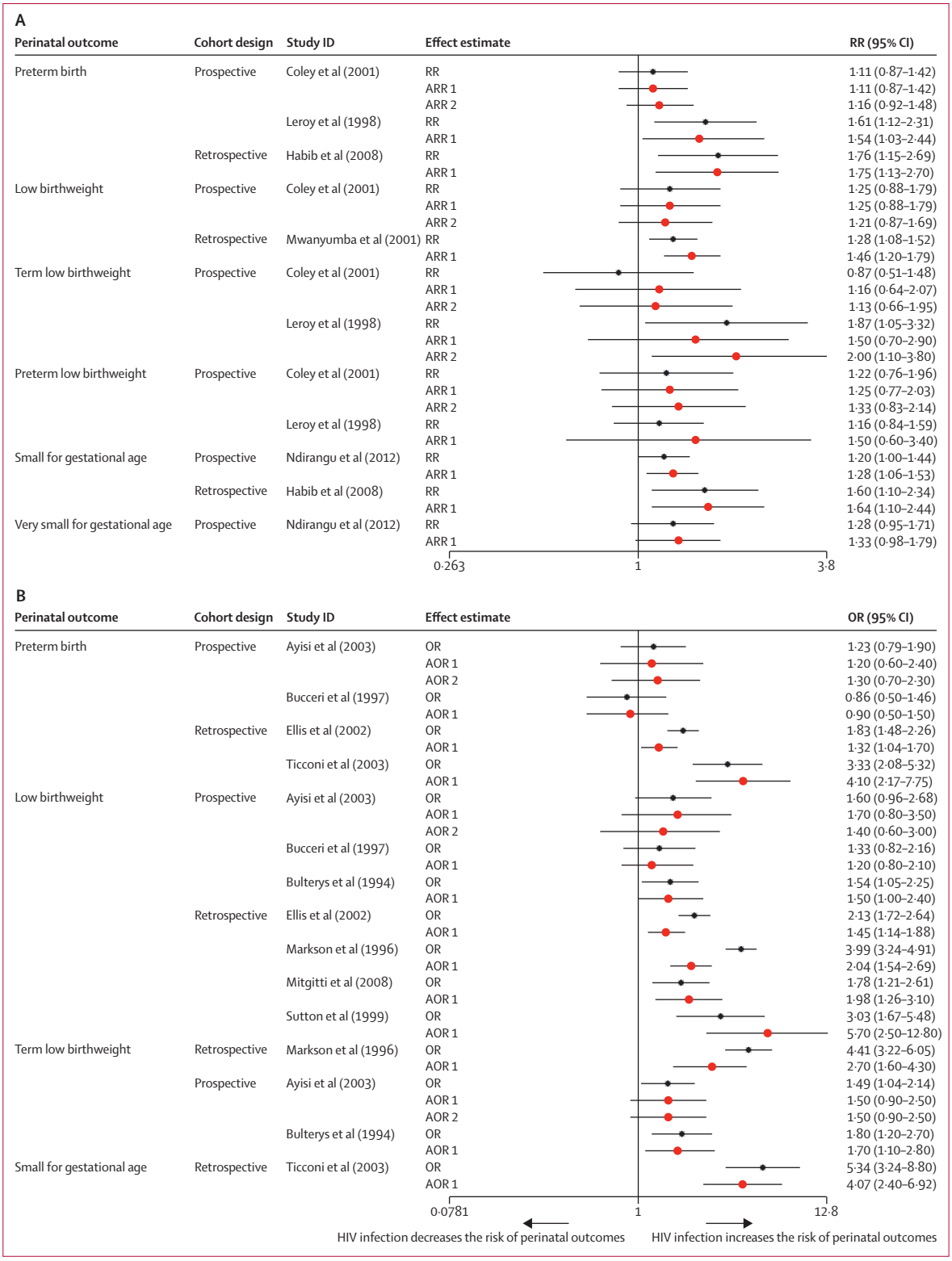

Figure 4: **Sensitivity analysis assessing the adjustment for confounding factors in studies of the association between maternal antiretroviral-naive HIV infection and perinatal outcomes**

RR estimates (A) and OR estimates (B). For all analyses, ARR 1 represents the multivariate analysis. For Coley et al (2001), ARR 2 represents the age-adjusted analysis. For Leroy et al (1998), ARR 2 represents the Mantel-Haenzel estimate adjusting for prematurity. For Ayisi et al (2003), AOR 1 represents the multivariate analysis in primigravidae and AOR 2 represents the multivariate analysis in multigravidae. Adjusted effect estimates are presented in red and black dots show unadjusted effect estimates. Studies are listed in alphabetical order of first author within each category. Covariate factors adjusted for are detailed in the appendix. RR=relative risk. ARR=adjusted relative risk. OR=odds ratio. AOR=adjusted odds ratio.

analysis allowed the effect estimate to reach statistical significance, and in another study,[17] the estimate lost significance (figure 4).

In the 2368 women assessed for the association between maternal HIV infection and very small for gestational age, one prospective study[14] in sub-Saharan Africa reported a positive, but non-significant, association between maternal HIV infection and very-small-for-gestational-age births (RR 1·28, 95% CI 0·95–1·71; figure 2). Adjustment for covariates did not change the effect estimate (figure 4).

125 women were included in the analysis of miscarriage. One prospective cohort study[45] in the Americas identified no association between maternal HIV infection and miscarriage (RR 1·40, 95% CI 0·37–5·36; figure 2). 1168 women were included in the analysis of stillbirth. The meta-analysis of two prospective cohort studies[20,25] in sub-Saharan Africa identified a significant association between maternal HIV infection and stillbirth (1·67, 1·05–2·66; figure 2); the only study to show an association on its own, was the average quality study that used mixed methods for gestational age assessment (appendix, p 22, 23).[20] 2667 women were included in the analysis of neonatal death. The meta-analysis of three prospective cohort studies in sub-Saharan Africa showed a positive but non-significant association of maternal HIV infection with neonatal deaths (1·68, 0·45–6·29), in the presence of substantial heterogeneity (I^2 89·9; appendix, p 80). One retrospective cohort study also identified no association with neonatal death (2·00, 0·61–6·54; figure 2).[27]

Discussion

HIV infection is strongly associated with increased risks of preterm birth, low birthweight, small for gestational age, and stillbirth, and weakly associated with term and preterm low birthweight. The evidence did not support an effect of maternal HIV infections on very preterm birth, very low birthweight, very small for gestational age, miscarriage, or neonatal death. Overall, the evidence is strongest and most consistent in sub-Saharan Africa, the region with the highest burden of maternal HIV infection, and largely persists irrespective of the study quality, method of gestational age determination, and controlling for confounding factors. Although only three studies[13,21,33] reported specific perinatal outcomes according to HIV disease stage, the risk of adverse perinatal outcomes increased with more advanced HIV disease.

Our analysis has several strengths. A systematic review and meta-analysis of observational studies generates the best possible evidence to assess the effect of maternal HIV-infection in women naive to antiretroviral therapy on perinatal outcomes. The scientific literature search included all study designs and no language or geographical restrictions. We clearly defined the outcomes of interest a priori and strictly applied the eligibility criteria to reduce misclassification bias. The random-effects model used accounted for heterogeneity, and several subgroup and sensitivity analyses explored unexplained heterogeneity and the presence and effect of underlying or residual bias and confounding. We assessed the effect of adjustment for confounding factors in individual studies. When applicable, the Peters' test confirmed an absence of publication bias and the systematic review was reported according to the PRISMA guidelines.[52] As 91% of HIV-positive pregnant women live in sub-Saharan Africa and most of the primary data in our review originated from that region, our findings are particularly relevant to those countries with the highest burden of maternal HIV infection.

A similar previous review[12] only included publications up to 1996, many of which were abstracts. Furthermore, this previous review used a fixed-effects model to pool the data despite substantial between-study heterogeneity; it also included fewer outcomes, which were poorly or not defined, and gestational age estimation methods were not assessed. By contrast, our systematic review and meta-analysis are based on up-to-date data (publications in 1989–2014), well defined outcomes, and advanced methods.

However, our review also has some limitations. Certain perinatal outcomes were only reported by a few studies, which precluded firm conclusions. Although inclusion of studies that did not define outcomes or defined outcomes differently to our protocol might have increased the power of our analyses, it would have compromised the validity of our study by introducing bias. Because of the limited reporting of unadjusted and adjusted effect estimates, only six outcomes were assessed in this sensitivity analysis. Furthermore, studies adjusted for various covariates and therefore a pooled analysis of adjusted estimates could not be done.[11] Residual confounding cannot be excluded. We could not assess the effect of certain important confounders (eg, maternal viral load and CD4 cell count), which are associated with adverse outcomes, because of limited measurement, adjustment, and reporting of these confounders in included studies. Furthermore, many studies did not mention how gestational age was established, and none used ultrasound scans at less than 14 weeks, which is the most accurate method of gestational age estimation.[56] This limitation might have contributed to misclassification bias and some of the heterogeneity observed because most of the outcomes depend on accurate estimation of gestational age. No distinction was made between spontaneous and caregiver initiated preterm birth. The methods and accuracy of birthweight measurements were not specifically assessed in our review. Finally, small-for-gestational-age and very-small-for-gestational-age cutoffs were based on various centile charts rather than international standards.[46] Similar challenges are encountered in many other studies assessing perinatal outcomes in low-income

and middle-income countries,[57] which emphasises the importance of using accurate and consistent methods of gestational age estimation[56] and international standards in future studies.[46]

Our study suggests that maternal HIV infection differentially affects individual perinatal outcomes. The effect of maternal HIV infection on preterm birth, low birthweight, small for gestational age, and stillbirth is clear, with significantly increased risks in both prospective and retrospective cohort studies. However, the effects on preterm birth, low birthweight, and small for gestational age did not translate into an effect on neonatal death, for which the data were highly heterogeneous. The evidence is weaker for term low birthweight and preterm low birthweight, where an effect of HIV infection was seen in retrospective, but not prospective cohort studies. Very preterm birth, very small for gestational age, and very low birthweight are associated with greater morbidity and mortality than preterm birth, small for gestational age, and low birthweight, respectively, but are less common and also less often reported, with only one study reporting each of these outcomes. One prospective study with 2368 participants reported no effect of maternal HIV infection on very preterm birth and very small for gestational age, and the retrospective study reporting very low birthweight had only 58 participants. Miscarriage was reported in only one prospective study with 125 participants. The limited evidence available for very preterm birth, very small for gestational age, very low birthweight, neonatal death, and miscarriage therefore prevents a definitive conclusion regarding an effect of maternal HIV infection on these outcomes.

How HIV infection results in these specific outcomes is unknown. The pathogenesis of preterm birth, small for gestational age, stillbirth, and neonatal death is complex and the underlying causes and clinical presentations are very diverse. No causes are identified in many cases.[5,9,58] Preterm birth, for example, is a heterogeneous syndrome associated with a wide range of clinical phenotypes, which might be maternal, fetal or placental in origin, that are associated with distinct prognoses.[58] Maternal HIV infection induces a chronic state of inflammation and immune activation, which might disrupt normal immunological processes of pregnancy maintenance.[59,60] HIV might have a direct effect on placental growth and function;[61] or the effects might result from intrauterine HIV infection of the infant.[62] Only a small proportion of mother-to-child transmission of HIV occurs before 36 weeks, which makes a role of in-utero HIV infection in pathogenesis less likely.[63] We did not assess whether HIV transmission itself was associated with perinatal outcomes in our study. Finally, adverse perinatal outcomes could result from poor general maternal health caused by HIV infection.[21] However, not all perinatal outcomes are affected equally by maternal HIV infection and although use of antiretroviral therapy in pregnancy improves maternal health[2] it seems to increase rates of at least some perinatal outcomes.[64,65] Future research efforts need to be directed towards the understanding of the biological mechanisms underlying adverse perinatal outcomes in the context of HIV infection.

The increases in preterm birth, small for gestational age, low birthweight, and stillbirth seen in maternal HIV infection suggest that maternal HIV infection is a major contributor to the worldwide burden of perinatal and child morbidity and mortality. The fact that 91% of HIV positive pregnant women live in sub-Saharan Africa means that the effects on the burden of disease are most pronounced in this region. In 2013, 32% of HIV-positive pregnant women in Africa did not receive any antiretroviral therapy in pregnancy[66] and were therefore exposed to a higher risk of preterm birth, low birthweight, small for gestational age, and stillbirth. New WHO guidance recommends that all pregnant women receive triple antiretroviral therapy, and zidovudine monotherapy is no longer recommended.[67] Although both treatment options have similar efficacy in preventing mother-to-child transmission of HIV, triple therapy provides additional maternal health benefits.[67] However, the effect of different antiretroviral therapy regimens on perinatal outcomes is unknown. Accumulating evidence suggests that triple therapy, as compared with zidovudine monotherapy or dual therapy, is associated with higher rates of adverse outcomes, including preterm birth, small for gestational age, low birthweight, and stillbirth.[64,65,68] Furthermore, the initiation of triple therapy before pregnancy or in the first trimester seems to confer a higher risk of preterm birth than initiation later in pregnancy.[64] The maternal and transmission-prevention benefits of triple antiretroviral therapy might therefore need to be balanced against an increase in adverse perinatal outcomes. An urgent need exists, therefore, to assess the effects of different antenatal antiretroviral therapy regimens on perinatal outcomes and all-cause neonatal and child morbidity and mortality.

Contributors

COOW wrote the systematic review protocol, assisted with the scientific literature search, screened the search results for relevant manuscripts and assessed their eligibility for the review, did the methodological quality assessment, extracted data, did the meta-analysis, sensitivity analyses, and subgroup analyses, interpreted the data, and wrote the first draft of the manuscript. SK designed and did the scientific literature search, and contributed to earlier drafts of the manuscript. SH provided statistical support and contributed to earlier drafts of the manuscript. RC assisted with screening of the search results for relevant manuscripts. SHK contributed to the coordination of the study, the assessment of eligibility, methodological quality, and extracted data, interpreted the data, and co-wrote the manuscript. JH conceived, designed and coordinated the study, co-wrote the systematic review protocol, assisted with the scientific literature search, the assessment of eligibility, methodological quality, and extracted data, interpreted the data, and co-wrote the manuscript. JH had full access to all the data in the study and had final responsibility for the decision to submit the manuscript for publication. All authors read and approved the final version of the manuscript.

Declaration of interests

We declare no competing interests.

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Appendix 3.1

HIV and perinatal outcome systematic review protocol

An analysis of the HIV-associated population attributable risk for adverse pregnancy outcome, in ART-naïve mothers. A systematic review and meta-analysis.

1. Background

As the 2015 Millennium Development Goal (MDG) deadline approaches, concerns about stagnating perinatal mortality and morbidity statistics are being expressed worldwide.[1–6] The Human Immunodeficiency Virus (HIV) epidemic, a known contributor to poor maternal[7–10] and fetal[11]outcome, continues to affect 34 million people (31.4 million-35.9 million) globally, 69% of which reside in sub-Saharan Africa.[12] Despite an overall decline in new HIV infections since 2001 (20% decline), annual trends in the Middle East and North African states have shown a 35% increase in the incidence of HIV.[12]

One of the major gains made during the HIV epidemic has been the decline in mother to child transmission (MTCT).[12]This decline has been attributed to the implementation of prevention of mother to child transmission (PMTCT) programs, in addition to the adoption of specific intrapartum guidelines and infant feeding practices.[12–14]In the developed world, the initiation of antiretroviral treatment (ART) has contributed to the reduction in MTCT rates to less than 1-2%.[14] Furthermore, PMTCT programs have facilitated the early diagnosis and treatment of pregnant women affected by the disease, which translates to improved perinatal maternal outcome and reduced maternal mortality rate.[9]Despite the benefits of ART, adverse pregnancy outcome in the presence of maternal HIV infection continues to persist.

Preterm birth (PTB), low birth weight (LBW) and stillbirth are widely acknowledged global causes of perinatal morbidity and mortality.[15–17]Preterm birth was the leading cause of neonatal death in 2010[6]accounting for 1.078 million (95%CI 0.916-1.325million) of all neonatal deaths (3.072 million),[18] while an estimated 3.2 million stillbirths occur annually.[16]The debate as to the role of maternal HIV infection as a risk factor for adverse pregnancy outcome is still ongoing.[19]Some studies have reported an increased incidence of adverse pregnancy outcome in HIV infected women,[20,21]while others dispute this finding.[22] Furthermore, numerous studies have observed an increase risk of adverse pregnancy outcome in women receiving ART during their pregnancy, but this has also been disputed,[23,24]with one paper stating that preterm birth rates in these women were similar to those found in the HIV negative population.[25]

The association between HIV and adverse pregnancy outcome is a contentious issue, as it would have over-arching implications for millions of HIV infected women. The fact that ART could potentially exacerbate this effect would mean that millions of neonatal deaths would have been caused by the only treatment we have available. This dilemma stems from the fact that to date we still do not know what effect the HIV virus has on pregnancy outcome, in comparison to healthy uninfected controls. Furthermore, we do not know whether ART exacerbates this effect or alleviates it. This knowledge would enable us to determine whether all ART regimens, including those said to have the highest risk of complications,[26] are truly associated with lower risks of adverse pregnancy outcome compared to HIV-positive women not on treatment.

Numerous studies have attempted to assess the risk of adverse pregnancy outcome in HIV infected women who are naïve to ART. However, many of these studies have been small with little statistical power,[27]have had biased methodologies,[20,21]have not measured or controlled known confounding factors,[21,28]have not reported vital information[21]and have reported different effect sizes[29–31]

Over the years systematic reviews and meta-analyses have enabled us to generate an approximation of an overall effect size of interest, through the combination of independent studies addressing the same effect.[32–35]In 1998, Brocklehurst et al conducted a systematic review and meta-analysis which assessed the association between maternal HIV infection and perinatal outcome.[19]They found that maternal HIV infection was associated with adverse perinatal outcome including, PTB (OR 1.83 95%CI 1.63-2.06), LBW (OR 2.09 95%CI 1.86-2.35), intra-uterine growth restriction (IUGR) (OR 1.7 95%CI 1.43-2.02) and stillbirth (OR 3.91 95% CI 2.65-5.77).[19]However, this association was deemed weak due to the inappropriate methodology used by the included studies, and the lack of control of confounding factors.[19]

HIV is known for its genomic variability, with different types (HIV-1 and HIV-2), subtypes (HIV-1: M, N, O, P and HIV-2: A-H),[36]sub-subtypes (e.g. A1-A4),[37]and recombinant forms (e.g. CRFs and URFs).[36]Different viral strains have been associated with different levels of virulence, different rates of mother to child transmission and different levels of resistance to ART.[36,37]However, the influence of HIV diversity on adverse pregnancy outcome has not been addressed in any review, including the Brocklehurst paper.

The variability of HIV, in addition to other causes of clinical heterogeneity (e.g. genetic factors, maternal nutritional status, clinical/immunological stage of HIV, difference in antenatal care availability and quality, as well as obstetric guidelines), and the methodological heterogeneity (cohort, case-control and controlled trials) results in a substantial amount of statistical variability which would not be taken into account when using the fixed-effect model[38–40] as performed by Brocklehurst et al.[19]Although numerous systematic reviews have been done looking at the influence of ART on pregnancy outcome,[41,42]no updated systematic review has been done addressing the influence of HIV on pregnancy outcome.

We aim to conduct a systemic review assessing the influence of untreated maternal HIV infection on specific pregnancy outcomes including, PTB, LBW, small for gestational age (SGA), intrauterine growth restriction (IUGR), and stillbirth. We propose that the heterogeneity within and between included studies would best be analysed with the use of the random-effects model.[34,35]The fixed-effect model assumes that there is homogeneity between the studies included in the review, and that any variation in the underlying effect size being investigated is due to sampling error.[38,40] Conversely, the random-effect model assumes that the underlying effect has a normal distribution,[34]with the mean representing the overall average effect.[34]In systematic reviews where there is no heterogeneity between studies, the use of the random-effects model and fixed-effects model yield similar results.[35] However, in studies where heterogeneity is present, the random-effect model incorporates both the clinical variability and the statistical variability due to sampling error, into the analysis,[40] which may produce different results.[34,35]Subgroup analyses will be used to assess the association between known confounders and specified outcomes. Population attributable risk for adverse outcomes will be determined and correlated with HIV subtypes identified in the region.

We aim to assess all studies and abstracts which were included in the Brocklehurst paper, in addition to studies addressing the same questions which have been published since. This would give us a larger sample of results to incorporate into our analysis, thus translating to the statistical power needed to estimate the most probable effect size.[35]Only when the true effect of maternal HIV infection on perinatal outcome is clarified, can we truly assess whether the introduction of ART has improved perinatal outcome.

2. Objectives of the review

2.1 Primary objective

The primary objective of this review is to answer the following question:

1. Do pregnant HIV-positive women, who are not exposed to any ART during the antenatal period, have an increased risk of adverse birth outcomes compared to HIV-negative women? The outcomes of interests include: PTB, LBW, SGA, IUGR and stillbirth.

2.2 Secondary objective

The secondary objectives of the review are to answer the following questions:

1. Do pregnant HIV-positive women, who are not exposed to ART during the antenatal period, have an increased risk of miscarriage, compared to HIV-negative women?
2. Do pregnant HIV-positive women, who are not exposed to ART during the antenatal period, have an increased risk of neonatal death (NND) compared to HIV-negative women?
3. Do pregnant HIV-positive women infected with different HIV strains have different risks of adverse pregnancy outcomes?

3. Methods

3.1 Inclusion and exclusion criteria

The eligibility criteria are formulated with guidance from the Cochrane Handbook for systematic reviews of interventions.[33] (www.cochrane.org/training/cochrane-handbook)

3.1.1 Study design

The review will analyse quantitative data obtained from primary research papers focused on HIV/AIDS in the pregnant human population. Study designs to be reviewed include observational studies (cohort, case-control and cross-sectional studies) as well as interventional studies (randomised controlled trial and controlled clinical trial).

3.1.2 Setting

The setting of the different studies will be taken into consideration, especially when comparing studies compiled in high-income countries versus middle and low-income countries as defined by the World-Bank-country-specific-income-status http://data.worldbank.org/country.

This will enable us to compare the influence of socio-economic status, quality and access to antenatal care facilities, as well as whether specific HIV subtypes found in different regions are associated with increased risk of adverse pregnancy outcome.

3.1.3 Population of interest

The review will include studies containing quantitative data on pregnant HIV-positive women. These participants may be of any ethnicity, social or educational status. Details pertaining to the maternal demography, pregnancy outcome and information regarding the newborn should be recorded, e.g. twin deliveries, birth-weight, gestational age at birth, mortality and morbidity regarding the baby. Studies containing HIV-positive and HIV-negative cohorts as only a subset of the entire study cohort will initially be included and discussed amongst the reviewers. After the discussion, a final decision will be made regarding the retention of the paper.

The study will be excluded if it is deemed irrelevant or unsuitable for the review. A cohort of HIV-negative pregnant women of any ethnic, social or educational background, from the same community as the HIV-positive participants, should serve as the control group.

Studies will be excluded if they contain the following:

- Ambiguous inclusion and exclusion criteria.
- No HIV-negative pregnant participants as the control group.
- HIV-positive women who have received more than an intrapartum dose of ART during their pregnancy.

3.1.4 Exposure of interest

The exposure of interest is the HIV infection, which is contracted either before or during pregnancy by any form of transmission (e.g. heterosexual, intravenous drug use etc.). A healthcare worker (a doctor, nurse or HIV counsellor) should have diagnosed or confirmed the participant's HIV status either before or during the period in which the study was conducted. If this is not done, the study should explain how HIV status was validated. Participants presenting with any clinical or biochemical stage of HIV, including AIDS will be included. Studies will not be excluded if HIV-positive pregnant participants only received ART before the pregnancy and its use was terminated before the pregnancy for any reason, (e.g. non-adherence) or a single ART dose was received at delivery.

Studies including HIV-positive pregnant participants who present with comorbidities or opportunistic infections will be included. Confounding factors will be assessed and taken into account during statistical analysis. Studies conducted on HIV-positive pregnant women that assess other exposures will be included and only relevant data will be extracted and synthesised.

3.2 Outcome measures

3.2.1 Primary outcomes

3.2.1.1 PTB and PPROM

In this review we will assess papers reporting PTB, defined as birth before 37^{+0}weeks gestation.[16]These will be categorised as follows: moderately preterm (33^{+0}-36^{+6}weeks), very preterm birth ($<32^{+0}$weeks) and extremely preterm birth ($<28^{+0}$weeks).[16]PPROM will be assessed and the data extracted from all studies.

3.2.1.2 LBW, SGA and IUGR

Studies will be assessed for the birth-weight of infants born to HIV-positive and negative mothers. LBW will be defined as a birth-weight <2500g[43]and Very Low Birth Weight (VLBW) will be defined as birth-weight <1500g[44]. We will also assess SGA, which for our purposes will be defined as birth-weight-for-gestational age $<10^{th}$ centile as well as IUGR, which will be defined as birth-weight-for gestational age $<3^{rd}$ centile.

3.2.1.3 Stillbirth and Fetal death:

The term "stillbirth" is used inconsistently in the literature, which may lead to misclassification of the variable of interest.[45]It is used interchangeably with fetal death,[45] which may further result in the exclusion of vital data if we restrict ourselves to just one term. Therefore, for the purpose of this review, we will extract stillbirth and fetal death data.

It will be defined as any third trimester delivery of a demised fetus with:

- ≥1000g birth-weight [45] or
- $\geq 24^{+0}$ completed weeks or
- ≥ 35cm body length [45]

3.2.2 Secondary outcomes

The secondary outcomes of interest include:

3.2.2.1 Neonatal death and miscarriage:

We aim to assess whether HIV-positive women have an increased risk of neonatal death or miscarriage compared to HIV-negative women.

Neonatal death will be defined as the death of the infant within 28 days of life and miscarriage will be defined as the spontaneous expulsion of the fetus before 24^{+0}weeks gestation.

3.2.2.2 Population specific HIV-subtypes

We will assess the influence of known HIV strains in each country where the retrieved studies are conducted. The different strains will be defined according to the type (HIV-1 and HIV-2), subtypes (HIV-1: M, N, O, P and HIV-2: A-H), sub-subtypes (e.g. A1-A4), and recombinant forms (e.g. CRFs and URFs). Different viral strains specific to each population will be correlated with observed levels of adverse pregnancy outcome including: PTB, LBW, SGA, IUGR, stillbirth, miscarriage and neonatal death and mother to child transmission rate.

3.3. Search methods for identification of studies

We will identify relevant studies through manual and electronic search strategies. The electronic database search will include: PubMed, MEDLINE, EMBASE, Global Health, and CINAHL from

January 1980 to March 2013. No methodological or language filters will be applied. Search terms will include Mesh headings and Emtree terms for pregnancy outcome, PTB, LBW, IUGR, SGA, stillbirth and HIV or ART in addition to free text words and possible synonyms. The bibliographies of the relevant retrieved articles will be examined and all relevant references will be retrieved either manually or electronically. An electronic search of the grey literature (Proquest dissertations and theses) will also be conducted to identify unpublished papers, theses, reports and conference abstracts relevant to the systematic review. Lastly a number of experts in the field will be contacted to enquire about additional papers that we may not have identified. All relevant material will be included in the systematic review.

All retrieved papers will be imported into Endnote reference manager and duplicated articles will be removed. A record will be made of all papers excluded in the de-duplication process.

3.4 Study collection and critical appraisal

The selection and critical appraisal of the papers will be conducted in 4 stages.

3.4.1 Stage 1: Title and abstract screens

Following the literature search, 2 individual reviewers will scan the titles and abstracts of all papers retrieved to identify whether they contain data that is relevant to the systematic review. Only papers reporting case-control, cohort, cross-sectional or interventional studies assessing perinatal outcome in HIV-positive and HIV-negative women will be included in this initial stage. All letters, comments, opinion pieces and case-reports will be excluded. Non-English publications will be considered and those deemed relevant to the review will be included. Any disagreement regarding inclusion at this stage will be discussed with a third reviewer, who will make the final decision. All excluded studies will be recorded.

3.4.2 Stage 2: Eligibility criteria

Two independent reviewers will assess each paper as to whether they meet the eligibility criteria. All studies which do not fulfil the eligibility criteria will be excluded. A record will be kept as to why each paper was excluded:

Inclusion criteria	Exclusion criteria
• HIV-positive pregnant women	• Ambiguous inclusion criteria
• HIV-negative pregnant control group	• > Stat dose of ART received during pregnancy
• Data collected on birth-weight or gestational age at delivery or stillbirth	• No control group

3.4.3 Stage 3: Quality appraisal of papers

Two reviewers will assess each paper according to a structured pre-formulated template. Numerous guidelines have been suggested to facilitate the improvement in the quality of reporting of observational studies (STROBE statement) and RCTs (CONSORT).[35]Although this improves the quality of the reporting, it does not necessarily translate to a good methodological quality.[46,47]To date there is no consensus regarding a quality assessment checklist for observational studies.[48]

We anticipate a wide range in methodological quality because of the inclusion of interventional studies as well as observational studies. Due to the vast difference in study methodology between observational and interventional studies we have chosen to use 2 different quality assessment tools, which are specific for each group.

The quality of the interventional studies (RCT and controlled studies) will be assessed according to the Cochrane risk of bias tool kit (Appendix 1). Study quality will be categorised as follows:

- Good quality: This will include studies which have fulfilled all 5 requirements of the risk assessment tool checklist and have no additional sources of bias.
- Average quality: This will include studies which have fulfilled between 2-4 of the 5 requirements of the risk assessment tool. Furthermore, they should not have any major methodological flaws, e.g. poor randomisation or additional sources of bias.

- Poor quality studies: Studies which only fulfil <2 of the requirements or contain a major methodological flaw.

An adaption of the Newcastle-Ottawa quality assessment tool will be used to assess all observational studies. Study quality will be categorised as follows:

- Good quality: Studies which meet all requirements (9stars).
- Average quality: Studies which achieve >2 stars in the "selection" section and "exposure" section, but < 9 overall.
- Poor quality: Studies which achieve ≤2 stars in both the "selection" section and the "exposure" section of the assessment tool.

Two independent reviewers will conduct this process, and any discrepancy in quality score will be discussed with a third reviewer, who will make the final decision.

A summary of the quality assessment results will be presented for each included study and correlated with the effect size observed in the study.

All studies will be combined according to the quality category which they have been assigned to e.g. good, average and low quality. Following data extraction, an overall aggregate effect size will be calculated for all primary and secondary outcomes in relation to different ART regimens and displayed as forest plots. Thereafter, the level of evidence will be graded according to the Grading of Recommendations Assessment, Development and Evaluation System (GRADE) http://www.gradeworkinggroup.org (Table 2).

Table 2: Grade of evidence: Levels of quality of a body of evidence in the GRADE approach	
Underlying methodology	**Quality rating**
Randomized trials; or double-upgraded observational studies.	High
Downgraded randomized trials; or upgraded observational studies.	Moderate
Double-downgraded randomized trials; or observational studies.	Low
Triple-downgraded randomized trials; or downgraded observational studies; or case series/case reports.	Very low

3.5 Data Extraction

Two independent reviewers will extract relevant data from the selected studies. A data collection sheet has been developed for the purpose of the review. It will allow reviewers to record data electronically into an Access-based database. The data extracted will include specific details including: study descriptors (e.g. journal title, author name, year of publication etc.), population information (e.g. maternal HIV status), outcome measures of interest, (e.g. MTCT, PTB, LBW, SGA, stillbirth) and confounding factors identified, non-adjusted and adjusted effect estimates, standard error, standard deviation as well as 95% CI. Data extracted will be compared between the two reviewers and any disagreement between authors will be resolved by discussion with the third reviewer.

3.6 Data synthesis

3.6.1 Summary of findings

A tabulated summary of all included studies will be presented with details regarding the following variables:[33]

- Study type
- Participants in each group (mother and infants)
- Maternal demographic and clinical characteristics
- Outcome measures
- Quality assessment score and aspects which the study did not fulfil.

3.6.2 Data analysis

Summary statistics will be compiled for individual studies irrespective of quality assessment score. All binary statistics will be recorded as relative risk ratios (RR), and results reported as odds ratios in retrieved studies will be converted to relative risk ratios.[33,34,49,50]Continuous data will be recorded as a mean. In addition, the population attributable risk fraction (PAR) for the adverse outcomes, namely LBW, SGA, PTB, PPROM and stillbirth, will be calculated for each study. If we assume that maternal HIV infection is a risk factor for the above mentioned adverse pregnancy outcomes, the PAR would enable us to better demonstrate the proportion of these outcomes, within the study populations, which would be modified or prevented if HIV infection were prevented.

We anticipate a heterogeneous sample of studies from the literature search; hence the null hypothesis will be tested using a random effects model.[34] We plan to conduct several meta-analyses of the aggregated data, with the aim of assessing the influence of HIV on the primary outcomes of interest. Forest plots will be used to demonstrate the patterns of effect. In instances where data cannot be pooled, a narrative summary will be produced.

3.6.3 Subgroup analysis

Subgroup analysis will be conducted to assessed the influence of possible covariates on the observed adverse pregnancy outcome as well as additional variables:

- Maternal demography: country, income status of country, alcohol use during pregnancy, smoking, advanced maternal age, teenage pregnancy, ethnicity, number of antenatal care receive and intravenous drug use.
- HIV stage: Immunological stage (mean $CD4^{+}$) or clinical stage of HIV (WHO/CDC).
- Mother to child transmission
- HIV subtypes

3.6.4 Unit of analysis

We aim to analyse the influence of maternal HIV infection on newborn outcome. Therefore, we will report information relating to both the mother and the infant.

3.6.5 Sensitivity analysis:

The sensitivity analysis aims to assess the robustness of the observed effect size.[51] We plan to conduct 5 sensitivity analyses including:

- Location of the study: low, middle and high-income country.
- Methodological quality: high quality versus average quality
- Study design: observational studies versus RCT
- Control for confounding factors: studies which used methods for controlling for known confounders and those which have not.
- The exclusion of studies with the largest effect size.

Meta-analyses will be conducted and differences between comparisons will be reported.

3.6.6. Assessment of heterogeneity

The statistical heterogeneity of the studies retrieved will be evaluated by comparing the study design and setting, in addition to Chi^2, τ^2 and I^2 statistic. The results will be assessed for clinical and statistical relevance.[33,34] The I^2 statistic will be used to test the null hypothesis of homogeneity and quantify the percentage of heterogeneity in effect size which occur as a consequence of between-study variability.[38,40] The result will be interpreted as: no heterogeneity (0%), low heterogeneity (25%), moderate heterogeneity (50%) and high heterogeneity (75%). [40,52]

In the event that a small number of studies are retrieved and included in the review, we will take the low statistical power of the I^2 statistic into consideration when we report our findings.[40]

3.6.7 Publication bias

Small study bias will be assessed with the use of funnel plots.[35]If any evidence of publication bias is identified. We will explore publication bias by looking for unpublished trials on trial registries.

Appendix 3.2

Specific search strategy.

Embase search strategy for “preterm birth or low birth weight or intrauterine growth restriction or stillbirth” and “HIV or ART”.

Search carried out on 20 February 2013 and rerun on 12 December 2014 (limited to the years 2013 or 2014 only) to include studies published between 1 January 1980 and 7 December 2014. Embase (OVID 1974 to 2013 Week 07):

1. Prematurity/
2. Premature labor/
3. Premature fetus membrane rupture/
4. Immature and premature labor/
5. ‘premature birth’.ti,ab.
6. ‘prematurity’.ti,ab.
7. 'gestational age at birth'.ti,ab.
8. 'gestational age at delivery'.ti,ab.
9. (PTB or PTBS).ti,ab.
10. (VPTB or VPTBs).ti,ab.
11. (pre-terms or preterms).ti,ab.
12. (pre-term adj (birth or births)).ti,ab.
13. (preterm adj (birth or births)).ti,ab.
14. (premature adj (birth or births)).ti,ab.
15. (PTL or PTLs).ti,ab.
16. (VPTL or VPTLs).ti,ab.
17. ((pre-term or preterm) adj (labor* or labour*)).ti,ab.
18. (pre-term adj obstetric adj labor*).ti,ab.
19. (pre-term adj obstetric adj labour*).ti,ab.
20. (preterm adj obstetric adj labor*).ti,ab.
21. (preterm adj obstetric adj labour*).ti,ab.
22. (premature adj (labor* or labour*)).ti,ab.
23. (premature adj obstetric adj labor*).ti,ab.
24. (premature adj obstetric adj labour*).ti,ab.
25. (PTD or PTDs).ti,ab.
26. (VPTD or VPTDs).ti,ab.
27. ((pre-term or preterm) adj deliver*).ti,ab.
28. ((pre-term or preterm) adj infant*).ti,ab.
29. (premature adj (deliver* or infant*)).ti,ab.
30. (PROM or PPROM).ti,ab.
31. 'preterm rupture of membranes'.ti,ab.
32. 'preterm rupture of fetal membranes'.ti,ab.
33. 'preterm rupture of foetal membranes'.ti,ab.
34. 'pre-term rupture of membranes'.ti,ab.
35. 'pre-term rupture of fetal membranes'.ti,ab.
36. 'pre-term rupture of foetal membranes'.ti,ab.
37. 'premature rupture of membranes'.ti,ab.
38. 'premature rupture of fetal membranes'.ti,ab.
39. 'premature rupture of foetal membranes'.ti,ab.
40. 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 or 26 or 27 or 28 or 29 or 30 or 31 or 32 or 33 or 34 or 35 or 36 or 37 or 38 or 39
41. Intrauterine growth retardation/

42. Small for date infant/
43. Low birth weight/
44. Very low birthweight/
45. Extremely low birth weight/
46. (IUGR or FGR).ti,ab.
47. ((intrauterine or intra-uterine) adj growth adj restriction).ti,ab.
48. ((intrauterine or intra-uterine) adj growth adj restricted).ti,ab.
49. ((intrauterine or intra-uterine) adj growth adj retardation).ti,ab.
50. 'fetal growth restriction'.ti,ab.
51. 'fetal growth restricted'.ti,ab.
52. 'fetal growth retardation'.ti,ab.
53. (SGA or SFGA).ti,ab.
54. 'small for gestational age'.ti,ab.
55. 'small-for-gestational-age'.ti,ab.
56. 'small-for-gestational age'.ti,ab.
57. 'small for gestation'.ti,ab.
58. 'small-for-gestation'.ti,ab.
59. (VSGA or SFD).ti,ab.
60. 'very-small-for-gestational-age'.ti,ab.
61. 'very-small-for-gestational age'.ti,ab.
62. 'small for dates'.ti,ab.
63. 'small-for-dates'.ti,ab.
64. 'weight for dates'.ti,ab.
65. 'weight for gestational age'.ti,ab.
66. 'weight for age at delivery'.ti,ab.
67. 'weight at delivery'.ti,ab.
68. 'birthweight for dates'.ti,ab.
69. 'birthweight for gestational age'.ti,ab.
70. 'birthweight for age at delivery'.ti,ab.
71. 'birth weight for dates'.ti,ab.
72. 'birth weight for gestational age'.ti,ab.
73. 'birth weight for age at delivery'.ti,ab.
74. 'birth-weight for dates'.ti,ab.
75. 'birth-weight for gestational age'.ti,ab.
76. 'birth-weight for age at delivery'.ti,ab.
77. LBW.ti,ab.
78. 'low BW'.ti,ab.
79. 'low birth weight'.ti,ab.
80. 'low birth-weight'.ti,ab.
81. 'low-birth weight'.ti,ab.
82. 'low-birth-weight'.ti,ab.
83. 'low birthweight'.ti,ab.
84. 'low-birthweight'.ti,ab.
85. 'lower BW'.ti,ab.
86. 'lower birth weight'.ti,ab.
87. 'lower birth-weight'.ti,ab.
88. 'lower-birth weight'.ti,ab.
89. 'lower-birth-weight'.ti,ab.
90. 'lower birthweight'.ti,ab.
91. 'lower-birthweight'.ti,ab.
92. 'reduced birth weight'.ti,ab.
93. 'reduced birthweight'.ti,ab.
94. 'reduced birth-weight'.ti,ab.
95. (VLBW or ELBW).ti,ab.

96. 'very-low birthweight'.ti,ab.
97. 'very-low birth weight'.ti,ab.
98. 'very-low birth-weight'.ti,ab.
99. 'very-low-birthweight'.ti,ab.
100. 'very-low-birth-weight'.ti,ab.
101. 'extremely-low birthweight'.ti,ab.
102. 'extremely-low birth weight'.ti,ab.
103. 'extremely-low birth-weight'.ti,ab.
104. 'extremely-low-birthweight'.ti,ab.
105. 'extremely-low-birth-weight'.ti,ab.
106. 41 or 42 or 43 or 44 or 45 or 46 or 47 or 48 or 49 or 50 or 51 or 52 or 53 or 54 or 55 or 56 or 57 or 58 or 59 or 60 or 61 or 62 or 63 or 64 or 65 or 66 or 67 or 68 or 69 or 70 or 71 or 72 or 73 or 74 or 75 or 76 or 77 or 78 or 79 or 80 or 81 or 82 or 83 or 84 or 85 or 86 or 87 or 88 or 89 or 90 or 91 or 92 or 93 or 94 or 95 or 96 or 97 or 98 or 99 or 100 or 101 or 102 or 103 or 104 or 105
107. Stillbirth/
108. Fetus death/
109. (stillbirth* or stillborn*).ti,ab.
110. (still adj (born* or birth*)).ti,ab.
111. (abortion* or miscarriage*).ti,ab.
112. (pregnancy adj (death* or loss* or demise* or mortalit*)).ti,ab.
113. (gestational adj (death* or loss* or demise* or mortalit*)).ti,ab.
114. (fetal adj (death* or loss* or demise* or mortalit*)).ti,ab.
115. (foetal adj (death* or loss* or demise* or mortalit*)).ti,ab.
116. (obstetric* adj (death* or loss* or demise* or mortalit*)).ti,ab.
117. (labor adj (death* or loss* or demise* or mortalit*)).ti,ab.
118. (birth adj (death* or loss* or demise* or mortalit*)).ti,ab.
119. (delivery adj (death* or loss* or demise* or mortalit*)).ti,ab.
120. (neonat* adj (death* or loss* or demise* or mortalit*)).ti,ab.
121. (newborn adj (death* or loss* or demise* or mortalit*)).ti,ab.
122. (new-born adj (death* or loss* or demise* or mortalit*)).ti,ab.
123. (new adj born adj death*).ti,ab.
124. (new adj born adj loss*).ti,ab.
125. (new adj born adj demise*).ti,ab.
126. (new adj born adj mortalit*).ti,ab.
127. (infant adj (death* or loss* or demise* or mortalit*)).ti,ab.
128. (reproductive adj (death* or loss* or demise* or mortalit*)).ti,ab.
129. (prelabor adj (death* or loss* or demise* or mortalit*)).ti,ab.
130. (pre-labor adj (death* or loss* or demise* or mortalit*)).ti,ab.
131. (intrauterine adj (death* or loss* or demise* or mortalit*)).ti,ab.
132. (intra-uterine adj (death* or loss* or demise* or mortalit*)).ti,ab.
133. (antenatal adj (death* or loss* or demise* or mortalit*)).ti,ab.
134. (ante-natal adj (death* or loss* or demise* or mortalit*)).ti,ab.
135. (prenatal adj (death* or loss* or demise* or mortalit*)).ti,ab.
136. (pre-natal adj (death* or loss* or demise* or mortalit*)).ti,ab.
137. (perinatal adj (death* or loss* or demise* or mortalit*)).ti,ab.
138. (peri-natal adj (death* or loss* or demise* or mortalit*)).ti,ab.
139. (neo-natal adj (death* or loss* or demise* or mortalit*)).ti,ab.
140. (postnatal adj (death* or loss* or demise* or mortalit*)).ti,ab.
141. (post-natal adj (death* or loss* or demise* or mortalit*)).ti,ab.
142. (antepartum adj (death* or loss* or demise* mortalit*)).ti,ab.
143. (ante-partum adj (death* or loss* or demise* or mortalit*)).ti,ab.
144. (intrapartum adj (death* or loss* or demise* or mortalit*)).ti,ab.
145. (intra-partum adj (death* or loss* or demise* or mortalit*)).ti,ab.
146. (peripartum adj (death* or loss* or demise* or mortalit*)).ti,ab.

147. (peri-partum adj (death* or loss* or demise* or mortalit*)).ti,ab.
148. (postpartum adj (death* or loss* or demise* or mortalit*)).ti,ab.
149. (post-partum adj (death* or loss* or demise* or mortalit*)).ti,ab.
150. 107 or 108 or 109 or 110 or 111 or 112 or 113 or 114 or 115 or 116 or 117 or 118 or 119 or 120 or 121 or 122 or 123 or 124 or 125 or 126 or 127 or 128 or 129 or 130 or 131 or 132 or 133 or 134 or 135 or 136 or 137 or 138 or 139 or 140 or 141 or 142 or 143 or 144 or 145 or 146 or 147 or 148 or 149
151. Human immunodeficiency virus/
152. Human immunodeficiency virus 1/
153. Human immunodeficiency virus 1 infection/
154. Human immunodeficiency virus 2/
155. Human immunodeficiency virus 2 infection/
156. Human immunodeficiency virus infection/
157. Human immunodeficiency virus infected patient/
158. Acquired immune deficiency syndrome/
159. Serodiagnosis/
160. HIV associated dementia/
161. HIV associated lipodystrophy/
162. HIV associated nephropathy/
163. AIDS related complex/
164. 'HIV seropositivity'.ti,ab.
165. 'HIV infection*'.ti,ab.
166. 'AIDS serodiagnosis'.ti,ab.
167. 'AIDS arteritis'.ti,ab.
168. 'AIDS-associated nephropathy'.ti,ab.
169. 'AIDS dementia complex'.ti,ab.
170. 'AIDS-related opportunistic infection*'.ti,ab.
171. 'AIDS-related lymphoma'.ti,ab.
172. (HIV or HIV-1 or HIV-type-1).ti,ab.
173. 'HTLV III'.ti,ab.
174. 'HTLV type III'.ti,ab.
175. (HTLV-III or HTLV-type-III or LAV or HTLV-III-LAV or LAV-HTLV-III).ti,ab.
176. (HIV-2 or HIV-type-2 or HIV-II or HTLV-IV or LAV-2).ti,ab.
177. (HIV-positive or HIV-1-positive or HIV-2-positive or HIV-infected).ti,ab.
178. (HIV-1-infected or HIV-type-1-infected or HTLV-III-infected or HTLV-type-III-infected or LAV-infected).ti,ab.
179. 'HTLV III-infected'.ti,ab.
180. 'HTLV type III-infected'.ti,ab.
181. (HTLV-III-LAV-infected or LAV-HTLV-III-infected or HIV-2-infected or HIV-type-2-infected or HIV-II-infected).ti,ab.
182. (HTLV-IV-infected or LAV-2-infected or HIV-infection*or HIV-1-infection* or HIV-type-1-infection* or HTLV-III-infection* or HTLV-type-III-infection*).ti,ab.
183. 'HTLV III-infection*'.ti,ab.
184. 'HTLV type III-infection*'.ti,ab.
185. (LAV-infection* or HTLV-III-LAV-infection* or LAV-HTLV-III-infection* or HIV-2-infection* or HIV-type-2-infection* or HIV-II-infection* or HTLV-IV-infection* or LAV-2-infection*).ti,ab.
186. 'Human Immunodeficiency Virus*'.ti,ab.
187. 'Human Immune Deficiency Virus*'.ti,ab.
188. 'Human T Cell Lymphotropic Virus Type III'.ti,ab.
189. 'Human T-Cell Lymphotropic Virus Type III'.ti,ab.
190. 'Human T Lymphotropic Virus Type III'.ti,ab.
191. 'Human T-Lymphotropic Virus Type III'.ti,ab.
192. 'Human T Lymphotropic Virus Type IV'.ti,ab.
193. 'Human T-Lymphotropic Virus Type IV'.ti,ab.
194. 'Human T Cell Leukemia Virus Type III'.ti,ab.

195. 'Human T-Cell Leukemia Virus Type III'.ti,ab.
196. 'Lymphadenopathy-Associated Virus*'.ti,ab.
197. 'Lymphadenopathy Associated Virus*'.ti,ab.
198. 'Acquired Immune Deficiency Syndrome'.ti,ab.
199. 'Acquired Immunodeficiency Syndrome'.ti,ab.
200. AIDS.ti,ab.
201. 151 or 152 or 153 or 154 or 155 or 156 or 157 or 158 or 159 or 160 or 161 or 162 or 163 or 164 or 165 or 166 or 167 or 168 or 169 or 170 or 171 or 172 or 173 or 174 or 175 or 176 or 177 or 178 or 179 or 180 or 181 or 182 or 183 or 184 or 185 or 186 or 187 or 188 or 189 or 190 or 191 or 192 or 193 or 194 or 195 or 196 or 197 or 198 or 199 or 200
202. Human immunodeficiency virus antibody/
203. Human immunodeficiency virus antigen/
204. exp Human immunodeficiency virus fusion inhibitor/
205. exp Human immunodeficiency virus proteinase inhibitor/
206. exp Anti human immunodeficiency virus agent/
207. exp Integrase inhibitor/
208. exp RNA directed DNA polymerase inhibitor/
209. Antiretrovirus agent/
210. Proteinase inhibitor/
211. (antiretroviral* or anti-retroviral*).ti,ab.
212. (antiretroviral adj (treatment* or therap* or regimen* or drug* or agent*)).ti,ab.
213. (anti-retroviral adj (treatment* or therap* or regimen* or drug* or agent*)).ti,ab.
214. (antiviral* or anti-viral*).ti,ab.
215. (antiviral adj (treatment* or therap* or regimen* or drug* or agent*)).ti,ab.
216. (anti-viral adj (treatment* or therap* or regimen* or drug* or agent*)).ti,ab.
217. (anti-HIV adj (treatment* or therap* or regimen* or drug* or agent*)).ti,ab.
218. (HIV adj (treatment* or therap* or regimen* or drug* or agent*)).ti,ab.
219. (anti-HIV-1 adj (treatment* or therap* or regimen* or drug* or agent*)).ti,ab.
220. (HIV-1 adj (treatment* or therap* or regimen* or drug* or agent*)).ti,ab.
221. (anti-HIV-2 adj (treatment* or therap* or regimen* or drug* or agent*)).ti,ab.
222. (HIV-2 adj (treatment* or therap* or regimen* or drug* or agent*)).ti,ab.
223. (anti-AIDS adj (treatment* or therap* or regimen* or drug* or agent*)).ti,ab.
224. (AIDS adj (treatment* or therap* or regimen* or drug* or agent*)).ti,ab.
225. (HAART or ARV or ARVs or cARV or cARVs).ti,ab.
226. (HAART-exposed or HAART-treated or Mega-HAART).ti,ab.
227. (ARV-exposed or ARV-treated or combination-ARV or combination-ARVs or combined-ARV or combined-ARVs).ti,ab.
228. (ART or Multi-ART or Triple-ART or cART or ART-exposed or ART-treated or combination-ART or combined-ART or sc-ART).ti,ab.
229. 'short-course-antiretroviral therap*'.ti,ab.
230. 'short-course-anti-retroviral therap*'.ti,ab.
231. (combination adj (treatment* or therap* or regimen* or drug* or agent*)).ti,ab.
232. (combined adj (treatment* or therap* or regimen* or drug* or agent*)).ti,ab.
233. (monotherap* or mono-therap*).ti,ab.
234. 'dual therap*'.ti,ab.
235. 'dual drug therap*'.ti,ab.
236. bitherap*.ti,ab.
237. (PI or PIs or PI-based or PI-boosted or PI-containing or PI-therap* or PI-treatment* or PI-regimen*).ti,ab.
238. (Ritonavir-boosted or NRTI or NRTIs or NRTI-based).ti,ab.
239. 'protease inhibitor*'.ti,ab.
240. (NRTI-containing or NRTI-therap* or NRTI-treatment* or NRTI-regimen*).ti,ab.
241. 'nucleoside reverse transcriptase inhibitor*'.ti,ab.
242. 'nucleoside analog reverse transcriptase inhibitor*'.ti,ab.

243. (NNRTI or NNRTIs or NNRTI-based or NNRTI-containing or NNRTI-therap* or NNRTI-treatment* or NNRTI-regimen*).ti,ab.
244. 'non nucleoside reverse transcriptase inhibitor*'.ti,ab.
245. 'non-nucleoside reverse transcriptase inhibitor*'.ti,ab.
246. 'nonnucleoside reverse transcriptase inhibitor*'.ti,ab.
247. 'non nucleoside analog reverse transcriptase inhibitor*'.ti,ab.
248. 'non-nucleoside analog reverse transcriptase inhibitor*'.ti,ab.
249. 'nonnucleoside analog reverse transcriptase inhibitor*'.ti,ab.
250. (NtRTI or NtRTIs or NtRTI-based or NtRTI-containing or NtRTI-therap* or NtRTI-treatment* or NtRTI-regimen*).ti,ab.
251. 'nucleotide reverse transcriptase inhibitor*'.ti,ab.
252. 'nucleotide analog reverse transcriptase inhibitor*'.ti,ab.
253. 'fusion inhibitor*'.ti,ab.
254. 'CCR5 receptor antagonist*'.ti,ab.
255. 'integrase inhibitor*'.ti,ab.
256. 'maturation inhibitor*'.ti,ab.
257. 'entry inhibitor*'.ti,ab.
258. (Abacavir or ABC).tw.
259. Abacavir/
260. Abacavir plus lamivudine/
261. Abacavir plus lamivudine plus zidovudine/
262. (Didanosine or ddI).tw.
263. Didanosine/
264. (Emtricitabine or FTC).tw.
265. Emtricitabine/
266. Efavirenz plus emtricitabine plus tenofovir disoproxil/
267. Emtricitabine plus rilpivirine plus tenofovir disoproxil/
268. Emtricitabine plus tenofovir disoproxil/
269. (Lamivudine or 3TC).tw.
270. Lamivudine/
271. Efavirenz plus lamivudine plus zidovudine/
272. Lamivudine plus nevirapine plus stavudine/
273. Lamivudine plus nevirapine plus tenofovir disoproxil/
274. Lamivudine plus nevirapine plus zidovudine/
275. Lamivudine plus stavudine/
276. Lamivudine plus tenofovir disoproxil/
277. Lamivudine plus zidovudine/
278. (Stavudine or d4T).tw.
279. Stavudine/
280. (Tenofovir or TFV or TDF).tw.
281. Tenofovir/
282. Cobicistat plus elvitegravir plus emtricitabine plus tenofovir disoproxil/
283. Tenofovir disoproxil/
284. Tenofovir 3 hexadecyloxypropyl ester/
285. Tenofovir alafenamide/
286. (Zidovudine or AZT or ZDV).tw.
287. Zidovudine/
288. Zidovudine 5' phosphate/
289. Zidovudine 5' triphosphate/
290. Zidovudine derivative/
291. Zidovudine glucuronide/
292. (Delavirdine or DLV).tw.
293. Delavirdine/
294. (Efavirenz or EFV).tw.

295. Efavirenz/
296. (Etravirine or ETR).tw.
297. Etravirine/
298. (Nevirapine or NVP).tw.
299. Nevirapine/
300. (Rilpivirine or RPV).tw.
301. Rilpivirine/
302. (Atazanavir or ATV).tw.
303. (Atazanavir/Ritonavir).tw.
304. (ATV/r).tw.
305. Atazanavir/
306. Atazanavir plus ritonavir/
307. (Darunavir or DRV).tw.
308. (Darunavir adj Ritonavir).tw.
309. (DRV adj r).tw.
310. Darunavir/
311. Darunavir plus ritonavir/
312. (Fosamprenavir or FPV).tw.
313. (Fosamprenavir adj Ritonavir).tw.
314. (FPV adj r).tw.
315. Fosamprenavir/
316. Fosamprenavir plus ritonavir/
317. (Indinavir or IDV).tw.
318. (Indinavir adj Ritonavir).tw.
319. (IDV adj r).tw.
320. Indinavir/
321. Indinavir plus ritonavir/
322. (Lopinavir or LPV).tw.
323. (Lopinavir adj Ritonavir).tw.
324. (LPV adj r).tw.
325. Lopinavir/
326. Lopinavir plus ritonavir/
327. (Nelfinavir or NFV).tw.
328. (Nelfinavir adj Ritonavir).tw.
329. (NFV adj r).tw.
330. Nelfinavir/
331. (Ritonavir or RTV).tw.
332. Ritonavir/
333. (Saquinavir or SQV).tw.
334. (Saquinavir adj Ritonavir).tw.
335. (SQV adj r).tw.
336. Saquinavir/
337. Ritonavir plus saquinavir/
338. (Tipranavir or TPV).tw.
339. (Tipranavir adj Ritonavir).tw.
340. (TPV adj r).tw.
341. Tipranavir/
342. Ritonavir plus tipranavir/
343. (Enfuvirtide or T-20).tw.
344. Enfuvirtide/
345. (Maraviroc or MVC).tw.
346. Maraviroc/
347. (Raltegravir or RAL).tw.
348. Raltegravir/

349. (Elvitegravir or EVG).tw.
350. Elvitegravir/
351. (Zalcitabine or ddC).tw.
352. Zalcitabine/
353. (Combivir or Trizivir or Kaletra or Epzicom or Kivexa or Truvada or Atripla).tw.
354. 202 or 203 or 204 or 305 or 206 or 207 or 208 or 209 or 210 or 211 or 212 or 213 or 214 or 215 or 216 or 217 or 218 or 219 or 220 or 221 or 222 or 223 or 224 or 225 or 226 or 227 or 228 or 229 or 230 or 231 or 232 or 233 or 234 or 235 or 236 or 237 or 238 or 239 or 240 or 241 or 242 or 243 or 244 or 245 or 246 or 247 or 248 or 249 or 250 or 251 or 252 or 253 or 254 or 255 or 256 or 257 or 258 or 259 or 260 or 261 or 262 or 263 or 264 or 265 or 266 or 267 or 268 or 269 or 270 or 271 or 272 or 273 or 274 or 275 or 276 or 277 or 278 or 279 or 280 or 281 or 282 or 283 or 284 or 285 or 286 or 287 or 288 or 289 or 290 or 291 or 292 or 293 or 294 or 295 or 296 or 297 or 298 or 299 or 300 or 301 or 302 or 303 or 304 or 305 or 306 or 307 or 308 or 309 or 310 or 311 or 312 or 313 or 314 or 315 or 316 or 317 or 318 or 319 or 320 or 321 or 322 or 323 or 324 or 325 or 326 or 327 or 328 or 329 or 330 or 331 or 332 or 333 or 334 or 335 or 336 or 337 or 338 or 339 or 340 or 341 or 342 or 343 or 344 or 345 or 346 or 347 or 348 or 349 or 350 or 351 or 352 or 353
355. 40 OR 106 OR 150
356. 201 OR 354
357. 355 AND 356
358. limit 357 to yr=1980-2013

Appendix 3.3

General search strategy

Embase search strategy for “pregnancy outcome” and “HIV or ART”.

Search carried out on 20 February 2013 and rerun on 12 December 2014 (limited to the years 2013 or 2014 only) to include studies published between 1 January 1980 and 7 December 2014.

1. Pregnancy outcome/
2. Pregnancy complication/
3. (pregnancy adj (outcome* or complication* or consequence* or characteristic* or event* or result* or problem* or morbidit* or sequelae)).ti,ab.
4. (gestational adj (outcome* or complication* or consequence* or characteristic* or event* or result* or problem* or morbidit* or sequelae)).ti,ab.
5. Fetus outcome/
6. (fetal adj (outcome* or complication* or consequence* or characteristic* or event* or result* or problem* or morbidit* or sequelae)).ti,ab.
7. (foetal adj (outcome* or complication* or consequence* or characteristic* or event* or result* or problem* or morbidit* or sequelae)).ti,ab.
8. (obstetric adj (outcome* or complication* or consequence* or characteristic* or event* or result* or problem* or morbidit* or sequelae)).ti,ab.
9. (obstetrical adj (outcome* or complication* or consequence* or characteristic* or event* or result* or problem* or morbidit* or sequelae)).ti,ab.
10. Labor complication/
11. (labor adj (outcome* or complication* or consequence* or characteristic* or event* or result* or problem* or morbidit* or sequelae)).ti,ab.
12. (labour adj (outcome* or complication* or consequence* or characteristic* or event* or result* or problem* or morbidit* or sequelae)).ti,ab.
13. (birth adj (outcome* or complication* or consequence* or characteristic* or event* or result* or problem* or morbidit* or sequelae)).ti,ab.
14. (delivery adj (outcome* or complication* or consequence* or characteristic* or event* or result* or problem* or morbidit* or sequelae)).ti,ab.
15. (neonate adj (outcome* or complication* or consequence* or characteristic* or event* or result* or problem* or morbidit* or sequelae)).ti,ab.
16. (newborn adj (outcome* or complication* or consequence* or characteristic* or event* or result* or problem* or morbidit* or sequelae)).ti,ab.
17. (new-born adj (outcome* or complication* or consequence* or characteristic* or event* or result* or problem* or morbidit* or sequelae)).ti,ab.
18. "new born outcome*".ti,ab.
19. "new born complication*".ti,ab.
20. "new born consequence*".ti,ab.
21. "new born characteristic*".ti,ab.
22. "new born event*".ti,ab.
23. "new born result*".ti,ab.
24. "new born problem*".ti,ab.
25. "new born morbidit*".ti,ab.
26. "new born sequelae*".ti,ab.
27. (infant adj (outcome* or complication* or consequence* or characteristic* or event* or result* or problem* or morbidit* or sequelae)).ti,ab.

28. (reproductive adj (outcome* or complication* or consequence* or characteristic* or event* or result* or problem* or morbidit* or sequelae)).ti,ab.
29. (prelabour adj (outcome* or complication* or consequence* or characteristic* or event* or result* or problem* or morbidit* or sequelae)).ti,ab.
30. (prelabor adj (outcome* or complication* or consequence* or characteristic* or event* or result* or problem* or morbidit* or sequelae)).ti,ab.
31. (pre-labour adj (outcome* or complication* or consequence* or characteristic* or event* or result* or problem* or morbidit* or sequelae)).ti,ab.
32. (pre-labor adj (outcome* or complication* or consequence* or characteristic* or event* or result* or problem* or morbidit* or sequelae)).ti,ab.
33. (intrauterine adj (outcome* or complication* or consequence* or characteristic* or event* or result* or problem* or morbidit* or sequelae)).ti,ab.
34. (intra-uterine adj (outcome* or complication* or consequence* or characteristic* or event* or result* or problem* or morbidit* or sequelae)).ti,ab.
35. (antenatal adj (outcome* or complication* or consequence* or characteristic* or event* or result* or problem* or morbidit* or sequelae)).ti,ab.
36. (ante-natal adj (outcome* or complication* or consequence* or characteristic* or event* or result* or problem* or morbidit* or sequelae)).ti,ab.
37. (prenatal adj (outcome* or complication* or consequence* or characteristic* or event* or result* or problem* or morbidit* or sequelae)).ti,ab.
38. (pre-natal adj (outcome* or complication* or consequence* or characteristic* or event* or result* or problem* or morbidit* or sequelae)).ti,ab.
39. Perinatal morbidity/
40. (perinatal adj (outcome* or complication* or consequence* or characteristic* or event* or result* or problem* or morbidit* or sequelae)).ti,ab.
41. (peri-natal adj (outcome* or complication* or consequence* or characteristic* or event* or result* or problem* or morbidit* or sequelae)).ti,ab.
42. (neonatal adj (outcome* or complication* or consequence* or characteristic* or event* or result* or problem* or morbidit* or sequelae)).ti,ab.
43. (neo-natal adj (outcome* or complication* or consequence* or characteristic* or event* or result* or problem* or morbidit* or sequelae)).ti,ab.
44. (postnatal adj (outcome* or complication* or consequence* or characteristic* or event* or result* or problem* or morbidit* or sequelae)).ti,ab.
45. (post-natal adj (outcome* or complication* or consequence* or characteristic* or event* or result* or problem* or morbidit* or sequelae)).ti,ab.
46. (antepartum adj (outcome* or complication* or consequence* or characteristic* or event* or result* or problem* or morbidit* or sequelae)).ti,ab.
47. (ante-partum adj (outcome* or complication* or consequence* or characteristic* or event* or result* or problem* or morbidit* or sequelae)).ti,ab.
48. (intrapartum adj (outcome* or complication* or consequence* or characteristic* or event* or result* or problem* or morbidit* or sequelae)).ti,ab.
49. (intra-partum adj (outcome* or complication* or consequence* or characteristic* or event* or result* or problem* or morbidit* or sequelae)).ti,ab.
50. (peripartum adj (outcome* or complication* or consequence* or characteristic* or event* or result* or problem* or morbidit* or sequelae)).ti,ab.
51. (peri-partum adj (outcome* or complication* or consequence* or characteristic* or event* or result* or problem* or morbidit* or sequelae)).ti,ab.
52. (postpartum adj (outcome* or complication* or consequence* or characteristic* or event* or result* or problem* or morbidit* or sequelae)).ti,ab.
53. (post-partum adj (outcome* or complication* or consequence* or characteristic* or event* or result* or problem* or morbidit* or sequelae)).ti,ab.
54. 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 or 26 or 27 or 28 or 29 or 30 or 31 or 32 or 33 or 34 or 35 or 36 or 37 or 38 or 39 or 40 or 41 or 42 or 43 or 44 or 45 or 46 or 47 or 48 or 49 or 50 or 51 or 52 or 53

55. Human immunodeficiency virus/
56. Human immunodeficiency virus 1/
57. Human immunodeficiency virus 1 infection/
58. Human immunodeficiency virus 2/
59. Human immunodeficiency virus 2 infection/
60. Human immunodeficiency virus infection/
61. Human immunodeficiency virus infected patient/
62. Acquired immune deficiency syndrome/
63. Serodiagnosis/
64. HIV associated dementia/
65. HIV associated lipodystrophy/
66. HIV associated nephropathy/
67. AIDS related complex/
68. 'HIV seropositivity'.ti,ab.
69. 'HIV infection*'.ti,ab.
70. 'AIDS serodiagnosis'.ti,ab.
71. 'AIDS arteritis'.ti,ab.
72. 'AIDS-associated nephropathy'.ti,ab.
73. 'AIDS dementia complex'.ti,ab.
74. 'AIDS-related opportunistic infection*'.ti,ab.
75. 'AIDS-related lymphoma'.ti,ab.
76. (HIV or HIV-1 or HIV-type-1).ti,ab.
77. 'HTLV III'.ti,ab.
78. 'HTLV type III'.ti,ab.
79. (HTLV-III or HTLV-type-III or LAV or HTLV-III-LAV or LAV-HTLV-III).ti,ab.
80. (HIV-2 or HIV-type-2 or HIV-II or HTLV-IV or LAV-20).ti,ab.
81. (HIV-positive or HIV-1-positive or HIV-2-positive or HIV-infected).ti,ab.
82. (HIV-1-infected or HIV-type-1-infected or HTLV-III-infected or HTLV-type-III-infected or LAV-infected).ti,ab.
83. 'HTLV III-infected'.ti,ab.
84. 'HTLV type III-infected'.ti,ab.
85. (HTLV-III-LAV-infected or LAV-HTLV-III-infected or HIV-2-infected or HIV-type-2-infected or HIV-II-infected).ti,ab.
86. (HTLV-IV-infected or LAV-2-infected or HIV-infection*or HIV-1-infection* or HIV-type-1-infection* or HTLV-III-infection* or HTLV-type-III-infection*).ti,ab.
87. 'HTLV III-infection*'.ti,ab.
88. 'HTLV type III-infection*'.ti,ab.
89. (LAV-infection* or HTLV-III-LAV-infection* or LAV-HTLV-III-infection* or HIV-2-infection* or HIV-type-2-infection* or HIV-II-infection* or HTLV-IV-infection* or LAV-2-infection*).ti,ab.
90. 'Human Immunodeficiency Virus*'.ti,ab.
91. 'Human Immune Deficiency Virus*'.ti,ab.
92. 'Human T Cell Lymphotropic Virus Type III'.ti,ab.
93. 'Human T-Cell Lymphotropic Virus Type III'.ti,ab.
94. 'Human T Lymphotropic Virus Type III'.ti,ab.
95. 'Human T-Lymphotropic Virus Type III'.ti,ab.
96. 'Human T Lymphotropic Virus Type IV'.ti,ab.
97. 'Human T-Lymphotropic Virus Type IV'.ti,ab.
98. 'Human T Cell Leukemia Virus Type III'.ti,ab.
99. 'Human T-Cell Leukemia Virus Type III'.ti,ab.
100. 'Lymphadenopathy-Associated Virus*'.ti,ab.
101. 'Lymphadenopathy Associated Virus*'.ti,ab.
102. 'Acquired Immune Deficiency Syndrome'.ti,ab.
103. 'Acquired Immunodeficiency Syndrome'.ti,ab.
104. AIDS.ti,ab.

105. 55 or 56 or 57 or 58 or 59 or 60 or 61 or 62 or 63 or 64 or 65 or 66 or 67 or 68 or 69 or 70 or 71 or 72 or 73 or 74 or 75 or 76 or 77 or 78 or 79 or 80 or 81 or 82 or 83 or 84 or 85 or 86 or 87 or 88 or 89 or 90 or 91 or 92 or 93 or 94 or 95 or 96 or 97 or 98 or 99 or 100 or 101 or 102 or 103 or 104
106. Human immunodeficiency virus antibody/
107. Human immunodeficiency virus antigen/
108. exp Human immunodeficiency virus fusion inhibitor/
109. exp Human immunodeficiency virus proteinase inhibitor/
110. exp Anti human immunodeficiency virus agent/
111. exp Integrase inhibitor/
112. exp RNA directed DNA polymerase inhibitor/
113. Antiretrovirus agent/
114. Proteinase inhibitor/
115. (antiretroviral* or anti-retroviral*).ti,ab.
116. (antiretroviral adj (treatment* or therap* or regimen* or drug* or agent*)).ti,ab.
117. (anti-retroviral adj (treatment* or therap* or regimen* or drug* or agent*)).ti,ab.
118. (antiviral* or anti-viral*).ti,ab.
119. (antiviral adj (treatment* or therap* or regimen* or drug* or agent*)).ti,ab.
120. (anti-viral adj (treatment* or therap* or regimen* or drug* or agent*)).ti,ab.
121. (anti-HIV adj (treatment* or therap* or regimen* or drug* or agent*)).ti,ab.
122. (HIV adj (treatment* or therap* or regimen* or drug* or agent*)).ti,ab.
123. (anti-HIV-1 adj (treatment* or therap* or regimen* or drug* or agent*)).ti,ab.
124. (HIV-1 adj (treatment* or therap* or regimen* or drug* or agent*)).ti,ab.
125. (anti-HIV-2 adj (treatment* or therap* or regimen* or drug* or agent*)).ti,ab.
126. (HIV-2 adj (treatment* or therap* or regimen* or drug* or agent*)).ti,ab.
127. (anti-AIDS adj (treatment* or therap* or regimen* or drug* or agent*)).ti,ab.
128. (AIDS adj (treatment* or therap* or regimen* or drug* or agent*)).ti,ab.
129. (HAART or ARV or ARVs or cARV or cARVs).ti,ab.
130. (HAART-exposed or HAART-treated or Mega-HAART).ti,ab.
131. (ARV-exposed or ARV-treated or combination-ARV or combination-ARVs or combined-ARV or combined-ARVs).ti,ab.
132. (ART or Multi-ART or Triple-ART or cART or ART-exposed or ART-treated or combination-ART or combined-ART or sc-ART).ti,ab.
133. 'short-course-antiretroviral therap*'.ti,ab.
134. 'short-course-anti-retroviral therap*'.ti,ab.
135. (combination adj (treatment* or therap* or regimen* or drug* or agent*)).ti,ab.
136. (combined adj (treatment* or therap* or regimen* or drug* or agent*)).ti,ab.
137. (monotherap* or mono-therap*).ti,ab.
138. 'dual therap*'.ti,ab.
139. 'dual drug therap*'.ti,ab.
140. bitherap*.ti,ab.
141. (PI or PIs or PI-based or PI-boosted or PI-containing or PI-therap* or PI-treatment* or PI-regimen*).ti,ab.
142. (Ritonavir-boosted or NRTI or NRTIs or NRTI-based).ti,ab.
143. 'protease inhibitor*'.ti,ab.
144. (NRTI-containing or NRTI-therap* or NRTI-treatment* or NRTI-regimen*).ti,ab.
145. 'nucleoside reverse transcriptase inhibitor*'.ti,ab.
146. 'nucleoside analog reverse transcriptase inhibitor*'.ti,ab.
147. (NNRTI or NNRTIs or NNRTI-based or NNRTI-containing or NNRTI-therap* or NNRTI-treatment* or NNRTI-regimen*).ti,ab.
148. 'non nucleoside reverse transcriptase inhibitor*'.ti,ab.
149. 'non-nucleoside reverse transcriptase inhibitor*'.ti,ab.
150. 'nonnucleoside reverse transcriptase inhibitor*'.ti,ab.
151. 'non nucleoside analog reverse transcriptase inhibitor*'.ti,ab.
152. 'non-nucleoside analog reverse transcriptase inhibitor*'.ti,ab.

153. 'nonnucleoside analog reverse transcriptase inhibitor*'.ti,ab.
154. (NtRTI or NtRTIs or NtRTI-based or NtRTI-containing or NtRTI-therap* or NtRTI-treatment* or NtRTI-regimen*).ti,ab.
155. 'nucleotide reverse transcriptase inhibitor*'.ti,ab.
156. 'nucleotide analog reverse transcriptase inhibitor*'.ti,ab.
157. 'fusion inhibitor*'.ti,ab.
158. 'CCR5 receptor antagonist*'.ti,ab.
159. 'integrase inhibitor*'.ti,ab.
160. 'maturation inhibitor*'.ti,ab.
161. 'entry inhibitor*'.ti,ab.
162. (Abacavir or ABC).tw.
163. Abacavir/
164. Abacavir plus lamivudine/
165. Abacavir plus lamivudine plus zidovudine/
166. (Didanosine or ddI).tw.
167. Didanosine/
168. (Emtricitabine or FTC).tw.
169. Emtricitabine/
170. Efavirenz plus emtricitabine plus tenofovir disoproxil/
171. Emtricitabine plus rilpivirine plus tenofovir disoproxil/
172. Emtricitabine plus tenofovir disoproxil/
173. (Lamivudine or 3TC).tw.
174. Lamivudine/
175. Efavirenz plus lamivudine plus zidovudine/
176. Lamivudine plus nevirapine plus stavudine/
177. Lamivudine plus nevirapine plus tenofovir disoproxil/
178. Lamivudine plus nevirapine plus zidovudine/
179. Lamivudine plus stavudine/
180. Lamivudine plus tenofovir disoproxil/
181. Lamivudine plus zidovudine/
182. (Stavudine or d4T).tw.
183. Stavudine/
184. (Tenofovir or TFV or TDF).tw.
185. Tenofovir/
186. Cobicistat plus elvitegravir plus emtricitabine plus tenofovir disoproxil/
187. Tenofovir disoproxil/
188. Tenofovir 3 hexadecyloxypropyl ester/
189. Tenofovir alafenamide/
190. (Zidovudine or AZT or ZDV).tw.
191. Zidovudine/
192. Zidovudine 5' phosphate/
193. Zidovudine 5' triphosphate/
194. Zidovudine derivative/
195. Zidovudine glucuronide/
196. (Delavirdine or DLV).tw.
197. Delavirdine/
198. (Efavirenz or EFV).tw.
199. Efavirenz/
200. (Etravirine or ETR).tw.
201. Etravirine/
202. (Nevirapine or NVP).tw.
203. Nevirapine/
204. (Rilpivirine or RPV).tw.
205. Rilpivirine/

206. (Atazanavir or ATV).tw.
207. (Atazanavir adj Ritonavir).tw.
208. (ATV adj r).tw.
209. Atazanavir/
210. Atazanavir plus ritonavir/
211. (Darunavir or DRV).tw.
212. (Darunavir adj Ritonavir).tw.
213. (DRV adj r).tw.
214. Darunavir/
215. Darunavir plus ritonavir/
216. (Fosamprenavir or FPV).tw.
217. (Fosamprenavir adj Ritonavir).tw.
218. (FPV adj r).tw.
219. Fosamprenavir/
220. Fosamprenavir plus ritonavir/
221. (Indinavir or IDV).tw.
222. (Indinavir adj Ritonavir).tw.
223. (IDV adj r).tw.
224. Indinavir/
225. Indinavir plus ritonavir/
226. (Lopinavir or LPV).tw.
227. (Lopinavir adj Ritonavir).tw.
228. (LPV adj r).tw.
229. Lopinavir/
230. Lopinavir plus ritonavir/
231. (Nelfinavir or NFV).tw.
232. (Nelfinavir adj Ritonavir).tw.
233. (NFV adj r).tw.
234. Nelfinavir/
235. (Ritonavir or RTV).tw.
236. Ritonavir/
237. (Saquinavir or SQV).tw.
238. (Saquinavir adj Ritonavir).tw.
239. (SQV adj r).tw.
240. Saquinavir/
241. Ritonavir plus saquinavir/
242. (Tipranavir or TPV).tw.
243. (Tipranavir adj Ritonavir).tw.
244. (TPV adj r).tw.
245. Tipranavir/
246. Ritonavir plus tipranavir/
247. (Enfuvirtide or T-20).tw.
248. Enfuvirtide/
249. (Maraviroc or MVC).tw.
250. Maraviroc/
251. (Raltegravir or RAL).tw.
252. Raltegravir/
253. (Elvitegravir or EVG).tw.
254. Elvitegravir/
255. (Zalcitabine or ddC).tw.
256. Zalcitabine/
257. (Combivir or Trizivir or Kaletra or Epzicom or Kivexa or Truvada or Atripla).tw.
258. 106 or 107 or 108 or 109 or 110 or 111 or 112 or 113 or 114 or 115 or 116 or 117 or 118 or 119 or 120 or 121 or 122 or 123 or 124 or 125 or 126 or 127 or 128 or 129 or 130 or 131 or 132 or 133 or

134 or 135 or 136 or 137 or 138 or 139 or 140 or 141 or 142 or 143 or 144 or 145 or 146 or 147 or 148 or 149 or 150 or 151 or 152 or 153 or 154 or 155 or 156 or 157 or 158 or 159 or 160 or 161 or 162 or 163 or 164 or 165 or 166 or 167 or 168 or 169 or 170 or 171 or 172 or 173 or 174 or 175 or 176 or 177 or 178 or 179 or 180 or 181 or 182 or 183 or 184 or 185 or 186 or 187 or 188 or 189 or 190 or 191 or 192 or 193 or 194 or 195 or 196 or 197 or 198 or 199 or 200 or 201 or 202 or 203 or 204 or 205 or 206 or 207 or 208 or 209 or 210 or 211 or 212 or 213 or 214 or 215 or 216 or 217 or 218 or 219 or 220 or 221 or 222 or 223 or 224 or 225 or 226 or 227 or 228 or 229 or 230 or 231 or 232 or 233 or 234 or 235 or 236 or 237 or 238 or 239 or 240 or 241 or 242 or 243 or 244 or 245 or 246 or 247 or 248 or 249 or 250 or 251 or 252 or 253 or 254 or 255 or 256 or 257
259. 105 or 258
260. 54 and 259
261. limit 260 to yr=1980-2013

Appendix 3.4

Adapted Newcastle-Ottawa quality assessment checklist for cohort studies included in the systematic review.

A study can be awarded a maximum of one point (for items indicated with an asterisk) for each numbered criterion within the "Selection" and "Outcomes" categories. A maximum of two points can be given for "Comparability".

Selection (maximum 4 points)

1) Representativeness of the exposed (HIV-positive women) cohort.

a) Truly representative of the average pregnant female population in the community. *

b) Somewhat representative of the average pregnant female in the community.

c) Selected group of users e.g. nurses, volunteers, teenage mothers.

d) No description of the derivation of the cohort.

2) Selection of the non-exposed (HIV-negative) cohort.

a) The HIV-negative population is drawn from the same community as the HIV-positive population. *

b) The HIV-negative population is drawn from a different source than the HIV-positive population.

c) No description of the derivation of the HIV-negative population.

3) Ascertainment of exposure.

a) HIV test conducted as part of the study. *

b) HIV status confirmed from secure medical records (e.g. hospital records). *

c) Structured interview-participant reported HIV-positive status.

d) Written self-report.

e) No description.

4) Demonstration that outcome of interest was not present at start of study.

a) Yes. *

b) No.

Comparability (maximum 2 points)

1) Comparability of cohorts on the basis of the analysis. In the analysis:

a) Study controls for BMI, smoking, parity, and maternal age. *

b) Study controls for any additional factor: prior history of adverse perinatal outcome, maternal hypertension, anemia, illicit drug or alcohol use during pregnancy. *

Outcome (maximum 3 points)

1) Is the assessment of the outcome valid?

a) Outcome was confirmed following clinical observation of outcome by clinician, midwife or trained birth attendant. *

b) Medical records. *

c) Self-report.

d) No description.

2) Was gestational age accurately assessed?

a) Yes, gestational age was determined according to early ultrasound (<14 weeks). *

b) Gestational age was determined by: late ultrasound (≥14 weeks' gestation) or last normal menstrual period or neonatal assessment, e.g. Ballard score, or a combination of methods.

c) Not reported or unspecified.

3) Adequacy of follow up of cohorts

a) Complete follow up - all subjects accounted for. *

b) Subjects lost to follow up unlikely to introduce bias - small number lost < 20 % follow up. *

c) Follow up rate < 80% (lost to follow-up> 20%) and no description of those lost.

d) No statement.

Appendix 3.5

Adapted Newcastle-Ottawa quality assessment checklist for case-control studies included in the systematic review.

A study can be awarded a maximum of one point (for items indicated with an asterisk) for each numbered criterion within the "Selection" and "Exposure" categories. A maximum of two points can be given for "Comparability".

Selection (maximum 5 points)

1) Is the case definition adequate?

a) Yes, the cases are defined as women who experience one of the following perinatal outcomes: PTB, VPTB, PPROM, LBW, VLBW, term-LBW, preterm-LBW, SGA, VSGA, MC, SB, and NND (as defined by the systematic review protocol).*

b) Yes, but definition is different to that which is specified in the HIV protocol.

c) No description.

2) Representativeness of the cases.

a) Consecutive or obviously representative series of cases.*

b) Potential for selection biases or not stated.

3) Selection of controls.

a) Community controls or hospital controls or matched control.*

b) Control selected from a different population to the case.

c) No description.

4) Definition of controls

a) No history of the following perinatal outcome at delivery: PTB, VPTB, PPROM, LBW, VLBW, term-LBW, preterm-LBW, SGA, VSGA, MC, SB, and NND (as defined by the systematic review protocol).*

b) Different definition to that which is specified in the HIV protocol or no description.

5) Was gestational age accurately assessed for cases and controls?

a) Yes, gestational age was determined according to early ultrasound (<14 weeks). *

b) Gestational age was determined by: late ultrasound or last normal menstrual period or clinically, e.g. Ballard score, or a combination of methods.

c) Not reported or unspecified.

Comparability (maximum 2 points)

1) Comparability of cases and controls on the basis of the design or analysis

a) Study controls for BMI, smoking, parity and maternal age. *

b) Study controls for any additional factor: prior history of adverse perinatal outcome, maternal hypertension, anemia, illicit drug or alcohol use during pregnancy. *

Exposure (maximum 3 points)

1) Is the assessment of exposure valid?

a) An HIV test performed as part of the study. *

b) Medical record of HIV status. *

c) Interview blinded to case/control status.

d) Interview not blinded to case/control status.

e) Written self-report.

f) No description.

2) Same method of ascertainment for cases and controls

a) Yes. *

b) No.

3) Non-response rate

a) Same rate (not more than 5% difference) for both groups. *

b) Non-respondents described.

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Appendix 4.1

ART and perinatal outcome systematic review protocol.

A comparative analysis of the risk of low birth weight, stillbirth and preterm birth identified in HIV-positive women who were on antiretroviral regimens during pregnancy: A systematic review of the current literature.

1. Background:

After three decades of being described as the worst public health disaster in history,[53]the Human Immunodeficiency Virus (HIV) is finally approaching an epidemiological transition.[54] This evolution would see it change from being an acute and deadly pandemic, currently affecting 34 million people globally,[12]to a chronic disease with a life expectancy surpassing a number of well-established chronic conditions.[54,55] This has occurred primarily because of the discovery and global distribution of antiretroviral therapy (ART).[55]

The use of ART in the context of pregnancy has been shown to not only improve maternal morbidity and mortality,[56]but also significantly reduce the risk of transmission of HIV from the mother to her baby.[RW.ERROR - Unable to find reference:13089]This discovery came at a time when the transmission rate of HIV from mother to child ranged between 25-48%,[57]with more than 50% of infected babies progressing to Acquired Immuno-Deficiency Syndrome (AIDS) or dying before their 6th birthday.[58]

In 1994, the Paediatric Aids Clinical Trial Group (PACTG 076) demonstrated that the use of ART in pregnancy could significantly reduce the vertical transmission of HIV.[59]This trial demonstrated a 67.5% reduction in mother to child transmission (MTCT), in women who had received antenatal Zidovudine.[59]Thereafter, numerous trials assessing new ART regimens for use in pregnancy were conducted,[60–62]and it soon became evident that any use of ART during the antenatal period provided a

gradient of effect, with triple therapy having the best effect (1.2%, 95%CI 0-2.5%), compared to dual therapy (3.8%, 95%CI 1.1-6.5%) and monotherapy (10.4% 95%CI 8.2-12.6%).[57]

Despite the obvious benefits associated with the use of ART in pregnancy, numerous studies have emerged with reports suggestive of a positive correlation between the in-utero exposure of the fetus to ART and adverse pregnancy outcome.[26,41,63]Although studies addressing the use of ART in the general non-pregnant population have yielded a substantial amount of knowledge regarding a wide range of adverse-drug-effects,[64]studies conducted in pregnant women exposed to ART are now also reporting an increased risk of premature birth (PTB), low birth weight (LBW), small for gestational age (SGA), stillbirth and neonatal death (NND).[26,65,66]

These reports have sparked a debate as to the safety of the use of ART in pregnancy,[41]and whether the severity of the HIV epidemic and our urgency to prevent the vertical transmission of the virus may have led to accelerated licensing of certain antiretroviral agents, irrespective of the gaps in our knowledge regarding their safety profile in pregnancy.[64]A large proportion of antiretroviral treatments, including Protease-inhibitors (PI, e.g. Lopinavir/Ritonavir) and Non-nucleoside-reverse-transcriptase-inhibitors (NNRTI, e.g. Nevirapine), were being administered to pregnant women for the purposes of PMTCT despite insufficient evidence validating the safety and efficacy of these antiretroviral agents.[64]

None of the antiretroviral agents in use today have received a category A rating (showing no risk to the fetus during the antenatal period) by the Food and Drug Administration (FDA).[64,67] However, commonly used agents such as Zidovudine have only been recommended for use if the potential benefits are shown to outweigh the risk (category C drug)[57,64,67] Nevirapine, which is commonly used both during labour and for post-exposure prophylaxis for the newborn infants, is currently rated as a category B drug (Animal studies fail to demonstrate a risk to the fetus).[57,67]

Multiple observational studies, many of which were conducted in Europe,[68–70]initially reported an increased risk of PTB in HIV-positive women on ART. The severity of the problem was emphasised

when it was estimated that 63 (95% CI 6-196) additional preterm deliveries would occur for every 100 HIV transmissions prevented through the use of Highly Active Antiretroviral Therapy (HAART), compared with the use of Zidovudine.[71]This would have a devastating effect on low and middle income countries which carry 90% of the global HIV burden[72]and lack the facilities to address the morbidity associated with prematurity.[1] Furthermore, studies have demonstrated that PTB is independently associated with a 2-3 fold increased risk of perinatal transmission[73,74]. This would result in an even bigger HIV burden in low and middle income settings, which would further exacerbate the crisis and impose additional strain on already overwhelmed healthcare systems.[75]

Numerous studies supporting these findings have suggested that the adverse pregnancy outcomes could be precipitated by the initiation of ART before conception[68,69,76]. However, other studies have reported a higher risk of adverse outcomes in cohorts where ART initiation occurred during pregnancy.[77,78]Some authors have even reported that no association between ART and adverse pregnancy outcome were observed, irrespective of when ART was initiated.[66,79–81] The toxicity associated with triple therapy (HAART) has also been proposed as a contributory factor[68,77,82]however, this was not observed elsewhere[79,83]with one study reporting that HAART reduced the risk of adverse pregnancy outcome to rates similar to those reported in HIV-negative women.[65,84]

The use of PI-based ART during pregnancy has been a contentious issue, with a number of studies suggesting that they are associated with the highest risk of adverse pregnancy outcome[73,77]while other authors oppose this premise.[23,80,85]Numerous Cochrane reviews have assessed the role of ART in the prevention of mother to child transmission.[86–88] However, the influence of ART on adverse pregnancy outcome is minimally described, with one review suggesting that an observed correlation between ART and LBW is possibly related to uncontrolled confounding factors[57]Due to the confounding and methodological bias associated with observational studies, the majority of the studies addressing this issue were not included in these reviews.

Kourtis et al (2007) conducted a systematic review which assessed the risk of PTB in HIV-positive women exposed to ART during the antenatal period.[41]This review included a fair number of observational studies, in comparison to the Cochrane reviews, but it could not shed any light on other types of adverse pregnancy outcome due to the limited scope of the research question. The review concluded that the use of ART during pregnancy does not increase the risk of PTB on a population level. This finding validated numerous previously published US-based studies with similar results.[83,89,90] However, the review suggested that the initiation of ART before conception may potentially contribute to an increased, albeit small, risk of PTB.[41]

In 2012, Chen et al (2012) published the largest surveillance study to date, which assessed the birth outcome in 33148 pregnant women in Botswana.[26]29.6% of these participants had a confirmed HIV-positive diagnosis and were found to have a higher risk of adverse pregnancy outcome compared to their HIV-negative counterparts.[26]These outcomes included; stillbirth [adjusted OR (aOR), 1.5; 95%CI 1.3-1.7], SGA (aOR 1.8; 95% CI 1.7-1.9), PTB (aOR 1.3; 95% CI 1.3-1.4) and NND (aOR 1.4; 95% CI 1.2-1.7).[26]

In the same cohort the initiation of HAART before conception, possibly because of immune failure, resulted in a significantly higher risk of PTB (aOR 1.2; 95% CI 1.1-1.4), SGA (aOR 1.8 95%CI 1.6-2.1) and stillbirth (aOR 1.5; 95% CI 1.2-1.8).[26]They also found that women continuing PI-based HAART from before conception had a 2 fold increased risk of PTB compared to those continuing non-PI based HAART (aOR 2.0; 95% CI 1.1-3.6).[26] Lastly, the antenatal initiation of HAART, possibly for PMTCT purposes, was associated with a significantly higher risk of stillbirth (aOR 2.5; 95% CI 1.6-3.9) PTB (aOR 1.4; 95% CI 1.2-1.8), SGA (aOR 1.3; 95%CI1.0-1.5) and NND (1.9% vs. 0.8% p=0.002) compared to the antenatal initiation of Zidovudine monotherapy.[26] These findings have reiterated the need for further research into the safety of ART, and have opened up a host of questions regarding the role of HIV as a contributing factors towards perinatal mortality.

A cure for HIV still remains elusive, which suggests that antiretroviral therapy will continue to be the main-stay of treatment in maternal HIV infection,[55,75]as well as the prevention of perinatal transmission.[75]Differences identified in adverse perinatal outcomes between continents, countries and specific sub-populations may be attributed to clinical or methodological heterogeneity.[41]However, they could also be due to factors specific to different genetic, ethnic, socio-economic and viral related factors, in addition to the different types of antiretroviral agents.[41]These differences could also mean that we have still not identified all the underlying covariates which attenuate the beneficial effects of ART. Conversely, we have yet to confirm whether these observed effects are not just a consequence of bias or confounding, but are secondary to ART-mediated transplacental toxicity.[91]

This question is even more pertinent now in light of recent WHO legislation recommending the prompt initiation of HAART for all pregnant patients with a $CD4^+$ count ≤ 350.[92]Even more so with developing countries steadily moving towards a policy of triple therapy regimens for all pregnant women, irrespective of $CD4^+$ count, as is current practice in developed countries.[75]Questions regarding the appropriate timing of ART initiation as well as the safety of different Antiretroviral agents need to be addressed and clarified in order to facilitate the improvement of perinatal outcome amongst the growing number of HIV-infected pregnant women.

2. Review questions

2.1 Primary objectives

The primary objective of this review is to answer 3 questions, including:

- Does the maternal use of antiretroviral therapy (ART) during the antenatal period result in an increased risk of adverse pregnancy outcome, including: PTB, PPROM LBW, SGA, intrauterine growth restriction (IUGR) and stillbirth?
- Do different ART regimens have similar levels of risk for adverse pregnancy outcomes including PTB, PPROM, LBW, SGA, IUGR and stillbirth?
- Does the time of initiation of ART, as well as the duration of antenatal antiretroviral treatment influence the risk of adverse pregnancy outcome? The specific points of initiation include: pre-conception, 1st trimester, 2nd trimester, 3rd trimester.

2.2 Secondary objectives

The secondary objectives of this review are to answer the following questions:

- Do HIV positive women, taking different ART regimens during the antenatal period have similar risks of neonatal death?
- Do HIV positive women taking different ART regimens during the antenatal period have similar risks of miscarriage?

3. Methods

3.1 Inclusion and exclusion criteria

The eligibility criteria have been developed according to guidelines stipulated in the Cochrane Handbook of Systematic reviews of interventions. [34]

3.1.1 Study design

The review will include observational (cohort, case-control and cross-sectional) and interventional (randomised controlled trials and controlled trials) studies containing quantitative data on foetal and neonatal pregnancy outcomes in HIV-positive women who have received ART during their pregnancy.

3.1.2 Setting

All participants included in the study should have been recruited from a healthcare facility. We will assess studies conducted in different countries with ranging economic statuses as defined by the World-Bank-country-specific income status http://data.worldbank.org/country. This will address the influence of socio-economic status, quality and access to antenatal healthcare within the different regions.

3.1.3 Population of interest

This review will include studies containing quantitative data on pregnant HIV-positive women of any clinical or immunological stage of HIV, ethnicity, social and educational status. The HIV status of the participant should either be diagnosed or confirmed during the study period. If this is not done, the

study should specify how the HIV status was confirmed. The diagnosis of HIV should be made or confirmed by a healthcare worker (nurse, medical doctor or HIV-counsellor)

Studies should contain quantitative data regarding the maternal demography, pregnancy outcome and newborn information, e.g. twin deliveries, birth-weight, gestational age at birth, and neonatal mortality. The studies should include an appropriate and clearly defined cohort as the control group or comparison. These participants should be drawn from the same community as the exposed population.
Studies which have included the population of interest as a subset of the entire study cohort, will initially be included. These studies will be discussed amongst the reviewers and a final decision regarding the relevance of the study to the review will be made. The study will be excluded if it is found to be irrelevant or inappropriate.

The control or comparison group in these studies should consist of pregnant HIV-positive women who are either not on any ART, or on a different ART regimen compared to the index group. Studies presenting data on participants who defaulted treatment will be included initially and assessed according to the number of days the fetus was exposed to the ART. Following discussion between the reviewers, a decision will be made regarding whether these studies should be included.

Studies will be excluded if they contain the following:

- Ambiguous eligibility criteria.
- An inappropriate control group or no control group (no ART/ no different ART)

3.1.4 Exposure of interest

The exposure of interest relates to the use of ART during the antenatal period in HIV-positive women. All studies should clearly define each class of ART used in the treatment regimen. If specific drug name or class is not specified then the study should specify whether the treatment regimen was monotherapy, dual therapy or triple therapy and when treatment was initiated.

Studies should contain enough detail to enable categorisation of pregnant women into the following groups (Table 1):

Table 1: Exposure of interest		
Absence or presence of ART	**Initiation of ART**	**Regimens**
Pregnant HIV-positive women who do not receive any ART during the antenatal period. This will be defined as <30 days of ART received). These participants may receive single dose ART at delivery.	Pre-conception	No ART
	First trimester (<14 weeks)	Monotherapy
	Second trimester (14 – 28 weeks)	Dual therapy
Pregnant HIV-positive women who receive more than 30 days of any ART during the antenatal period.	Third trimester (> 28 weeks)	Combination • Triple NRTI • PI + NRTI • NNRTI + NRTI • PI + NRTI + NNRTI

Studies which include participants who receive any of the following FDA approved antiretroviral agent's during the course of their pregnancy will be assessed (Table 2):

Table 2: FDA approved Antiretroviral agents	
Class of Antiretroviral	**Drug names**
Nucleoside reverse transcriptase inhibitors (NRTI)	Zidovudine, didanosine, stavudine, lamivudine, abacavir, tenofovir, combivir (lamivudine & zidovudine), trizivir (abacavir/lamivudine/zidovudine), emtricitabine, truvada (emtricitabine & tenofovir combination), epzicom (abacavir & lamivudine).
Non-nucleoside reverse transcriptase inhibitors (NNRTI)	Nevirapine, delaviridine, efavirenz, etravirine, rilpivirine.
Protease inhibitors (PI)	Saquinavir, indinavir, ritonavir, nelfinavir, amprenavir, lopinavir/ritonavir, atazanavir, fosamprenavir, tipranavir, darunavir
Entry inhibitors	Enfuvirtide, maraviroc
Integrase inhibitors	Raltegravir

Studies will be excluded if they contain the following:

- An inappropriate control group,
- The ART group only receives as a single ART dose at delivery or for <30 days duration,
- A study that assesses participants who receive antenatal ART, in addition to another treatment e.g. anti-malaria treatment or anti-tuberculosis treatment. The only exception to this rule is if all the groups in the study are taking the treatment and the ART regimen is different between the different groups e.g. multivitamins taken during pregnancy,
- Studies that have included participants with concomitant conditions e.g. syphilis, will initially be included into the review. These papers will be discussed by the reviewers and a final decision will be made regarding their retention or exclusion from the study.

3.2 Outcome measures

3.2.1 Primary outcomes

The primary outcomes of interest include: PTB, PPROM, LBW, SGA, IUGR and stillbirth in HIV-positive women who receive different ART regimens during the antenatal period.

3.2.1.1 PTB and PPROM

For the purposes of this review we will include studies which define PTB as birth before 37^{+0} completed weeks of gestation.[16]These will be categorised as follows: moderately preterm (33^{+0}-36^{+7}weeks), very preterm birth (<32^{+0}weeks) and extremely preterm birth (<28^{+0}weeks).[16]PPROM will also be assessed, and studies which report that women underwent tocolysis will also be included.

3.2.1.2 LBW, SGA and IUGR

We will extract data related to the birth-weight of the newborns, born to mothers in respective ART-categories. LBW will be defined as a birth-weight <2500g[43] and Very Low Birth Weight (VLBW) will be defined as birth-weight <1500g[44]. We will also assess SGA, which for our purposes will be defined as birth-weight-for-gestational age <10^{th} centile as well as IUGR, which will be defined as birth-weight-for gestational age <3^{rd} centile.

3.2.1.3 Stillbirth and Fetal death

To avoid missing the variable of interest because of misclassification,[45]we have opted to use the terms stillbirth as well as fetal death to assess the incidence of stillbirth in the different ART groups. Stillbirth and fetal death will be defined as any third trimester delivery of a demised fetus with: ≥1000g birth-weight [45] or ≥24^{+0} completed weeks [45] or ≥ 35cm body length [45]

3.2.2 Secondary outcomes

The secondary outcomes of interest include: neonatal death and miscarriage. We aim to assess whether use of certain ART regimens contribute to an increased risk of neonatal death and miscarriage in HIV-positive women. Neonatal death will be defined as the death of the infant within 28 days of life and miscarriage will be defined as the spontaneous expulsion of the fetus before the 24^{+0th} week of pregnancy.

3.3 Search methods for identification of studies

We will identify relevant studies through manual and electronic search strategies. The electronic database search will include: PubMed, MEDLINE, EMBASE Global Health, and CINAHL from January 1980 to March 2013. No methodological or language filters will be applied. Search terms will include Mesh headings and Emtree terms for pregnancy outcome, PTB, LBW, IUGR, SGA, stillbirth

and HIV or ART in addition to free text words and possible synonyms. The bibliographies of the relevant retrieved articles will be examined and all relevant references will be retrieved either manually or electronically. An electronic search of the grey literature (e.g. Proquest dissertations and theses) will also be conducted to identify unpublished papers, theses, reports and conference abstracts relevant to the systematic review. Lastly a number of experts in the field will be contacted to enquire about additional papers that we may not have identified. All relevant material will be included in the systematic review.

All retrieved papers will be imported into Endnote reference manager and all duplicated articles will be removed. A record will be made of all papers excluded in the de-duplication process.

3.4 Study collection and critical appraisal

The selection and critical appraisal of the papers will be conducted in 4 stages.

3.4.1 Stage 1: Title and abstract screens

Following the literature search, 2 individual reviewers will scan the titles and abstracts of all papers retrieved to identify whether they contain data that is relevant to the systematic review. Only papers reporting case-control, cohort, cross-sectional or interventional studies assessing perinatal outcome in HIV-positive women who received ART during the pregnancy will be included in this initial stage. All letters, comments, opinion pieces and case-reports will be excluded. Non-English publications will be considered and those deemed relevant to the review will be included. Any disagreement regarding inclusion at this stage will be discussed with a third reviewer, who will make the final decision. A record of all excluded studies will be made.

3.4.2 Stage 2: Eligibility criteria

Two independent reviewers will assess each paper as to whether they meet the eligibility criteria. All studies which do not fulfil the eligibility criteria will be excluded. A record will be kept as to why each paper was excluded.

Table 3: Inclusion and exclusion criteria	
Inclusion criteria	**Exclusion criteria**
• HIV-positive pregnant women receiving ART during the antenatal period • Appropriate control: HIV-positive pregnant women on ART for < 30days or on a different ART for >30days • Specification of ART treatment- drug name OR whether monotherapy/ dual therapy/triple therapy OR PI-base versus NRTI based etc. • Data collected on birth-weight or gestational age at delivery or stillbirth	• Ambiguous inclusion criteria • Additional treatment taken by mother e.g. anti-malaria treatment • Concomitant disease e.g. Malaria • ART group only received single dose of ART at delivery. • ART received during pregnancy is for < 30 days • No control group

3.4.3 Stage 3: Quality appraisal of papers

We anticipate a wide range in methodological quality because of the inclusion of interventional studies as well as observational studies. Due to the vast difference in study methodology between observational and interventional studies we have chosen to use two different quality assessment tools, which are specific for each group.

The quality of the interventional studies (RCT and controlled studies) will be assessed according to the Cochrane risk of bias tool kit (Appendix 1). Study quality will be categorised as follows:

- Good quality: This will include studies which have fulfilled all 5 requirements of the risk assessment tool checklist and have no additional sources of bias.
- Average quality: This will include studies which have fulfilled between 2-4 of the 5 requirements of the risk assessment tool. Furthermore, they should not have any major methodological flaws, e.g. poor randomisation or additional sources of bias.
- Poor quality studies: Studies which only fulfil <2 of the requirements or contain a major methodological flaw.

An adapted version of the Newcastle-Ottawa quality assessment tool will be used to assess all observational studies. Study quality will be categorised as follows:

- Good quality: Studies which meet all requirements (9stars).

- Average quality: Studies which achieve >2 stars in the "selection" section and "exposure/outcome" section, but <9 overall.
- Poor quality: Studies which achieve ≤2 stars in both the "selection" section and the "exposure/outcome" section of the assessment tool.

Two independent reviewers will conduct this process, and any discrepancy in quality score will be discussed with a third reviewer, who will make the final decision.

A summary of the quality assessment results will be presented for each included study and correlated with the effect size observed in the study. All studies will be combined according to the quality category which they have been assigned to e.g. good, average and low quality.

Following data extraction, an overall aggregate effect size will be calculated for all primary and secondary outcomes in relation to different ART regimens and displayed as forest plots. Thereafter, the level of evidence will be graded according to the Grading of Recommendations Assessment, Development and Evaluation System (GRADE) (Table 4). http://www.gradeworkinggroup.org

Table 4: Grade of evidence: Levels of quality of a body of evidence in the GRADE approach

Underlying methodology	**Quality rating**
Randomized trials; or double-upgraded observational studies.	High
Downgraded randomized trials; or upgraded observational studies.	Moderate
Double-downgraded randomized trials; or observational studies.	Low
Triple-downgraded randomized trials; or downgraded observational studies; or case series/case reports.	Very low

3.5 Data Extraction

Two independent reviewers will extract data from each paper and it will be recorded in a pre-formulated electronic Access-based database. The data extracted will include details related to the study descriptors (e.g. journal title, author name, year of publication etc.), population information (e.g. country), outcome measures of interest, (MTCT, PTB, LBW, SGA, IUGR, stillbirth and different treatment regimens) and confounding factors identified. Data extracted will be compared between the two reviewers and any disagreement between authors will be resolved by discussion with the third reviewer.

3.6 Data synthesis

3.6.1 Summary of findings

A tabulated summary of all included studies will be presented with details regarding the following variables[33]:

- Study type
- Participants in each group (mothers and infants)
- Maternal demographic and clinical characteristics
- ART regimen being investigated
- Outcome measures
- Quality assessment score and aspects which the study did not fulfil

3.6.2 Data analysis

Summary statistics will be compiled for individual studies irrespective of quality assessment score. All binary statistics will be recorded as relative risk ratios (RR), and results reported as odds ratios in retrieved studies will be converted to relative risk ratio. Continuous data will be recorded as a mean and the number needed to harm will be calculated for each primary and secondary outcome and comparisons will be made between different ART regimens. Depending on the heterogeneity of the studies included, the random effects model will be used to combine the different groups of studies and determine an overall effect size. As we expect a heterogeneous sample of studies, with the clinical heterogeneity potentially influencing the effect size to a large extent, we plan to conduct several meta-analyses of the aggregated data as well as narrative syntheses, with the aim of assessing the influence of ART on the primary outcomes of interest. Forest plots will be used to demonstrate the patterns of effect.[33,93,94]

3.6.3 Subgroup analysis

Subgroup analyses will be conducted to assess the influence of possible covariates on the observed adverse pregnancy outcome as well as additional variables:

- Maternal demography: country, income status of country alcohol use during pregnancy, smoking, advanced maternal age, teenage pregnancy, ethnicity, number of antenatal visits and intravenous drug use.
- HIV stage: Immunological (mean $CD4^+$count) or clinical stage of HIV (WHO/CDC).
- Mother to child transmission rate.
- The most frequent HIV subtypes identified in each country
- Population attributable risk will be assessed for adverse pregnancy outcome in HIV-positive women on ART in different countries, compared to HIV-positive women in the same country who are not on treatment.

3.6.4 Unit of analysis

We aim to analyse the influence of ART on the newborn outcome. Therefore, we will report information relating to both the mother and the infant.

3.6.5 Sensitivity analysis

Sensitivity analysis aims to identify whether any of the studies is driving the effect size:

- Location of the study: low, middle and high income country
- Methodological quality: high quality versus average quality
- Study design: RCT versus observational studies
- The exclusion of studies with the largest effect size.
- Control for confounding factors: studies which controlled for confounding factors versus studies which did not control for confounding factors.

Meta-analyses will be conducted and differences between comparisons will be reported.

3.6.6 Assessment of heterogeneity

The statistical heterogeneity of the studies retrieved will be assess visually by assessing the overlap of the confidence interval, as well as with the tau^2 statistic, the chi^2 statistic and the I^2 statistic.[33,34,51]The I^2 results will be interpreted as follows: no heterogeneity (0%), low heterogeneity (25%), moderate

heterogeneity (50%) and high heterogeneity (75%).[34]If statistical significance is observed, we will assess the studies for the possible causes of heterogeneity.

3.6.7 Publication bias

Small trial publication bias will be assessed with the use of funnel plots.[51]If any evidence of publication bias is identified. We will explore publication bias by looking for unpublished trials on trial registries.

Appendix 4.2.

Critical appraisal of RCTs: the Cochrane Collaboration's tool for assessing risk of bias[95]

Sequence generation	Describe the method used to generate the allocation sequence in sufficient detail to allow an assessment of whether it should produce comparable groups	The investigators describe a random component in the sequence generation process such as: • Referring to a random number table, using a computer random number generator, coin tossing, shuffling cards or envelopes, throwing dice, drawing of lots, minimization. • Minimization may be implemented without a random element, and this is considered to be equivalent to being random.	**Yes:** **Give reason**
		The investigators describe a non-random component in the sequence generation process. Usually, the description would involve some systematic, non-random approach, for example: • Sequence generated by odd or even date of birth, by some rule based on date (or day) of admission, by some rule based on hospital or clinic record number. • Other non-random approaches happen much less frequently than the systematic approaches mentioned above and tend to be obvious. They usually involve judgment or some method of non-random categorization of participants, for example: allocation by judgment of the clinician, by preference of the participant, based on the results of a laboratory test or a series of tests, by availability of the intervention	**No:** **Give reason**
		• Insufficient information about the sequence generation process to permit judgment of 'Yes' or 'No'.	**Unclear**
Allocation concealment	Describe the method used to conceal the allocation sequence in sufficient detail to determine	Participants and investigators enrolling participants could not foresee assignment because one of the following, or an equivalent method, was used to conceal allocation:	**Yes**

	whether intervention allocations could have been foreseen in advance of, or during, enrolment.	• Central allocation (including telephone, web-based, and pharmacy, controlled, randomization). • Sequentially numbered drug containers of identical appearance. • Sequentially numbered, opaque, sealed envelopes.	
		Participants or investigators enrolling participants could possibly foresee assignments and thus introduce selection bias, such as allocation based on: • Using an open random allocation schedule (e.g. a list of random numbers). • Assignment envelopes were used without appropriate safeguards (e.g. if envelopes were unsealed or non-opaque or not sequentially numbered). • Alternation or rotation. • Date of birth. • Case record number. • Any other explicitly unconcealed procedure.	**No**
		Insufficient information to permit judgment of 'Yes' or 'No'. This is usually the case if the method of concealment is not described or not described in sufficient detail to allow a definite judgment. For example, if the use of assignment envelopes is described, but it remains unclear whether envelopes were sequentially numbered, opaque and sealed.	**Unclear**
Blinding (participants, personnel and outcome assessors)	Describe all measures used, if any, to blind study participants and personnel from knowledge of which intervention a participant received. Provide any information relating to whether the intended blinding was effective.	Any one of the following: • No blinding, but the review authors judge that the outcome and the outcome measurement are not likely to be influenced by lack of blinding. • Blinding of participants and key study personnel ensured, and unlikely that the blinding could have been broken. • Either participants or some key study personnel were not blinded, but outcome assessment was blinded and the non-blinding of others unlikely to introduce bias.	**Yes**
		Any one of the following: • No blinding or incomplete blinding, and the outcome or outcome measurement is likely to be influenced by lack of blinding. • Blinding of key study participants and personnel attempted, but likely that the blinding could have been broken. • Either participants or some key study personnel were not blinded, and the	**N0**

		non-blinding of others likely to introduce bias.	
		Any one of the following: • Insufficient information to permit judgment of 'Yes' or 'No'. • The study did not address this outcome.	**Unclear**
Incomplete outcome data	Describe the completeness of outcome data for each main outcome, including attrition and exclusions from the analysis. State whether attrition and exclusions were reported, the numbers in each intervention group (compared with total randomised participants), reasons for attrition or exclusions where reported, and any re-inclusions in analyses performed by the review authors.	Any one of the following: • No missing outcome data. • Reasons for missing outcome data unlikely to be related to true outcome (for survival data, censoring unlikely to be introducing bias), • Missing outcome data balanced in numbers across intervention groups, with similar reasons for missing data across groups. • For dichotomous outcome data, the proportion of missing outcomes compared with observed event risk not enough to have a clinically relevant impact on the intervention effect estimate. • For continuous outcome data, plausible effect size (difference in means or standardized difference in means), missing outcomes not enough to have a clinically relevant impact on observed effect size. • Missing data have been imputed using appropriate methods.	**Yes**
		Any one of the following: • Reason for missing outcome data likely to be related to true outcome, with either imbalance in numbers or reasons for missing data across intervention groups. • For dichotomous outcome data, the proportion of missing outcomes compared with observed event risk enough to induce clinically relevant bias in intervention effect estimate. • For continuous outcome data, plausible effect size (difference in means or standardized difference in means) among missing outcomes enough to induce clinically relevant bias in observed effect size. • 'As-treated' analysis done with substantial departure of the intervention received from that assigned at randomization. • Potentially inappropriate application of simple imputation.	**N0**
		Any one of the following: • Insufficient reporting of attrition/exclusions to permit judgment of 'Yes' or	**Unclear**

		'No' (e.g. number randomized not stated, no reasons for missing data provided). • The study did not address this outcome.	
Selective outcome reporting.	State how the possibility of selective outcome reporting was examined by the review authors, and what was found.	Any of the following: • The study protocol is available and all of the study's pre-specified (primary and secondary) outcomes that are of interest in the review have been reported in the pre-specified way. • The study protocol is not available but it is clear that the published reports include all expected outcomes, including those that were pre-specified (convincing text of this nature may be uncommon).	**Yes**
		Any one of the following: • Not all of the study's pre-specified primary outcomes have been reported. • One or more primary outcomes are reported using measurements, analysis methods or subsets of the data (e.g. subscales) that were not pre-specified. • One or more reported primary outcomes were not pre-specified (unless clear justification for their reporting is provided, such as an unexpected adverse effect). • One or more outcomes of interest in the review are reported incompletely so that they cannot be entered in a meta-analysis. • The study report fails to include results for a key outcome that would be expected to have been reported for such a study.	**No**
		Insufficient information to permit judgment of 'Yes' or 'No'. It is likely that the majority of studies will fall into this category.	**Unclear**
Other sources of bias Is the paper free of other bias?	State any important concerns about bias not addressed in the other domains in the tool. If particular questions/ entries were pre-specified in the review's protocol, responses should be provided for each question/entry.	The study appears to be free of other sources of bias	**Yes**
		There is at least one important risk of bias. For example, the study:	**No**

		• Had a potential source of bias related to the specific study design used. • Stopped early due to some data-dependent process (including a formal-stopping rule). • Had extreme baseline imbalance. • Has been claimed to have been fraudulent • Had some other problem.	
		Criteria for the judgment of 'UNCLEAR' (uncertain risk of bias). There may be a risk of bias, but there is either: • Insufficient information to assess whether an important risk of bias exists. • Insufficient rationale or evidence that an identified problem will introduce bias.	**Unclear**

Appendix 4.3

Classification of studies included in ART systematic review, according to the Cochrane risk of bias assessment checklists[95]

Summary assessments of the risk of bias for each important outcome (across domains) within and across studies			
Risk of bias	Interpretation	Within a study	Across studies
Low risk of bias	Plausible bias unlikely to seriously alter the results.	Low risk of bias for all key domains.	Most information is from studies at low risk of bias.
Unclear risk of bias	Plausible bias that raises some doubt about the results.	Unclear risk of bias for one or more key domains.	Most information is from studies at low or unclear risk of bias.
High risk of bias	Plausible bias that seriously weakens confidence in the results.	High risk of bias for one or more key domains.	The proportion of information from studies at high risk of bias is sufficient to affect the interpretation of results.

Appendix 4.4

Antenatal and intrapartum ART received in trials included in ART systematic review

Study	Treatment group (Intrapartum treatment)	Antiretroviral regimens (Intrapartum treatment)	Antiretroviral class received by cohort (Intrapartum treatment)	Antiretroviral drugs (dose) (Intrapartum treatment)
Connor et al 1994[59]	1. Antenatal initiation of placebo	Placebo	None	Placebo
	2. Antenatal initiation of NRTI-based monotherapy (& intrapartum NRTI-based monotherapy)	Monotherapy (& intrapartum monotherapy)	NRTI (& intrapartum NRTI)	Zidovudine (100mg po 5 times daily) (& intrapartum zidovudine 2mg/kg for 1 hr followed by 1mg/kg until delivery - intravenous)
Sperling et al 1998[96]	1. Antenatal initiation of placebo	Placebo	None	Placebo
	2. Antenatal initiation of NRTI-based monotherapy (& intrapartum NRTI-based monotherapy)	Monotherapy (& intrapartum monotherapy)	NRTI (& intrapartum NRTI)	Zidovudine (100mg po 5 times daily) (& intrapartum zidovudine 2mg/kg for 1 hr followed by 1mg/kg until delivery - intravenous)
Tubiana et al 2013[97]	1. Antenatal initiation of boosted PI-based monotherapy (& intrapartum NRTI-based monotherapy)	Monotherapy (& intrapartum monotherapy)	Boosted PI (& intrapartum NRTI)	Lopinavir/ritonavir (400/100mg twice daily) (& intrapartum zidovudine - intravenous)

	2. Antenatal initiation of boosted PI-based HAART (& intrapartum NRTI-based monotherapy)	HAART (& intrapartum monotherapy)	Boosted PI & 2 NRTI (& intrapartum NRTI)	Lopinavir/ritonavir (400/100mg twice daily) & zidovudine (300mg twice daily) & lamivudine (150mg twice daily) (& intrapartum zidovudine - intravenous)
Powis et al 2011[98]	1. Antenatal initiation of NRTI-based HAART	HAART	3 NRTI	Abacavir (300mg po twice daily) & zidovudine (300mg po twice daily) & lamivudine (150mg po twice daily)
	2. Antenatal initiation of boosted PI-based HAART	HAART	Boosted PI & 2 NRTI	Lopinavir/ritonavir (400mg/100mg po twice daily) & zidovudine (300mg po twice daily) & lamivudine (150mg po twice daily
Natureeba et al 2014[99]	1. Antenatal initiation of boosted PI-based HAART	HAART	Boosted PI & 2 NRTI	Lopinavir/ritonavir (400mg/100mg po twice daily until 30 weeks' gestation, increased to 600mg/150mg po twice daily thereafter) & zidovudine (300mg twice daily) & lamivudine (150mg twice daily). Tenofovir in case of zidovudine intolerance.
	2. Antenatal initiation of NNRTI-based HAART	HAART	NNRTI & 2 NRTI	Efavirenz (600mg once daily) & zidovudine (300mg twice daily) & lamivudine (150mg twice daily). Tenofovir in case of zidovudine intolerance.
Koss et al 2014[100]	1. Antenatal initiation of boosted PI-based HAART	HAART	Boosted PI & 2 NRTI	Lopinavir/ritonavir (400mg/100mg po twice daily until 30 weeks' gestation, increased to 600mg/150mg po twice daily thereafter) & zidovudine (300mg twice daily) & lamivudine (150mg twice daily). Tenofovir in case of zidovudine intolerance.

	2. Antenatal initiation of NNRTI-based HAART	HAART	NNRTI & 2 NRTI	Efavirenz (600mg once daily) & zidovudine (300mg twice daily) & lamivudine (150mg twice daily). Tenofovir in case of zidovudine intolerance.
The Kesho Bora Study Group et al 2011[101]	1. Antenatal initiation of NRTI-based monotherapy (& intrapartum single-dose NNRTI-based dual therapy)	Monotherapy (& intrapartum dual therapy)	NRTI (& intrapartum NRTI & NNRTI)	Zidovudine (300mg po twice daily) [& intrapartum single-dose zidovudine (600mg po) & single-dose nevirapine (200mg po)]
	2. Antenatal initiation of boosted PI-based HAART	HAART	Boosted PI & 2 NRTI	Lopinavir/ritonavir (400mg/100mg po twice daily) & zidovudine (300mg po twice daily) & lamivudine (150mg po twice daily)
Fowler et al 2016 - period 1 and 2[102]	1. Antenatal initiation of NRTI-based monotherapy (& intrapartum single-dose NNRTI-based monotherapy)	Monotherapy (& intrapartum monotherapy)	NRTI (& intrapartum NNRTI)	Zidovudine (300mg po twice daily) [& intrapartum single-dose nevirapine (200mg po)]
	2. Antenatal initiation of NRTI-based PI-HAART	HAART	Boosted PI & 2NRTI	Lopinavir/ritonavir [(400mg/100mg po twice daily) from 14 to <28 weeks' gestation and (600mg/150mg po twice daily) ≥ 28 weeks' gestation] & zidovudine (300mg po twice daily) & lamivudine (150mg po twice daily)
Fowler et al 2016 - period 2 only[102]	1. Antenatal initiation of NRTI-based monotherapy (& intrapartum single-dose NNRTI-based monotherapy)	Monotherapy (& intrapartum monotherapy)	NRTI (& intrapartum NNRTI)	Zidovudine (300mg po twice daily) [& intrapartum single-dose nevirapine (200mg po)]

	2. Antenatal initiation of NRTI-based PI HAART	HAART	Boosted PI & 2NRTI	Lopinavir/ritonavir [(400mg/100mg po twice daily) from 14 to <28 weeks' gestation and (600mg/150mg po twice daily) ≥ 28 weeks' gestation] & zidovudine (300mg po twice daily) & lamivudine (150mg po twice daily)
	3. Antenatal initiation of NtRTI-based PI HAART	HAART	Boosted PI & 1 NRTI & 1 NtRTI	Lopinavir/ritonavir [(400mg/100mg po twice daily) from 14 to <28 weeks' gestation and (600mg/150mg po twice daily) ≥ 28 weeks' gestation] & emtricitabine-tenofovir disoproxil fumarate (200mg/300mg po once daily)
Shapiro et al 2010[103]	1. Antenatal initiation of NRTI-based HAART	HAART	3 NRTI	Abacavir (300mg po twice daily) & zidovudine (300mg po twice daily) & lamivudine (150mg po twice daily)
	2. Antenatal initiation of boosted PI-based HAART	HAART	Boosted PI & 2 NRTI	Lopinavir/ritonavir (400mg/100mg po twice daily) & zidovudine (300mg po twice daily) & lamivudine (150mg po twice daily)
Lallemant et al 2015[104]	1. Antenatal initiation of NRTI-based monotherapy (& intrapartum NRTI-based monotherapy & placebo)	Monotherapy (& intrapartum monotherapy & placebo)	NRTI (& intrapartum NRTI & placebo)	Zidovudine (300mg po twice daily) (& intrapartum zidovudine 300mg po three-hourly & placebo)
	2. Antenatal initiation of NRTI-based monotherapy (& intrapartum NNRTI-based dual therapy)	Monotherapy (& intrapartum dual therapy)	NRTI (& intrapartum NRTI & NNRTI)	Zidovudine (300mg po twice daily) (& intrapartum zidovudine 300mg po three-hourly & single-dose nevirapine 200mg po)
	3. Antenatal initiation of boosted PI-based dual therapy (& intrapartum NRTI-based monotherapy)	Dual therapy (& intrapartum monotherapy)	Boosted PI & 1 NRTI (& intrapartum NRTI)	Lopinavir/ritonavir (400mg/100mg po twice daily) & zidovudine (300mg po twice daily) (& intrapartum zidovudine 300mg po three-hourly)

Appendix 4.5

Demographic factors not significant between treatment groups in trials included in ART systematic review

Study	Reference	Trial	Correction of confounders	Demographic factors not significant between groups
Connor et al. 1994[59]	N Engl J Med.1994; 331(18): 1173-80	PACTG 076	RCT	Between group differences were not assessed for statistical significance
Sperling et al. 1998[96]	AIDS 1998; 12(14): 1805-1813	PACTG 076	RCT	No demographic characteristics reported
Tubiana et al. 2013[97]	Clin Infect Dis. 2013; 57(6): 891-902	ANRS 135 PRIMEVA	RCT	Maternal age, country of birth, marital status, tobacco use, gestational age at booking, screening and enrollment, gravidity, HIV diagnosis, history of antiretroviral therapy, resistance-associated mutations, HIV subtype, $CD4^+$ T cell percentage, $CD4^+$ T-lymphocyte count, plasma HIV-1 RNA.
Natureeba et al. 2014[99]	J Infect Dis 2014; 210(12): 1938-45	PROMOTE	RCT	Maternal age, gestational age at recruitment, gravidity, bed net ownership, receiving TMP-SMX prophylaxis, hemoglobin level, $CD4^+$ T-lymphocyte count, HIV RNA load, WHO stage of HIV disease
Koss et al. 2014[100]	J Acquir Immune Defic Syndr. 2014; 67(2): 128-135	PROMOTE	RCT	Maternal age, education, socio-economic status, gravidity, prior preterm delivery, gestational age at recruitment, receiving TMP-SMX prophylaxis, BMI, systolic blood pressure, anemia, $CD4^+$ T-lymphocyte count, HIV RNA load
Shapiro et al. 2010[103]	N Engl J Med. 2010; 362(24): 2282-2294	Mma Bana Study	RCT	Between group differences were not assessed for statistical significance

Powis et al. 2011[98]	J Infect Dis. 2011; 204(4): 506-514	Mma Bana Study	Self-reported maternal income	Maternal age, gravidity, HIV-1 RNA, $CD4^+$ T-lymphocyte count, hemoglobin, gestational age at study enrollment, median time on HAART prior to delivery, enrollment site, marital status, education, employment, income and electricity in home.
The Kesho Bora Study Group et al. 2011[101]	Lancet Infect Dis. 2011; 11(3): 171-180	Kesho Bora Study	RCT	Mode of delivery, duration of receipt of antiretrovirals from enrollment until delivery, adherence (no missed doses before delivery)
Fowler et al. 2016 – (period 1 and 2)[102]	N Engl J Med 2016; 375(18): 1726-1737	PROMISE	RCT	Between group differences were not assessed for statistical significance
Fowler et al. 2016 – (period 2 only)[102]	N Engl J Med 2016; 375(18): 1726-1737	PROMISE	RCT	Between group differences were not assessed for statistical significance
Lallemant et al. 2015[104]	AIDS 2015; 29(18): 2497-2507	PHPT-5	RCT	Between group differences were not assessed for statistical significance

Appendix 4.6

$CD4^+$ T-lymphocyte count, viral load distribution and breastfeeding in trials included om ART systematic review

Study	Antenatal treatment group (Intrapartum treatment)	$CD4^+$ T-lymphocyte count (cells/µL)			Plasma HIV-1 RNA (copies/mL)		Breastfeeding
		Threshold for enrollment	Distribution at enrollment	Distribution at delivery	Distribution at enrollment	Distribution at delivery	
Connor et al 1994[59]	Placebo	>200	200 – 500 = 40.9%, >500 = 59.1%	Unspecified	Unspecified	Unspecified	Only 1 mother breastfed so these MTCT estimates are probably due to intrauterine/intrapartum transmission
	NRTI-based monotherapy (& intrapartum NRTI-based monotherapy)	>200	200 – 500 = 40.6%, >500 = 59.4%	Unspecified	Unspecified	Unspecified	
Sperling et al 1998[96]	Placebo	>200	200 – 500 = 40.9%, >500 = 59.1%	Unspecified	Unspecified	Unspecified	Only 1 mother breastfed so these MTCT estimates are probably due to intrauterine/intrapartum transmission.
	NRTI-based monotherapy (& intrapartum NRTI-based monotherapy)	>200	200 – 500 = 40.6%, >500 = 59.4%	Unspecified	Unspecified	Unspecified	

Tubiana et al 2013[97]	Boosted PI-based monotherapy (& intrapartum NRTI-based monotherapy)	≥350	Unspecified	Unspecified	Unspecified	<50 = 78.3%, <200 = 92.8%	Unspecified
	Boosted PI-based HAART (& intrapartum NRTI-based monotherapy)	≥350	Unspecified	Unspecified	Unspecified	<50 = 97.2%, <200 = 97.2%	Unspecified
Powis et al 2011[98]	NRTI-based HAART	≥200	Entire cohort: 200 – 399 = 50.6%, 400 – 599 = 34.2%, ≧600 = 15.3%	Unspecified	Entire cohort: <10,000 = 48.3%, 10,000 – <100,000 = 37.1% ≧ 100,000 = 14.6%	Unspecified	97% of women breastfed in total, 93% of whom breastfed exclusively
	Boosted PI-based HAART	≥ 200	Entire cohort: 200 – 399 = 50.6%, 400 – 599 = 34.2%, ≧600 = 15.3%	Unspecified	Entire cohort: <10,000 = 48.3%, 10,000 – <100,000 = 37.1%, ≧100,000 = 14.6%	Unspecified	
Natureeba et al 2014[99]	Boosted PI-based HAART	Any $CD4^+$ T-lymphocyte count	Entire cohort: <200 = 14.3%, ≧200 = 85.7%	Unspecified	Entire cohort: <1000 = 15.4%, 1000 – 10,000 = 27.1%, 10,000 – 100,000 = 39.4% ≧100,000 = 15.4%	≤ 400 = 97.7%	96.7% of women breastfed (59.6% did so exclusively)
	NNRTI-based HAART	Any $CD4^+$ T-lymphocyte count	Entire cohort: <200 = 14.3%, ≧200 = 85.7%	Unspecified	Entire cohort: <1000 = 15.4%, 1000 – 10,000 = 27.1%, 10,000 – 100,000 = 39.4% ≧100,000 = 15.4%	≤ 400 = 86.0%	98.7% of women breastfed (60% did so exclusively)
Koss et al 2014[100]	Boosted PI-based HAART	Any $CD4^+$ T-lymphocyte count	Entire cohort: <200 = 14.3%, ≧200 = 85.7%	Unspecified	Entire cohort: <1000 = 15.4%, 1000 – 10,000 = 27.1%, 10,000 – 100,000 = 39.4% ≧100,000 = 15.4%	≤ 400 = 97.7%	96.7% of women breastfed (59.6% did so exclusively)

	NNRTI-based HAART	Any $CD4^+$ T-lymphocyte count	Entire cohort: <200 = 14.3%, ≧200 = 85.7%	Unspecified	Entire cohort: <1000 = 15.4%, 1000 – 10,000 = 27.1%, 10,000 – 100,000 = 39.4% ≧100,000 = 15.4%	≤ 400 = 86.0%	98.7% of women breastfed (60% did so exclusively)
The Kesho Bora Study Group et al 2011[101]	NRTI-based monotherapy (& intrapartum single-dose NNRTI-based dual therapy)	200 – 500	Unspecified	Unspecified	Unspecified	<300 = 29.8%	Exclusive breastfeeding = 44% Ever breastfed = 78%
	Boosted PI-based HAART	200 – 500	Unspecified	Unspecified	Unspecified	<300 = 63.8%	Exclusive breastfeeding = 45% Ever breastfed = 77%
Fowler et al 2016 - period 1 and 2[102]	NRTI-based monotherapy (& intrapartum single-dose NNRTI-based monotherapy)	≥350 or country specific threshold if that threshold is higher	350 – <499 = 41.7%, 500 – <750 = 42.0%, >750 = 16.3%,	Unspecified	<LLQ = 4.4% LLQ – 399 = 9.6% 400 – 999 = 7.4% 1000 – 9999 = 37.4% 10000 – 99999 = 34.7% 100000 – 199999 = 3.9% ≥ 200000 = 2.5%	<LLQ = 10.7% LLQ – 399 = 5.4% 400 – 999 = 28.5% 1000 – 9999 = 37.9% 10000 – 99999 = 16.2% 100000 – 199999 = 1.0% ≥ 200000 = 0.2%	Unspecified - depending on breastfeeding or formula-feeding setting for follow-on study.
Fowler et al 2016 - period 1 and 2[102]	NRTI-based PI-HAART	≥350 or country specific threshold if that threshold is higher	350 – <499 = 42.4%, 500 – <750 = 42.3%, >750 = 15.2%,	Unspecified	<LLQ = 5.3% LLQ – 399 = 5.9% 400 – 999 = 6.8% 1000 – 9999 = 37.3% 10000 – 99999 = 37.0% 100000 – 199999 = 4.6% ≥ 200000 = 3.2%	<LLQ = 57.9% LLQ – 399 = 10.3% 400 – 999 = 18.9% 1000 – 9999 = 7.4% 10000 – 99999 = 4.1%, 100000 – 199999 = 1.0% ≥ 200000 = 0.4%	Unspecified - depending on breastfeeding or formula-feeding setting for follow-on study.

Fowler et al 2016 - period 2 only[102]	NRTI-based monotherapy (& intrapartum single-dose NNRTI-based monotherapy)	≥ 350 or country specific threshold if that threshold is higher	350 – <499 = 42.6%, 500 – <750 = 41.2%, >750 = 16.2%,	Unspecified	<LLQ = 2.4% LLQ – 399 = 10.5% 400 – 999 = 8.5% 1000 – 9999 = 36.5% 10000 – 99999 = 35.3% 100000 – 199999 = 3.4% ≥ 200000 = 3.4%	<LLQ = 9.6% LLQ – 399 = 6.1% 400 – 999 = 32.1% 1000 – 9999 = 33.0% 10000 – 99999 = 17.0%, 100000 – 199999 = 1.9% ≥ 200000 = 0.3%	Unspecified - depending on breastfeeding or formula-feeding setting for follow-on study.
	NRTI-based PI HAART	≥ 350 or country specific threshold if that threshold is higher	350 – <499 = 37.1%, 500 – <750 = 47.3%, > 750 = 15.6%,	Unspecified	<LLQ = 4.6% LLQ – 399 = 6.8% 400 – 999 = 6.8% 1000 – 9999 = 39.8% 10000 – 99999 = 35.1% 100000 – 199999 = 3.4% ≥ 200000 = 3.4%	<LLQ = 52.0% LLQ – 399 = 12.8% 400 – 999 = 24.9% 1000 – 9999 = 5.6% 10000 – 99999 = 3.1%, 100000 – 199999 = 1.6% ≥ 200000 = 0.0%	Unspecified - depending on breastfeeding or formula-feeding setting for follow-on study.
	NtRTI-based PI HAART	≥ 350 or country specific threshold if that threshold is higher	350 – <499 = 41.1%, 500 – <750 = 40.4%, > 750 = 18.5%,	Unspecified	<LLQ = 3.9% LLQ – 399 = 6.2% 400 – 999 = 6.2% 1000 – 9999 = 36.5% 10000 – 99999 = 39.4% 100000 – 199999 = 5.4% ≥ 200000 = 2.5%	<LLQ = 41.1% LLQ – 399 = 16.1% 400 – 999 = 26.0% 1000 – 9999 = 9.5% 10000 – 99999 = 4.9%, 100000 – 199999 = 1.3% ≥ 200000 = 1.0%	Unspecified - depending on breastfeeding or formula-feeding setting for follow-on study.
Shapiro et al 2010[103]	NRTI-based HAART	≥ 200	Unspecified	Unspecified	<1000 = 14.7%, 1000 – 100,000 = 69.8%, ≧100,000 = 15.4%	<400 = 96%	97% of women breastfed in total, 93% of whom breastfed exclusively.
	Boosted PI-based HAART	≥ 200	Unspecified	Unspecified	<1000 = 17.1%, 1000 – 100,000 = 69.8%, ≧100,000 = 13.1%	<400 = 93%	
Lallemant et al 2015[204]	NRTI-based monotherapy (& intrapartum NRTI-based monotherapy & placebo)	Before 4 June 2010 = ≥ 250, after 4 June 2010 = ≥ 350.	Unspecified	Unspecified	Unspecified	Unspecified	Entire cohort: 1 woman breastfed

	NRTI-based monotherapy (& intrapartum NNRTI-based dual therapy)	Before 4 June 2010 = ≥ 250 cells/μL, after 4 June 2010 = ≥ 350	Unspecified	Unspecified	Unspecified	Unspecified
	Boosted PI-based dual therapy (& intrapartum NRTI-based monotherapy)	Before 4 June 2010 = ≥ 250, after 4 June 2010 = ≥ 350	Unspecified	Unspecified	Unspecified	Unspecified

Abbreviation: Highly Active Antiretroviral Treatment, LLQ = Lower limit of quantification of the assay, NNRTI = Non-Nucleoside Reverse Transcriptase Inhibitors, NRTI = Nucleoside Reverse Transcriptase Inhibitors (NRTIs), NtRTI = Nucleotide Reverse Transcriptase Inhibitors, PI = Protease Inhibitor, RNA = ribonucleic acid.

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Appendix 5.1

WHO clinical staging of HIV/AIDS for adults and adolescents with confirmed HIV infection[105].

Table 3. WHO clinical staging of HIV/AIDS for adults and adolescents with confirmed HIV infection[i]

Clinical stage 1
Asymptomatic Persistent generalized lymphadenopathy
Clinical stage 2
Moderate unexplained weight loss (<10% of presumed or measured body weight)[i] Recurrent respiratory tract infections sinusitis, tonsillitis, otitis media and pharyngitis) Herpes zoster Angular cheilitis Recurrent oral ulceration Papular pruritic eruptions Seborrhoeic dermatitis Fungal nail infections
Clinical stage 3
Unexplained[i] severe weight loss (>10% of presumed or measured body weight) Unexplained chronic diarrhoea for longer than one month Unexplained persistent fever (above 37.6°C intermittent or constant, for longer than one month) Persistent oral candidiasis Oral hairy leukoplakia Pulmonary tuberculosis (current) Severe bacterial infections (such as pneumonia, empyema, pyomyositis, bone or joint infection, meningitis or bacteraemia) Acute necrotizing ulcerative stomatitis, gingivitis or periodontitis Unexplained anaemia (<8 g/dl), neutropaenia (<0.5 × 10^9 per litre) or chronic thrombocytopaenia (<50 × 10^9 per litre)
Clinical stage 4[ii]
HIV wasting syndrome Pneumocystis pneumonia Recurrent severe bacterial pneumonia Chronic herpes simplex infection (orolabial, genital or anorectal of more than one month's duration or visceral at any site) Oesophageal candidiasis (or candidiasis of trachea, bronchi or lungs)
Extrapulmonary tuberculosis Kaposi's sarcoma Cytomegalovirus infection (retinitis or infection of other organs) Central nervous system toxoplasmosis HIV encephalopathy Extrapulmonary cryptococcosis including meningitis Disseminated non-tuberculous mycobacterial infection Progressive multifocal leukoencephalopathy Chronic cryptosporidiosis (with diarrhoed) Chronic isosporiasis Disseminated mycosis (coccidiomycosis or histoplasmosis) Recurrent non-typhoidal Salmonella bacteraemia Lymphoma (cerebral or B-cell non-Hodgkin) or other solid HIV-associated tumours Invasive cervical carcinoma Atypical disseminated leishmaniasis Symptomatic HIV-associated nephropathy or symptomatic HIV-associated cardiomyopathy

i Unexplained refers to where the condition is not explained by other causes.

ii Some additional specific conditions can also be included in regional classifications (such as reactivation of American trypanosomiasis [meningoencephalitis and/or myocarditis]) in the WHO Region of the Americas and disseminated penicilliosis in Asia).

Appendix 5.2

MIHS and SHAPOSSA study protocol

The analysis of immunological mechanisms underlying preterm delivery and intra-uterine growth restriction in HIV-positive and HIV-negative women in South Africa.

1. Project title

The analysis of immunological mechanisms underlying preterm delivery and intra-uterine growth restriction in HIV-positive and HIV-negative women in South Africa.

2. Background

The HIV pandemic has been one of the biggest public health challenges that Africa has had to address since it was first described by Gottlieb et al in 1981[106]. It has resulted in the escalation of maternal mortality rates and has been a major obstruction to numerous African countries' efforts of achieving the millennium development goals[107]. In 2011 the overall HIV prevalence in South African was estimated at approximately 10.6% with a total number of 5.38 million people living with HIV, the highest number of HIV positive people in any country[108,109]. One-fifth of South African women in their reproductive ages are HIV positive, with the national prevalence amongst antenatal women rising from 29.3% in 2008 to an estimated 30.2% (95% CI of 29.39-30.91) in 2010[108]. The introduction of the prevention of mother to child transmission (PMTCT) program in South Africa has resulted in a significant decline in the HIV transmission rate, with current rates standing at 3.5% at 6 weeks after birth and a 95% coverage rate of affected mothers[101].

Pregnancy is a state of maternal immune tolerance to a semi-allogeneic fetus which expresses a large amount of paternal antigens[110,111]. One of the immunological factors enabling this symbiotic relationship is the decline in Th1 cytokines (IFN-γ and IL-2) and a concurrent increase in Th2 cytokines

(IL-4 and IL10), the so-called Th1 to Th2 shift[112] The conceptus' survival and optimal growth is dependent on placental function following adequate trophoblast invasion of the endometrium and maternal spiral arteries[110]. Placentation is a complex process regulated by mechanisms involving T cells, Natural Killer (NK) cells and monocytes[103.] Failure of adequate placentation has been shown to result in placental insufficiency leading to conditions including: intrauterine growth restriction (IUGR), preterm birth (PTB), hypoxic-ischaemic injury, hypertension and fetal demise[113].(8)

HIV infection has also been shown to be associated with a Th1 to Th2 shift, as well as reduced circulating $CD4^+$ T cell and increased $CD8^+$ T cell levels[114,115]. Furthermore, T cell activation is a hallmark of HIV pathogenesis and is closely associated with the progression of the disease[114]. The introduction of highly active antiretroviral therapy (HAART) does not only delay disease progression from HIV to AIDS, but is also associated with an increase in $CD4^+$ count. However, studies have also demonstrated that HAART counteracts the Th1 to Th2 shift seen in normal pregnancy.[114]. HIV infection in association with antiretroviral drugs has thus been associated with increased inflammatory markers, increased type-1 immune response, increased $CD4^+$ and $CD8^+$ T cell activation and increased adverse pregnancy outcomes compared to uninfected pregnancies[116,117].

The role of T regulatory (Treg) cells in pregnancy is currently being investigated following evidence that a normal pregnancy is associated with a 2-fold increase in the frequency of these suppressor cells[118]. Treg cells have been shown to suppress type-1 immune response, prevent auto-immunity, prevent graft rejection and are essential to the maintenance of allograft tolerance[118,119]. This has been followed by numerous studies suggesting that adverse pregnancy outcomes including pre-eclampsia, recurrent miscarriage and fetal loss are associated with low maternal levels of Treg cells[118]. HIV-positive women have been shown to have similar Treg levels in early pregnancy compared to their HIV-negative counterparts[117]. However, while there is a significant increase in Treg cell levels during the second trimester in HIV-negative women, this Treg cell expansion is not seen in HIV-positive women[117]. This finding, in association with the knowledge that maximal trophoblast invasion occurs during the second

trimester, raises questions as to whether there is an association with this phenomenon and the increased rate of spontaneous miscarriages in HIV-positive women. Decreased Treg cell levels have also been reported in women with SLE, with authors suggesting that the low Treg frequency may be the underlying explanation for the high rate of pregnancy complications in this group of women[119].

Numerous studies, mostly conducted in the developed world, have shown increased rates of IUGR, small for gestational age (SGA), low birth weight (LBW), and PTB associated with maternal HIV infection[19]. PTB and LBW are known risk factors for intellectual disability, cerebral palsy, visual and auditory deficits[17,120]. These remain key outcomes used worldwide for evaluating adverse perinatal health for both the mother and child [17]. 96% of LBW infants live in developing countries, with 1/5 of this cohort residing in sub-Saharan Africa.[17] This is further complicated by the fact that 11% of all newborns in developing countries are born with LBW as a consequence of IUGR, which is a 6 times higher rate than that observed in developed countries.[113] IUGR has been shown to indirectly increase mortality and morbidity through adverse neonatal outcomes including, hypoglycemia, sepsis, hyperbilirubinemias, seizures, polycythemia, and birth asphyxia[113].

Despite the fact that sub-Saharan Africa carries the greatest burden of HIV and adverse pregnancy outcome (PTB and IUGR) in the world, very little has been done in this region to identify if HIV and ART are truly associated with poor pregnancy outcome, and what proportion of the present adverse-outcome burden is attributable to antenatal exposure to HIV and ART. Furthermore, studies which have been conducted in this region have used inaccurate methods of gestational age estimation (e.g. Last normal menstrual period (LNMP) or Ballard score) and fetal growth monitoring (symphysis-pubis measurement) due to the unavailability of ultrasonography facilities, thus resulting in inconsistent and inaccurate results.

Moreover, despite the many advances made with regards to the treatment of HIV-infected pregnant women and PMTCT, little is known about the mechanisms by which HIV infection causes IUGR and

PTB, even in the absence of MTCT, which makes it difficult to develop targeted, preventative and interventional strategies. The issue is further complicated by controversy over whether HAART itself contributes to the IUGR and PTB rate[81,85]. However, no studies have tried to define the mechanisms by which HIV or HAART lead to IUGR or PTB.

Given the profound effect of HIV infection on the immune response and the immunological and inflammatory processes involved in successful placentation and PTB, we hypothesize that it is through an immunological mechanism that HIV affects immunological processes involved in successful placentation and adaption associated with pregnancy maintenance, thus resulting in IUGR and preterm birth. These changes may involve both the innate and adaptive arms of the immune response. Hence, we plan to characterize the immune responses associated with HIV infection in pregnancies affected by IUGR and preterm birth. Elucidation of the cellular immune phenotypes associated with preterm birth and IUGR in HIV infection may lead to new therapeutic interventions with immune-modulating agents such as cytokines (IL-10, IL-4), antibodies (anti-TNFα, anti-IFN-γ, anti-IL-6) and fusion proteins (e.g. CTLA4-Ig). Immunological phenotypes associated with preterm birth and IUGR in HIV infection may also shed light on the broader immunological mechanisms underlying these conditions in general, as well as their associations with other systemic infections, e.g. malaria. Immune mechanisms correlating with MTCT of HIV and HAART treatment may further lead to interventions preventing MTCT and the adverse effects of HAART.

We propose to conduct a longitudinal observational study of HIV-positive and HIV-negative pregnant women in South Africa. Accurate gestational age estimations and fetal growth monitoring will be conducted by ultrasound scans which will be used, in conjunction with socio-economic and clinical data, to assess the proportion of the IUGR and PTB incidence in South Africa that can be attributed to the presence of HIV and ART (population attributable risk), inform the development of an effective predictive model for PTB and IUGR and evaluate the causal effect of assigned ART on pregnancy outcome. Furthermore, soluble biomarkers, as well as cellular immune phenotypes associated with

IUGR and PTB in HIV infection will be determined with a view to develop targeted, preventative and interventional strategies.

3. Hypothesis

- H_1 (Alternative Hypothesis): HIV infection and antiretroviral drugs in pregnant women cause alterations in immunological markers, which are associated with preterm birth and IUGR.
- H_0 (Null Hypothesis): HIV infection and antiretroviral drugs in pregnant women do not cause alterations in immunological markers and are not associated with preterm birth and IUGR.

4. Project aim and specific objectives

4.1 Aims

Aim 1:

The first aim of the proposed study is to investigate whether HIV or antiretroviral drugs cause changes in the immunological profile of HIV-positive pregnant women. Furthermore, we aim to identify whether these immunological changes correlate with PTB and IUGR.

Aim 2:

The second aim of this study is to investigate whether in-utero exposure of the fetus to HIV or ART is associated with adverse pregnancy outcomes in South Africa, and identify clinical and socio-economic factors which are predictive of PTB and IUGR in a low-income setting with a high HIV prevalence.

4.2 Objectives

4.2.1 Objective 1

Assess the incidence of IUGR and Preterm birth in HIV-positive and HIV-negative pregnant women in a resource-poor setting.

We propose to conduct a cohort study of 3000 women presenting to the Chris Hani Baragwanath hospital (CHBH) with singleton pregnancies. As CHBH is a referral hospital we expect that this sample will not be representative of the population, thus a purposive sampling method will be employed to

recruit 3000 women in total, while ensuring that this includes a minimum of 500 HIV-positive participants.

4.2.1.1 The primary outcome

To determine the incidence of IUGR and PTB in HIV-positive and HIV-negative pregnant women. Despite evidence demonstrating the syndromic nature of PTB and its association to multiple independent aetiological factors[121] (20) a large proportion of cases of preterm birth are inaccurately classified as being due to 'preterm spontaneous parturition'. Thus all preterm births identified in this cohort will be categorized using a new classification system developed by Villar et al.(2012) [114]

4.2.1.2 Secondary outcomes:

Outcome 1

We will assess the incidence and population attributable risk of adverse pregnancy outcome in HIV-positive and HIV- negative women presenting to the CHBH in Soweto, South Africa. Careful analysis will be conducted to:

- Identify socio-economic and clinical factors associated with adverse pregnancy outcome, including PTB and IUGR, in HIV-positive and HIV-negative pregnant women presenting to CHBH.
- Formulate a model using identified clinical and socio-economic variables, which can be used in the clinical setting to predict the probability of IUGR and PTB in South African women.
- Estimate the causal association between ART use and adverse pregnancy outcome (PTB and IUGR), using a semiparametric model.

Outcome 2:

Within the HIV-positive cohort, sub-group analyses will be performed assessing the relationship between maternal viral load, $CD4^+$ count, antiretroviral regimen, disease progression, mother to child transmission rate and adverse pregnancy outcome.

During the analysis of the primary and secondary outcomes, careful analysis will be performed on the dataset to exclude the role of known confounders, such as number of antenatal visits, previous LBW, previous/current hypertensive disorder, maternal nutritional status (BMI etc), parity, diabetes, smoking, alcohol, drug use, co-infections (e.g. TB), other STI and bleeding during pregnancy.

4.2.2 Objective 2

Collect maternal and newborn biological samples.

Maternal blood samples will be collected during the 1st trimester, 2nd trimester and 3rd trimester of pregnancy, with maternal and cord blood being collected at delivery. All samples will be collected and stored using protocols and tracking systems developed for the Fetal Growth Study (FGS): Investigating maternal factors associated with Fetal growth and delivery outcomes (ethics reference number: M120524), which is currently being headed by Professor Shane Norris and the Developmental Pathways for Health Research Unit (DPHRU) in Johannesburg, South Africa.

Blood samples will be separated and stored as whole blood, plasma and peripheral blood mononuclear cells (PBMCs), for measurement of soluble factors, antibody assays, cellular essays, ELISPOT assays and intracellular cytokine staining. Biological testing will be elaborated on in objective 3 and 4.

4.2.3 Objective 3:

Identify immune markers in pregnancy which correlate with IUGR and PTB.

Soluble inflammatory mediators and cellular immune phenotypes within the maternal and cord blood which are associated with IUGR and preterm birth will be determined. Inflammatory markers and cellular immune phenotypes to be assessed include:

1. FACS analysis:

- Major lymphocyte subsets: $CD4^+$ T cells, $CD8^+$ T cells, Regulatory T cells ($CD3^+$ $CD4^+$ $CD25^+$ $CD127^+$ $FOXP3^+$), γδT cells, NK cells ($CD16^+CD56^+$) and B cells ($CD20^+$) .
- To determine the expression of activation and memory molecules: HLA-DR^+, $CD38^+$, $CD45RO^+$, $CD45RA^+$, $CD27^+$, $CD127^+$, $CD69^+$, $CD62L^+$, PD-1^+, $CD244^+$.
- Intracellular cytokine staining (ICS).

2. Plasma: ELISA assays:

- Cytokines: IL-1, IL-2, IL-4, IL-6, IL-8, IL-10, IL-16, IL-18, IFN-γ, TNF-α, G-CSF, MMIF, neopterin.
- Chemokines: MCP-1, RANTES, ACDNAP-78.

- Additional markers: CTLA-4, β2-microglobulin, CRP, PAF, MMP8, angiogenin, relaxin, HCG, PAPP-A, activin-A, Inhibin, prolactin, PP-13, SP1, endoglin, thrombin-antithrombin III complex.

Analysis of immune markers

The above-mentioned immune markers will be correlated to perinatal outcome by univariate and multivariate analysis under the following headings:

1. Maternal HIV status.
2. Preterm birth versus term deliveries.
3. IUGR vs. standard growth patterns and macrosomia.
4. HIV-positive cohorts:
    - Stage of disease (WHO staging system).
    - Antiretroviral treatment received during pregnancy versus no treatment.
    - Initiation of antiretroviral treatment at different stages of pregnancy.
    - Type of antiretroviral treatment regimen received during pregnancy
    - Associated opportunistic infections (e.g. Tuberculosis).

4.2.4 Objective 4

Nested HIV Immunology Study: HIV-specific T cell response in pregnancy.

HIV is associated with persistent immune activation, progressive $CD4^+$ lymphopenia and defective HIV-specific $CD8^+$ response[122,123]. HIV, as well as pregnancy have both been shown to induce expansion of the T-regulatory cell subset of the lymphocyte population.[118,124]. This may be beneficial to both conditions by diminishing HIV-induced generalised T cell activation, thus slowing both $CD4^+$ exhaustion and disease progression, in addition to facilitating the preservation of Fetal-maternal tolerance. However, the expansion in Treg cells may abate the $CD4^+$ and $CD8^+$ T- cell response to HIV-specific epitopes thus contributing to the prevention of viral eradication[125].

We propose to conduct a nested longitudinal descriptive cohort study of 20 HIV positive women which evaluates the changes in the HIV-specific immune response during pregnancy. 20ml of peripheral blood will be collected from each participant at each visit, in addition to what is currently being collected and 1 sample will be collected at 6 weeks postnatal. Tests to be conducted include:

Appendices

1. ELISPOT assays

This is a sensitive method of characterizing and quantifying HIV specific $CD4^+$ and $CD8^+$ T cell response. $CD4^+$ and $CD8^+$ T cells work together to establish an effective HIV specific immune response to the virus which correlates with suppression of viral replication.[126](25) A panel of 410 overlapping peptides spanning the entire HIV-1 clade C genome will be used to screen for HIV-specific T cell responses.

2. HLA typing

DNA for HLA class I typing will be isolated from whole blood collected during the 1st trimester (booking visit<14wks). The relationship between HLA type and the observed HIV specific $CD4^+$ and $CD8^+$ T cell response, viral load, antiretroviral drug treatment, $CD4^+$ count, T-regulatory cell population (percentage, count and functionality) and expression of immune activation markers will be analysed and correlated with rate of disease progression and risk of PTB and IUGR.

3. Control sample

The HIV-specific $CD4^+$ and $CD8^+$ T cell response is dependent on an individual's HLA type and specific sequence variation of the infecting virus[127].(26) We propose that a postnatal sample from each participant will serve as the best control for this cohort. A pre-pregnancy sample would be the preferred control sample, but recruitment into the study is only possible following a positive pregnancy test, thus eliminating the possibility of attaining a pre-pregnancy sample. A maternal peripheral blood sample will be collected at 6 weeks post-delivery from each of the 20 participants and an ELISPOT assay will be done. We will compare postnatal results with similar variables assessed during pregnancy with the aim of determining the influence of pregnancy on the maternal HIV specific T cell response.

4. Viral RNA and DNA extraction and sequencing to detect selection of $CD8^+$ T cell escape mutations.

Studies have suggested that the HIV virus adapts to HLA-restricted $CD8^+$ T cell responses by selecting escape mutations which result in the virus evading $CD8^+$ T cell responses[128]. Viral RNA and/or DNA will be isolated and sequenced from maternal plasma collected at each visit. We will assess the relationship between the HLA type, observed CTL responses and selection of escape mutations

within the context of pregnancy. This will be correlated with the maternal viral load, antiretroviral drug treatment, $CD4^+$ count, disease progression, mother to child transmission rate and adverse pregnancy outcome including PTB and IUGR.

5. Experimental design and methods

This research aims to conduct 3 studies including:

- The Maternal Immunology Study
- The HIV Immunology Study
- The Soweto HIV and ART pregnancy study: The influence of HIV and Antiretroviral treatment on pregnancy outcome in Soweto, South Africa. This study will be abbreviated as the SHAPOSSA Study

5.1 Setting

This is a collaboration between Professor Shane Norris, "Fetal Growth Study (FGS): Investigating maternal factors associated with fetal growth and delivery outcomes" (ethics reference: M120524) at the DPHRU, Chris Hani Baragwanath Hospital, Johannesburg, South Africa, The INTERBIO-21st Project, Nuffield Department of Obstetrics and Gynecology, and Professor Phillip Goulder who is based at the Peter Medawar Building for Pathogen Research, Oxford University, UK.

5.2 Study population

5.2.1 Population description

Maternal	Cohort study	Sample size	600
		Age	18 years and older
		Gestational age at booking	<14weeks
		HIV positive women	181
		HIV-negative women	419
		Recruitment site	Baragwanath and Zola hospitals
		Recruitment time frame	2 years

Immunology Study Population		Treatment	Antiretroviral treatment and any treatment deemed necessary by attending Obstetrician.
	Nested study	**HIV Immunology Study**	
		Sample size	20 HIV-positive pregnant participants of the Maternal Immunology Study cohort.
SHAPOSSA Study population	Cohort study	Sample size	3000
		Age	18 years and older
		Gestational age at booking	<14weeks
		HIV-positive women	900
		HIV-negative women	2100
		Recruitment site	Baragwanath hospital
		Recruitment time frame	3 years
		Treatment	Antiretroviral treatment and any treatment deemed necessary by attending Obstetrician

5.2.2 Inclusion and exclusion criteria

Maternal Immunology Study	**Inclusion criteria:**
	Pregnancy must have been conceived naturally
	Dating scan at Zola or Baragwanath hospitals
	BMI<35
	Gestational age <14weeks at booking
	Women must be at least 18 years old
	Exclusion criteria:
	Multiple pregnancies
	BMI>35
HIV Immunology Study	Only HIV-positive women from the Maternal Immunology Study cohort will be invited to participate in this nested study.
SHAPOSSA Study	≥ 18 years of age
	< 14 weeks' gestation age at booking
	Singleton pregnancy
	Women residing in Soweto
	Black South African females
	Exclusion criteria:
	Multiple pregnancy

	Fetal abnormalities detected
	Intellectually or physically disabled women

Patient recruitment per year

Maternal and HIV Immunology study				
Recruitment rate: 25/month	**Booking <14 weeks**	**2nd Trimester 24-28 weeks**	**3rd Trimester 34-38 weeks**	**Delivery (~40weeks)**
Year 1 (April 2013 – March 2014)	300	250	175	150
Year 2 (April 2014 – March 2015)	300	300	300	300
Year 3 (April 2015 – March 2015)	-	50	125	150
Total	600	600	600	600

SHAPOSSA study			
Recruitment rate: 80/month	**Booking**	**Delivery (~40wks)**	**Total deliveries**
Year 1: May 2013 – April 2014	960	640	-
Year 2: May 2014 – April 2015	960	960	1600
Year 3: May 2015 – April 2016	960	960	2560
Year 4: May 2016 – June 2016	120	440	3000
Total	3000	3000	

5.3 Study design

Maternal Immunology Study cohort and SHAPOSSA study: this is an analytical, longitudinal cohort study of black women from Soweto. Women will be recruited from the Chris Hani Baragwanath Hospital and the DPHR Unit, where they will undergo a dating scan at <14 weeks. They will then be followed up with standard clinical and 2D ultrasound assessment every 5 weeks. Preterm infants will be classified according to the new classification system: Villar J et al, *Am J Obstet Gynecol* Oct 25 2011 and IUGR will be classified according to the new INTERGROWTH small for gestational age growth charts.

The SHAPOSSA study is an addition to the FGS. The SHAPOSSA study aims to use all socio-economic and clinical data derived from the FGS study, to assess the influence of HIV-infection and ART on pregnancy outcome. Furthermore, in addition to the data being collected by the FGS study, we will collect HIV-specific clinical information from all HIV-positive women included in the FGS cohort (Appendix 2).

HIV Immunology Study: this nested, analytical descriptive, prospective longitudinal cohort study will recruit HIV-positive women from those included in the Maternal Immunology Study.

5.3.1 SHAPOSSA study

5.3.1.1 Exposure of interest and determination of HIV-status

The exposure of interest includes maternal HIV infection, diagnosed pre-pregnancy or during the antenatal period, and maternal exposure to ART during the antenatal period. As all women will be recruited at < 14 weeks' gestation, we expect that all HIV-positive participants not yet on treatment will be initiated promptly at the booking visit as specified by the South African PMTCT regimens, unless the patient opts out of HIV testing or refuses treatment.[129]

The self-reported maternal HIV-status, in conjunction to the HIV-status documented in maternal antenatal card will be used to determine HIV-exposure status. The viral load and $CD4^+$ count of all included participants will be obtained from the National Health Laboratory System, which will serve

as an independent verification of HIV Status and will be used for statistical and epidemiological analysis. Following confirmation of HIV status, participants will be asked to bring their ART to their follow-up visit thus allowing the capturing of details regarding the participants' ART regimen.

5.3.1.2 Outcome of interest

The following outcomes of interest will be assessed in HIV-positive and HIV-negative pregnant women:

Primary outcomes of interest

1. PTB and premature prelabour rupture of membrane (PPROM)
    - PTB defined as birth > 24^{+0} but <37^{+0} weeks' gestation,[130] which will be categorised as follows:
    - Moderately PTB (34^{+0}-36^{+6} weeks),
    - Very PTB (28^{+0} -<33^{+6} weeks) and
    - Extremely preterm birth (24^{+0} - < 28^{+0} weeks).[16]
2. A distinction will be made between iatrogenic and spontaneous PTB.
3. LBW, VLBW, SGA and IUGR

The birth-weight of infants born to HIV-positive and negative mothers will be assessed and infants will be classified as following:

- LBW, defined as a birth-weight <2500g[43]
- Very low birth weight (VLBW) will be defined as birth-weight <1500g[44]
- SGA, defined as birth-weight-for-gestational age <10^{th} centile of the INTERGROWTH-21^{st} neonatal standard.[130]
- IUGR, a diagnosis made on ultrasound examination, will be defined as an estimated fetal weight (EFW) below the 10^{th} percentile and an umbilical artery pulsatility index (PI)> 2 standard deviations.[131]

Secondary outcomes of interest

1. Miscarriage, which will be defined as the spontaneous expulsion of the fetus < 24^{+0} weeks' gestation.
2. Stillbirth, which will be defined as any second or third trimester delivery of a demised fetus at ≥24^{+0} weeks
3. Neonatal death will be defined as the death of the infant within the first 28 days of life.

5.3.1.3 Study conduct

Participants who volunteer to participate in the FGS will be followed up with serial ultrasound growth scans on a ±6 weekly basis, where biological samples, clinical and socio-economic data will be collected. The additional HIV-specific data which will be collected from all included HIV-positive women will be collected at any one of these follow-up visits.

FGS Antenatal visit	Recruitment	1st visit	2nd visit	3rd visit	4th visit	Delivery
Gestational age	< 14 weeks	20 weeks	26 weeks	32 weeks	38 weeks	>38 weeks

5.3.1.4 Sample size calculation and power

As the sample size is already decided a priori (3000), we sought to establish the power of the study based on different effect sizes. We acknowledge the fact that literature on the rate of PTB amongst HIV-positive and HIV-negative mothers are scanty, especially in Africa. A study by Blencowe et al suggested a 39% increase in the rate of PTB between HIV-positive and HIV-negative mothers, although this difference was not statistically significant.[132] Another study by Chen et al reported a 30 – 50% increased risk of PTB in HIV-positive mothers when compared to HIV-negative mothers and this was statistically significant.[26] In this study they report the prevalence of PTB as 17.2% in HIV-negative mothers and 23.7% in HIV-positive mothers.[26] We have thus based our power calculation on the Chen et al findings and assumed the prevalence rate for PTB in HIV-negative women to be 15%. We have then calculated the power achieved if we were to detect a difference of 5%, 10% and 15% between HIV-positive and negative women.

Sample size = 3000 subjects (Outcome = Preterm delivery)

Outcome = Preterm delivery			
Rate in HIV-negative mothers	Rate in HIV positive mothers	Effect size	Power achieved
0.15	0.20	5%	76.2%
	0.25	10%	99.8%
	0.30	15%	100%

Similarly, for IUGR there is limited literature on the prevalence amongst HIV-positive and HIV-negative women. A study by Ndirangu et al approximates a prevalence rate for IUGR in South Africa of 7.9% in HIV-negative women.[29] We have thus assumed a prevalence rate of 5% for HIV-negative mothers and calculated the power achieved for effect sizes of 5%, 10% and 15%.

Outcome = IUGR			
Rate in HIV negative mothers	Rate in HIV positive mothers	Effect size	Power achieved
0.05	0.10	5%	96.88%
	0.15	10%	100%
	0.20	15%	100%

5.3.1.5 Statistical Analysis

Logistic regression: The odds of PTB and IUGR in HIV-positive and HIV-negative mothers will be compared using univariable and multivariable logistic regression, when adjusting for other covariates like maternal age, parity, number of antenatal visits and other obstetric diagnoses during pregnancy. The role of ARV's in PTB and IUGR and whether it modifies how HIV status relates to PTB and IUGR will also be explored. Survival analyses of the rates of IUGR and PTB by HIV status and other covariates will also be explored. Tests of association for categorical variables will be performed using a Chi-square test or Fishers exact test as appropriate.

Structural equation modeling will be used to explore how various socio-economic and clinical determinants interact to influence pregnancy outcome (PTB and IUGR) in HIV-positive and HIV-negative women. Targeted maximum likelihood estimation (T-MLE), a new technique developed by

Professor MJ Van der Laan, Berkeley University, will be used to evaluate the causal effects of assigned ART treatment on risk of adverse pregnancy outcome.[133] Unlike regression analysis, which enables the analysis of relationships between variables without suggesting that the relationship is causal,[134] TMLE allows the author to make causal inferences between two specific variables of interest.[135] This analysis will be done through a collaborative effort between Oxford University and University of California at Berkeley. Statistical significance will be assessed at the 5% level and all analyses will be undertaken using STATA version 13 and R statistical analytical software.

5.3.1.6 Data handling and record keeping

All study data will be entered into a database (SHAPOSSA Access data file) and a study specific participant number will identify participants. The participant's name and any identifiable details will not be included in any study data electronic file.

5.3.1.9 Funding

The Nuffield Department of Obstetrics & Gynaecology (NDOG) will provide funding for the printing of consent forms and a research technician who will undertake data capturing. Clinical and socio-economic data will be collected by the FGS team, which is being funded by the DPHRU.

5.3.2 Maternal Immunology Study and HIV immunology Study

5.3.2.1 Sample collection

Supplies for blood collection:

	Booking visit <14 weeks	***24-28 weeks***	***34-38 weeks***
Maternal Immunology Study	Maternal blood	Maternal blood	Maternal blood
	2x10ml EDTA collection tube Blood collection set 4x Nunc cryovials 1x 5ml collection tube 4x 2ml Nalgene sterile tubes	2x10ml EDTA collection tube Blood collection set 4x Nunc cryovials 4x 2ml Nalgene sterile tubes	2x10ml EDTA collection tube Blood collection set 4x Nunc cryovials 4x 2ml Nalgene sterile tubes
In addition to the above-mentioned supplies, the following supplies will be used to collect additional samples from participants included in the nested HIV Immunology Study:			
HIV Immunology Study	***Booking visit <14 weeks***	***24-28 weeks and 34-38 weeks and delivery***	***Postnatal sample (control sample)***
	2x10ml EDTA collection tube 4x Nunc cryovials 1x 5ml collection tube 4x 2ml Nalgene sterile tubes	2x10ml EDTA collection tube 4x Nunc cryovials 1x 5ml collection tube 4x 2ml Nalgene sterile tubes	2x10ml EDTA collection tubes 4x Nunc cryovials 1x 5ml collection tube 4x 2ml Nalgene sterile tubes

Blood collection and blood volume required:

- Maternal blood samples will be collected by a trained phlebotomist, midwife or research assistant.
- Cord blood should be collected by a physician, a trained midwife or research assistant. Cord blood must be collected within 30 minutes of delivery of the placenta.
- Processing of the cord blood should be completed within 4 hours of blood collection to avoid getting a poor-quality specimen or haemolysis. However, if this is unachievable, it should be processed to up to 12 hours after collection.

Summary of blood volume required:

	Booking	***24-28 weeks***	***34-38 weeks***	***Delivery***	***Postnatal blood***
Maternal Immunology Study participants					
Total blood volume per visit	20 ml	20 ml	20 ml	None	None
HIV Immunology Study participants					
Maternal Immunology Study component	20ml	20ml	20ml	None	None
HIV Immunology Study component	20ml	20ml	20ml	20ml	20ml
Total maternal blood volume	40ml	40ml	40ml	20ml	20ml

The above samples will be taken in addition to the samples taken as part of the Fetal Growth Study protocol (ethics reference M120524):

Maternal blood:

- Booking (<14 week): 16ml
- Second trimester visit (24-28weeks): 30ml
- Third trimester visit (34-38 weeks): No blood collection
- Delivery: No blood collected

5.3.2.2 Sample collection flow diagram

	Maternal Immunology Study	**HIV Immunology Study**
Contact point	• Participants present to Chris Hani Baragwanath Hospital with a suspicion of pregnancy. • Pregnancy test is positive.	• HIV-positive participants who have met inclusion criteria for Maternal Immunology Study are invited to participate in nested study.
Enrollment	• Discuss Maternal Immunology Study with patient. • Consent forms are signed. • Complete: contact details sheet/ family pedigree. • Book patient for ultrasound.	• Discuss HIV Immunology Study with patient. • Consent forms are signed. • Complete :contact details sheet/family pedigree. • Book patient for ultrasound.
Booking: <14 weeks	• ANC: Booking bloods: HIV, $CD4^+$, Hb, Syphilis, Rh, Tetanus toxoid • Dating scan • 20ml venous blood taken (2x10ml EDTA tube)	• ANC: Booking bloods: HIV, $CD4^+$, Hb, Syphilis, Rh, Tetanus toxoid • Dating scan • 40ml venous blood taken (4x10ml EDTA tube)

	• DNA extraction: remove 3ml of blood from collected sample and process as per DNA extraction processing. (see below).	• DNA extraction: remove 3ml of blood from collected sample and process as per DNA extraction processing. (see below).
24-28 weeks	• Fetal growth scan. • 20ml venous blood (2x10ml EDTA tube).	• Fetal growth scan. • 40ml venous blood (4x10ml EDTA tube).
34-38 weeks	• Fetal growth scan • 20ml venous blood (2x10ml EDTA tube).	• Fetal growth scan • 40ml venous blood (2x10ml EDTA tube)
Delivery	None	• Maternal venous blood: 20ml (2x10ml EDTA tube).
Postnatal	None.	• Maternal blood: 20ml (2x10ml EDTA tube).

5.3.2.3 Sample processing on site

Local laboratory

All blood samples will be collected from Zola hospital and Chris Hani Baragwanath hospital by the transport service carrying study participants to the DPHRU. A research nurse or assistant based at Zola hospital and Chris Hani Baragwanath hospital should ensure that all samples are loaded onto the bus. All blood samples should be kept in the fridge (2-8°C) at the hospital while awaiting transportation. All samples must be transferred to DPHRU as soon as possible or within 12hr of collection.

Blood separation and cryopreservation

This process is the manner in which plasma and peripheral blood mononuclear cells are isolated from fresh blood samples.

Before starting:

1. Fill a plastic bucket with 2% Virkon solution for emersion of plastic waste except tips. Following use, soak items overnight on the draining board, not in the cabinet.
2. Premix R10 solution:
    - Take 40ml of RPMI-1640.
    - Add 4ml of heat inactivated, filtered fetal calf serum (FCS).
    - Add 0.4ml of L-glutamine.
    - Add 0.4ml of Penicillin + Streptomycin.
    - Label with R10, your name and date of preparation.

- Store this solution for 4 weeks at 4°C.

3. Take out the R10 from the refrigerator at the start of the procedure to warm at room temperature.
4. Keep everything inside the cabinet as sterile as possible.
5. Spray everything with 70% ethanol before placing in the hood.
6. Label three 50ml Falcon tubes (Label fourth Falcon tube for DNA extraction at booking visit)
7. Check that the Nunc cryovials, Nalgene sterile tubes and 5ml collection tube found in sample kit are correctly pre-labelled with bar-coded stickers.
8. Ensure that the freezing container (Mr Frosty) must be charged with neat isopropanol (Prolabo) and left at 4ºC for 1 hour before any cryovials are added to it.

Isolation of plasma

1. Invert the EDTA tubes with whole blood several times to ensure the blood is thoroughly mixed.
2. Spray the EDTA tubes with 70% ethanol before transferring the blood to the hood.
3. Only booking sample: Take 3ml of blood and put in the first 50ml Falcon tube, put tube aside for DNA extraction (see below).
4. Take the remaining (17ml) of blood and place in the second 50ml Falcon tube.
5. Spin at 894g for 10min to obtain plasma layer.
6. Use Pasteur pipette to remove plasma and place in Nalgene sterile tubes (1-1.8ml of plasma in each Nalgene tube).
7. At least 4ml of plasma (4 Nalgene tubes) is required and this amount can normally be obtained from 2-3 EDTA tubes.
8. Nalgene tubes containing plasma should be **stored at -80°C immediately.**
9. Complete the e-form.

Isolation of PBMC

1. Measure how much blood is left and how much lymphoprep and PBS is required: 1:1:1 ratio (e.g. If you have 5ml of blood remaining, you will need 5ml of lymphoprep: 5ml of PBS).
2. Put lymphoprep (Ficoll) at the bottom of the third empty 50ml Falcon tube.
3. Take PBS and put into the 2nd Falcon tube containing the remaining buffy coat and red blood cell layer (Total PBS-blood solution should be in a 1:1 ratio).
4. Mix well.
5. Layer the blood-PBS solution **SLOWLY** onto the lymphoprep (Ficoll).
6. Centrifuge **IMMEDIATELY at room temperature (RT)** for 30 minutes at 250g with slow start and no brake. (Acceleration=1, deceleration=2)
7. Check centrifuge buckets for leaks before and after spin.

8. Add blood collection tubes into liquid waste container and immerse in 2% Virkon.
9. Transfer mononuclear cells to 4th 50ml Falcon tube using a Pasteur pipette (mononuclear layer is found between the PBS and lymphoprep - Please avoid taking red blood cells however, don't worry if some of the lymphoprep taken up as well because the washes will remove it).

Wash PBMC

1. Add plain RPMI to buffy coat until it reaches the 45ml mark of Falcon tube.
2. Mix well.
3. Centrifuge at 894g at RT for 10minutes. Always check if there is a pellet at the bottom of the tube after spinning.
4. Discard the supernatant into the bucket with Virkon- making sure you don't remove the cells.
5. Add 1ml of RPMI using a Pasteur pipette and mix gently to re-suspend cells.
6. Add RPMI until the level reaches the 45ml of mark of Falcon tube.
7. Centrifuge at 894g for 5 min at RT.
8. Discard the supernatant into the bucket of Virkon –do not remove cells.
9. Add 1ml of RPMI using a Pasteur pipette and mix gently to resuspend cells.
10. Add RPMI until the level reaches the 45ml mark of Falcon tube
11. Centrifuge at 894g for 5 min at RT.
12. Discard the supernatant into the bucket of Virkon – do not remove cells.
13. Scrape the tube along the rack to break up the cell pellet.
14. Re-suspend the cells by adding 1ml of R10 with a Pasteur pipette and mix gently.
15. Add R10 until total volume reaches 10ml.
16. Count cells.

Counting the cells

1. Take 20µl cell solution and put in a well of a 96 well plate.
2. Add 20µl of Trypan blue (Sigma) to the 20µl of cell solution (in the 96 well plate).
3. Mix thoroughly.
4. Slowly pipette 10µl of this mix of cells into the counting chamber.
5. Count the number of cells at the top left and bottom right of the chamber grid.
6. Calculate the total number of cells in the cell suspension:

Add the number of cells in the two 16 squares x 10 000 x volume of medium. The average recovery using this technique with a 10ml blood sample ranges from 8 to 10 million cells. A consistent recovery rate below 4 million cells implies that the technique is not being performed successfully.

Freezing the cells

Preparation of cryovials:

1. Centrifuge the cell solution to be frozen at 894g for 10minutes.
2. Check that the Nunc cryovials are correctly pre-labelled with bar-coded stickers.
3. Chill the vials in fridge while cells are spinning down.
4. Prepare 40ml of freezing solution:
    - 20ml of filtered, heat-inactivated fetal calf serum (FCS, Gibco).
    - 4ml of 10% DMSO (Sigma)
    - 16ml of R10 (see above).

This gives a final concentration of 50% FCS, 10% DMSO and 40% R10.

You should prepare enough for 1ml of freezing solution per vial.

5. After the spin: discard of the supernatant into the Virkon bucket.
6. Add freezing solution to the cells dropwise and slowly.
7. Cells are added to cryovials so that each contains 5 million cells in a total volume of 1ml freezing mix.
8. Once the cells are in the freezing solution place them in the Mr Frosty stratacooler box immediately, since DMSO is toxic to cells.

Freezing

1. The cryovials which contain re-suspended cells must now be frozen according to a protocol which aims to lower the vial temperature in stages.
2. When the cryovials are added to the Mr Frosty the container must be immediately placed in a -80°C freezer overnight (at least 12 hours at -80°C is recommended).
3. After that the freezing container is taken rapidly to liquid nitrogen storage containers which should be in the same laboratory space.
4. The vials are then rapidly placed in coded racks and loaded into the vapour phase of liquid nitrogen (Forma Scientific).

DNA extraction from whole blood:

Take 3ml of blood intended for DNA extraction:

1. Label a 50ml falcon tube with patient ID, date, DNA, amount of blood taken (3ml).
2. Add 9ml of red blood cell lysis solution.
3. Incubate for 10 minutes at room temperature (RT) and mix periodically.
4. Centrifuge at 894g for 10min.
5. Discard supernatant.
6. Add 3ml of white cell lysis solution and mix to re-suspend the pellet.

7. Transfer solution into 5ml storage container.
8. Store at room temperature.

ELISPOT assay:

Before you begin:

IMPORTANT - Keep everything inside the cabinet as sterile as possible. Make sure to spray everything with 70% ethanol before placing in the hood.

If you are not using all the wells on the plate, coat the whole plate and wash the rows you need + 1 at step 2 Day 1, and then continue only with the wells you effectively use.

Prepare premix **Blocking buffer** solution:

1. 5ml of PBS.
2. Add 5ml of heat inactivated, filtered fetal calf serum (FCS), to give a 1% solution (5ml).
3. Label solution as blocking buffer and the date of preparation.
4. Store this solution for 4 weeks at 4°C.

Day before you start:

1. Coat a 96-well polyvinylidene difluoride plate (Millipore).
2. Label the plate base with your initials and date of coating.
3. Turn light off in the hood. Take 25µl of anti-IFN-γ Antibody (Ab) (ELISPOT reagent #1 – green top) and add to 50ml PBS. Vortex the solution well (This is enough for 5 plates however, make less if you have fewer plates).
4. Add 100µl of the Ab solution (step 3) into each well using the multi-channel pipette.
5. Coated plates must be incubated at least overnight at 4°C (refrigerator). They can be coated up to 1 month in advance and kept at 4°C.

Day 1

1. Label the plate on the lid and base with: patient ID, date, number of cells added per well (50,000 for matrix and 100,000 for confirmation, your initials, show position of positive and negative controls on the lid).
2. Empty wells and wash the plate 6x with 100µl/well blocking buffer using the multi-channel pipette. A Virkon-free container should be used to empty washes into and paper towel placed underneath to catch splashes.

3. Add 50µl of R10 to each well of the ELISPOT plate using the multi-channel pipette. (The plate should be left in the fridge at 4°C until the peptides and the cells are added, this prevents the wells from drying out, max 24 hours)
4. Add 10µl of each OLP's pool / individual peptide (40µg/ml) and 2x (wells) 10µl of PHA (250µg/ml) as positive control. Use a fresh pipette tip each time. Each plate should have 2 PHA positive control wells and 4 negative control wells (where cells only are added). Keep plate in the fridge at 4ºC until cells are added.
5. The cells are added in 100µl of R10 using a multi-channel pipette. Use a fresh pipette tip each time. The cells should be diluted to give 50,000 cells/well for the matrix plate and 100,000 cells/well for the confirmation plate.
6. Incubate plate overnight at 37°C with 5% CO_2. (14-16 hours is ideal). Plates can be developed after 20 hours' maximum in emergencies – IMPORTANT: if Day 2 is on Saturday, wash as detailed in Day 2(1) and add 200µl/well PBS, leave until Monday in the fridge at 4°C).

Day 2

1. Discard cells and wash the plate 6x with 100µl/well PBS using a multi-channel pipette. Try not to touch the plate with the tips, if you do, change the tips. A Virkon-free container should be used to empty washes into and paper towel placed underneath to catch splashes (This is to ensure that disinfectant does not get into the wells and affect the assay).
2. **Turn light off in the hood.** Add **5µl biotinylated IFN-γAb (Elispot reagent #2- yellow top)** to **10ml of PBS (from the original bottle)**. **Vortex solution well and make up fresh every time.**
3. Add **100µL of the Ab solution (step 2)** to each well using a multi-channel pipette **(no need to change tip each time).**
4. **Wrap** the plate in paper towel to protect from the light and leave in the hood to **incubate at room temperature (RT) for 90 minutes.**
5. Empty wells and wash the plate **6x** with **100µL/well PBS (from the glass bottle, self-made PBS)** using a multi-channel pipettor. **(no need to change tip each time).**
6. **Turn light off in the hood.** Add **5uL of Streptavidin-alkaline phosphatase conjugate (Elispot reagent #3-white top)** to **10mL PBS**. **Vortex solution well and make up fresh every time.**
7. Add **100µL of the Streptavidin conjugate solution (step 6)** to each well using a multi-channel pipette **(no need to change tip each time).**
8. Incubate in the hood for **45 minutes, wrapped paper towel** to protect from light, **at room temperature.**
9. Wash the plate as detailed in (5).
10. Make substrate colour solution (per plate):
11. 10ml sterile distilled water

12. 400µL buffer
13. 100µl BCIP (colour solution A, clear) and 100µl NBT (colour solution B, yellow)
14. Add **100µl of the substrate colour solution (step 10)** to each well using a multi-channel pipette **(no need to change tip each time).**
15. Incubate plate **at room temperature in the light** until the blue spots appear. This usually takes 15-20 minutes. Stop the reaction before the background comes up in the negative wells.
16. Discard the colour reagents in the sink and stop colouring process by washing the plate with water. Wash at least **6x with tap water.**
17. Leave to dry on tissue paper.
18. Place 2% Virkon in the bucket you put the waste cells into and leave to soak overnight.
19. Spray inside of plate lid with ethanol and ensure plate is dry before storing.

6. Storage and shipping

Once the biological samples are collected and processed, they will initially be stored at the DPHRU in South Africa. At regular intervals batches of samples will be shipped to the Peter Medawar Building, Oxford, UK. This process will continue throughout the period of recruitment and sample collection until all the samples have been shipped to Oxford. Samples will be stored at the Peter Medawar Building and will be used to conduct the assays stipulated in the protocol. Samples may be stored for 20 years, i.e. until 2035.

Packaging and shipping**:**

When sending biological samples to Oxford for long term storage, the shipment must meet international shipping regulations. To do this, the package must contain three components:

1. Primary container- must be leak-proof. *
2. Secondary container- must be leak-proof. *
3. Outer packaging- this must be rigid packaging which is large enough to hold the secondary container(s).

* Either the primary or secondary container must able to withstand pressures of 95kPA

Primary Container

The tubes supplied in the sample collection kits are the primary containers. These tubes must be stored in cryoboxes with each cryobox containing one sample type e.g. all maternal buffy coat samples or all cord blood plasma samples. In the lid of the cryobox you need to place absorbent material, there must be sufficient absorbent material to absorb the contents of each tube in the box in the event of a leak.

Secondary Container

Specimen bags ('BioBag') are secondary containers for shipping biological samples. The cryoboxes containing the primary samples are placed in the specimen bags. If absorbent material has not been placed in the top of the cryobox on top of the samples, then sufficient absorbent material should be placed in the bag to absorb the contents of each primary container in the event of a leak.

Outer Packaging

This will be a polystyrene box large enough to hold the secondary containers as well as sufficient dry ice to keep the samples frozen whilst they are in transit. The paperwork outlining the contents of the shipment must also be within the outer packaging. The package cannot exceed 4 litres or 4kg of biological samples.

<u>Paperwork</u>

Each package requires the appropriate paperwork before it can be sent. This includes:

1. Export licence allowing biological samples to be shipped to the UK.
2. Manifest – states the shipper's and receiver's address, number of packages, details of the contents, the weight and the value of the package.
3. Airway bill.
4. Miscellaneous dangerous substances label on the outer packaging (for dry ice).
5. UN3373 Label marking for infectious substances of category B.

7. Immunological analysis at Oxford

All processing of samples will be conducted at the Peter Medawar Building for Pathogen Research, which houses high-quality molecular biology laboratory space at category III containment level suitable for HIV research. The facility will enable us to conduct the experiments detailed in Objectives 3 & 4. Maternal blood and cord blood will be analysed as follows:

7.1 Fluorescence-activated cell sorting analysis (FACS)

FACS staining will be performed on the cryopreserved peripheral blood mononuclear cells (PBMCs) with naïve, memory and activated lymphocyte subsets being identified using the following panels of monoclonal antibodies: anti-CD8-FITC, anti-CD38-PE, anti-CD4-PeCY7, anti-CD3-ECD, anti-IL-17A-PE, anti-Human CD20, anti-62L-APC, anti-CD45RA-PE, anti-Human gamma delta TCR, anti-CD69-FITC,anti-CD27-APC, anti-CD45RO-PE, anti-HLA-DR-APC,anti-CD244-Alexa Fluoro. T regulatory cells will be identified by the extracellular staining of PBMCs with anti-CD4-APC, anti-CD8-PE-anti-CD25-PE, anti-CD3-PERCP, anti-CD45RA-PECy7, and anti-CD127-PE. These cells will then be fixed, permeabilised and blocked with normal rat serum. This will be followed by intracellular staining with anti-FOXP3-APC. Natural Killer cells will be identified using: anti-CD56-APC, anti-CD16-PE. All samples will be analysed using a BD LSR II flow cytometer and the resulting will be analysed using a FlowJO software.

7.2 Quantification of cytokines by ELISA

Serum samples will be assayed for the following cytokines: IL-1, IL-2, IL-4, IL-6, IL-8, IL-10, IL-16, IL-18, IFN-γ, TNF-α, G-CSF, MMIF, and neopterin.

7.3 Quantification of acute phase proteins and hormones

The following acute phase proteins and hormones will be quantified: CTLA-4, β2-microglobulin, CRP, PAF, MMP8, angiogenin, relaxin, HCG, PAPP-A, activin-A, Inhibin, prolactin, PP-13, SP1, endoglin, thrombin-antithrombin III complex.

8. Ethical considerations

This study protocol will be submitted to the University of the Witwatersrand, Human Research Ethics Committee (HREC) for ethical consideration and the University of Oxford Tropical Research Ethics Committee (OXTREC) for ethical consideration. A patient information sheet and DNA information sheet describing relevant information will be supplied to each participant in addition to information sheets provided by the Fetal Growth Study.

A consent form will be signed by all participants enrolled in the Maternal Immunology study and an additional consent form will be signed by all participants who volunteer to participate in the HIV Immunology Study.

We plan to submit a request for an amendment to our approved study protocol from University of the Witwatersrand, Human Research Ethics Committee (HREC) (Protocol M130134), and the University of Oxford Tropical Research Ethics Committee (OXTREC) (OXTREC 1008-13) for permission to use all research data collected by the FGS study for statistical analysis as well as to collect additional clinical information from all HIV-positive women included in the FGS.

Study staff will ensure that the participants' right to privacy, anonymity and confidentiality will always be maintained, and consent will be obtained from all HIV-positive participants who supply additional HIV-related clinical information. An amendment has been made to our current HIV consent form, which requests for access to any HIV-specific clinical data collected from all HIV-positive women. (Appendix 3)

9. Plans for Research dissemination

We intend to disseminate the results of our study locally and nationally in the United Kingdom and South Africa, as well as internationally. Results will be presented at local, national and international medical and scientific conferences and published in international peer-reviewed medical and scientific journals.

10. Study timetable

Month	Ethics amendment	SHAPOSSA Study ethics amendment	SHAPOSSA study recruitment	Learn processing method	Sample collection	Specimen analysis	Statistical analysis	Writing up of dissertation	Submission of articles for publication	DPhil thesis submission and oral examination
May 2013	X									
Oct 2012 - March 2013				X						
June 2013 - June 2014		X	X		X					
July 2014 - June 2015			X		X	X				
July 2015 - September 2015			X				X	X	X	
January 2016			X				X	X	X	X

Appendix 5.3

MRC/WITS DEVELOPMENTAL PATHWAYS FOR HEALTH RESEARCH UNIT

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SHAPOSSA Questionnaire

	Section 1	
	Participant Identification	
1.1	INTERBIO-21st/SFG/3G PTID number:	
1.2	Date of birth:	D D M M Y Y
1.3	Date of interview:	D D M M Y Y
1.4	Gestational age at this visit:	Days: ☐☐ Post-delivery: ☐ Weeks: ☐

	Section 2			
	HIV-status			
2.1	HIV-status:	Positive: ☐ Negative: ☐		
2.2	Date tested HIV-positive:	D D M M Y Y		
2.3	Test used to confirm HIV status:	Rapid test: ☐ ELISA test: ☐ Unknown: ☐		
2.4	CD4 count in this pregnancy	Absolute CD4 count (10^6/L)	CD4 %	Date of test (DD/MM/YYYY)
	Pre-pregnancy test			
	Tests during pregnancy			
2.5	Viral load	Copies/ml	Date of test (DD/MM/YYYY)	
	Pre-pregnancy test			
	Tests during pregnancy			

2.6	WHO Stage of disease currently, choose from options below:			
	Clinical stage 1:		Clinical stage 4 (AIDS):	
	Clinical stage 2:		Not available	
	Clinical stage 3:			

	Section 3			
	Antiretroviral treatment			
3.1	Is she on antiretroviral treatment:		Yes: ☐ No: ☐	
3.2	If yes, which treatment is she receiving: please choose from options below:			
	FDC (TDF + FTC/3TC + EFV)		AZT + 3TC + EFV	
	AZT		TDF+ FTC+ LPV/RTV	
	TDF+ FTC+ NVP		Other	
3.3	If other, please specify:			
3.4	When was this treatment started:		D D M M Y Y	
3.5	During this pregnancy, has she received any different ART treatment prior to the above-mentioned treatment regimen:		Yes: ☐ No: ☐	
3.6	If yes, on what date was the ART treatment changed to the current treatment:		D D M M Y Y	
3.7	What treatment was she receiving, please choose from options below:			
	FDC (TDF + FTC/3TC +EFV)		AZT + 3TC + EFV	
	AZT		LPV/RTV or any other Protease Inhibitor based ART	
	TDF+ FTC+ NVP		Other	
3.8	If other, please specify:			
3.9	Why was treatment changed, please choose from options below:			
	Because of pregnancy		Treatment failure	
	Drug side effect		Other	
3.10	If other, please specify:			

	Section 4			
	Tuberculosis			
4.1	Has she been tested for TB in the last year:		Yes: ☐ No: ☐	
4.2	What was the result of TB test :		Positive: ☐ Negative: ☐ Unknown: ☐	
4.3	Has she received treatment for TB during this pregnancy:		Yes: ☐ No: ☐	
4.4	If yes, when was treatment initiated:		D D M M Y Y	
4.5	Is she still receiving treatment for TB:		Yes: ☐ No: ☐	
4.6	If no, when did the treatment stop:		D D M M Y Y	
4.7	What TB treatment is she currently, or was she previously on. Please choose from options below:			
	Isoniazid Preventive Therapy (IPT)		Isoniazid	
	Rifampicin		Pyrazinamide	
	Ethambutol		Other	
4.8	If other, please specify:			

	Section 5	
	Previous pregnancies	
5.1	Number of previous pregnancies (Including miscarriage & TOP):	
For each previous pregnancy, complete a " Previous pregnancy outcome form".		

Previous pregnancy outcome form

	Section 6: Pregnancy			
6.1	Pregnancy number:	☐ of ☐		
6.2	Year of delivery/miscarriage/TOP:			
6.3	HIV-status during pregnancy, please choose from options below: Positive: ☐ Negative: ☐ Unknown: ☐			
6.4	Did she receive ART during this pregnancy:	Yes: ☐ No: ☐		
6.5	Specify ART treatment, please choose from options below:			
	Same as current treatment	☐	AZT + 3TC + EFV	☐
	FDC (TDF + FTC/3TC +EFV)	☐	LPV/RTV or any other Protease Inhibitor based ART	☐
	AZT	☐	Other	☐
	TDF+ FTC+ NVP	☐	If other, specify:	
6.6	Was she diagnosed with TB during this pregnancy:	Yes: ☐ No: ☐		
6.7	Did she receive TB treatment during pregnancy:	Yes: ☐ No: ☐		
6.8	What TB treatment did she receive, please choose from options below:			
	Isoniazid Preventive Therapy (IPT)	☐	Isoniazid	☐
	Rifampicin	☐	Pyrazinamide	☐
	Ethambutol	☐	Other	☐
6.9	Comorbidities and problems during pregnancy or delivery:	Yes : ☐ No: ☐		
6.10	If yes please, specify:			
6.11	Mode of delivery:	Vaginal: ☐ Caesarean section: ☐		
6.12	Gestational age at birth, enter months *or* weeks and days:	Months: ☐		
		Weeks: ☐ Days: ☐		

6.13	How was gestational age determined:		LNMP: ☐ Early U/S (<14wks) ☐ Late U/S (>14wks) ☐ New-born assessment ☐	
6.14	Birth weight (g)		☐ Unknown: ☐	
6.15	Pregnancy outcome, please choose from options below:			
	Live births	☐	Stillbirth ($\geq 24^{+0}$ weeks)	☐
	Miscarriage ($< 24^{+0}$)	☐	Termination of pregnancy	☐
	Ectopic pregnancy	☐		☐
6.16	Was the newborn admitted to high care or ICU after delivery:		Yes: ☐ No: ☐	
6.17	If yes, for how many days			
6.18	Is the child still living:		Yes: ☐ No: ☐	
6.19	If no, at what age did child pass away, enter years and months *or* weeks and days:		Year: Y Y Month: M M Weeks: W W Days: D D	
6.20	HIV status of child, please choose from options below: Positive: ☐ Negative: ☐ Unknown: ☐			
6.21	If child is positive, is the child receiving ART treatment?		Yes: ☐ No: ☐	

Appendix 5.4

Oxford Tropical Research Ethics Committee

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Dr Joris Hemelaar
Nuffield Department of Obstetrics & Gynaecology
The Women's Centre
John Radcliffe Hospital
Oxford University

08 November 2013

Dear Dr Hemelaar

Full Title of Study: The analysis of immunological mechanisms underlying preterm delivery and intrauterine growth restriction in HIV-positive and HIV-negative women in South Africa.

OXTREC Reference: 1008-13

Thank you for your letters of the 28 October 2013 and of the 06 November 2013, requesting an amendment to the revised document(s):

Documentation:	Version:	Date:
Protocol	V5.0	06/11/13
PIS	V5.0	06/11/13
SHAPOSSA questionnaire	V1.0	06/11/13

I am pleased to be able to take the Chairman's action and approve this extension.

Yours sincerely,

pp J Gerencser

Dr Mary Warrell
OXTREC Chairman

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Appendix 5.5

University
of the Witwatersrand,
Johannesburg

Human Research Ethics Committee (Medical)
(formerly Committee for Research on Human Subjects (Medical))
Secretariat: Research Office, Room SH10005, 10th floor, Senate House • Telephone: +27 11 717-1234 • Fax: +27 11 339-5708
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15 January 2014

Dr Opope O Wedi
Principal Investigator
Maternal and Immunology Study
Nuffield Department of
Obstetrics & Gynaecology
University of Oxford

Sent by email to: opope.wedi@st-hughs.ox.ac.uk

Dear Dr Wedi

RE: Protocol M130134: "The Analysis of Immunological Mechanisms Underlying Pre-term Delivery and Intra-Uterine Growth Restriction in HIV-Positive and HIV-Negative Women in South Africa
Protocol Amendment

This letter serves to confirm that the Chairman of the Human Research Ethics Committee (Medical) has reviewed and approved the following amendments on the abovementioned protocol as detailed in your letters dated 6th October 2013 and 10 December 2013:

- Protocol Version 5, 6th November 2013
- Participant Information and Consent Form Version 5, 6th November 2013
- SHAPOSSA Questionnaire Version 2, 09 December 2013

Thank you for keeping us informed and updated.

Yours sincerely

Anisa Keshav
Administrator
Human Research Ethics Committee (Medical)

Appendix 5.6

Appendix: Perinatal outcomes

Variable	Total (N=632)	HIV-negative (N = 403)	HIV-positive (N = 229)	P-value
Perinatal outcomes				
Very low birthweight, n (%)	22 (3.9%)	14 (3.9%)	8 (3.8%)	0.962
Term birth with low birthweight, n (%)	38 (6.7%)	23 (6.4%)	15 (7.1%)	0.728
Preterm birth with low birthweight, n (%)	77 (13.5%)	44 (12.2%)	33 (15.7%)	0.239
Spontaneous preterm birth, n (%)	91 (14.4%)	56 (13.9%)	35 (15.3%)	0.642
Very preterm birth, n (%)	66 (10.6%)	44 (11.1%)	22 (9.7%)	0.594
Spontaneous very preterm birth, n (%)	42 (6.7%)	26 (6.6%)	16 (7.1%)	0.811
Very small for gestational age, n (%)	24 (4.2%)	13 (3.6%)	11 (5.3%)	0.348

a = Wilcoxon rank-sum (Mann-Whitney) test

Gender = 616

Preterm birth = 632

Very preterm birth = 625

Preterm low birthweight = 570

Low birthweight = 570

Small for gestational age = 458

Very small for gestational age = 458

* = statistically significant, P < 0.05; ** P<0.01; ***P<0.001

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