

## **PROTOCOL**

# **HIV INCIDENCE RATES, SOCIO-BEHAVIOURAL AND BIOLOGICAL HIV RISK FACTORS, HIV TRANSMISSION RATES and ACCEPTABILITY OF PrEP DURING PREGNANCY AND/OR POST-NATALLY IN KWAZULU NATAL**

**Version 1.1, dated 3 March 2017**

**D Moodley<sup>1,2</sup>, Q Abdool Karim<sup>2</sup>, K Naidoo<sup>3</sup>, L Pillay<sup>3</sup>, N Samsunder<sup>2</sup>, N Yende<sup>2</sup>**

### **INSTITUTIONS:**

- 1. Women's Health and HIV Research Unit, School of Clinical Medicine, University of KwaZulu-Natal**
- 2. Centre for the AIDS Programme of Research in South Africa (CAPRISA)**
- 3. Paediatrics and Child Health, School of Clinical Medicine, University of KwaZulu-Natal**

## **SYNOPSIS**

Maternal HIV acquisition during pregnancy and breastfeeding is known to occur. In early studies (2005-2012) in South Africa, HIV seroconversion rates among pregnant and breastfeeding women ranged from 1.3% to 3.3%. The current comprehensive HIV prevention strategy comprises proven biomedical and behavioural interventions to protect HIV negative women from acquiring HIV. This includes risk reduction counselling, condom promotion, and screening and treatment of STIs. Use of tenofovir in combination with emtricitabine (PrEP) (a nucleotide reverse transcriptase inhibitor) by HIV uninfected persons is now included in combination with safer sex practices as pre-exposure prophylaxis (PrEP) to reduce the risk of sexually acquired HIV-1 in adults.

Notwithstanding the WHO recommendation of PrEP use in all populations at high risk of acquiring HIV, and a systematic review of the safety of PrEP in pregnant and lactating women (Mofenson et al, 2016 in press), there is concern by the South African Department of Health and the Medicines Control Council about its use for pregnant and breastfeeding women due to limited safety data in this population that remains excluded from prevention science research. Demonstration projects are planned to determine the safety of use of Truvada as PrEP in HIV negative pregnant and breastfeeding women. However, even if Truvada is determined to be safe for pregnant and breastfeeding women, PrEP would only be available for populations at substantial risk as defined by an incidence of HIV infection that is sufficiently high (>3 per 100py) to make the availability of PrEP potentially cost-effective. With limited resources, financial constraints and competing demands within an overburdened health system, governments may choose to target populations in whom one would see the greatest impact. The challenge lies in the lack of accurate epidemiological data that could either dictate the need for immediate resource allocation for optimal implementation of a combination HIV prevention strategy that includes PrEP or prioritising and strengthening other components of the combination prevention package without PrEP. Although current PMTCT programs promote repeat HIV testing during pregnancy and postnatally to identify women who seroconvert and require immediate antiretroviral treatment, data collected by the District Health Information System are inadequate to determine HIV incidence and accurately identify high risk antenatal and breastfeeding populations.

### **Aim:**

To determine the incidence rate of HIV infections among women during pregnancy and/or postnatally, risk factors for HIV acquisition, rate of mother-to-child transmission of HIV during seroconversion and acceptability of PrEP in KwaZulu-Natal.

**Objectives:**

- To determine the incidence rate of HIV during the index pregnancy and postnatally as defined by a negative HIV rapid test at an initial visit and a positive result at a subsequent visit.
- To determine the association between socio-behavioural and biological characteristics including sexually transmitted infections in pregnancy and post-delivery HIV acquisition.
- To determine the incidence rate of mother-to-child transmission of HIV among a cohort of antenatal attendees as an outcome of seroconversion during the index pregnancy and breastfeeding.
- To describe clinical outcomes of children born to women who have seroconverted during pregnancy or during breastfeeding.
- To determine the acceptability of PrEP use during pregnancy and/or post-natally
- To compare the incidence rate generated prospectively with statistically computed incidence rates using routinely available cross-sectional data from this setting

**Study Setting and Design:**

This prospective cohort study will be conducted in Umlazi, an urban township in KwaZulu-Natal with an antenatal HIV prevalence of 36%. The study will be conducted at the antenatal and infant immunisation departments at each of 3 primary health clinics, Section D, Section H and Section U21 clinics.

**Study Population:**

Pregnant women, who register for antenatal care at the designated primary health clinics and test negative for HIV at their first visit will be screened for study participation. An average of 45 HIV negative pregnant women register at each of the clinics per month.

**Sample Size:**

An average of 45 HIV negative pregnant women are expected to be enrolled at each of the 3 clinics per month. We assume an incidence rate of 5% during pregnancy and post-delivery. Each woman will be followed for a period of 15 months (6 months during pregnancy and 9 months post-delivery).

Sample size	Precision from 5%	Expected women-years of follow-up	Expected number of HIV infections
1825	1%	2281	114
1000	1.4%	1250	63
811	1.5%	1014	51
457	2%	571	28

If we assume that age of the woman will be a significant predictor of HIV infection, with younger women showing 50% higher risk of HIV infection compared to older women. A sample size >1000 will suffice.

**Study Period and Time points:**

The study will be conducted over a period of 2 years. Antenatal clinic attendees who test negative for HIV at their first antenatal visit will be enrolled. Study participants will be followed longitudinally over a period of 15 months (up to 6 months during pregnancy and 9 months post-delivery). As per the 2015 Maternity Care Guidelines, women will be retested for HIV during pregnancy between 28 and 34 weeks gestation and post-delivery at 3, 6 and 9 months in order to determine the incidence of HIV infection during pregnancy and breastfeeding.

## 1. BACKGROUND

Between 2009 and 2015, countries like Burundi, Ghana, Malawi, South Africa and United Republic of Tanzania reported a 20-46% reduction in the number of new infections among women, however an estimated 750 000 women of child-bearing age are newly infected with HIV per year (UNAIDS, 2016). In a meta-analysis of 19 cohort studies in Sub-Saharan Africa spanning 22 803 person years, the incidence in pregnancy and postpartum was 4.7 (95%CI 3.3-6.1)/100 women years and 2.9 (95%CI 1.8-4.0)/100 women years respectively (Drake 2014). In a population-based evaluation (2011-2012), Dinh et al confirmed the high HIV seroconversion rates among pregnant women attending public health facilities in South Africa; 3.3% (95% CI: 2.8%-3.8%) of women seroconverted in pregnancy (Dinh 2015). Evidence for HIV acquisition during pregnancy and post-delivery is also supported by a household survey conducted in Kenya, South Africa and Malawi. HIV incidence rates during pregnancy and breastfeeding among women who were recently pregnant were 3.8/100py, 3.2/100py and 0.9/100py respectively (Huerga 2016). Among women who tested positive during the survey, 37.5% reportedly acquired HIV during their recent pregnancy or during breastfeeding. Studies comparing HIV incidence during pregnancy or postpartum to non-pregnant women concluded that the risk of HIV acquisition (HR 1.3; 95%CI 0.5-2.1) and (HR 1.1; 95%CI 0.6-1.6) was not significantly different ( $p=0.92$ ) (Drake 2014). From this meta-analysis of 19 cohort studies in Sub Saharan Africa one can conclude that that compared to other high risk populations, pregnant and lactating women are at similar risk or perhaps higher (3.8; 95%CI 3.0-4.6) per 100py than Sex Workers (2.7/100py) and discordant couples (2-3.6/100py) (Drake et al). This is not surprising given that unprotected sex in high burden HIV settings could result in both pregnancy and HIV acquisition.

### RISK FACTORS FOR HIV ACQUISITION IN PREGNANCY AND POST-DELIVERY

A combination of socio-behavioural and biological characteristics makes women in general more susceptible to HIV infection. In recent intervention studies in search of an effective female controlled HIV prevention method, younger women (<24 years), older partners (>5 year age difference), unprotected sex and presence for any of the other sexually transmitted infections were consistently and significantly associated with risk for HIV infection (Ramjee 2016; McKinnon 2016).

#### Behavioural Risk for HIV Acquisition in Pregnancy and Post-delivery

Inconsistent or lack of condom use or lack of hormonal contraceptive methods is commonly associated with the high rate of unintended pregnancies and HIV incidence among young women in sub-Saharan Africa (Ramjee 2016). As in non-pregnant women, older partners and unprotected sex are also common among HIV infected pregnant women (Kharsany 2016). In the KwaZulu-Natal HIV Incidence study involving 2835 pregnant women, the majority (63%) who tested HIV negative at their first antenatal visit continued to engage in unprotected sex during pregnancy and post-delivery, with

abstinence during pregnancy reported among 17% of the women (Chetty 2012). A behavioural intervention study in a South African urban community reported similar high risk sexual behaviour among postpartum women (Maman 2014). Inconsistent condom use was reported for 45% of the sexually active women at the 14 week post-delivery visit, increasing to 59% at the 9 months visit post-delivery. Furthermore, inconsistent condom use was more common among HIV uninfected women at 14 weeks and at 9 months. It is also becoming evident that women are more vulnerable in pregnancy not only as a result of their behaviour but the increased promiscuous behaviour of the sexual partner during pregnancy and post-delivery. In Malawi, where HIV incidence in pregnancy is 4.0/100 person years (95% CI 2.2-7.2), sexual activity decreased in late pregnancy and the early postpartum period (62%) and women noticed an increase in multiple partner relationships by their partner their late pregnancy and postpartum periods (Keating 2012). In south-west Nigeria and in other African countries, traditional norms impose abstinence during pregnancy and in the postpartum period (Moodley 2009; Cleland, 1999). In a community-based study in Nigeria, while it is common for men to have extramarital relationships (42.1%), the chances of extramarital relationships in the abstinence period postpartum was higher than during pregnancy (42.1% vs 48%  $p < 0.001$ ) (Lawoyin 2002). Male partner multiple sexual partners, alcohol consumption and intimate partner violence are less common but remain important risk factors for HIV acquisition for women during pregnancy (Peltzer 2013).

#### Biological Risk for HIV Acquisition in Pregnancy and Post-delivery

The increased level of progesterone during pregnancy is hypothesised to alter genital mucosal structure and local inflammatory responses, both increasing HIV susceptibility during pregnancy (Morrison 2014; Sheffield 2009). Moreover, the prevalence of other sexually transmitted infections in pregnancy is an indicator for unprotected sex during pregnancy and if untreated could increase risk of HIV acquisition in pregnancy and postpartum through catalysing localised immune responses and disruptive changes to the genital mucosal integrity (Moodley 2015; Morrison 2014). In a cohort study in western Kenya, 1304 HIV seronegative pregnant women were studied until 9 months postpartum (Kinuthia 2015). The HIV incidence rate was 2.31/100 person-years (95% CI 0.71–4.10) and incident HIV was associated with syphilis (HR 9.18, 95% CI 2.15–39.3), chlamydia (HR 4.49, 95% CI 1.34–15.0), bacterial vaginosis (HR 2.91, 95% CI 1.25–6.76), and yeast infection (HR 3.46, 95% CI 1.46–8.19) in addition to behavioural risk factors.

Two recent studies in KwaZulu-Natal provide more persuasive evidence for why women in sub-Saharan Africa are at higher risk for HIV infection than women living in the USA. Women whose vaginal microbiome included more than 1% of *P. Bivia* had the highest levels of genital inflammation and the highest likelihood of becoming infected with HIV. These studies further elaborated that alterations to the healthy Lactobacilli dominated vaginal microbiome by other sexually transmitted bacterial pathogens such as *Gardnerella* and *Prevotella* induce a local inflammatory response that enhanced HIV acquisition.

Although, such studies have not been conducted in pregnant and lactating women, disruptions in the vaginal microbiota during pregnancy and post-delivery have been documented notably with adverse pregnancy outcomes (McIntyre 2015; Romero 2014; DiGiulio 2015).

## HIV PREVENTION STRATEGY FOR PREGNANT AND POSTNATAL WOMEN

The current comprehensive HIV prevention strategy comprises proven biomedical and behavioural interventions that is designed to protect HIV negative women from acquiring HIV. This includes risk reduction counselling, condom promotion, and screening and treatment of STIs. The use of Tenofovir in combination with emtricitabine (a nucleotide reverse transcriptase inhibitor) by HIV uninfected persons (PrEP) is now included as combination prevention to reduce the risk of sexually acquired HIV-1 in adults at high risk (WHO 2015). PrEP is recommended for all populations at substantial risk of HIV infection. Substantial risk is defined as an HIV incidence rate  $>3\%$ /per annum. Cost-benefit analysis studies of PrEP demonstrate the incremental cost-effectiveness in populations where the incidence rates is  $\geq 3\%$ .

Limited resources, financial constraints and competing demands within an overburdened health system preclude new evidence based interventions to be automatically provided to the general population. Instead governments may choose to target populations in whom one would see the greatest impact. The challenge lies in the lack of accurate epidemiological data that could either dictate the need for immediate resource allocation for optimal implementation of a combination HIV prevention strategy that includes PrEP or prioritising and strengthening other components of the combination prevention package without PrEP.

Although current PMTCT programs promote repeat HIV testing during pregnancy and postnatally to identify women who seroconvert and require immediate antiretroviral treatment, data collected by the District Health Information System are inadequate to determine HIV incidence and accurately identify high risk antenatal populations.

### **2. AIM:**

To measure the incidence rate of HIV infections among women during pregnancy and postnatally, determine risk factors for HIV acquisition and the rate of mother-to-child transmission of HIV during seroconversion.

### **3. OBJECTIVES:**

- To determine the incidence rate of HIV during the index pregnancy and postnatally as defined by a negative HIV rapid test at an initial visit and a positive result at a subsequent visit.
- To determine the association between socio-behavioural and biological characteristics including sexually transmitted infections in pregnancy and post-delivery HIV acquisition.
- To determine the incidence rate of mother-to-child transmission of HIV among a cohort of antenatal attendees as an outcome of seroconversion during the index pregnancy and breastfeeding.
- To describe clinical outcomes of children born to women who have seroconverted during pregnancy or during breastfeeding.
- To determine the acceptability of PrEP use during pregnancy and/or post-natally
- To compare the incidence rate generated prospectively with statistically computed incidence rates using routinely available cross-sectional data from this setting

### **4. STUDY SETTING AND DESIGN**

This prospective cohort study will be undertaken in Umlazi, an urban township in KwaZulu-Natal that has an antenatal HIV prevalence of 36%. The study will be conducted at the antenatal and infant immunisation departments at each of 3 primary health clinics (Section D, Section H and Section U21). An average of 45 new HIV negative pregnant women utilize each of these clinics per month.

### **5. STANDARD OF CARE HIV COUNSELLING AND TESTING (Maternity Care Guidelines 2015)**

- All women attending antenatal care should be given routine information about HIV testing and the PMTCT programme, with a group information session, followed by individual counselling for women who have never tested, or have previously tested negative.
- Verbal consent must be obtained before testing. A woman may refuse an HIV test (opt-out).
- A rapid test will be performed on a finger prick sample of blood. If the test is positive, a second rapid HIV test using a test kit from a different supplier will be performed on a second finger prick sample. If both tests are positive, the woman is confirmed HIV positive.
- If the first rapid test is positive and the second rapid test is negative, then a laboratory ELISA test performed on a venepuncture blood sample. The healthcare

provider should explain the reason for the laboratory test, and the woman should be asked to return for the ELISA results. Results should be obtained within a week.

- Post-test counselling should be offered to both HIV positive and HIV negative women.
- Women who opt-out of HIV testing should have individual 'post refusal' counselling, and HIV testing offered at each antenatal visit.

### **Women with a confirmed positive result**

- Should be clinically staged, and have blood taken for CD4 count, and serum creatinine.
- All women should be started on HAART (Fixed Dose Combination TDF/FTC/EFV).
- Women are to return after 7 days for results.

### **Women who test negative**

- All women who test HIV negative are to be offered repeat HIV testing after 3 months, and/or around 32 weeks gestation, and every 3 months while breastfeeding.

## **5. STUDY METHODOLOGY**

### **5.1. Study Period**

The study will be conducted over a period of 2 years. Antenatal clinic attendees who test negative for HIV at their first antenatal visit will be enrolled. Study participants will be followed longitudinally over a period of 18 months (6 months during pregnancy and 12 months post-delivery). As per the 2015 Maternity Care Guidelines, women will be retested for HIV during pregnancy between 28 and 34 weeks gestation and post-delivery at 3, 6 and 9 months in order to determine the incidence rate of HIV infection during pregnancy and/or breastfeeding.

## 5.2 Study Participants

### Sample Size

An average of 45 HIV negative pregnant women are expected to be enrolled at each of the 3 clinics per month. We assume an incidence rate of 5% during pregnancy and post-delivery. Each woman will be followed for a period of 15 months.

Sample size	Precision from 5%	Expected women-years of follow-up	Expected number of HIV infections
1825	1%	2281	114
1000	1.4%	1250	63
811	1.5%	1014	51
457	2%	571	28

If we assume that age of the woman will be a significant predictor of HIV infection, with younger women showing 50% higher risk of HIV infection compared to older women. A sample size >1000 will suffice.

### Inclusion Criteria:

- Pregnant
- Registered for antenatal care at Study Facility
- HIV test seronegative
- Willing to provide informed consent

### Exclusion Criteria:

- HIV test seropositive
- In labour or with obstetric complications that require referral to regional hospital
- Does not intend continuing routine antenatal care at any of the three identified facilities
- Does not intend to reside in the Umlazi area for the period of the study

## 5.3 Study Procedures

### Screening and Study Informed Consent:

All first visit antenatal attendees who have registered for antenatal care and received standard HIV Counselling and Testing by Department of Health (DOH) staff will be screened for eligibility (inclusion and exclusion criteria – Appendix 1). Women meeting eligibility criteria will be asked to provide written informed consent for participation in this study (Appendix 2). Consent for storage of biological specimens will be included in the main study consent. Women can choose not have their specimens stored for further research.

### Study Registration:

Participants who have provided study consent will be registered in the study enrolment system. A unique Study Identification Number (SID) will be issued to each participant and documented in an enrolment log. An appointment card with a SID and participant's South African ID number will be used to verify data and specimen collection for each participant at each visit.

### Administration of Demographic and Sexual-Behavioural Questionnaires:

A Research Assistant (behavioural science graduate) will complete both demographic (Appendix 3) and sexual-behavioural questionnaires (Appendix 4) at entry. The sexual-behavioural questionnaire will also be administered at subsequent visits.

### Clinical Screening for STIs, Rapid Syphilis Test and Rapid HIV-1 Test:

The Research Nurse (RN) will perform all study related clinical investigations. Participants will be screened for sexually transmitted infections as per the National Guidelines for STI Management and treated accordingly. The Research Nurse will also perform the repeat syphilis test in pregnancy, and at the 6 and 9 months postpartum visits. She will perform the Rapid HIV test (by a finger-prick) as per Maternity Care Guidelines and in addition collect 4ml of blood (EDTA tube-plasma) for a full blood count and a CD4 count. An additional 5ml blood (SST tube-serum) for laboratory testing of STIs viz. syphilis and Hepatitis B. For the purpose of storage, 8ml of blood (2 EDTA tubes) will also be collected. The latter sample will be used for further laboratory confirmation of HIV and syphilis results. The blood specimen will also be used to prepare a card of dry blood spots and then subsequently processed for storage as aliquots of plasma and cell pellets.

### Repeat HIV-1 Test:

The Rapid Anti-HIV (1&2) Test Card (Advanced Quality, InTec PRODUCTS, INC.USA) will be used as a screening test for HIV at the study visit. Women who test positive will have a 2<sup>nd</sup> POC test (TRILINE HIV-1/2 Rapid Test, ABON Biopharm INC, China) to confirm infection. Blood sample sent to the laboratory for storage will be used to confirm HIV infection if POC test results are positive. All previously stored specimens for women who seroconvert will be tested for HIV by DNA-PCR.

### Laboratory Testing for Vaginal Infections and Sexually Transmitted infections:

Three vaginal swabs will be collected at enrolment, subsequent study antenatal visit, and at 3 and 9 months postnatally. The swabs will be transported to the laboratory for further processing and storage. The first swab will be used to make a slide smear to determine the Nugent Score for the diagnosis of BV. The remaining 2 swabs will be stored for further investigations. Depending on the availability of funding, stored specimens will be tested for *C trachomata*, *N gonorrhoea*, *Prevotella bivia*; *Gardnerella* and *T vaginalis* at the end of the study period.

### Participant Clinical Care at Primary Health Clinic:

Depending on the HIV status, women will be referred to a relevant health care worker in the PHC for appropriate care. Briefly, all women will receive post-test counselling, receive immediate antiretroviral treatment if HIV positive, receive ongoing antenatal care if

pregnant, receive risk reduction counselling if HIV negative, and receive appropriate infant feeding advice according to her HIV status.

### Schedule of Events for Women Enrolled in the HIV Incidence Study

*Note: Study visits coincide with routine Antenatal and Child Immunisation Visits*

	ENTRY	Wk 28-34	LABOUR/ DELIVERY	Wk 14	Wk 26	WK 38 (EXIT VISIT)
Informed Consent	X					
Study Registration	X					
Demographic Questionnaire	X					
Sexual-Behavioural Questionnaire	X	X		X	X	X
Clinical screening for STIs	X	X		X	X	X
Full Blood Count (test for anaemia)	4ml			4ml		4ml
<b>CD4 count (Baseline and Exit value)</b>						
Syphilis, HepB* (Lab tests)	5ml*	5ml			5ml	5ml*
Rapid Syphilis Test	X	X			X	X
HIV-1 Rapid Test (finger prick)		X		X	X	X
<b>Vaginal pH</b>	X	X			X	X
<b>Vaginal Swab (3)</b>	X	X			X	X
Breast Milk Sample (If HIV infected)				20ml	20ml	20ml
HIV test for baby (if mother is HIV infected)				X	X	X
Stored EDTA Plasma, DBS, Cell Pellet	8ml	8ml		8ml	8ml	8ml
<b>TOTAL BLOOD VOLUME</b>	<b>17ml</b>	<b>13ml</b>		<b>12ml</b>	<b>13ml</b>	<b>17ml</b>

### 5.3 Data Management

*Data Collection:*

The following data will be collected in the antenatal clinic:

- Date of This Visit
- South African Identity Number (Baseline only)
- Age (Baseline only)
- Gravidity (Baseline only)
- Gestational Age
- Date of Last HIV Test:
- HIV Result at the Last Test
- Place of Last Test
- Clinical Symptoms of STI
- Treatment of STI
- Syphilis Result
- HIV Test Result
- Blood Sample Collected
- Vaginal swab done

The following data will be collected in the postnatal clinic (child immunisation clinic)

- Date of This Visit
- Pregnancy Outcome
- Date of Last HIV Test:
- HIV Result at the Last Test
- Place of Last Test
- Clinical Symptoms of STI
- Treatment of STI
- Syphilis Result
- HIV Test Result
- Blood Sample Collected
- Vaginal Swab Done
- Infant Feeding Practice (Breastfeeding)
- DNA PCR Test for HIV exposed infants (mothers with incident HIV infections)
- Clinical Outcomes of HIV exposed infants (mothers with incident HIV infections)

**STUDY PLAN**

Enrolment will occur over a period of 10 months (Jan-Oct 2017), and the last pregnant participant enrolled in the 10<sup>th</sup> month is expected to deliver sometime in the 13<sup>th</sup> month (Jan 2018). Follow-up will be completed in the 22<sup>nd</sup> month (Oct 2018). Overall, the study is expected to be completed within 2 years.

## REFERENCES

- Chetty V, Moodley D, Chaturgoon A. Evaluation of a 4th generation rapid HIV test for earlier and reliable detection of HIV infection in pregnancy. *J Clin Virol*. 2012 Jun;54(2):180-4. doi: 10.1016/j.jcv.2012.02.021. Epub 2012 Mar 22
- Cleland JG, Ali MM, Capo-Chichi V (1999) Post-partum sexual abstinence in West Africa: implications for AIDS-control and family planning programmes.
- DiGiulio DB, Callahan BJ, McMurdie PJ, et al. Temporal and spatial variation of the human microbiota during pregnancy. *Proc Natl Acad Sci U S A*. 2015 Sep 1;112(35):11060-5. doi: 10.1073/pnas.1502875112. Epub 2015 Aug 17.
- Dinh T-H, Delaney KP, Goga A, Jackson D, Lombard C, Woldesenbet S, et al. Impact of Maternal HIV Seroconversion during Pregnancy on Early Mother to Child Transmission of HIV (MTCT) Measured at 4-8 Weeks Postpartum in South Africa. 2011-2012: A National Population-Based Evaluation. *PLoS ONE* 2015; 10(5): e0125525. doi:10.1371/journal.pone.0125525
- Drake AL, Wagner A, Richardson B, John-Stewart G. Incident HIV during pregnancy and postpartum and risk of mother-to-child HIV transmission: a systematic review and meta-analysis. *PLoS Med*. 2014; 11:e1001608. doi: 10.1371/journal.pmed.1001608 PMID: 24586123
- Huerga H, Van Cutsem G, Ben Farhat J, et al. Who needs to be targeted for HIV testing and treatment in KwaZulu-Natal? Results from a population-based survey. *J Acquir Immune Defic Syndr*. 2016 May 26. [Epub ahead of print] PMID: 27243903
- Keating MA, Hamela G, Miller WC, Moses A, Hoffman IF, Hosseinipour MC. High HIV incidence and sexual behavior change among pregnant women in Lilongwe, Malawi: implications for the risk of HIV acquisition. *PLoS One*. 2012;7(6):e39109. doi: 10.1371/journal.pone.0039109. Epub 2012 Jun 29.
- Kharsany AB, Frohlich JA, Yende-Zuma N, et al. Trends in HIV Prevalence in Pregnant Women in Rural South Africa. *J Acquir Immune Defic Syndr*. 2015 Nov 1;70(3):289-95. doi: 10.1097/QAI.0000000000000761
- Kinuthia J, Drake AL, Matemo D, Richardson BA, Zeh C, Osborn L, Overbaugh J, McClelland RS, John-Stewart G. HIV acquisition during pregnancy and postpartum is associated with genital infections and partnership characteristics. *AIDS*. 2015 Sep 24;29(15):2025-33
- Lawoyin TO, Larsen U. Male sexual behaviour during wife's pregnancy and postpartum abstinence period in Oyo State, Nigeria. *J Biosoc Sci* 2002; 34: 51–63.
- MacIntyre DA, Chandiramani M, Lee YS, et al. The vaginal microbiome during pregnancy and the postpartum period in a European population. *Sci Rep*. 2015 Mar 11;5:8988. doi: 10.1038/srep08988.

Maman S, Moodley D, McNaughton-Reyes HL, et al. Efficacy of enhanced HIV counseling for risk reduction during pregnancy and in the postpartum period: a randomized controlled trial. *PLoS One*. 2014 May 13;9(5):e97092. doi: 10.1371/journal.pone.0097092. eCollection 2014

McKinnon LR, Karim QA. Factors Driving the HIV Epidemic in Southern Africa. *Curr HIV/AIDS Rep*. 2016 Jun;13(3):158-69.

Moodley D, Esterhuizen TM, Pather T, Chetty V, Ngaleka L (2009) High HIV incidence during pregnancy: compelling reason for repeat HIV testing. *AIDS* 23: 1255–1259.

Moodley D, Moodley P, Sebitloane M, Soowamber D, McNaughton-Reyes H, Groves A, Maman S. High Prevalence and Incidence of Asymptomatic Sexually Transmitted Infections during Pregnancy and Postdelivery in KwaZulu Natal, South Africa. *Sexually Transmitted Diseases*. 2015 January; 42(1):43-47.DOI:

Morrison C, Fichorova RN, Mauck C, Chen PL, Kwok C, Chipato T, Salata R, Doncel GF. Cervical inflammation and immunity associated with hormonal contraception, pregnancy, and HIV-1 seroconversion. *J Acquir Immune Defic Syndr*. 2014 Jun 1;66(2):109-17. doi: 10.1097/QAI.

National Department of Health, South Africa 2015 Guidelines for Maternity Care in South Africa A manual for clinics, community health centres and district hospitals. 2015.

Peltzer K, Mlambo G. Sexual HIV risk behaviour and associated factors among pregnant women in Mpumalanga, South Africa. *BMC Pregnancy Childbirth*. 2013 Mar 4;13:57. doi: 10.1186/1471-2393-13-57.

Ramjee G, Moonsamy S, Abbai NS, Wand H. Individual and Population Level Impact of Key HIV Risk Factors on HIV Incidence Rates in Durban, South Africa. *PLoS One*. 2016 Apr 22;11(4):e0153969.

Romero R, Hassan SS, Gajer P, Tarca AL, Fadrosh DW, Nikita L, Galuppi M, Lamont RF, Chaemsaitong P, Miranda J, Chaiworapongsa T, Ravel J. The composition and stability of the vaginal microbiota of normal pregnant women is different from that of non-pregnant women. *Microbiome*. 2014 Feb 3;2(1):4. doi: 10.1186/2049-2618-2-4

Sheffield JS, Wendel GD Jr, McIntire DD, Norgard MV. The effect of progesterone levels and pregnancy on HIV-1 coreceptor expression. *Reprod Sci*. 2009 Jan;16(1):20-31. doi: 10.1177/19

UNAIDS. 2016. The Incredible Journey of the Global Plan towards the elimination of new HIV infections among children by 2015 and keeping their mothers alive

World Health Organisation; 2015. Guideline on when to start antiretroviral therapy and on pre-exposure prophylaxis for HIV. Geneva.