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Factors associated with pregnancy in women taking part in a phase III microbocide trial in Johannesburg

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A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfillment of the requirements for the degree of Master of Science in Medicine in the field of Epidemiology and Biostatistics

09 May 2011

DECLARATION

I, Sibongile Walaza (8460842) declare that this research report is my own. It is being submitted for the degree of Master of Science in Medicine in the field of Epidemiology and Biostatistics at the University of the Witwatersrand, Johannesburg. This report has not been submitted before for any other degree or examination at this or any other University.

Signature



Date 10 May 2011

To my children, Vuyo and Sazi, this would not have been possible without the sacrifices that you have had to make in terms of competing demands on my time. Thank you for your patience and understanding.

ETHICS COMMITEE APPROVAL

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
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CLEARANCE CERTIFICATE	**M090461**
PROJECT	Factors Associated with Pregnancy in Women Taking Part in a Phase III Microbicide Clinical Trial in Johannesburg
INVESTIGATORS	Dr Sibongile Walaza.
DEPARTMENT	School of Public Health
DATE CONSIDERED	09.04.29
DECISION OF THE COMMITTEE*	Approved uncontionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 09.04.29 **CHAIRPERSON** .....
(Professor P E Cleaton Jones)

*Guidelines for written 'informed consent' attached where applicable

cc: Supervisor : Dr J Moyes

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

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ABSTRACT

Introduction

This was a secondary data analysis of a prospective cohort of women enrolled in a phase III microbicide trial between October 2005 and August 2008. The study aimed to assess the pregnancy incidence rates and factors associated with pregnancy in women using barrier method and hormonal contraception, enrolled in the trial.

Methods

A total of 2508 participants were enrolled in the trial and followed up for up to 12 months. Of these 2437 were included in the pregnancy incidence analysis and 2171 participants were included in the multivariate analysis. Data on the main exposure, contraception, were collected by structured interview. The main outcome of interest was pregnancy, which was measured by detection of human chorionic gonadotrophin in urine using Quick Vue® test and confirmed by laboratory based testing. The incidence rate of pregnancy was calculated as number of pregnancies per 100 women years of follow up. Kaplan Meier Survival analysis was used to determine average time to first pregnancy. Univariate and multivariate analyses were conducted using Cox regression models to asses the factors associated with incident pregnancies. Data was analysed using Stata® version 10.

Results

A total of 2248 women years of follow up were recorded. A total of 238 pregnancies occurred resulting in pregnancy incidence of 11 per 100 women-years of follow up (95% CI: 9.32 to 12.02). The incidence of pregnancy increased with time in the study; 98 per

100 women years of follow up (95% CI: 85.09 to 112.35) in the last 3 months compared to 2 per 100 women-years of follow up (95% CI: 0.94 to 2.92) in the first 3 months of follow up. Older age and hormonal contraception use were significantly associated with a decreased risk of pregnancy. Women 35 years and older were 49% less likely to fall pregnant compared to those who were younger than 25 years, adjusted hazard ratio (AHR) 0.51(95% CI: 0.30 to 0.88, p=0.016). Women who used hormonal contraception had a reduced risk of falling pregnant AHR 0.66(95% CI: 0.46 to 0.94, p=0.02). There was no difference between the two types of hormonal contraception (injectable vs oral) with respect to pregnancy risk.

Conclusion:

The incidence of pregnancy increased with time in the study. Women who used hormonal contraception and who were older were less at risk of pregnancy. There was no significant difference in pregnancy risk by type of hormonal contraception (i.e. oral contraception vs injectable contraception) used.

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GLOSSARY OF ABBREVIATIONS

AHR	Adjusted Hazards Ratio
AOR	Adjusted Odds Ratio
ART	Anti retroviral treatment
BMI	Body Mass Index
CS	Cellulose Sulfate
CTU	Clinical Trials Unit
CI	Confidence interval
CRF	Case record forms
DMPA	Depot medroxyprogesterone acetate
FHI	Family Health International
HIV	Human Immunodeficiency Virus
HR	Hazards Ratio
HPTN	HIV Prevention Trial Network
HSV 2	Herpes Simplex Virus type 2
hCG	Human chorionic gonadotropin
HREC	Human Research Ethics Committee
GCP	Good clinical practice
ICH	International Conference for Harmonisation
MDP	Microbicide Development Programme
MRC	Medical Research Council
MIRA	Methods for Improving Reproductive Health in Africa
OC	Oral contraception
OR	Odd Ratio
PCR	Polymerase Chain Reaction
PrEP	Pre - exposure prophylaxis
RHRU	Reproductive Health and HIV Research Unit
STI	Sexually Transmitted Infection
UK	United Kingdom (UK)

1. INTRODUCTION

1.1. Background

The acquisition of HIV infection is an endpoint in all HIV prevention trials and recruiting people into these trials requires participants who are at risk for HIV infection. Efficacy and effectiveness trials of vaginal microbicides as HIV prevention technologies recruit young women whose risk of HIV infection also puts them at risk for pregnancy[1]. Incident pregnancy in an efficacy trial of an investigational product, that has not undergone safety testing in pregnancy, leads to interruption of the product use and loss to follow up in the trial. It may also have implications for the outcome of the pregnancy. This loss to follow up may require increasing the sample size, which may already be large in these efficacy trials[2]. Information on different methods of contraception and pregnancy counseling is provided to trial participants and some trial sites provide contraception and pregnancy testing is done at regular intervals. Despite this, participants continue to fall pregnant and high pregnancy rates have been reported in some trial cohorts[1,3,4]. Understanding factors associated with pregnancy in HIV prevention trials will assist in developing strategies for pregnancy prevention in the context of HIV prevention trials. This analysis aimed to identify risk factors for pregnancy, in order to help with the targeting of counseling of potential trial participants.

1.2. Literature review: Pregnancy in HIV prevention trials:

Some vaginal microbicide trials have experienced rates as high as 64 pregnancies per 100 person years (range 16 and 64 per 100 woman years)[1]. A number of trials testing

different vaginal microbicide products and other HIV prevention strategies completed recently have reported the following pregnancy rates:

- Some of the lowest pregnancy incidence rates were reported in the Carraguard trial. In the treatment arm 6.6 pregnancies per 100 woman years were reported and 8.2 pregnancies per 100 woman years in the control group[5].
- Three randomised placebo controlled trials, Methods for Improving Reproductive Health in Africa (MIRA) trial[6], HIV Prevention Trial Network (HPTN) 039[7] and HPTN 035[8] reported similar pregnancy incidence rates. The HPTN 039 trial which assessed the effect of acyclovir on HIV -1 acquisition in HSV 2 seropositive women reported pregnancy incidence rates of 13.2 per 100 women years of follow up[7]. Similar pregnancy incidence rates were reported in the HPTN 035 trial of a vaginal gel 11.3 per 100 woman years[8]. The MIRA trial which tested the diaphragm and gel for the prevention of HIV acquisition in Southern African women, reported pregnancy incidence rates of 13.1 % and 13.2 % in the intervention and control groups respectively[6].
- The Cellulose Sulfate (CS) trial, which evaluated the safety and effectiveness of 6% Cellulose Sulfate vaginal gel in preventing vaginal acquisition of HIV, *Neisseria gonorrhoea* and *Chlamydia trachomatis* infection, enrolled 1398 women from 3 African and 2 Indian sites. This trial reported pregnancy incidence rates of 21.8 in the CS group and 23.1 in the placebo group[9]. In a parallel phase III trial of CS in Lagos, Nigeria[10] that enrolled 1644 HIV negative women, the pregnancy incidence was 29 and 28 per 100 woman years in the CS and placebo groups, respectively .

- Another phase III trial evaluating the effectiveness of SAVVY vaginal gel (C31G) in preventing acquisition of HIV infection among women at high risk in Ghana reported much higher pregnancy incidence rates. This trial enrolled 2142 women with mean age of 22.7 years , the pregnancy incidence rates were 42.5 per 100 person years and 43.7 per 100 person years in the SAVVY and placebo groups respectively[11] .
- The highest reported pregnancy incidence rates were in a phase 2 trial conducted by Family Health International (FHI) in three sites (Ghana, Cameroon and Nigeria), evaluating the safety and preliminary effectiveness of daily dose Tenofivir Disoproxil Fumarate as an oral pre - exposure prophylaxis (PrEP) in preventing HIV infection in women . The reported pregnancy incidence was 52 per 100 woman years[12].

The large number of pregnancies in women participating in HIV vaccine trials suggest that, in future, trials looking at high risk women take into account possible pregnancy incidence when sample size is determined[2;13,14]. Even though these trials reported high pregnancy rates, the majority did not report or identify any factors associated with pregnancy.

1.3. Factors associated with pregnancy:

Reid et al[15] analysed the risk factors associated with pregnancy among 1358 HIV negative HSV-2 seropositive women from 3 African countries who had participated in HPTN 039 trial[7]. Oral contraception, injectables and the intra-uterine device (IUD) were associated with a decreased risk of falling pregnant, adjusted hazard ratio (AHR) of 0,

31 (95% CI: 0.21 to 0.46), 0.13 (95% CI: 0.07 to 0.22) and 0.27 (95% CI: 0.08 to 0.87) respectively. Younger age was associated with increased risk of pregnancy[15].

Understanding factors associated with pregnancy may also apply to treatment trials such as HIV treatment trials. A prospective cohort study by Homsy et al[16] analysed factors associated with pregnancy in a cohort of 733 women from rural Uganda, who initiated antiretroviral treatment (ART). The overall pregnancy incidence was 8.2 per 100 woman-years, peaking at 11.7 per 100 women years after ART initiation. Younger age (HR = 2.71 per 10 year decrease, 95% CI: 2.95 to 3.78, p<0.001), having a body mass index (BMI) ≥ 18.5 (HR = 1.09, 95% CI: 1.01 to 1.18, p=0.024) and not using condoms consistently in the last 3 months (HR 1.79, 95% CI: 1.02 to 3.13, p= 0.04) were independently associated with pregnancy[16].

1.4. Pregnancy and safety in clinical trials

The safety of participants in clinical research is of prime importance. Concerns about unknown effects of an investigational agent on a foetus and potential risks to the future reproductive capacity of the female participants necessitate caution when enrolling women into HIV prevention clinical trials[1,2,13] Recently completed and current prevention trials are testing products that have never been tested in pregnancy or have limited data available on safety in pregnancy. Even though active ingredients of most candidates are not systemically absorbed, the foetus / embryo may still be exposed due to passage of product to the uterus via the cervical canal[17].

High pregnancy rates in the trials of these investigational products accentuated the importance of establishing safeguards to protect the pregnant woman and the foetus[1]. Different trials have adopted different methods to optimize safety. For HIV prevention trials, the strategy adopted has been to ensure that the study protocol prevents exposure of pregnant women to drug if clinically important harm from the use of the product during pregnancy cannot be ruled out.

Trials prevent exposure to product by either precluding pregnancy among participants or prohibiting product use during pregnancy[1]. A number of safety and efficacy microbicide trials[9,10,12,18] and other HIV prevention trials[15] required that women stop using the investigational product when they became pregnant and resume product use after pregnancy. Strict implementation of this strategy is not always achievable because pregnancies are not identifiable as soon as they occur[1]. By the time human chorionic gonadotropin (hCG) is detected by most pregnancy tests, it is several weeks after fertilization[19]. Wilcox et al, reported that in about 10% of clinical pregnancies, implantation occurred after the 1st day of the next expected period. If trial protocols suggest testing for pregnancy intermittently it is possible that some pregnancies may remain unrecognized for weeks to months after conception. Thus exposure may be prolonged by participants who miss their follow up visits and continue to use the investigational product for extended periods of time. This strategy will fail to eliminate exposure during early gestation, specifically weeks 2 to 6, a time when the foetus is most vulnerable to teratogenic effects[1].

As a result of these safety concerns, some trials attempted to preclude pregnancy among enrolled participants by excluding participants who were planning to fall pregnant within a certain period[9,10,12,18] and or required that participants use a reliable form of contraception throughout the duration of their participation in the trial.

Recommendations from a meeting hosted by Family Health International (FHI) on pregnancy in microbicide trials held in November 2005 suggested that future vaginal microbicide trials must ensure that women have access to contraception either at the trial site or are referred to other providers. In addition effective methods of contraception such as the IUCD, injectable contraception, implants and in some areas, oral contraception must be easily accessible to participants[2,14]. Implementing this strategy does not guarantee success in preventing pregnancy as providing contraception does not necessarily mean that participants will use these consistently. In addition contraception is not widely used or acceptable in all communities and this could be a barrier to use even if sites do provide contraception.

There are a number of factors that are associated with contraception use and uptake, including increased level of education, being married, employed, high parity, desire for birth spacing and religion[3;20,21,22;23,24,25]. Kibuuka et al[3], described the pattern of contraception use in a multi-site phase I/IIa HIV vaccine trial in East Africa. Pregnancy during the vaccination period resulted in discontinuation of further vaccination. The majority, 58.3%, of enrolled women reported using hormonal contraception. Married women were more likely to use hormonal contraception compared to single, separated

or widowed women, OR 3.3 (95% CI: 1.34 to 7.93) and less likely to use condoms, OR 0.3 (95% CI: 0.12 to 0.97).The pregnancy rate was 8.9%. Of those women who became pregnant, 78% had reported using hormonal contraception[3].

Being employed, a student, having ever been pregnant and number of sexual partners in the past 12 months were described as factors associated with contraception use in South African youth[26] [AOR 1.8 (95% CI: 1.3 to 2.6), AOR 1.9 (95% CI: 1.3 to 2.7), AOR 1.9 (95% CI: 1.5 to 2.5), AOR 0.7 (95% CI:0.5-0.9), respectively][26]

1.5. Contraception types, efficacy and safety of types.

The risk of pregnancy among typical users of highly effective contraception i.e IUCD, hormonal injections, sterilization and hormone implants, is 3% or less in the first year of use[27]. Oral contraception and barrier methods (diaphragm and condoms) are less effective in preventing pregnancy so the use of these methods in clinical trials may be limited. Participants on the injectable contraception were found to be less likely to fall pregnant compared to those using barrier methods or oral contraception[20,15,28]. In a secondary data analysis of 5224 women enrolled in a prospective cohort study to evaluate the association between hormonal contraception and HIV acquisition, Steiner et al[28] described the pregnancy risk among oral contraception, injectable contraception and condom users in Uganda, Zimbabwe and Thailand. The overall 12 month cumulative probability of pregnancy for injectable contraception users was lower than oral [0.6% (95% CI: 0.3 to 1.0) and 9.5% (95% CI: 8.1 to 11.0) respectively]. Women in Thailand experienced lower pregnancy risk with condom use compared to women from

Uganda and Zimbabwe [18.4%(95% CI: 11.1 to 25.7), 29.5 (95% CI: 25.7 to 33.4) and 23% (95% CI: 19.4 to 27.2) respectively][28].

Condoms have been shown to be less adequate in reducing pregnancy, especially in women with a high frequency of sex acts[1]. Skoler et al[29], estimated a 12 month cumulative probability that a woman engaging in 20 coital sex acts per month will become pregnant, was 51%; given a 90% rate of condom use and no other contraception[29].

Even though increasing the use of effective contraception in clinical trials has advantages in preventing pregnancies, their use presents other challenges by affecting trial outcomes. The use of hormonal contraception may alter the susceptibility of vaginal and cervical mucosa to the local effects of the study product, which may complicate safety assessment[1]. Injectable hormonal contraception causes non-menstrual bleeding which could affect assessment of product safety, making it difficult to assign bleeding as product-related in the presence of injectable progesterone. Depot medroxyprogesterone acetate (DMPA) may induce heavier, prolonged or irregular bleeding in some women, especially in the first year of use. These bleeding side effects could also have negative effects on contraception adherence rates[30].

1.6. Diagnosing pregnancy in vaginal microbicide efficacy and effectiveness trials: sub clinical pregnancies

Pregnancy in clinical trials should be detected as early as possible to avoid exposure to the product whilst pregnant. To ensure this, most trials perform pregnancy tests on a monthly basis. More frequent testing could mean that some sub-clinical pregnancies are

diagnosed, these are pregnancies that would normally end around the time of a normal menstrual period and so would not have been identified in a normal setting[1,19]. The literature suggests that up to 70% of conceptions may be lost prior to term and the majority of these occur prior to the time of the missed menstrual period[31]. Wilcox et al[19] studied the risk of early pregnancy loss by collecting daily urine specimens from 221 healthy women who were attempting to conceive. The study identified 198 pregnancies by an increase in the hCG level near the expected time of implantation. Of these, 22% ended before pregnancy was detected clinically. Monthly pregnancy testing may result in a higher rate of diagnosis of sub-clinical pregnancies. This could in turn lead to unnecessary censoring of women[32] from a study and might require larger sample sizes for efficacy studies to accommodate this early censoring.

In the HPTN 039 trial, 59/228 (25.9%) of participants that tested positive for pregnancy at follow up, were negative on repeat testing at the next monthly visit, with 41/59 (69%) reported as miscarriages[15].

1.7. The impact of pregnancy in the interpretation of trial data

High pregnancy rates in HIV prevention trials pose a number of challenges in interpretation of trial data, such as the following:

1.7.1. Reducing the maximum detectable effectiveness of product and power to detect the lower effectiveness level

The trial's ability to detect a difference may be affected when pregnant participants stop product use, either because product is interrupted or discontinued by the study, or if the participants decide to stop using product of their own choice due to pregnancy[1].

Time off product due to pregnancy adversely affects the study's power in trials whose sample size and power calculations may not account for this time off product. In addition, where the possibility of pregnancy is taken into account and higher than expected pregnancy rates occur, this will affect the ability of the trial to detect a difference between treatment arms[1,4;11;33,34,35].

In the CS trial in Nigeria, pregnancy in both the placebo and CS groups was the primary reason for product discontinuation, accounting for 54% of product discontinuations, but because many women did not carry pregnancies to term, time off due to pregnancy[10] was only 4.48% of total observed person-time. In the SAVVY Nigeria trial, even though the most common reason for product interruption was running out of gel, pregnancy caused longer interruptions and accounted for 50% of all observed person-time off product, about 5% of total time in both groups[36].

The SAVVY Ghana trial reported the longest time off product use due to pregnancy, with the median amount of time off product use, due to pregnancy, being 2 months. This resulted in 10% of the total time off product being due to pregnancy[11].

Raymond et al[1] gave an example to demonstrate the effect of non-use of product on the power and study size in a trial that is designed to have 80% power to detect 50% effectiveness in reducing HIV acquisition. Assuming product is used 80% of the time and where the 12 month cumulative probability of pregnancy is 40% and each pregnancy lasts 3 months, then approximately 10% of follow up time off product would be due to pregnancy. This scenario would increase the total amount of time off product due to pregnancy from 20% to 28%, reduce effectiveness to 45% and increase the

number of incident HIV infections required to demonstrate an effect in an intent to treat analysis by 33%[1] This increase will be a challenge even in countries with high HIV incidence.

Also, in an intention to treat analysis the effect size decreases because pregnant women who stop using product have a likelihood of seroconversion that is comparable to that of the control group. Censored observations reduce the total number of participants left at risk for the event under study[37].

1.7.2. Introducing bias in estimating effectiveness

It maybe possible to introduce bias if there are different pregnancy rates between the experimental and placebo groups, for example, if the product being evaluated has contraceptive properties. If the risk of HIV acquisition changes with pregnancy as suggested by some investigators[38], the observed effect could be a result of the product's direct ability to prevent HIV or its indirect impact on changes in HIV risk associated with pregnancy[1,32].

Gray et al[38] reported higher HIV incidence rate ratios during pregnancy compared to non-pregnant and non-lactating women (2.03, 95% CI: 1.33 to 3.11). The HIV incident rate ratio was also higher during pregnancy compared to the period of breastfeeding (1.76, 95% CI: 1.05 to 2.94)[38].

A trial with a product that has a highly effective contraceptive effect but has no direct effect on HIV acquisition, could give a result suggestive of effect if the risk of acquiring

HIV increases disproportionately in the control group, due to increased pregnancy rates in the control group[1].

If on the other hand pregnancy is associated with decreased risk of HIV acquisition, an efficacious product that is also contraceptive, resulting in low pregnancy rate in the active arm could appear ineffective[1].

1.7.3. Sexual behaviour change in pregnancy

The investigators for the SAVVY Ghana trial did an interim analysis in 2006 that showed a change in sexual behavior of pregnant participants. After approximately 75% of the expected person-time accrued in the trial, there were 713 women with at least one pregnancy. Of these, 636 had self-reported information regarding sexual activity and condom use pre- and post-pregnancy detection. The pre-pregnancy mean number of vaginal sex acts in the last 7 days was 6.4; the mean dropped to 5.1 at the post-pregnancy follow-up visit. This difference of 1.3 sex acts (95% CI: 0.88 to 1.67) was significant using the Wilcoxon signed ranks test ($p<0.001$)[39].

A stratified analysis by pregnancy test result at the next follow-up visit also showed that the reduction in number of sex acts was greater in women who continued with pregnancy. The women who were no longer pregnant at the follow up visit reported an average reduction of 0.7 acts (95% CI: 0.22 to 1.27; $p=0.009$). However, the women who were still pregnant at follow up reported an average reduction of 1.9 acts (95% CI: 1.28 to 2.47; $p <0.001$).

This kind of change in sexual behavior could affect a woman's risk of acquiring HIV, which could in turn lead to statistical issues including loss of study power and difficulties in the interpretation of study results.

The SAVVY Ghana trial subsequently reported a low incidence of HIV (1.09 per 100 person years, 95% CI: 0.63 to1.74) and was closed prematurely because it would have required revision of sample size for it to be adequately powered to show a significant difference between the trial arms[11].

1.8. Statement of problem:

Pregnancy in vaginal microbicide trials is an issue because of safety concerns about unknown effects of an investigational product to both the pregnant woman and the fetus. In addition, high pregnancy rates among women in HIV prevention trials can undermine the statistical measures of effectiveness and safety. The number of pregnancies in a clinical trial influences the time contributed by the person who is pregnant, which affects the outcome and interpretation of the trial results due to a change in sample size and power to detect effectiveness. The reduction in use of product during pregnancy reduces both the maximum detectable effectiveness of product and the power to detect this effectiveness. Therefore, predictors of pregnancy are important to explore and document for researchers to maximize efforts to reduce the incidence of pregnancy in the trial population.

1.9. Justification for the study:

At a microbicide conference held in Cape Town in 2006, high pregnancy incident rates were highlighted as a concern in the conduct of microbicide trials[2;14]. Interpretation of trial results, validity, safety issues, logistics of diagnosing pregnancy, management and care of the pregnant participants are some of the issues that have to be considered with increasing numbers of pregnancies in these trials[2,14;40;40;41.]

As southern African countries are still experiencing high incidences of HIV, countries such as South Africa are considered ideal places to conduct trials where HIV acquisition is a trial end point. Factors that are locally relevant that may help trials to predict the incidence of pregnancy and consequently decrease this incidence, make local data of high importance.

This analysis aims to assess if women using barrier methods and hormonal contraception who became pregnant in the trial shared any common characteristics. In addition this analysis will attempt to describe differences, in particular type of contraception used, between those women who became pregnant on the trial and those who did not. If women who became pregnant shared common characteristics, this information could be used to help with the targeting of recruitment of potential participants and counseling of trial participants.

1.10. Research Question:

What is the incidence of pregnancies and factors associated with pregnancy in women using barrier methods and hormonal contraception who took part in a phase III vaginal microbicide trial in Johannesburg?

1.11. Null Hypothesis:

There is no difference in demographic factors, education and partner types between women using barrier methods and hormonal contraception who became pregnant and those who did not fall pregnant during their period of participation in a phase III microbicide trial.

1.12. Objectives:

- To determine the incidence of pregnancy in participants who took part in a phase III vaginal microbicide trial at the Johannesburg site.
- To describe the factors that were associated with incident pregnancies in women using barrier methods and hormonal contraception

2. METHODOLOGY

This chapter details the study methodology which includes an overview of the primary study, data collection, data entry, cleaning and methods for analysis. Details of the specimen collection methods and testing are also described.

2.1. Research Setting

The Reproductive Health and HIV Research Unit (RHRU) was one of six partner sites that participated in the Microbicides Development Programme (MDP) clinical trial. The primary study, the MDP301 trial, was a multicentre randomized double blind placebo controlled phase III trial. The primary objective of the trial was to assess the efficacy and safety of 0,5% and 2% PRO 2000/5 vaginal microbicide compared to placebo in preventing vaginally acquired HIV infection. The 2% arm was discontinued in February 2008. Between October 2005 and August 2008, the RHRU sites recruited and enrolled 2508 HIV negative, sexually active women who were 18 years and above. Each woman was followed up monthly for a period of up to 12 months.

2.2. Study Design

This was a secondary data analysis of data collected in the MDP 301 trial. This analysis will look at a cohort of women, in a prospective manner, to determine the incidence of pregnancy and the risk factors associated with pregnancy in women using barrier methods and hormonal contraception. Even though the trial was designed for 12 months of follow up, of the 2508 women enrolled in the study period, not all women would have completed a full year of follow up at the time of this analysis. A total of 2437

women completed at least one follow up visit and so were included in the analysis of incident pregnancy.

2.3. Study population

The MDP 301 trial recruited women from Orange Farm and Soweto which are large townships south of Johannesburg, South Africa. Women were recruited from local primary health care clinics and referred to designated study clinics for screening.

To be enrolled in the study, women had to be 18 years and above, sexually active at enrolment and likely to be sexually active during follow up, willing to undergo HIV testing at 12 week intervals (including receiving HIV results), HIV negative at screening, willing to use the study gel as instructed and undergo regular speculum examinations and screening for genital tract infections, willing to test for pregnancy at monthly visits, willing to receive health education on condoms and willing and able to give informed consent.

A woman could not be enrolled if she was allergic to latex, likely to have sex more than 14 times a week on a regular basis, had a grade 3 clinical or laboratory abnormality or was participating in another HIV prevention trial.

Intending to fall pregnant was not an exclusion criterion and women were informed during the informed consent process that product use would be discontinued or interrupted if they became pregnant during follow up. Participants were counselled on effective contraception and initially were referred to local clinics for contraception; later in the trial, contraception was provided at the site.

Once enrolled, each woman was scheduled for monthly follow up visits and followed up for a total period of up to 52 weeks. If a participant missed a scheduled visit, 3 telephonic contacts and a home visit were made to contact that participant and reschedule the visit. These attempts were made for each missed monthly visit. All participants who did not return to the study before the end of their one-year follow up period were considered lost to follow up.

2.3.1. Trial Procedures at follow up

Each monthly follow up visit included an interview, gel collection and pregnancy testing. Clinical visits were scheduled at 12-week intervals with a visit window period of 2 weeks on either side of this 12-week interval. Clinical visit procedures included HIV and pregnancy testing, collection of swabs for STI testing from all participants and bloods for the safety profile testing for the first 500 enrolled patients. Participants who were symptomatic for STI at any visit were treated syndromically at the visit and those who had positive results from STI screening at clinical visits were called back to collect treatment.

2.3.1.1. Gel Use

Based on their sexual activity, each woman was provided with enough gel supply at monthly visits to cover each sexual act until the next visit and was encouraged to come back for more gel supplies between visits when necessary. Following the review of data accrued by end January 2008, the independent data monitoring committee recommended that the 2% arm be discontinued on grounds of futility. Women remaining

on 2% arm were withdrawn from gel use but were asked to attend the clinical visits at week 4,12,24,40 and 52.

2.3.1.2. Management of pregnancy outcomes

At each monthly visit a pregnancy test was conducted and results given to the participants. Gel use was interrupted at the time of a positive pregnancy and options available were discussed with each woman. Participants who opted to continue with pregnancy were scheduled for quarterly visits. Participants who opted for termination of pregnancy were referred to a facility that provides termination of pregnancy. Women were allowed to resume gel use after a pregnancy was completed, and a pregnancy test was confirmed negative .

2.4. Measurement

2.4.1. Measurement of Outcome Variable

The main outcome of interest was pregnancy. Pregnancy status was assessed for each enrolled woman on a monthly basis by testing urine using a Quick Vue® rapid test. To validate the study site test, 5% of all urine samples tested at the site were sent to the reference laboratory, for confirmation using the Quick Vue qualitative one step hCG combo test.

Pregnancy tests at the clinical trial site were performed by the research nurses, who were trained in the testing procedure. All positive tests were confirmed with serum qualitative hCG tests.

Time to first positive pregnancy test after enrolment was defined as time to failure.

Only one pregnancy was considered for each woman.

pregnancy outcomes was not included in this analysis.

2.4.2. Exposure variables

The main exposure variable was type of contraception used. Contraception use was confirmed at each monthly visit, through structured interviews. Type of contraception used was classified to oral contraception, injectable (DMPA and Nur-isterate), barrier method (male or female condoms, diaphragm), natural rhythm, IUCD and traditional methods (oral and other).Only women on hormonal contraception and barrier method were included in the regression model. Contraception use at follow up was allocated as the type of contraception that was reported by the woman at the visit when pregnancy was diagnosed. The analysis did not take into account the change in contraception use over time and how long each woman had been on a particular method before they became pregnant.

Time on the study was also considered as a risk factor for pregnancy.

2.4.3. Sources of Bias and Confounders

Selection bias could play a role as participants for the main trial were volunteers and women who chose to participate could be different to the general population. Trial targets for follow up were 85% for each visit and women who were lost to follow up could be different from those who continued trial participation until the end. The study excluded HIV positive women; this group might be systematically different from the HIV negative women thus making extrapolation to the general population difficult.

Confounders in the relationship between type of contraception used and pregnancy could include age, in that younger women are more likely to consider pregnancy[23] and

religion as some religions could influence choice of contraception. Additional confounders would include level of education, partner type and marital status[16;23,25]. The only possible effect modifier for consideration is the study product, 0.5%, and 2% PRO2000/5 gels. If the product had contraceptive properties, this could have affected the risk of pregnancy differently between participants on PRO2000/5 and placebo. Literature suggests that PRO2000/5 does not have any contraceptive properties [18,42]. It was therefore assumed that the risk of pregnancy remained the same for participants on active product and those on placebo.

2.4.4. Other Risk Factors

Data on intercurrent illness like vomiting, that could have interfered with absorption of oral contraception was not collected as part of the main trial and was therefore not possible to analyse. Data on concurrent medication that could have interfered with absorption of oral contraception was not included in the analysis.

2.5. Data Management:

2.5.1. Data Collection

Data were collected through structured interviews by trained research nurses and community health workers using case record forms (CRF). Demographic data, which included age, employment status, level of education and religion were collected at the screening visit. The screening visit was a maximum of 6 weeks before enrolment into the study. Data on contraception use and sexual behaviour were collected at the screening visit and again at the enrolment visit. Sexual behavior and contraception histories were updated at the monthly gel collection visits and at the quarterly clinical

visits. Inconsistencies in CRFs were dealt with at the data collection level by research nurses who did quality checks on all the forms completed.

2.5.2. Data Entry

Data for the main trial were double data entered into an MS SQL database. Data were verified to see if the two entries were corresponding before saving each record. Inconsistencies picked up at data entry were dealt with by raising queries for the clinic staff to correct the errors before data entry. Quarterly monitoring visits were conducted on the site and a proportion of case record forms were reviewed for accuracy of data and to ensure that the study was conducted according to International Conference on Harmonization- Good Clinical Practice (ICH-GCP) guidelines.

2.5.3. Staff training:

To ensure standardized data collection, all the staff members who were responsible for data collection and entry received training conducted by the MRC UK's Clinical Trials Unit (CTU) and by the site. Training included interviewing skills, completion of CRFs, specimen collection and testing and interpretation of pregnancy results.

2.5.4. Data extraction:

Four data sets were extracted for this analysis. The data sets comprised of demographic data, sexual behavior data, participant follow up and pregnancy results.

2.5.5. Data cleaning:

The MDP database had a number of in-built quality checks that ensured that high quality data was produced. Participant's identification numbers were verified before study records were captured, completed CRFs were checked after each visit by quality control personnel before being captured.

After extraction each datatable was individually cleaned. Variables were described, tabulated, cross-tabulated, summarized and browsed through to check for inconsistencies and outliers. Data cleaning included range checks across data and removing extreme values which were biologically implausible.

The variable "age" was compared to the date of birth variable to confirm if age recorded was correct.

2.5.6. Missing values

Missing values were identified and are reported in the results sections.

2.6. Data processing and data analysis methods

The data was managed and analysed using STATA® version 10. For the analysis, the different datatables were merged into one dataset using the study ID as a unique identifier.

2.6.1. Regrouping of variables

For the process of data analysis the following regroupings were done:

Age

Age was re-grouped into a categorical variable and the categories used were age 18 to 24 years, 25 to 34 years, 35 to 44 years, 45 years and above. This grouping put

younger women, who maybe more likely to fall pregnant, into an appropriate group. The age groups 35–44 years and above 45 years were collapsed into one age group, 35 years and above, because there were very few participants that were aged 45 years and over.

Contraception used:

Type of contraception used was regrouped into a binary group hormonal contraception (oral or injectable) and barrier methods (condoms and diaphragm) for the incidence rates for pregnancy by type of contraception used analysis. For the incidence rates for pregnancy by type of hormonal contraception used analysis, contraception was divided into the binary group injectable contraception and oral contraception as the exposures.

Level of education:

This was regrouped to the following categories: No education, completed primary education, completed secondary education and completed tertiary education.

No education was added to the primary education group due to small numbers in the no education group. Also, completed secondary education and tertiary were combined into one group in the univariate and multivariate analysis due to small numbers in the completed tertiary education group.

Employment status:

This was regrouped to a binary outcome - employed and not employed.

Religion

Religion was regrouped into a categorical nominal variable - Christian, Zionist, other (Muslim, Shembe, Hindu, Jehova's witness and African traditional) and None.

Type of partner was grouped to a binary outcome (long-term stable partner and other).

2.6.2. Descriptive Statistics

Descriptive statistics were used to describe frequencies and proportions of possible risk factors for pregnancy i.e age, type of sexual partner, level of education, employment status, religion and type of contraception used in the cohort. The distribution of risk factors at baseline was compared between those who became pregnant and those who did not. To assess group differences, categorical variables were presented with numbers, percentages in each category and a Chi squared or Fishers exact test were used, as appropriate, to assess statistical significance.

2.6.3. Analytical statistics

All results were presented with a significance level and a 95% confidence interval. Any result with $P<0.05$ was considered statistically significant.

2.6.3.1. Incidence rate

The incidence rate of pregnancy was calculated as number of pregnancies per 100 woman-years of follow up, i.e. calculated as number of new pregnancies/total woman-years of follow up multiplied by 100.

2.6.3.2. Survival time analysis

For this analysis, pregnancy test results were categorised into a binary outcome. Time to first positive pregnancy test after enrolment was defined as time to failure and data were set to survival time data. A survival analysis of cumulative probability of having the outcome at any one point in time and the median time to occurrence of the outcome was conducted. The Kaplan Meier survival analysis was used to determine the time to first pregnancy. These results were plotted on a Kaplan Meier curve and groups (hormonal compared to barrier method, injectable compared to oral contraception) compared by the log rank test.

2.6.3.3. Univariate Analysis

The hazard rate for pregnancy was calculated using a Cox regression model. Cox regression allowed for analysis of time-varying outcome variables in a prospective cohort study design.

A univariate analysis for each potential confounder was done and hazard ratios with p values of less than 0.15 for each confounder were then included in the multivariate model.

2.6.3.4. Multivariate Analysis

Variables with a p value of 0.15 on Cox univariate analysis were individually added into the final multivariate Cox regression model, observing changes in hazards ratios to assess for potential confounders. Variables that were considered for inclusion into the

model were age, level of education, religion, employment status, type of contraception and type of partner.

2.7. Ethical Considerations

The protocol and consent forms for the primary study were approved by the University of the Witwatersrand Human Research Ethics Committee (HREC) and the protocol for secondary data analysis was reviewed and approved by the HREC, reference M090467. All participants signed informed consent in their preferred language (isiZulu, seSotho or English). Consent was confirmed verbally at each follow up visit. To maintain confidentiality, participant records and results were only identified by unique identifiers and not by participant name. Participants who were diagnosed with a STI were treated at the study site and those who fell pregnant or who acquired HIV were referred to health facilities close to where they lived for further care.

Participants were not paid for participation in the trial but were re-imbursed for transport costs for each visit attended.

3. RESULTS

3.1. Participants

Between October 2005 to August 2008, 2508 women were enrolled in the MDP301 trial. Figure one illustrates the flow of participants that were included or excluded in this analysis. Participants that contributed at least one follow up visit were included in this analysis. Of the 2508 enrolled participants, 2489 were included in the description of baseline characteristics and 2437 were included in the survival analysis. The main reasons for exclusion were women who did not attend a follow up visit. Of the 2437 patients included in the survival analysis, 238 (10%) participants became pregnant during their period of follow up. Of the 2437 participants included in the survival analysis, 2171(hormonal contraception and barrier method users) were included in the multivariate analysis

3.2. Missing data

Of the 337 participants who did not contribute to the multivariate analysis, 266 were using methods of contraception other than a barrier method or a hormonal method(IUCD, tubal ligation and traditional methods), 5 participants were missing data on the type of contraception used at follow up, 47 only contributed data for the enrolment visit and 19 were co-enrolments in other HIV prevention trials (or between the RHRU's two clinical sites). The demographic features of those excluded from the final analysis were similar to those of the main cohort. The median age was 31 SD (10) years, 317(84%) were unemployed and 213(57%) Christian At the time of data extraction, 1 236 participants were still in follow up (and so did not contribute a full year

of follow up) and 209 had stopped participating in the trial early. Of the 209 that stopped participation in the trial, 23/209 (11%) had relocated, 22/209 (11%) withdrew consent for various reasons, 3/209(1%) withdrew when 2% arm of the gel was discontinued, 3/209 (1%) died and 158/209 (76%) were lost to follow up. None of those who withdrew consent were because of pregnancy.

Data from women who prematurely withdrew from the study or who were lost to follow up were censored on the date of the last pregnancy test result.

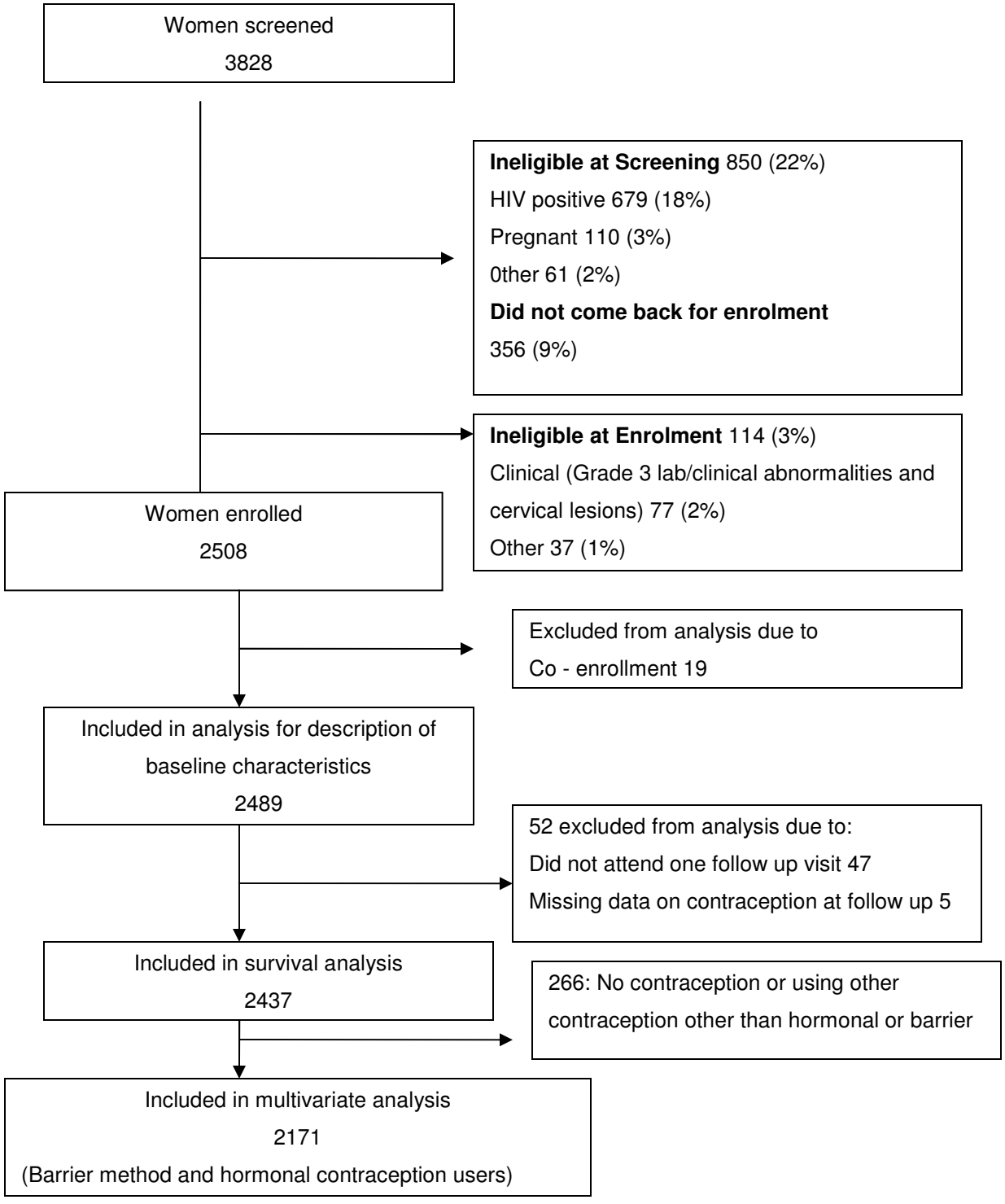


Figure 1: Flow of participants enrolled in the MDP trial, Johannesburg 2005 - 2008, from screening to inclusion in the final analysis

3.3. Descriptive data

The baseline demographic characteristics of the participants enrolled were compared in two groups, those who became pregnant and those who did not become pregnant (table 3.1). With the exception of age, where women who became pregnant were younger than those who did not, the two groups were similar with respect to demographic characteristics. The mean age of women participating in the study was 27.30 (SD 8) years. The majority of enrolled patients 1269/2489 (51%) were between 18 and 24 years. The reported rates of contraception use at enrolment were high, 94% (2333/2489) of women reported using contraception; with the majority 55% (1373/2489) reporting hormonal contraception use. Barrier methods were more commonly used by women who became pregnant than those who did not fall pregnant 59% (140/239) and 32% (712/2250) respectively $p<0.001$.

Table 3 1: Baseline characteristics of women in the MDP trial: comparing those who became pregnant during the study and those who did not become pregnant, Johannesburg 2005 to 2008

	All women N (%) 2489	Did not become pregnant N (%) 2250	Became pregnant N (%) 239	P- value
Age				
18-24	1269(51)	1126(50)	143(60)	<0.001
25-34	770(31)	694(31)	76 (32)	
>35	450 (18)	430(19)	20(8)	
Level of Education				
No Education	87(3)	85(4)	2(1)	0.191
Completed Primary	1317 (53)			
School		1191 (53)	126 (53)	
Completed Secondary	1036 (42)			
School		931 (41)	105(44)	
Completed a tertiary		43 (2)	6(2)	
Qualification	49(2)			
Employment Status				
Not Employed	2146 (86)	1935(86)	211(88)	0.34
Employed	343(14)	315(14)	28(12)	
Type of Contraception Used				
No contraception	156(6)	141(6)	15(6)	<0.001
Barrier Method	852(34)	712(32)	140(59)	
Hormonal	1373(55)	1289(57)	84(35)	
Other(IUCD and	108(4)	108(5)	0	
Sterilized)				
Religion				
None	312 (12)	277 (12)	35 (15)	0.23
Christian	1419 (57)	1296 (58)	123 (51)	
Zionist	365 (15)	330 (15)	35 (15)	
Other	393 (16)	347 (15)	46 (19)	
Type of Partner				
Long term stable	2437 (98)	2202 (98)	235 (98)	0.64
Casual partner	52(2)	48(2)	4(2)	

3.4. *Pregnancy incidence and survival time*

The 2437 enrolled women included in the analysis provided 2248 woman-years of follow up. A total of 238 incident cases of pregnancy were recorded in this group. One pregnant participant was excluded from the analysis because she did not have data on contraception at follow up. Three of the women had two pregnancies each. The overall pregnancy incidence was 11 per 100 woman-years of follow up (95% CI: 9.32 to 12.02). The incidence rate of pregnancy was highest in the last 3 months of follow up, 98 per 100 woman-years of follow up (95% CI: 85.09 to 112.35) compared to 2 per 100 woman-years of follow up in the first 3 months (95% CI: 0.94 to 2.92) (table 3.2 and figure 2).

Table 3.2 Pregnancy incidence rates at 3 monthly intervals of follow up, in women enrolled in the MDP trial, Johannesburg 2005-2008.

Follow up time period (months)	Incident pregnancies	Woman-years of follow up	Incidence rate (per 100 woman-years)	95% C I
0–3	12	724	1.66	0.94 - 2.92
3-6	18	693	2.6	1.64 - 4.12
6-9	9	628	1.43	0.75 - 2.76
9-12	199	204.07	98	85.09 - 112.35
Total	**238**	**2248.07**	**10.590**	**9.32 - 12.02**

Table 3.3 shows pregnancy incidence rates and unadjusted incidence rate ratios of pregnancy by type of contraception used during follow up. The incidence rate of pregnancy was higher in those women using barrier methods of contraception, 11 per 100 woman-years of follow up (95% CI: 7.78 to 14.34) compared to those women using hormonal contraception, 7 per 100 woman-years of follow up (95% CI: 6.07 to 8.72). Relative to barrier method users, the incidence rate ratio of pregnancy was lower in those who reported hormonal contraception use 0.69(95% CI: 0.48 to 0.91, p= 0.045).

Table 3.3 Incidence rates for pregnancy by type of contraception used (hormonal compared to barrier method) at follow up in women enrolled in MDP trial, Johannesburg, 2005-2008

Type of contraception	Incident pregnancies N	Woman years to follow-up	Incidence per 100 woman years (95% CI)	Rate Ratio	p-value
Barrier Method	41	388	11 (7.78 - 14.34)	1	0.045
Hormonal	117	1608	7 (6.07 - 8.72)	0.69 (0.48 – 0.91)	
Total	**158**	**1996**	**8 (6.77 – 9.25)**		

For those women who used hormonal contraception, there was no difference in pregnancy incidence rates between oral contraception and injectable contraception users, 8 per 100 woman-years (95% CI: 5.78 to10.67) and 7 per 100 woman-years (95% CI: 5.59 to 8.76) respectively. The rate ratio of pregnancy was lower in those using injectable contraception, 0.89 (95% CI: 0.60 to 1.34) but this result was not significant (p = 0.54) (table 3.4).

Table 3.4: Incidence rates for pregnancy by type of hormonal contraception used at follow up in women enrolled in MDP trial, Johannesburg 2005 - 2008

Type of contraception	Incident pregnancies	Woman years to follow-up	Incidence per 100 woman years(95%CI)	Rate Ratio relative to oral	P value
Oral	41	522	8 (5.78 – 10.67)	1	0.548
Injectables	76	1086	7 (5.59 - 8.76)	0.89 (0.60 - 1.34)	
Total	**117**	**1608**	**7 (6.07 - 8.72)**		

3.5. Survival time outcomes

The Kaplan Meier curve in figure 2 illustrates the survival time to first pregnancy in the 2437 participants that were included in the analysis.

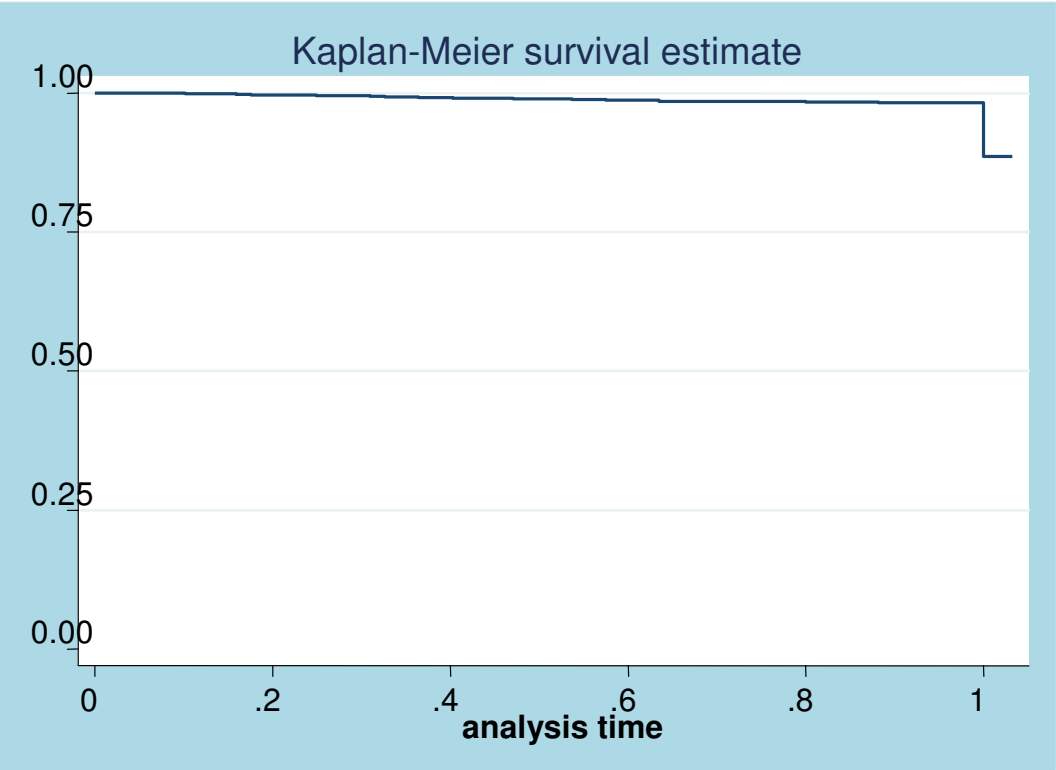
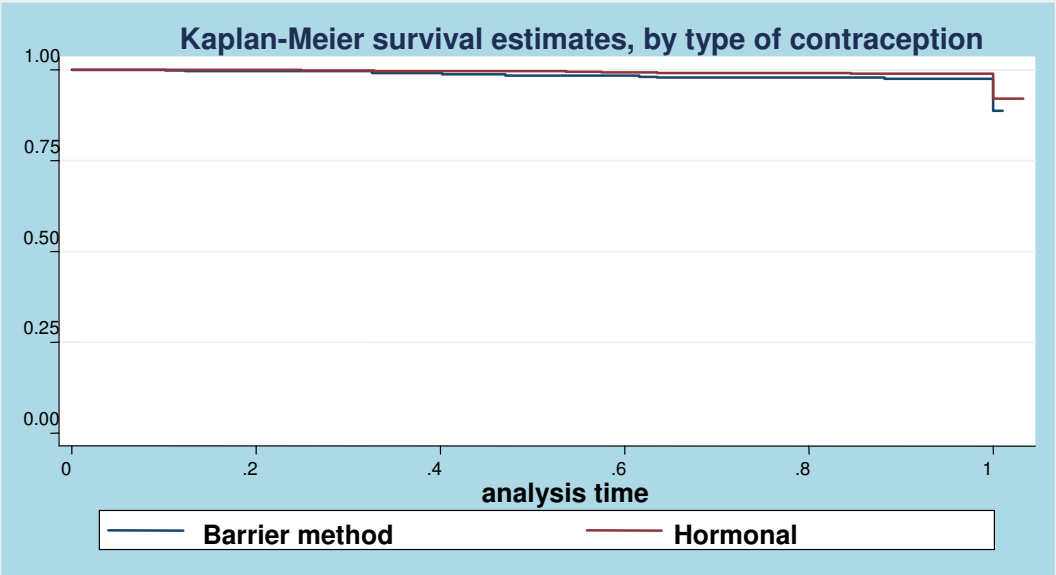


Figure 2: Kaplan Meier curve for survival to first pregnancy at follow up in women enrolled in a microbicide trial, Johannesburg 2005-2008

The Kaplan Meier curves for survival time to pregnancy comparing, firstly, any hormonal contraception to barrier methods and secondly, injectable contraception to oral contraception, are illustrated in figures 3a and 3b respectively. The log rank test for equality showed a significant difference in survival time to pregnancy in participants using barrier methods compared to hormonal contraception and no difference in survival

time to pregnancy in participants using oral contraception compared to injectable contraception (table 3.5).



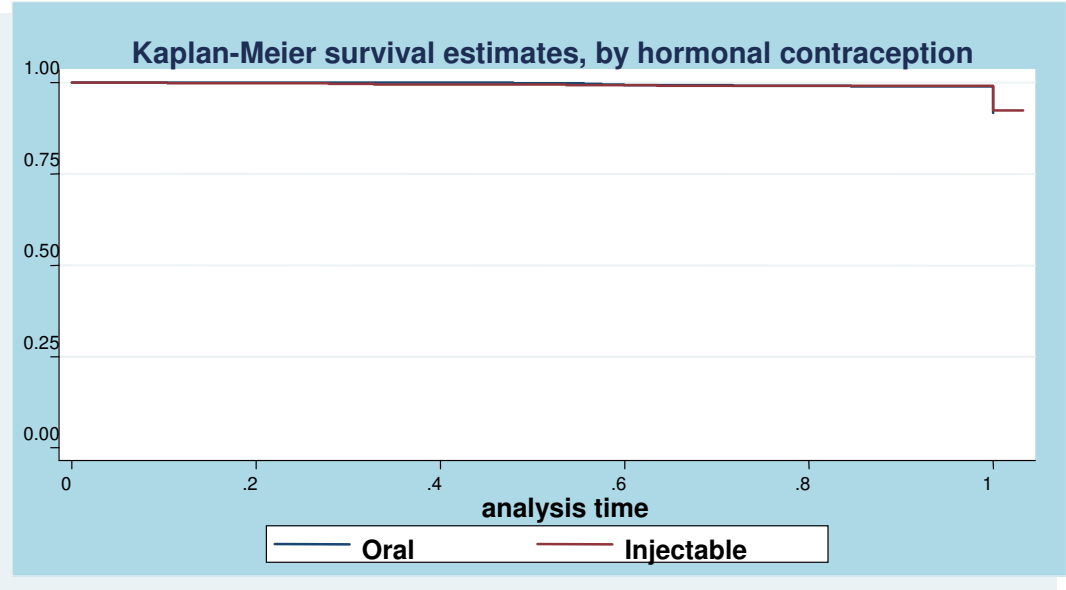


Figure 3(a) and 3 (b): Kaplan- Meier curves for survival to pregnancy by type of contraception used during follow up in women enrolled in a microbicide trial , Johannesburg 2005-2008

Table 3.5 Log rank test for equality of survival times measured for pregnancy in women enrolled in MDP trial, Johannesburg 2005-2008

Variable	*Expected Events*	*Observed events*	*Chi 2*	*P value*
Type of contraception				
Barrier Method	30.49	41	4.78	0.028
Hormonal	127.51	117		
Hormonal Contraception				
Oral	39	41	0.23	0.63
Injectable	78	76		

3.6. Univariate and Multivariate analysis of factors associated with pregnancy.

For the analysis comparing hormonal contraception to barrier methods, of the 2171 participants that reported use of these methods at follow up, 80% (1740 / 2171) were on hormonal contraception(table 3.6).

The univariate analysis for association between type of contraception used and pregnancy demonstrated a hazard ratio (HR) of 0.68 (95%CI: 0. 48 to 0.97; p= 0.04). There was no significant difference in risk of pregnancy between the hormonal contraception (oral vs injectable) users, HR 0.91 (95% CI: 0.63 to 1.33, p=0.64).

Age group was the only possible confounder that fitted the criteria for inclusion into the multivariate model, p< 0.15.

Being 35 years and older reduced the risk of falling pregnant during follow up by 47%, HR 0.53 (95% C.I 0.31 to 0.92; p=0.024), compared to women who were younger than 25 years. (table 3.6)..

When age was included in the multivariate model for the association of type of contraception used and pregnancy, the univariate HR of 0.68 (95% CI: 0 .48 - 0.97; p=0.04) changed very slightly to an adjusted HR (AHR) 0.66(95% CI: 0.46 to 0.94, p=0.02).

Adjusting for type of contraception used, those who were 35 years and older were 49% less likely to fall pregnant compared to those who were younger than 25 years, AHR 0.51(95%CI: 0.30 to 0.88, p=0.016).

Education and employment status were not associated with pregnancy risk.

Table 3.6 Univariate and multivariate analysis results for factors associated with pregnancy at follow up in women enrolled in MDP trial, Johannesburg 2005-2008

	Univariate analysis		Multivariate analysis	
	Hazards Ratio (95% confidence interval)	**P value**	**Hazard Ratio (95% confidence interval)**	**P value**
Age				
18-24	1		1	
25-34	0.80(0.56 - 1.13)	0.20	0.81 (0.57 - 1.14)	0.24
>35	0.53(0.31 - 0.92)	0.024	0.51 (0.30 - 0.88)	0.016
Contraception Type				
Barrier	1		1	
Hormonal	0.68(0 . 48 - 0.97)	0.04	0.66(0.46 - 0.94)	0.02
Level of Education				
Primary education orl less	1			
Completed Secondary school	1.19 (0.92 -1.54)	0.17		
Employment Status				
Unemployed	1			
Employed	0.92 (0.57 - 1.49)	0.74		
Religion				
None	1			
Christian	0.86(0 .55 - 1.35)	0.50		
Zionist	0.71 (0 .38 - 1.30)	0.30		
Other	0.94 (0.54 - 1.62)	0.81		
Type of Partner				
Casual partner	1			
Stable/long term Partner	1.18(0.38 - 3.70)	0.78		

3.7. Test for the assumptions of a Cox regression model

The assumption of a Cox regression model is that, the proportion of hazards is constant over time. A test for this assumption was done in STATA® version 10. The observed Kaplan Meir survival curves were close and parallel to the Cox predicted curves, illustrating that the assumption was not violated(figure 4)

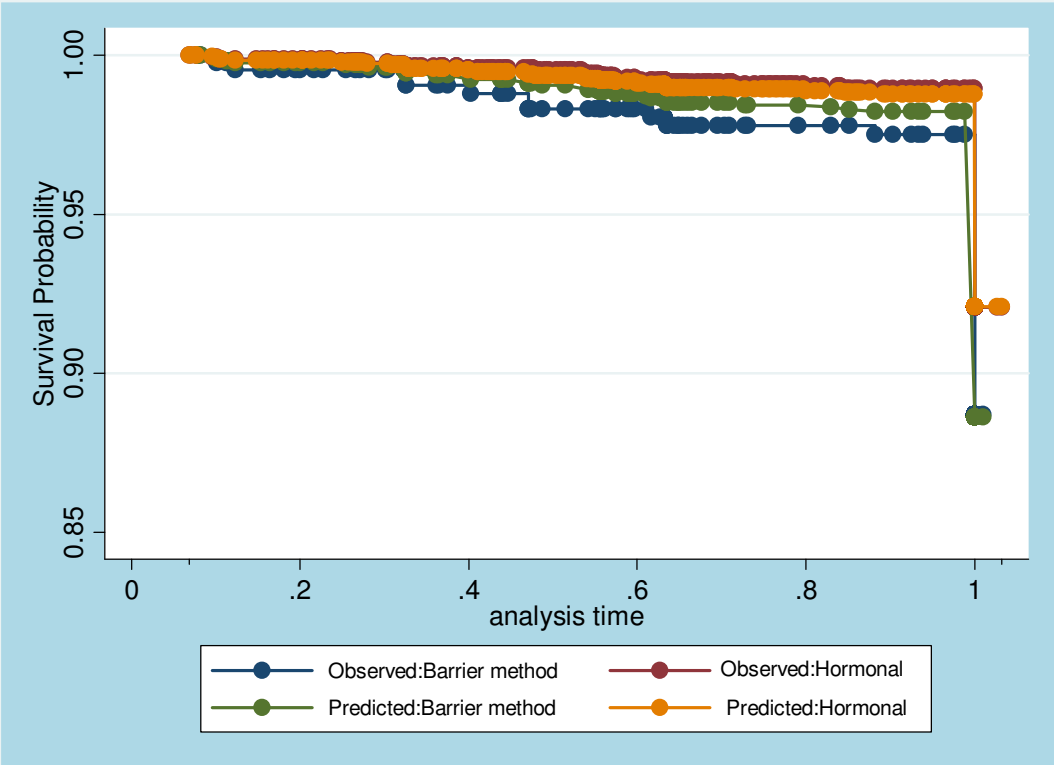


Figure 4. Test for assumptions of Cox regression Model applied to Cox model for the assessment of risk in women enrolled in MDP trial, Johannesburg 2005 to 2008.

4. DISCUSSION

This analysis revealed a significant difference in pregnancy incidence rate between women who used hormonal contraception and those who used barrier methods. Relative to barrier method users, women on hormonal contraception had a significantly decreased risk of falling pregnant. However, there was no significant difference in pregnancy incidence rates between women who used injectable contraception and those who used oral contraception. In addition younger age was associated with incident pregnancies. The incidence of pregnancy increased with increasing time of participation in the trial, with highest pregnancy incidence recorded in the last 3 months of follow up.

At baseline there was no significant difference in demographic features between those who became pregnant during follow up and those who did not. A high proportion of women reported contraception use in both groups. Hormonal contraception was the most common method of contraception (53%) used, followed by condom use (34%). This finding was similar to other HIV prevention trials[5,6]. In contrast, the two SAVVY trials[36,11] and the CS[10] trial reported higher proportions of participants using condoms than hormonal contraception (71% vs 9%; 47% vs 14% and 56% vs 17%) respectively. The possible explanation for this difference is that this study only included women from South Africa, where contraception is easily available and recruitment activities focused on family planning clinics.

4.1. Pregnancy incidence

The study found that the overall pregnancy incidence was 11 per 100 woman-years of follow up (95% CI: 9.32 to 12.02) This was similar to the pregnancy rates reported in some of the HIV prevention trials: 13.2 per 100 woman-years in the HPTN 039 trial [15] and 11.3 per 100 woman-years in the HPTN 035 trial[8]. However, much higher incidence rates were reported in some trials. The Ghana SAVVY trial reported pregnancy incidence rates of 42.5 per 100 woman years and 43.7 per 100 woman years in the SAVVY and placebo groups respectively[11]. Incidence rates as high as 52 per 100 woman-years reported in the FHI Tenofivir Disoproxil Fumarate trial[12]. One possible explanation for the difference in pregnancy incidence in these trials could be that both the SAVVY and the FHI trials reported a higher proportion of women using condoms as compared to hormonal contraception. The other explanation could be that the FHI Tenofivir trial recruited women with multiple partners and therefore could have engaged in a higher number of sex acts.

This study showed that there was an increase in pregnancy rates with increasing duration of study participation. The incidence rate of pregnancy was highest in the last 3 months of follow up, 98 per 100 (95% CI: 85 to 112) woman-years of follow up. One possible explanation for the increase in pregnancy incidence rates with increasing time could be due to changes in a woman's desire to have children during the study period. Also because of the fact that participants were counseled against pregnancy during follow up, they might have planned to attempt pregnancy later in the trial with the hope that they would only conceive after the year's participation in the trial was completed. It is also possible that the intensity of counseling messages decreased with increasing

duration of participation in the trial, with the participants who were perceived to be adherent to study requirements receiving fewer, less focused counseling messages on contraception or staff spending less time on counseling.

The results of this study also showed that there was a difference between pregnancy incidence rates in women who reported hormonal contraception use at follow up compared to those on barrier methods. However, there was no significant difference in pregnancy incidence between the two types of hormonal contraception (oral vs injectable) users. This is contrary to other studies that have reported substantially increased protection from non-user dependent methods, such as injectable contraception methods, compared to oral contraception [28,15]. This maybe due to good counseling on adherence.

Because information on change in contraception use was not analysed and because not all participants received contraception at the study site, it is possible that adherence was not good even in those who reported non user-dependent methods. This highlights the importance of having systems to monitor adherence to contraception use.

4.2. Factors associated with pregnancy

This study found that hormonal contraception use reduced the risk of falling pregnant. The association between the types of contraception used at follow up was significant at univariate level, with women on hormonal contraception being 32%, (HR 0.68, CI: 0. 48 - 0.97) less likely to fall pregnant compared to those who used barrier methods. After

controlling for age, the risk of falling pregnant in those who used hormonal contraception, at follow up, was further reduced to 34% less, AHR 0.66 (95% CI: 0.46 to 0.94, p=0.02), compared to those who used barrier methods. The association between age and hormonal contraception supports findings from other HIV prevention studies[15,28].

In univariate and multivariate analysis there was decreased pregnancy incidence with increasing age. In univariate analysis women aged 35 years and older had 47% (HR 0.53; 95% CI: 0.31 to 0.92; p=0.024) less risk of falling pregnant compared to women younger than 25 years. This association remained significant after controlling for type of contraception used. This finding was similar to those from other studies[15;16;38;40].

There was no association between level of education and risk of falling pregnant. This is contrary to what has been reported in literature where education was protective. A sensitivity analysis, looking at completed secondary education as a reference was conducted; this analysis did not change the result.

4.3. Strengths of the study

A prospective cohort study is a good study design to estimate incidence of a common outcome such as pregnancy in HIV prevention trials. The study has a large sample size, which allowed estimation of tight confidence intervals. For example, the confidence intervals around the pregnancy incidence is narrow in this analysis, suggesting that the

true pregnancy incidence lies between 9.32 and 12.02 per 100 woman-years, which is very close to 11 per 100 woman-years of follow up. Overall follow up was good (91%) which resulted in a high number of woman-years to follow up, again allowing for accurate estimates of effect.

Additional strengths of this study included the use of consistent and standardised enrollment and data collection protocols that occur within the context of clinical trials with adequate resources. Pregnancy testing was done regularly and consistently, the screening pregnancy test is sensitive and the confirmatory test was done in a laboratory setting and is considered very specific. This allowed accurate estimation of the outcome variable.

4.4. Limitations

4.4.1. Definition of exposure

For the analysis, a change in type of contraception used was not considered and the duration of time on a particular type of contraception was not analysed. Since not all participants who reported hormonal contraception use received contraception at the trial site, it was not possible for the investigators to accurately verify information given by participants about contraception use. Also condom use was self reported and the analysis to determine how often these were used was not done. There was no biological assessment for confirmation of hormonal levels to measure consistent use of hormonal contraception.

4.4.2. Other limitations

The data for the analysis was extracted before the end of the trial and this led to a decrease in the number of participants contributing a complete year of follow up. This could have underestimated the pregnancy incidence.

Contribution to time off product due to pregnancy was not analysed, therefore it was not possible to assess whether pregnancy in this cohort contributed to a significant amount of time off product use and so how this may have affected the power of the study to detect a difference in the three arms of treatment.

The analysis was limited to participants using hormonal and barrier method of contraception, this meant that the full set of reasons for pregnancy were not explored.

4.4.3. Possible sources of bias

Selection bias might have been introduced because participants volunteered to participate in the trial and those who chose to participate might have been systematically different from those who chose not to participate.

The study focused recruitment activities at family planning clinics, so the results may be biased towards people with access to family planning and who therefore have a higher proportion of hormonal contraception users than the general population.

Loss to follow up although low (10 %), may have introduced bias during the trial leading to an underestimation of the pregnancy incidence rate. Women who were lost to follow up may have been different to those who remained in follow up, with a likely scenario that women who knew they were pregnant may not have returned to the study as all participants were counseled about the investigators' concerns around pregnancies.

4.4.4. Possible source of confounding

Possible confounders included age, religion and level of education. Information on these was collected by structured interviews during the trial and these were controlled for during the analysis by the regression model.

This was a secondary data analysis, thus data on variables that are important such as information on parity and whether participants were married or cohabiting was not collected as part of the main trial and these could not be analysed or controlled for in the analysis. Other studies reported increased hormonal contraception use in married women compared to single women. Kibuuka et al[3] reported that married women were more likely to use hormonal contraception compared to single, separated or widowed women OR 3.3 (95% CI: 1.34 to 7.93) and were less likely to use condoms OR 0.3 (95% CI: 0.12 to 0.97).

4.5. Conclusion

Even though there is limited published data on factors associated with pregnancy in HIV prevention trials, this study corroborated previous findings with regards to the significance of hormonal contraception use and participant's age as factors associated with pregnancy.

Despite hormonal contraception use being protective, there was still a significant number of participants that became pregnant who had reported using these methods at follow up. In reality, reported contraception use does not necessarily translate into prevention of pregnancy. Provision of contraception by the trial site may improve adherence and lead to more consistent use, as trial staff may well have more time to counsel women than staff in a busy primary health care clinic.

Focus group discussions and or interviews with a sample of participants who become pregnant and those who do not become pregnant in the trials could provide valuable information for better understanding of additional factors associated with pregnancy.

While testing of investigational products in pregnancy is still being considered, HIV treatment and prevention trials will need to consider innovative measures to improve access to reliable contraception and to ensure regular and, more importantly, persistent use of reliable methods to prevent the pregnancies that may occur later in the trial as happened in the MDP301 trial. This may include specific counseling sessions for women in the second part of the year's participation, group sessions on contraception and pregnancy. The innovative use of technology, such as videos, may help to decrease the load of this additional counseling on study staff. Particular attention should be focused on women who are younger than 25 years and using condoms as the only method of contraception.

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Ref Type: Conference Proceeding

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Ref Type: Report

Site name: ----------PRE-PRINT----------		Date of visit: / / DD /MM / YYYY
Screening number:	Initials:	

Interviewer: read the questions to the volunteers verbatim and allocate the correct answer from the selection, unless it is indicated that you read out each answer to the volunteer as well. Boxed type is instructions to the interviewer. Type in italics is to be read to the volunteer.

Please try to answer these questions accurately as the answers to them are very important to the outcome of the study. Remember that the information you give us is confidential and will only be used for the purposes of this study.

Section 1: Family planning

1 Are you currently using any method of family planning? Yes → Q. 1a

No → Q. 1b

1a If yes, which of the following methods are you using?

		Natural/rhythm	→ Q. 2
Pills		Foam/jelly/spermicide	→ Q. 2
Diaphragm		Injectable Nur-Isterate	→ Q. 2
Injectable Depo-Provera		Injectable other	→ Q. 2
IUCD		Norplant implant	→ Q. 2
Condom (male or female)		Traditional vaginal	→ Q. 2
Traditional oral		Traditional other	→ Q. 2
Sterilisation			→ Q. 2
Other	Specify_____ _____		→ Q. 2

1b If no, why are you not using any method of family planning?

		Breastfeeding
Wanting to become pregnant		Not sexually active
Menopause		Sterilised (participant or partner)
Other	Specify_____	

2 How many days ago was the first day of your last menstrual period? [List number, 99 if more than 3 months or 00 if menstruating now]

2a Was this period when you expected it to be? Yes

Refer for clinical assessment No

Section 2: Sexual activity and condom use

Signature	Print name	Date

Site name: ----------PRE-PRINT----------		Date of visit: / / DD /MM / YYYY
Screening number:	Initials:	

Interviewer: please spend some time ensuring that the volunteer understands what is termed by a sex act: one sex act is "penetrative vaginal sex that may or may not end with ejaculation".

Also – don't forget to probe for EXACT NUMBERS

"I am now going to ask you about your sexual activity and condom use. Please answer accurately as the responses are very important to the study results."

3	How many days ago did you last have sex?	1 (includes yesterday, last night and today)	2 (the day before yesterday)	→ Q. 4
		3	4	→ Q. 4
		5	6	→ Q. 4
		7		→ Q. 4
			1-4 weeks	→ Q. 9
			More than 4 weeks	→ Q. 13

4	How many times have you had sex in the last week?	[list number of times or 77 if unsure]
5	How many different people have you had sex with in the last week?	[list number or 77 if unsure]

Ensure that the volunteer understands what we mean by each category of partner. 1*) Long-term stable relationships* include some/most of the following characteristics: official marriage, traditional marriage, bride price paid, man known to and accepted by woman's family, have children together, live together, long-term relationship, man provides regular financial/material support, may be cohabiting or non-cohabiting. 2) *One-off sexual encounter* refers to: only had sex once, and not likely to have sex with the same person again (e.g. one-time sex at party or while travelling, in direct exchange for money or other payment, no commitments involved). The category of other types of partner are those who are not part of a long-term stable relationship, but someone the woman has seen more than once, any other kind of partner who doesn't fit into the first 2 categories.

5a	How many of these partners were:	Long term stable relationships	
		One-off sexual encounters	
		Other types of partner	

Interviewer: check that the total of the answers given in question 5a is the same as the answer given in question 5 and rectify if necessary.

Signature	Print name	Date

Site name: ----------PRE-PRINT----------		Date of visit: / / DD /MM / YYYY
Screening number:	Initials:	

Interviewer: only fill this table in with participants who HAVE had sex in the last 1 week.

6. **"Now I am going to ask you some more detailed questions about your condom use each time you had sex in the last week."**

Interviewer: each question (row) refers to a particular sex act. Go through all columns for the individual sex act before moving on to the next row. Write the number corresponding to the answer code for each column in the box in each cell. Remind the participant about the definition of a 'sex act' and allow enough time for the participant to carefully consider each answer.

Sex acts	**Partner**	**Condom**
Sex acts in the last week	What **type** of partner was this act with?	Did you use a condom during this sex act?
Codes	1=long-term stable relationship 2=one off sexual encounter, 3=other type of partner 8=don't remember	1=yes 2=no 8=don't remember
1 last sex act		
2 sex act before that		
3 sex act before that		
4 etc.		
5		
6		
7		
8		
9		
10		

Interviewer: read each of the responses to questions 7 and 8 to the participant so she can choose the one that most accurately reflects her answer

7 How confident are you that the responses you gave to the detailed questions about sex, condom and gel use in the last week are accurate?

Signature	Print name	Date

Site name: ----------PRE-PRINT----------		Date of visit: / / DD /MM / YYYY
Screening number:	Initials:	

Very confident they are accurate

Quite confident but I may have mistaken some details

Not very confident about the accuracy of my answers

Not at all confident about the accuracy of my answers

8 How often was a condom used when you had sex in the last week?

Always Most of the time

Sometimes Never → Q 12

Interviewer: only fill this table in with participants who have NOT have sex in the last 1 week but who HAVE had sex in the last 4 weeks. You should only fill in one table for each respondent: either 6 or 9

9. "***Now I am going to ask you some more detailed questions about your condom use each time you had sex in the last 4 weeks. Please answer the questions for each time you had sex.***"

Interviewer: each question (row) refers to a particular sex act. Go through all columns for the individual sex act before moving on to the next row. Write the number corresponding to the answer code for each column in the box in each cell. Remind the participant about the definition of a 'sex act' and allow enough time for the participant to carefully consider each answer.

Sex acts	**Partner**	**Condom**
Sex acts in the last 4 weeks	What **type** of partner was this act with?	Did you use a condom during this sex act?
Codes	1=long-term sexual relationship, 2=one off sexual encounter, 3=other type of partner 8=don't remember	1=yes 2=no 8=don't remember
1 last sex act		
2 sex act before that		
3 sex act before that		
4 etc.		
5		
6		
7		
8		

Signature	Print name	Date

Site name: ----------PRE-PRINT----------		Date of visit: / / DD /MM / YYYY
Screening number:	Initials:	

9		
10		

Interviewer: read each of the responses to questions 10 and 11 to the participant so she can choose the one that most accurately reflects her answer

10 How confident are you that the responses you gave to the detailed questions about sex, condom and gel use in the last 4 weeks are accurate?

Very certain they are accurate

Quite certain but I may have mistaken some details

Not very certain about the accuracy of my answers

Not at all certain about the accuracy of my answers

11 How often was a condom used when you had sex in the last 4 weeks?

Always Most of the time

Sometimes Never

12 In the last 4 weeks have you had sex whilst you were menstruating? Yes

No

Section 3: Other products and practices

"Some women insert products into their vaginas for a variety of reasons, such as cleaning inside the vagina, or drying or lubricating the vagina before sex. The next questions are about this".

13 In the last week have you inserted anything other than the study gel (excluding water/fingers) into your vagina? Yes

No → Q. 14

13a Why did you insert this other thing? To clean the vagina To lubricate the vagina

To dry the vagina Specify_____

Other _____

_____

13b If yes, how many times did you do this?

More than once per day Once per day

Less than once per day but more than once in the week Once in the week

Don't remember

Signature	Print name	Date

Site name: ----------PRE-PRINT----------		Date of visit: / / DD /MM / YYYY
Screening number:	Initials:	

13c	What time of day did you normally do this?	Morning	Afternoon	
		Evening		
13d	When in relation to sex did you normally do this?	After sex	Before sex	
			Some other time	

13e	PROBE FOR MULTIPLE ANSWERS What did you insert?	Disinfectant	Creams	
		Vaseline	Dry cloth	
		Herbs	Wet cloth	
			Lemon	
		Other	Specify:	

"Some women have anal sex. The next question refers to this practice".

Interviewer: spend some time making sure the participant understands what anal sex is: "penetrative anal sex that may or may not end with ejaculation".

14	Have you had anal sex in the last 4 weeks?		Yes	
			No	→ Q. 15
14a	Did you use a condom?	Always	Most of the time	
		Sometimes	Never	

Section 4: Pregnancy test and clinical symptoms

15	Has urine sample been collected?		Yes	
			No	→ Q. 15a
15a	If not, why not?		Not indicated in protocol schedule	
			Not clinically indicated	
			Not possible to obtain urine specimen	
			Other (specify_____)	

Signature	Print name	Date

Site name: ----------PRE-PRINT----------		Date of visit:
Screening number:	Initials:	/ / DD /MM / YYYY

Interviewer code

Comments

Signature	Print name	Date