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HIV INCIDENCE, SEXUAL AND REPRODUCTIVE HEALTH AMONG HIGH-RISK FEMALES RECRUITED FOR PARTICIPATION IN HIV PREVENTION TRIALS IN TANZANIA

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Cover illustration: Women seated outside brothel rooms along a passageway in Dar es Salaam.

HIV Incidence, Sexual and Reproductive Health among High-Risk Females Recruited for Participation in HIV Prevention Trials in Tanzania

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

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To our beloved son, Malcolm.

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ABSTRACT

Background: Testing and evaluating new HIV prevention products such as HIV vaccines, requires identifying, enrolling and retaining cohorts of HIV high risk individuals. This thesis describes the recruitment and follow up of high-risk females for participation in HIV prevention trials in Tanzania. Specifically, the thesis describes a cohort of female sex workers (FSWs) in Dar es Salaam recruited for participation in an upcoming HIV vaccine trial- known as PrEPVacc vaccine trial, as well as changes in sexual risky behaviours in a similarly high-risk cohort from northern Tanzania.

Methods: Between October and December 2018, FSWs aged 18–45 years were recruited using a Respondent Driven Sampling method. They were screened for eligibility and 700 of them were enrolled into the PrEPVacc registration cohort in Dar es Salaam. At screening and at 3 monthly follow-up visits, social demographics, HIV risk behavioural assessments and collection of blood samples for HIV testing were done. Qualitative interviews were conducted to explore contraceptive use. Data from a separate cohort of high-risk women from Northern Tanzania was used to explore changes in sexual behaviour and its association with HIV incidence.

Results: The baseline HIV prevalence in the PrEPVacc registration cohort was 7.6% (59/773, 95% CI; 5.8%-9.7%). There were 21 HIV seroconversions over a 12 month follow up period. The HIV incidence rate was 3.45 per 100 person-years-at-risk (PYR) (95% CI; 2.25-5.28/100 PYR). The HIV incidence rate was higher among FSWs aged 18-24 years (4.31/100PYR), those using illicit drugs (4.25/100PYR) and those diagnosed with either Syphilis or Hepatitis B/C virus (10.04/100 PYR). (**Study I**).

Nearly half (49%) of high-risk women enrolled in the cohorts from northern Tanzania did not change their sexual behaviour practices after 12 months, while 25% had higher risk practices after 12 months of follow up. The proportion of women reporting multiple partners, transactional sex and high-risk sex practices declined at each 3 months visit (33%, 43% and 47% reduction in odds per visit respectively, p for linear trend <0.001 for all). There was no evidence of an effect of change in sexual behaviour on HIV rate after adjusting for other factors (adjusted odds ratio (aOR) 0.88 95%CI 0.39-2.01, P=0.76) (**Study II**).

In the PrEPVacc registration cohort, awareness of HIV pre-exposure prophylaxis (PrEP) increased from 67% at cohort enrolment to 97% after 12 months (p<0.001). Willingness to use PrEP was high at both time points (98% vs 96% p=0.84). Only 8% (57/700) of the FSWs reported to have ever initiated PrEP use over the 12 months follow-up period. Use of PrEP was independently associated with: marital status i.e married/cohabiting (aOR) 4.19; 95%CI 1.44-12.18) or separated/divorced/widowed (aOR 2.38; 95%CI 1.17-4.83) and engaging in sex with a HIV infected partner (aOR 3.98;1.20-13.15) (**Study III**).

FSWs in the PrEPVacc registration cohort reported that sex work impedes good contraceptive behaviour because; FSWs felt unable to negotiate consistent condom use, avoided health services due to stigma, missed monthly contraceptive supplies because of inconvenient clinic operating hours and skipped contraceptive pills when intoxicated after taking alcohol. Financial hardships related to child rearing and painful abortion experiences influenced FSWs' commitment to good contraceptive behaviour (**Study IV**).

Conclusions: The high HIV incidence among FSWs in the PrEPVacc registration cohort demonstrates that this population is suitable for participation in HIV vaccine trials. The HIV incidence may decline over time because of reduction in sexual risky behaviour practices and increased PrEP uptake.

LIST OF SCIENTIFIC PAPERS

I. **Faini D*,** Msafiri F*, Munseri PJ, Bakari M, Lyamuya E, Sandström E, Biberfeld G, Nilsson C, Hanson C, Aboud S. The Prevalence, Incidence and Risk factors for HIV among Female Sex Workers – a cohort being prepared for a Phase IIb HIV Vaccine Trial in Dar es Salaam, Tanzania.
in Manuscript
**Authors contributed equally to the work*

II. **Faini D**, Hanson C, Baisley K, Kapiga S, Hayes R. Sexual behaviour, changes in sexual behaviour and associated factors among women at high risk of HIV participating in feasibility studies for prevention trials in Tanzania.
PloS one 2020 15:4 e023176

I. **Faini D,** Munseri P, Sandstrom E, Hanson C, Bakari M and the PrEPVacc Study Team. Awareness, willingness and use of HIV pre-exposure prophylaxis among female sex workers in Dar es Salaam.
In Manuscript

I. Faini D, Munseri P, Bakari M, Sandström E, Faxelid E*, Hanson C*. "I did not plan to have a baby. This is the outcome of our work": a qualitative study exploring unintended pregnancy among female sex workers
BMC women's health 2020 20:1 267.
**Authors contributed equally to the work*

CONTENTS

LIST OF ABBREVIATIONS

AIDS	Acquired Immuno Deficiency Syndrome
aOR	Adjusted Odds Ratio
aRR	Adjusted Rate Ratio
ART	Antiretroviral Therapy
AUDIT	Alcohol Use Disorders Identification Test
CI	Confidence Interval
DEFF	Design Effect
DNA	Deoxyribonucleic Acid
ELISA	Enzyme-linked Immunosorbent Assay
FSW	Female sex worker
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HIV	Human Immunodeficiency Virus
HPTN	HIV Prevention Trials Network
HSV-2	Herpes simplex virus type 2
HVTN	HIV Vaccine Trials Network
KI	Karolinska Institutet
LTFUP	Loss to follow up
MUHAS	Muhimbili University of Health and Allied Sciences
MVA	Modified vaccinia Ankara
OR	Odds Ratio
PrEP	Pre-Exposure Prophylaxis
RDS	Respondent Driven Sampling
RR	Rate ratio
Sida	Swedish International Development Agency
STI	Sexually Transmitted Infection
TAF/FTC	Tenofovir Alafenamide and Emtricitabine
TasP	Treatment as prevention
TDF/FTC	Tenofovir Disoproxil Fumarate
UNAIDS	Joint United Nations Programme on HIV/AIDS

1 INTRODUCTION

Globally, substantial success has been made in the reduction of HIV/AIDS associated morbidity and mortality, following the wide spread use of antiretroviral therapy. However, there has been slow progress in the reduction of new HIV infections. The UNAIDS goal of reducing the number of new HIV infections to 500,000 globally by 2020 was not achieved. An estimated 1.7 million people were newly infected with HIV in 2019, more than three times the UNAIDS goal (1). The sub-Saharan Africa region disproportionately continues to bear the highest global burden of the pandemic and contributed 59% of all new HIV infections in 2019. HIV infections rates are high among women living in sub-Saharan Africa and in high risk population groups which include, men who have sex with men, people who inject drugs, as well as sex workers and their clients (1). Hence highly effective HIV prevention interventions especially those that offer control to females are essential so as to reduce HIV incidence and curb the HIV epidemic.

The HIV prevention intervention profile has been evolving rapidly in the past two decades. These include use of condoms, voluntary medical male circumcision, risk reduction counselling, testing and treatment of sexually transmitted infections as well as interventions that address structural risk factors e.g., stigma. At the centre of biomedical HIV prevention interventions is the use of antiretroviral based prevention i.e. Treatment for Prevention (TasP) and Pre-Exposure Prophylaxis (PrEP). However, there are some challenges with the use of antiretroviral therapy in order to achieve HIV epidemic control (2). First, broad implementation of universal HIV testing and treatment face logistical and financial challenges (3, 4). Second, attaining population level viral suppression through universal treatment is unlikely to achieve HIV elimination at a population level because access to HIV care is often limited in HIV high-risk populations who are largely responsible for the HIV transmission (4, 5). And third, even when PrEP is accessible, uptake may be low because some of the available highly effective PrEP agents don't work or are not desired by HIV high-risk population groups because of poor adherence, stigma or side effects associated with the use of PrEP (6, 7). Therefore, the use of antiretroviral therapy as prevention will not be sufficient to reduce the number of new HIV infections (8-10). Thus, research to accelerate the development and efficacy testing of a long lasting, preventive vaccine remains a priority in controlling the HIV epidemic (6, 8, 10-12).

Of all the HIV vaccine trials conducted worldwide to date, seven have progressed to Phase III efficacy trials (8, 13-19). The RV144 Thailand study of (ALVAC-prime, VaxGen rgp120/alum boost) is the only trial ever to show modest efficacy of 31% (95% CI 1%–52%) (16). Outcomes from the HIV vaccine trials have highlighted that the identification of key immunological correlates for protection against HIV and genetic variability of the HIV virus are the key challenge for the development of a HIV vaccine (6, 20, 21). Apart from this, there is also a

challenge in identification and inclusion of populations with high HIV incidence for evaluating vaccine efficacy (21-23).

This thesis describes the epidemiological considerations in recruiting, enrolling and following up participants in cohorts being prepared for participation in HIV prevention trials. The focus of this thesis is high-risk females which includes women self-identifying as sex workers as well as those not self-identifying as sex workers even though they sell sex to supplement their income. Together these high-risk women make a suitable population of HIV prevention trials because they experience high-risk sexual exposures from multiple sexual partners. Both qualitative and quantitative research methods were utilized to answer the research questions in this thesis.

2 LITERATURE REVIEW

2.1 HIV EPIDEMIOLOGY

2.1.1 The global burden of HIV

The global HIV trend shows that the pandemic is declining but the disease burden is still high. Although the HIV associated mortality and morbidity are declining, the number of new HIV infections is still high globally. In 2019, an estimated 38 million people were living with HIV worldwide and about 4500 people were newly infected with HIV every day (1).

The burden of HIV is disproportionately distributed by geographical location, gender and in high-risk population groups. Eastern and southern Africa contributes the highest burden with 20.7 million people living with HIV in the region. Women and girls in sub-Saharan Africa accounted for 59% of all new HIV infections in the region in 2019 (1). This may reflect that the HIV prevention interventions have disproportionately not reached this population or that the available interventions have not worked.

Globally, 62% of all new HIV infections in adults occur among most-at risk populations also known as key populations (1). HIV Key populations include adolescent girls and young women, men who have sex with men, sex workers and people who inject drugs. Female sex workers have the highest HIV infection rate attributable to heterosexual transmission. A meta-analysis that included studies published between 2007 and 2011 from 50 low-and middle income countries reported an overall HIV prevalence among female sex workers to be 12% (24). In this meta-analysis, the pooled odds ratio for HIV infection among female sex workers compared to women of reproductive age was 13.5 (24). Another meta-analysis that included data between 2006-2017 reported that female sex workers living in Eastern and Southern Africa have the highest HIV prevalence estimated at 33.3% (5). As of 2019, the UNAIDS estimated HIV prevalence among female sex workers in this region to range from 10.4% in Eritrea, 33% in Uganda to 57.7% in South Africa (1). **Figure 1** shows a map of the world with the 2019 UNAIDS estimates of HIV prevalence among female sex workers.

Encouragingly, the number of new HIV infections has been decreasing globally over the past decade. This decline of new HIV infections has been attributed to the 38% reduction in new HIV infection among women in southern Africa. This reduction is higher than the global average of 28% reduction in new HIV infections (1). Therefore, controlling the heterosexual HIV transmission in women in southern Africa especially among female sex workers is the key to curb the HIV pandemic as this population account for the largest global HIV burden.

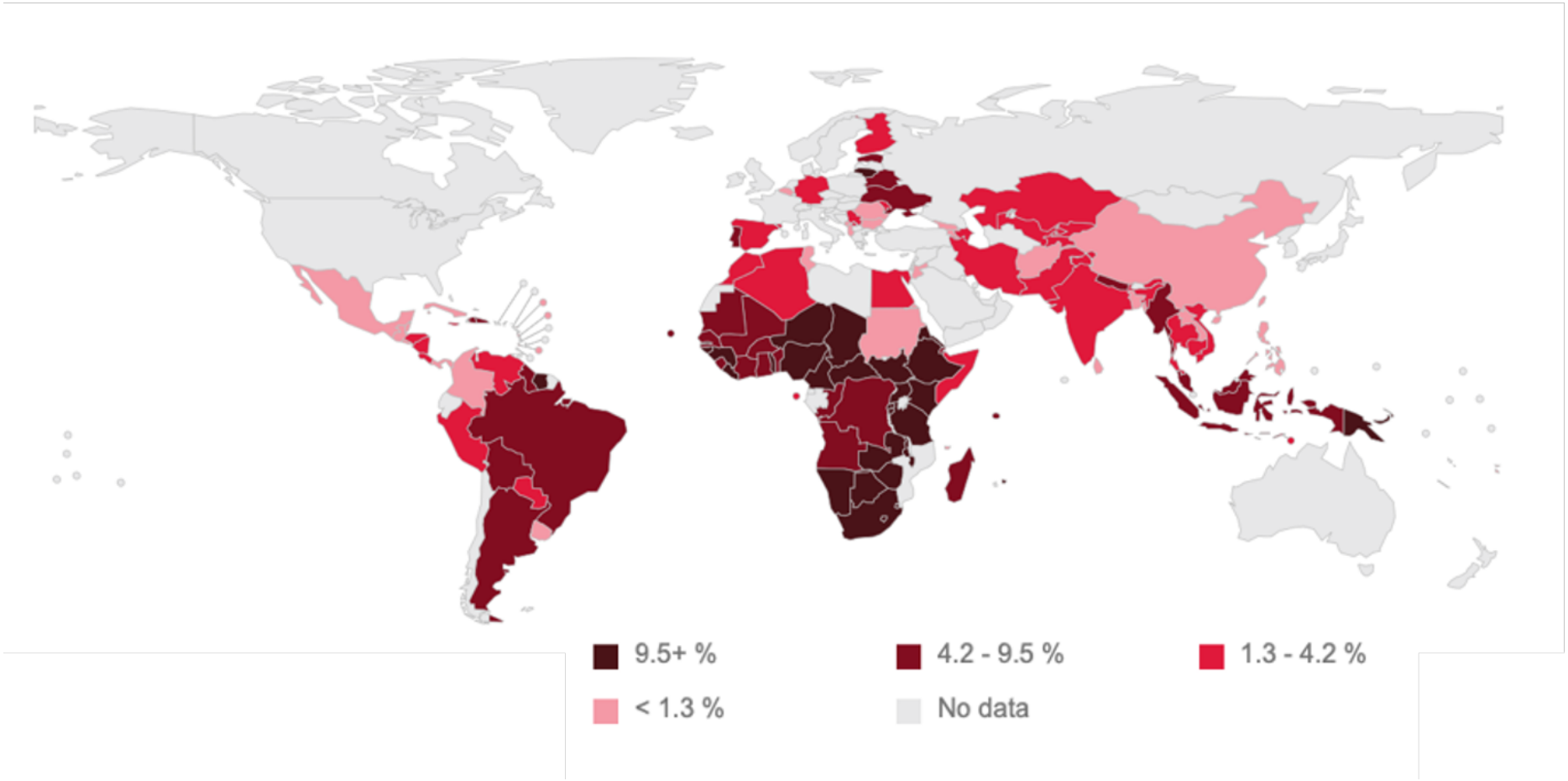


Figure 1 Estimates of HIV prevalence among female sex workers in 2019.

Source UNAIDS: https://aidsinfo.unaids.org

2.1.2 The epidemiology of HIV in Tanzania

The overall HIV epidemic in Tanzania has had an encouraging downward trend in the last decade, following the wider national coverage of antiretroviral treatment programmes as well as other interventions. The HIV prevalence among adults aged 15 to 49 years has decreased from 5.7% in 2007/2008 to 4.8% in 2016/2017 (25, 26). However, the declining trend has disproportionately been less among women compared to men within the same age groups. For instance, HIV prevalence declined from 4.6% to 3.1% among men and from 6.6% to 6.4% among women between 2007/2008 and 2016/2017. Although the increased life expectancy attributed to the wide availability of antiretroviral therapy may explain the small decline in the HIV prevalence across the years, higher HIV infection rate among females may account for the disproportionate and persistent higher HIV prevalence among women.

In the year 2019, there were 1,700,000 people living with HIV in Tanzania and an estimated 69,000 people were newly infected with HIV (27). The HIV incidence rate in the adult population is estimated to be 1.27 infections per 1000 people (27). This incidence rate is above the goal of less than 1 per 1,000 adults per year needed to attain HIV epidemic control in the country (28-30). However, Tanzania is among countries to have nearly achieved the 2020 UNAIDS 90-90-90 goal. As of 2019, 83% of Tanzanian living with HIV were aware of their HIV status, 75% of those aware of their status were on antiretroviral therapy and 69% of those on treatment were virally suppressed (27).

The HIV epidemic in Tanzania is concentrated in certain regions where the prevalence of HIV infection is higher. For example, the prevalence of HIV infection varies from 0.3% in Lindi region to 11.4% in Njombe region (25). The high HIV prevalence in Njombe region is largely caused by the sex-trade along the Tanzania-Zambia highway, a major long-distance truck route. HIV prevalence is also high in the Lake zone regions (e.g., Mwanza 7.2%, Geita 5%) because of the multiple and concurrent sexual partnerships among the fisher-folk community (31). The HIV prevalence in Dar es salaam, the business capital of Tanzania is close to the national average (4.3% versus 4.7%). The HIV prevalence in Dar es Salaam is of public health interest in the national fight against HIV because the city is densely populated and has the largest number of HIV high-risk population group in the country (32, 33). **Figure 2** shows a map of Tanzania with HIV prevalence across different regions.

Other than geographical variation, HIV prevalence in Tanzania is higher among key population groups. Key population groups in Tanzania include, sex workers, men who have sex with men and people who inject drugs. HIV prevalence among the high-risk groups in different parts of the country is estimated to range from 8% to 42% among people who inject drugs (27, 34, 35), 15% to 41% among female sex workers (36-38) and 16% to 31% among men who have sex

with men (39-42). A study conducted in five regions in Tanzania estimated that 5.6% of females and 1.3% of males aged 15-49 years are female sex workers and men-who -have sex with men respectively (33). Moreover, there is evidence indicating an overlap of sexual behaviours between members of the key population groups and the overall general population. Studies indicate that at least half of men who have sex with men in Tanzania have concurrent female sexual partners (39, 42)

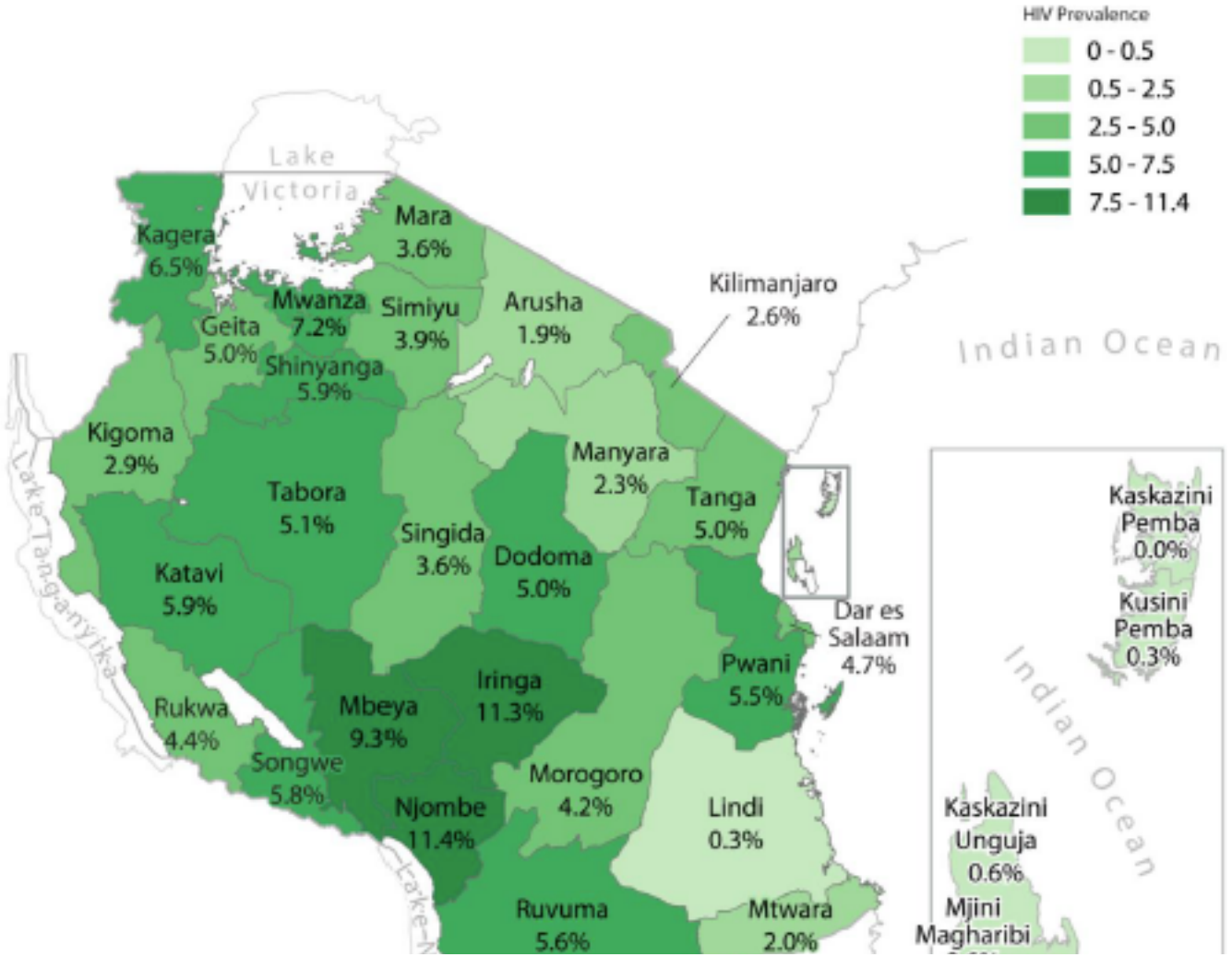


Figure 2 HIV prevalence among adults aged 15 years and older, by region in Tanzania

Source: Tanzania HIV Impact Survey 2016-2017

2.1.2.1 *Sex work context in Tanzania*

Sex work, generally defined as exchange of sex for money, goods or other favours (43, 44), is diverse and operates in various contexts. There are many types of sex work but generally divided into "direct" and "indirect" sex work (43). "Direct" sex workers are those whose primary mode of income is through exchanging sex for a fee. "Indirect" sex workers sell sex to supplement an income from another lowly paying occupation and many do not self-identify as a sex worker. A qualitative study of female sex workers in Tanzania found that there were four main forms of commercial sex reported: (a) *waziwazi* (open or frank) prostitution in which women work from their own rooms within a community, (b) employed women supplementing their salaries, (c) brothel girls, and (d) streetwalkers (45). There is also a great of variety in the social context for selling sex. In Tanzania, sex work based in entertainment places (bars and/or night clubs) is most dominant (33). Sex work in lodges and on the streets is also common. While some sell sex through formal organized groups (in brothels), others work independently and others solicit sex through mobile phones. Women working in bars, guesthouses and recreational facilities (bar maids) who supplement their income by offering sex in return for money or gifts, form a subset of high-risk women in the country (46). These female bar maids often act as indirect sex workers but may not self-identify as a sex worker in contrast to bar based female sex workers (46-54).

On the other hand, female sex workers have different kind of partnerships. Partners may include (a) regular clients - someone who pays to have sex with the sex-worker on a regular basis, i.e., daily, weekly, monthly (b) non-regular/ casual partner with whom only a sex worker has a single sexual encounter or several sexual encounters over a short period of time, without the expectation of a relationship (c) husband, boyfriend, or steady partner who is someone that the female sex worker regularly has sex with, without receiving any cash payments (d) transactional partner with whom sex is exchanged for a favour but not for money e.g, a partner who may buy her drinks at a bar, a *bodaboda* driver who gives her a ride or a watch guard who alerts her on police raids.

Sex work is criminalized according to the Tanzanian Penal Code (55). Selling and buying of sex and sex-work related activities such as facilitating sales, brothel ownership or pimping are considered illegal Arrest, police raids and extortion of sex workers are not uncommon in Tanzania. Nevertheless, the Tanzanian National HIV Strategic Plan recognizes the need to reach out to sex workers and provide sexual reproductive health and HIV services (56).

2.2 DETERMINANTS OF HIV RISK AMONG FEMALE SEX WORKERS

The heightened risk for HIV acquisition among female sex workers is driven by variety of behavioural, structural and biomedical factors (5, 7, 24, 57-59).

2.2.1 Behavioural risk factors

Female sex workers experience high HIV risk exposure through the high number of sexual partners. The HIV risk is increased because some of the sexual partners may be HIV infected and not virally suppressed. The report of number, type and time frame of female sex workers' partners in literature is highly varied. In Tanzania, more than half of female sex workers reported having two to four sexual partners on the last day they worked (36, 54) and an average of five partners per week (37, 60) . However, it is important to note that female sex workers engage in sex work intermittently and that they occasionally take break from sex work (7).

The use of condoms among female sex workers is low and highly dependent on the type of sexual partner. Consistent condom use is generally higher with paying regular or non-regular/ casual partners as compared to steady/intimate partners (36, 45, 61-63). Condom use at last sex is often used as a proxy measure for consistent condom use because of the recall bias and over-reporting of condom use in studies (64). Estimate of condom use at last sex among female sex workers is estimated to be 80% globally (1) and 72% in Tanzania(27, 36).

Many female sex workers are unable to consistently use condoms because of client refusals. Clients may be forceful and sexually assault the sex worker or may offer higher pay to forgo condom use (37, 58, 65). Inconsistent condom use is also particularly high among younger female sex workers (44, 66), probably because of undeveloped condom negotiating skills or greater financial vulnerability. In surveys among female sex workers in Tanzania, consistent condom use was reported by 16%-74% of the sex workers (36, 37, 60).

Substance use including hazardous alcohol drinking and illicit drug use are common among female sex workers. In Tanzania, 76% of female sex workers surveyed who use alcohol, reported having consumed alcohol while conducting sex work (36). Use of substance among female sex workers heighten their risk-taking behaviour, reduce their ability to negotiate and correctly use condoms and is associated with increased sexual and physical abuse (67, 68).

2.2.2 Biomedical risk factors

The high prevalence of sexually transmitted infections among female sex workers and the synergistic relation between HIV infection and other sexually transmitted infections compounds the HIV risk in this population (69). In Tanzania, high rates of sexually transmitted infections have been reported among high-risk women including female sex workers working

in major cities and in towns around major transit routes. These include Herpes simplex virus type 2, Chlamydia infection, bacterial vaginosis and genital ulcers with reported hazard ratio (adjusted) for HIV incidence of up to 4.3, 5.2, 2.1 and 2.7 respectively (47, 48, 70, 71).

Female sex workers who also use injectable illicit drugs have a higher risk of HIV infection. This is because of the increased parenteral HIV exposure from shared injection equipment. Female sex workers who are intoxicated after injecting drugs are also more likely to engage in high-risk sex and less likely to use condoms (35, 72). Needle sharing and unprotected sex practices further increases their risk to Hepatitis C virus infection as well as other sexually transmitted infections.

2.2.3 Structural risk factors

Structural factors play a central part in HIV epidemic among sex-workers. There is an overall consensus in the literature from the past decade suggesting that even though high-risk sexual behaviours play a significant role in increasing new HIV infection in this population, interventions targeting only the behavioural and biomedical risk factors have had only a modest effect because structural factors underpin the context in which these behavioural and biomedical risks operate. Structural factors include poverty, stigma, human rights violation, gender inequality, violence against women, criminalization of sex work, inappropriate legislation and lack of political will to ensure access to preventive health, HIV services (7, 11, 24, 73-75).

In a systematic review of structural risk factors for HIV infection among female sex workers globally, Shannon and colleagues demonstrated the interrelationship of structural factors with behavioural and biological factors to influence HIV risk (57). The authors showed criminalization, sex work related stigma, gender-based violence and poverty were associated with increased inconsistent condom use and HIV infection, while seasonal migration to areas of high burden increased their HIV vulnerability. A study conducted in Iringa Tanzania reported the impact of stigma, discrimination and violence among female sex workers on impeding engagement of female sex workers with HIV services (37). Similarly, qualitative studies done in Iringa Tanzania have reported stigma against sex workers seeking ante-natal services (76, 77).

Mobility is also a structural determinant of HIV among female sex workers as it can limit their access to and extent of using HIV services when they move across several locations. Female sex workers mobility can also have an impact on HIV epidemiology depending on the local epidemics of the settings they moved into. Female sex workers commonly move to another location in search of seasonal-sex trade opportunities or access a wider potential market for

clients in case of festivals. For example, female sex workers participating in a qualitative study in Northern Tanzania reported travelling away from their usual work locations for periods of one week to a month for better pay, some travelling to higher HIV prevalence setting such as fishing sites, mining or highways (31, 48). Indeed, in that the study, female sex workers reported that most men at the fishing sites preferred and paid higher for condom-less sex and therefore increase risk of HIV transmission.

2.3 CONTRACEPTIVE USE AND UNINTENDED PREGNANCY AMONG FEMALE SEX WORKERS

Access to reproductive healthcare including contraceptives, is an essential component of the comprehensive HIV prevention services for female sex workers. Nevertheless, several studies have reported a large unmet contraceptive need in this population (60, 76, 78). This unmet need has been evident from the high rates of unintended pregnancy in this population (79-83), high abortion rates (79, 81, 83, 84) and the expressed desire to prevent future pregnancies (77, 85). Access to family planning offers an opportunity for female sex workers to harness other HIV interventions including PrEP, condoms, risk reduction counselling, diagnosis and treatment of sexually transmitted infections.

2.3.1 Contraceptive use among female sex workers

The use of dual methods of contraception i. e condoms together with a modern non-barrier contraceptive method among female sex workers living in Dar es Salaam is very low, at 5.3% (60). This is lower than the 40% reported in other cities in sub-Saharan Africa (86, 87). Among female sex workers, the use of injectable and oral contraceptive pills methods is much more prevalent as compared to the use of long-acting reversible contraceptives such as Intra-uterine devices and Implants (88, 89). Although all the contraceptive methods are effective with correct use, dual contraceptive use with long-acting reversible contraceptives are advocated for female sex workers (90). This is because of their adherence advantage as they are less prone to incorrect use, discontinuation or frequent switching (12, 80, 87).

2.3.2 Unintended pregnancy among female sex workers

Incidence of pregnancy among HIV high risk females is reported to range from 12-18 per 100 person-years of exposure in Tanzania (52, 91) and up to 20 per 100 person years of exposure in other African settings (92, 93). Most of the pregnancy among female sex workers are reported to be unintended or unplanned. In a study among female sex workers in Uganda, 65% reported their pregnancy to have been unplanned (82). Factors associated with unintended pregnancy among female sex workers include being impregnant by a casual (non-emotional) partner, experiences of rape and substance abuse (78, 82, 83).

Unintended pregnancy among female sex workers is of public health concern because of the economic and social implications which may intensify the HIV risk associated with sex work. For instance, because of the financial burden associated with child rearing expenses, money

becomes a powerful incentive for forgoing condom use or engaging in other forms of high-risk sex (77, 94, 95). Also, unintended pregnancy that ends up in abortion contribute to the maternal mortality burden (96).

Thus, unintended pregnancy among female sex worker can be a proxy measure for poor sexual health as it may reveal lack of access to contraceptive services. It may also reflect challenges in adhering to the available contraceptive methods e.g., failure to negotiate consistent condom use and failure to adhere to daily pill taking. These challenges are explored and discussed in this thesis. A reflection on how existing barriers to contraceptive use among female sex workers and how these can inform better ways to deliver services in this population is provided in the later sections of this thesis.

2.4 BIOMEDICAL HIV PREVENTION INTERVENTIONS

Table 1 provides a summary of the available biomedical HIV prevention interventions, evidence for their efficacy and status of implementation where applicable. Globally, the portfolio of biomedical HIV prevention interventions is rapidly changing (6, 97). Biomedical HIV prevention interventions include; male and female condoms, medical male circumcision, testing and treatment of sexually transmitted infections, immediate initiation of antiretrovirals among infected persons so as to reduce their infectiousness (Treatment as Prevention, also known as TasP), the use of antiretrovirals among uninfected individuals to prevent acquiring infection (Pre-Exposure Prophylaxis, also known as PrEP) as well as use of antiretrovirals following inadvertent HIV exposure (Post exposure Prophylaxis, also known as PEP). The use of antiretroviral drugs as PrEP can either be as oral tablets, long acting injectables, vaginal rings, vaginal or rectal gels. Of all the available HIV prevention interventions, this section will mainly focus on PrEP and HIV vaccine products as they are relevant in this PhD thesis.

The use of daily oral PrEP pill consisting of tenofovir disoproxil fumarate in combination with emtricitabine (also known as Truvada©,) has been shown to be safe and efficacious for HIV prevention in both men and women (98-103). Efficacy trials which included diverse high-risk populations (including female sex workers) have found PrEP to reduce HIV incidence by over 90% when used consistently (100-102, 104-106). Following these evidence, in 2015 the World Health Organization recommended Truvada to be used among populations with an HIV incidence greater than 3 per 100 person years of observation (107). Another tenofovir based antiretroviral therapy that has been recently approved for use as an oral PrEP is Descovy©, which contains emtricitabine and tenofovir alafenamide. Recent efficacy trials (*The Discover Trial*) showed that the use of Descovy was non inferior to Truvada and that Descovy had less renal and bone toxicity compared to Truvada (108). However, Descovy has not been sufficiently assessed among heterosexual females living in sub-Saharan Africa. Descovy would be tested for the first time as part of the PrEPVacc vaccine trial which is described in this thesis.

Apart from the daily oral PrEP formulation, there are other long-acting PrEP products available or under research. Dapivirine vaginal ring is an antiretroviral based PrEP that has been found to be safe, efficacious and acceptable in two phase III trials. This is a silicon vaginal ring that is worn for a month and contains the dapivirine antiretroviral drug. The use of the dapivirine ring was found to reduce HIV infection risk by 30% to 91% depending on adherence (109-112). In 2020, the use of the dapivirine vaginal ring was recommended by European Medicine Agency and the World Health Organization for use among women aged 18 and older particularly in southern Africa. Other long-acting PrEP products includes a 8 -weekly injectable Caboltegavir which has been shown to be safe and effective in a Phase III trial (113-116). Islatravir is a long acting PrEP which has completed phase II trials assessing its use as a monthly oral tablet and trials are ongoing assessing its use as an yearly implant (117).

There are three ongoing HIV vaccine efficacy trials evaluating vaccine immunogens designed elicit protective responses against global HIV-1 subtypes. The *Imbokodo* study (HVTN 705) is a Phase IIb vaccine efficacy trial evaluating the safety and efficacu of a prime regimen consisting of Ad26.Mos4.HIV and aluminium-phosphate adjuvanted Clade C gp 140 in reducing HIV incidence among women at high HIV risk in Southern (118). The *Mosaico* study (HVTN 706) is a Phase III vaccine efficacy trial that evaluates the same immunogens (used in the *Imbokodo* study) among men having sex with men and transgender individuals in America and Europe (119). The third trial is the *PrEPVacc trial* a Phase IIb which aims to compare two HIV vaccine products DNA-HIV-PT123+ AIDSVAX and DNA, modified vaccinia Ankara Virus and envelope protein plus adjuvant alongside two pre-exposure prophylaxis (Descovy and Truvada) (120). The major part of this thesis will discuss the preparation of a cohort of high-risk females recruited for participation in the PrEPVacc vaccine trial at Dar -es Salaam site.

In summary, by the end of the year 2020, there were several ongoing efficacy trials evaluating biomedical HIV prevention products. These include HIV vaccine products, passively infused monoclonal antibodies and antiretroviral based PrEP in different formulations i.e. oral pill, injectables, vaginal ring and implants. **Figure 3** provides a summary of some of the ongoing efficacy trials of biomedical products for HIV prevention. The level of efficacy varies and the evidence for these products is constantly evolving. In these studies, the trial design includes combining a previous established efficacious biomedical product (such as Truvada as an oral PrEP) with new or partially effective product. This combination is ethically required as a standard prevention package so as to minimize HIV risk among study volunteers. As a result, designs of HIV vaccine trials include an active control arm whereby participants receive a high efficacious PrEP agent instead of a placebo. This design results in reduction of HIV incidence events and hence lower the statistical power of the study needed to detect efficacy of the vaccine product. This thesis will highlight some of the problems and methodological considerations in the current designs of HIV vaccine trials in this era of available highly effective PrEP.

Table 1 Summary of HIV biomedical intervention and supporting evidence

Intervention	Evidence of efficacy
Male condoms	Up to 80% efficacy when used correctly and consistently (121).
Female condoms	No data from HIV efficacy trials
Testing and treatment of other sexually transmitted Infections	There is strong evidence from observation studies on an increased risk of HIV in the presence of a sexual transmitted infection(122); However there is lack of evidence from randomized clinical trials on the effect of treatment of sexually transmitted infections in reducing the risk of HIV infection (9, 123, 124).
Post Exposure prophylaxis	Effectiveness has been established from observational studies and are supported by studies in non-human primates (125). Randomized clinical trials in humans are unethical.
Treatment as prevention (TasP)	96% protective efficacy among sero-discordant partners (126). Mixed trial results on the effect of "universal test and treat" strategy in reducing HIV incidence at population level (2, 3, 127, 128).
Antiretrovirals drugs as pre-exposure prophylaxis (PrEP)	
Tenofovir based oral daily PrEP	Up to ~96% efficacy when used consistently (100, 104-106). Recommended for use in high-risk populations
Tenofovir 1% topical vaginal gel	2% to 54% efficacy depending on adherence (105, 129, 130). No subsequent trials or licensure in progress.
Dapivirine Vaginal ring (Long-acting PrEP)	30%-92% efficacy depending on adherence (109-112). Recommended for women 18 years and older.
Caboltegravir Injectable (Long-acting PrEP)	66% -89% more effective than Truvada in preventing HIV infection (113, 114).
HIV vaccine candidates	
ALVAC-HIV + AIDSVAX subtype B/E gp120/alum vaccine regimen	HVTN 702 (RV144 Thai Trial) Estimated vaccine efficacy of 61% through 12 months and vaccine efficacy of 31% through 42 months (16, 131).The modified vaccine regime for the subtype C epidemic in southern Africa was stopped in 2020 because non efficacy.
Broad Neutralizing antibodies (bNab)	A proof of concept trial as demonstrated that long term infusion with broad neutralizing antibodies (VRCO1) is safe, tolerable and prevents HIV acquisition (132, 133).

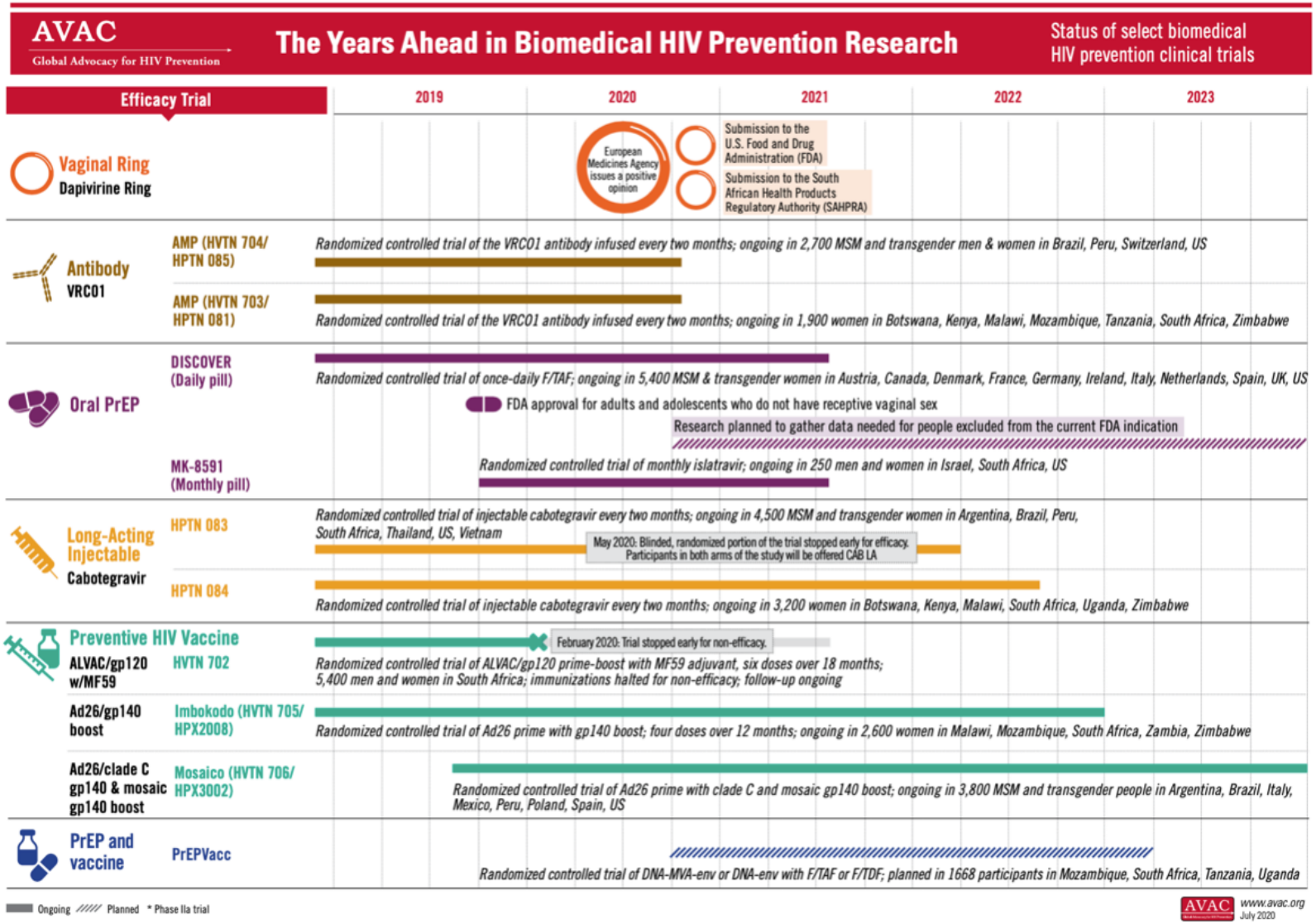


Figure 3 Summary of biomedical HIV prevention clinical trials as of November 2020

Source: AVAC: Global Advocacy for HIV Prevention
https://www.avac.org/infographic/years-ahead-hiv-prevention-research

2.5 HIV VACCINE TRIALS IN TANZANIA: LESSONS LEARNT AND KNOWLEDGE GAPS

Tanzania began to prepare for the conduct of HIV vaccine trials in the early 1990s. Preparation included recruitment and assessment of a police officers' cohort for participation in HIV vaccine trials (134). Laboratory infrastructure including virological and immunological capacity for evaluating vaccine immunogenicity were established at the Muhimbili University of Health and Allied Science (MUHAS) with support from the Swedish International Development Agency (Sida). This was done together with the training of clinical researchers, laboratory scientists, pharmacists and socio-behavioural scientists. Over the years, the MUHAS HIV Vaccine Clinical Trial Unit was established as a research site for the conduct of HIV vaccine trials.

A total of five Phase I/II HIV vaccine trials assessing safety and immunogenicity of vaccine products have been conducted at the MUHAS HIV Vaccine Clinical Trial Unit between 2007 and 2015. These include HIVIS-03, HIVIS-06, TaMoVac I, TamoVac I+gp140 and TaMoVac II (135-142).

The HIVIS-03 study was a Phase I/II randomized, double-blinded placebo- controlled clinical trial conducted from 2007 to 2010. The study enrolled 60 healthy, HIV uninfected adults. Participants were randomised into three arms to receive either three doses of a multiclade, multigene HIV-1 DNA plasmid vaccine intradermally (low dose), intramuscularly (higher dose) or a placebo followed by two boosts of a heterologous HIV-1, modified vaccinia virus Ankara (MVA) boost. The study found that priming with a low intradermal dose elicited a higher and broader cell-mediated immune response as compared to the higher priming dose given intramuscularly (136).

The HIVS-06 study was a Phase II study conducted from March to May 2012. The study intended to explore the durability of the immune response from the second HIV-1 MVA boost received in HIVIS-03 and the effect of a late, third boost given three years later. In the trial, twenty volunteers from HIVIS-03 who had received the HIV-DNA prime/HIV-MVA boost regime described above were enrolled. They were given a third boost of HIV-1 MVA, three years after the last boost had been received. The study found that potent antibody and cellular immune responses persisted for three years in 74% of vaccinees. It also found that the third dose of HIV-MVA significantly boosted both antibody and cellular immune responses to a similar level as that achieved after the recipient of a second HIV-MVA dose (141).

The TaMoVac I study was a Phase IIa randomized, placebo controlled doubled blind trial conducted from 2008-2012. The study enrolled 129 participants who included police officers and prison services officers. The trial explored whether HIV-DNA priming can be simplified by reducing the number of injections and compared the administration of a combined versus separate plasmid pools. Participants were randomized to three arms to receive either 5 injections of HIV-DNA (1000 μg total dose of 3 Env and 2 Gag encoding plasmids) or two simplified regimes of 2 injections of HIV-DNA (600 μg total dose of Env- and Gag-encoding plasmids) administered separately or combined. The study found that the simplified regime comprising of 2 injections of low HIV-DNA dose of combined HIV-DNA plasmids was as efficient as the standard regime of 5 injections which had a total dose of 1000 μg (137).

The TaMoVac I+gp140 was conducted in 2012 whereby, forty volunteers (thirty-five vaccinees and five placebo recipients) from the TaMoVac I study were given two boosts of a subtype C CN54rgp140 envelope protein adjuvanted in glucopyranosyl lipid. The study intended to evaluate the safety and impact of the boost given after 30 to 71 weeks after the last HIV-MVA vaccination. The study found enhanced binding antibody responses and Env-specific cell mediated immune response among vaccinees but there was no increase in the antibody-dependent cellular cytotoxicity (140).

The TaMoVac II included 191 healthy, HIV uninfected vaccine recipients and was conducted between 2012 and 2015. The trial aimed to assess safety and immunogenicity of DNA priming administered using an electroporation and whether this method would reduce the number of vaccination doses needed to achieve immune response. Participants were first randomized to two arms to receive a HIV- DNA prime with or without an intradermal electroporation. They were concurrently randomised to receive HIV-MVA boost given together with or without the subtype C CN54rgp140 envelope protein adjuvanted in glucopyranosyl lipid. The study found that the intradermal electroporation increased DNA induced Gag response but had no impact on the Env-specific responses. There was enhanced antibody responses among those who received HIV-MVA with rgp140/ glucopyranosyl lipid A (139).

In summary, findings from the HIV vaccine trials conducted at MUHAS showed that the DNA prime and the MVA boosting strategy was safe and highly immunogenic. The vaccines were further optimized by simplifying the DNA regimen, applying novel delivery methods and adopting better boosting strategies.

A side from the clinical trials, auxiliary social-behavioural studies which were conducted during the trials showed that there was high willingness among individuals to participate in

HIV vaccine trials. Majority of the participants were motivated by altruistic reasons of being part of HIV vaccine development while others participated because they believed that the trial would offer protection against HIV infection (143-145). Reasons for declining participation included personal fears and negative influences by significant others including sexual partners, friends and immediate family members (145-147).

Another notable experience from the HIVIS-03 trial, was the low recruitment and poor retention of female volunteers (148). This was because the study required that a volunteer should not conceive during the entire study duration. During the study, four volunteers (4.5% of all screened) became pregnant and had to exit the study. Based on this experience, this thesis will discuss on facilitator and barriers to contraceptive use among female sex workers enrolled in a registration cohort being prepared for participation in the PrEPVacc vaccine trial.

Through all the HIV vaccine trials, Tanzania has had a platform to train laboratory and clinical research personnel to conduct advanced HIV vaccine trials. The laboratory capacity built over time has accorded MUHAS the infrastructure to conduct all necessary laboratory analyses needed to support Phase II HIV vaccine trial locally. The MUHAS HIV vaccine clinical trial unit established during these trials will be used to conduct the PrEPVacc vaccine trial discussed in this thesis.

3 AIMS AND OBJECTIVES OF THE THESIS

3.1 AIM

The overall aim of this thesis was to determine the feasibility of recruiting female sex workers for participation in HIV vaccine trials. The PhD studies intended to determine the HIV incidence and associated behavioural risk factors for HIV acquisition as well as to assess reproductive health needs among HIV high-risk females participating in HIV preventions trials in Tanzania.

3.2 SPECIFIC OBJECTIVES

1. To estimate the HIV prevalence, the one-year HIV incidence and factors associated with HIV sero conversion among female sex workers in Dar es Salaam, Tanzania.
2. To describe the changes in sexual behaviours and factors associated with risky behaviour changes after one-year of follow-up among women enrolled in HIV prevention trials in Northern Tanzania.
3. To assess awareness, willingness and use of HIV pre-exposure prophylaxis among female sex workers in Dar es Salaam, Tanzania.
4. To explore experiences of unintended pregnancy and contraceptive use among female sex workers in Dar es Salaam, Tanzania.

4 THESIS FRAMEWORK

The conceptual framework for this thesis addresses research questions in two key areas—*HIV risk* and *HIV prevention interventions* among female sex workers (**Figure 4 and 5).** The research questions, interpretation of results and description of ethical considerations are discussed with reference to the three levels of HIV risk among female sex workers—behavioural, biomedical/biological and structural risks. On the other hand, the thesis discussed behavioural, biomedical and structural HIV prevention interventions among female sex workers, although an emphasis is made on biomedical HIV prevention interventions.

The research studies in this thesis explored the heightened risk of HIV acquisition among female sex workers that is driven by a combination of behavioural, biomedical and structural risk factors which transcend those for the general population (7, 24, 57). Data collected across the studies included variables from all three risk levels. The research studies also explored behavioural, biomedical and structural HIV prevention interventions (58, 149). The HIV prevention interventions for female sex workers discussed in this thesis include; use of antiretroviral based for HIV pre-exposure prophylaxis (PrEP), screening and treatment of sexually transmitted infections (as biomedical intervention), consistent condom use, risk reduction counselling and HIV testing (as behavioural intervention) and access to sex-worker friendly reproductive health services (as structural intervention).

Paper I is the central paper that provides an estimate of the HIV burden among female sex workers (prevalence and incidence) and associated behavioural, biomedical/biological and structural risk factors. The paper explored the contribution of behavioural factors (e.g., number of sexual partners), biomedical factors (e.g., co infection with sexually transmitted infections) and structural factors (e.g., sexual violence).

Paper II is linked to the HIV behavioural risk factors and the behavioural interventions. This paper assessed sexual risky behaviour practices and explored if reduction in risky behaviours occurs over time among women receiving risk-reduction counselling. It further explored if the reduction in risk behaviours is associated with a reduction in the HIV incidence.

Paper III explored PrEP as a biomedical intervention. The paper explored PrEP awareness, willingness and use among female sex workers. It also explored determinants of PrEP use in this population.

Finally, Paper IV mainly explored structural risk factors that influence sex workers' capacity to choose and use products to protect against unintended pregnancy as well as sexually transmitted infection including HIV. This paper explored how factors such as sex-work stigma in health care settings impede access to contraceptives and how sex workers fail to negotiate consistent condom use because of gendered-power imbalance.

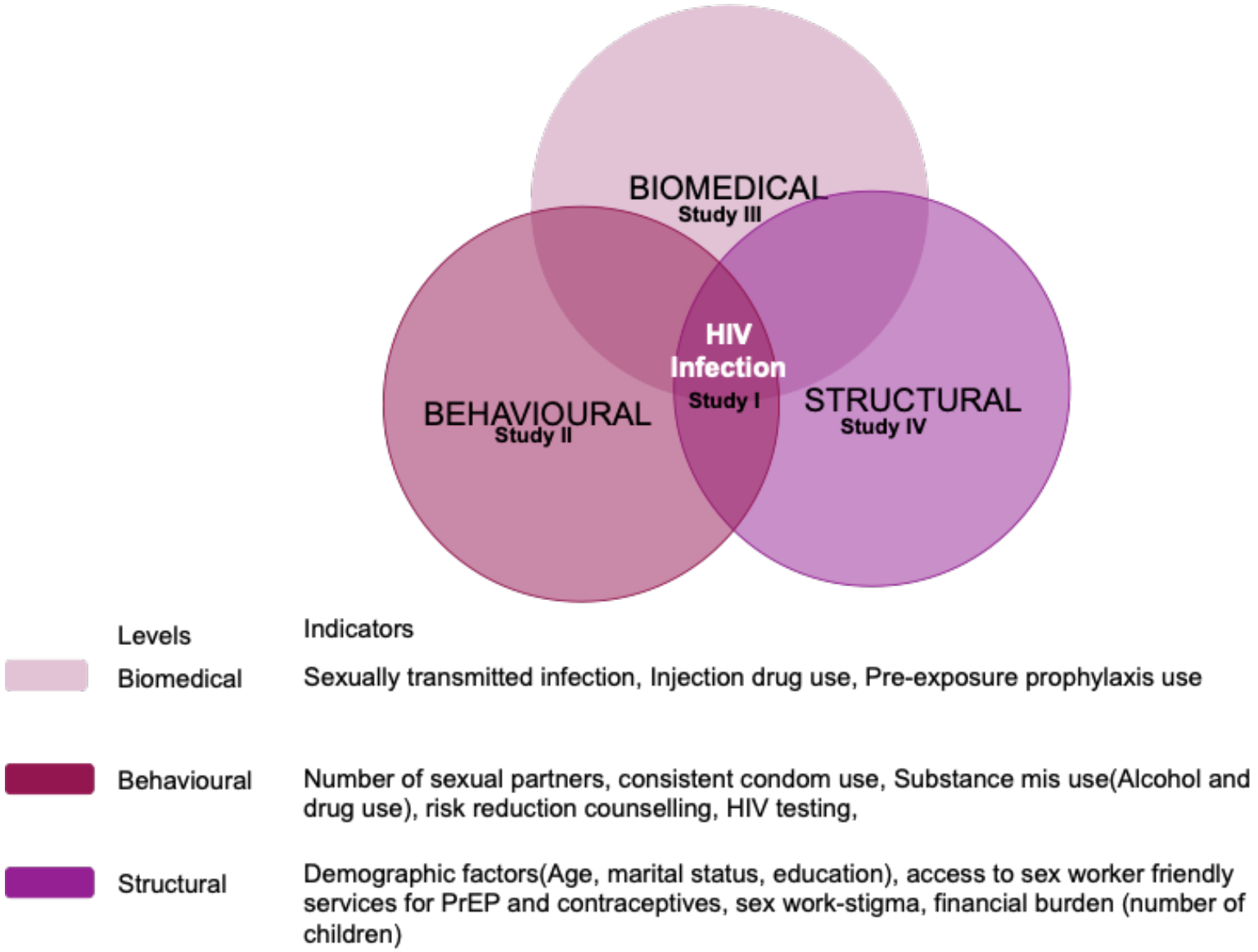


Figure 4 Thesis framework linking PhD studies to different domains of HIV risks and HIV prevention interventions.

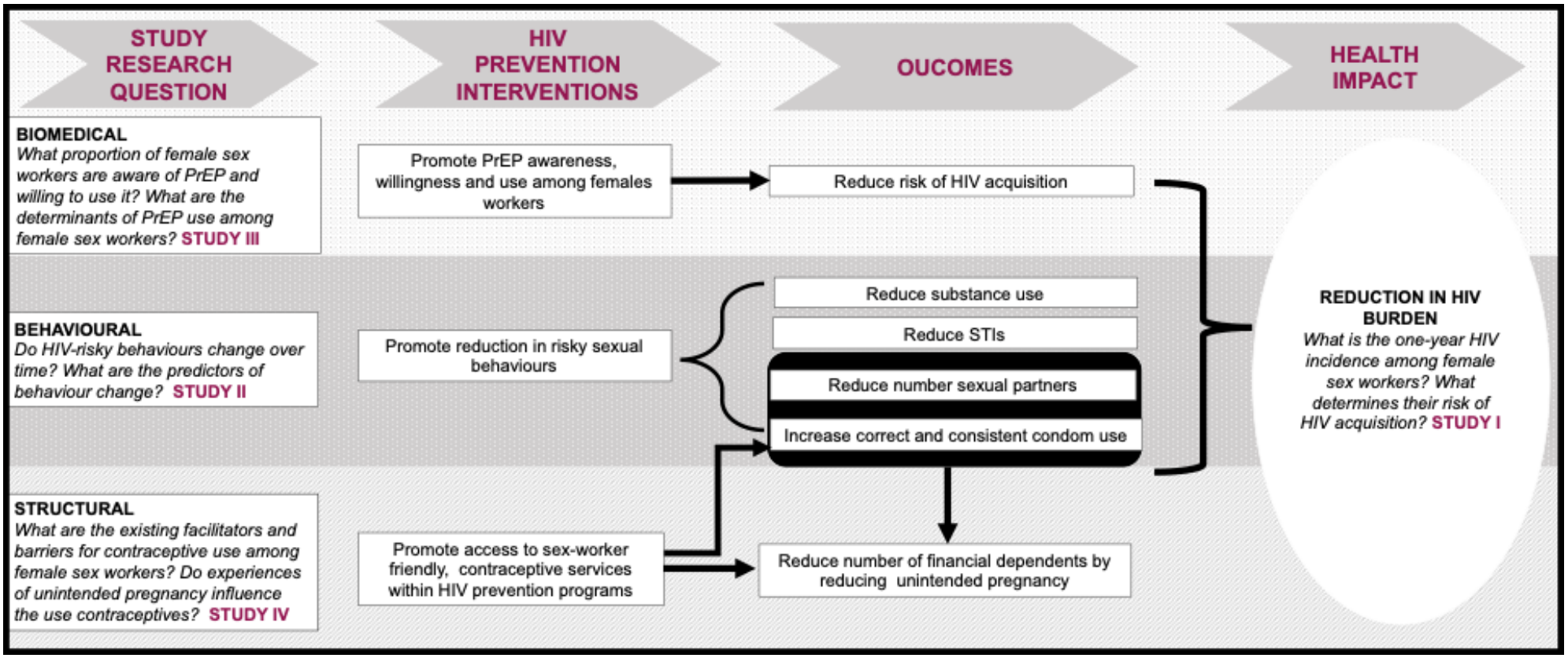


Figure 5 Thesis framework liking the study research questions to the levels of HIV prevention interventions among female sex workers

5 MATERIALS AND METHODS

5.1 OVERVIEW

The PhD research studies included in this thesis utilized both qualitative and quantitative research methods. **Figure 6** below provides a summary of the research objectives, study populations, study design and sample size used for each paper. The study populations for the PhD studies included HIV high-risk females enrolled in cohorts being prepared for participation in HIV prevention trials in different parts of the country. Study I, III and IV were conducted among female sex workers recruited between 2018-2020 into the PrEPVacc registration cohort in Dar es Salaam, Tanzania. Study II was conducted among high-risk women recruited between 2008-2010 into the microbicide and vaccine feasibility studies in northern Tanzania. The map of Tanzania in **Figure 7** shows the study areas for the parent cohorts of the different PhD studies.

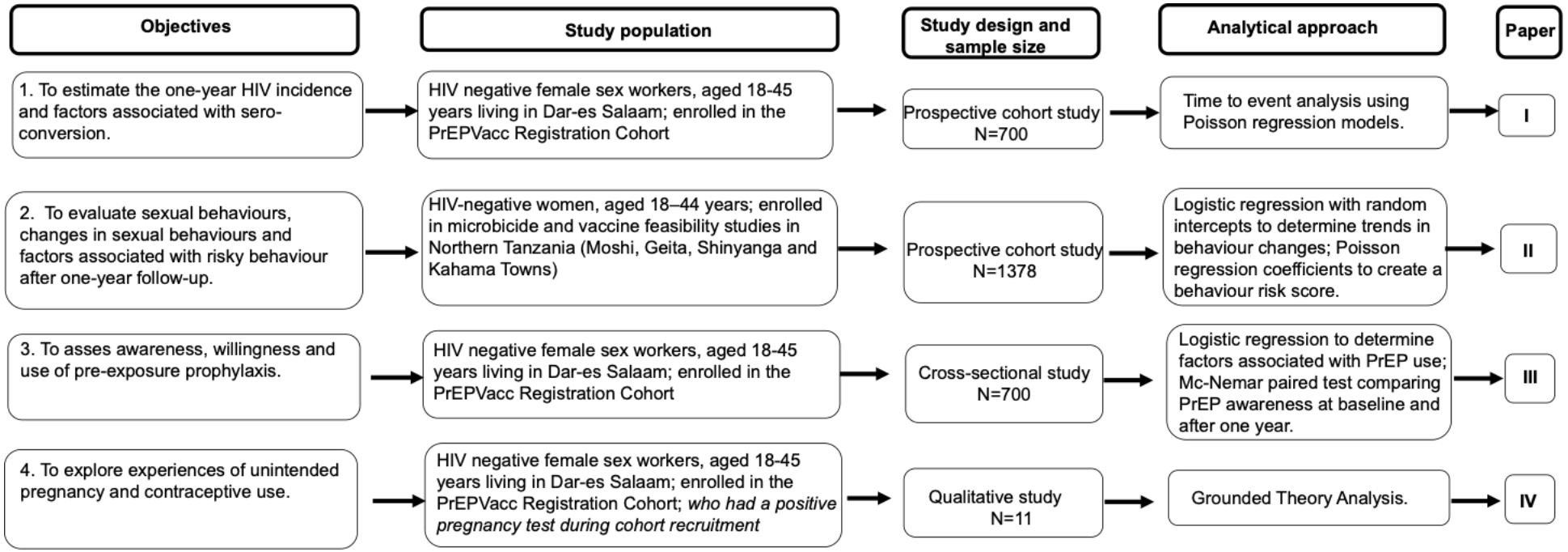


Figure 6 Summary of study objectives, study population, study design, study sample and data analysis methods

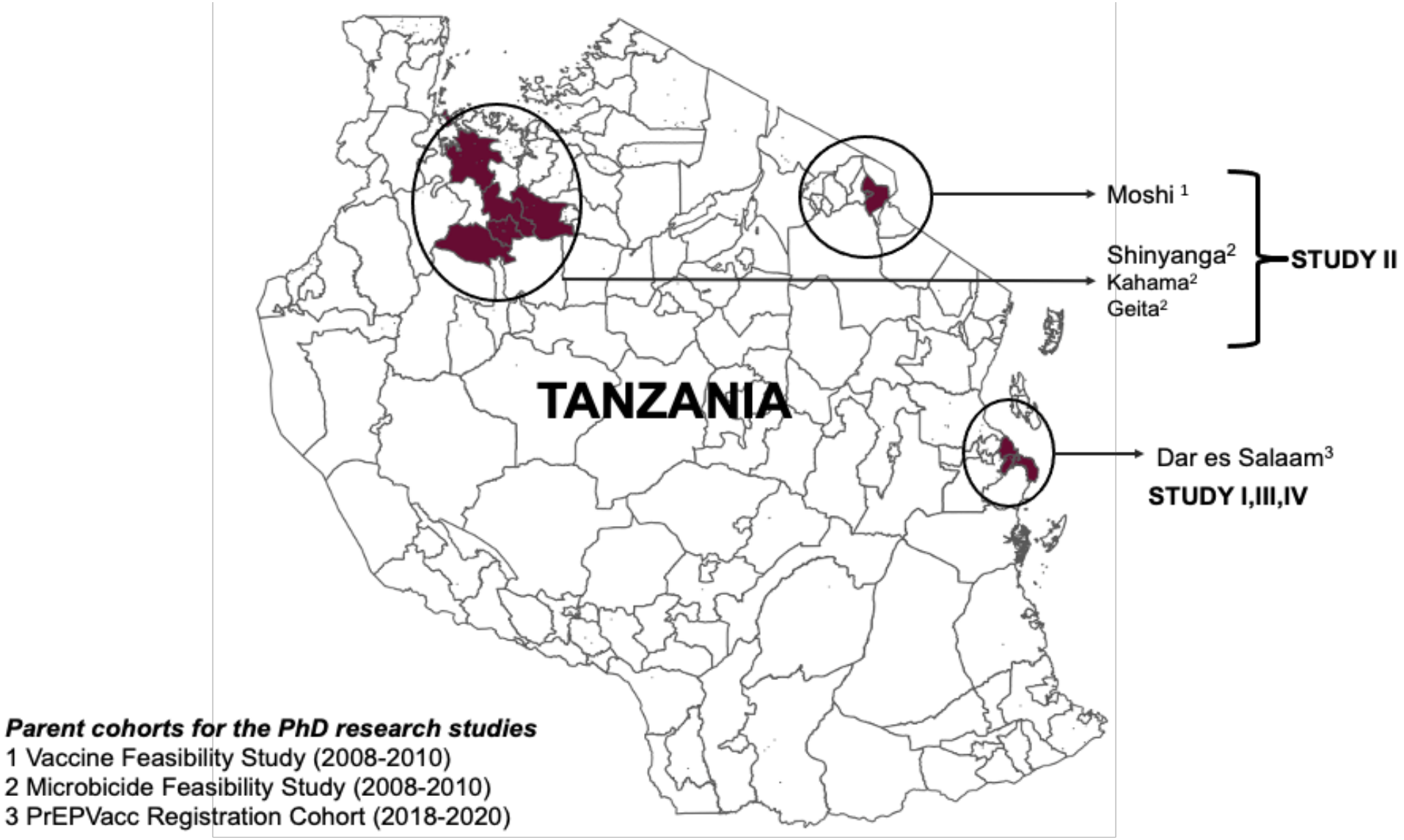


Figure 7 Map of Tanzania showing the study areas

Vaccine and microbicides feasibility studies: Kapiga *et al PLoS One (2013)*
The PrEPVacc registration cohort: *ClinicalTrials.gov. (NCT04066881)*

5.2 STUDY SETTING

This section provides a description of parent cohorts from which the PhD studies were conducted. The description on the PrEPVacc study is provided in sections **5.2.1** whereas the Microbicide and Vaccine feasibility cohort study conducted in Northern Tanzania is described in sections **5.2.2.**

Three PhD studies (Study I, II and IV) were conducted as part of a HIV vaccine trial preparation cohort, i.e the PrEPVacc registration cohort in Dar es Salaam (120, 150) while Study II was conducted as part of the microbicides and vaccine preparedness cohort studies conducted in Northern Tanzania (71).

5.2.1 The PrEPVacc registration cohort

Studies I, III and IV were conducted within the PrEPVacc registration cohort — a vaccine preparedness study at MUHAS, Dar es Salaam Tanzania.

PrEPVacc is a collaborative partnership between European and African research and academic institutions, funded through the European and Developing Countries Clinical Trials Partnership (EDCTP). The PrEPVacc research has two components, first the registration cohort — in which the PhD project was nested and second, the PrEPVacc HIV vaccine trial.

The PrEPVacc vaccine trial is a multi-centre, phase IIb, three-arm, two-stage randomized trial that will evaluate the effectiveness of combining pre-exposure prophylaxis (PrEP) with two HIV vaccines in preventing new HIV infections (121). The PrEPVacc vaccine trial sites include Masaka (Uganda), Maputo (Mozambique), Durban and Verulam (South Africa), Mbeya and Dar es Salaam (Tanzania).

The PrEPVacc trial will have two-stage randomization. The first will be a three-arm prospective 1:1:1 randomisation comparing two experimental vaccine combination regimens with a placebo control. Participants will receive either a combination of the DNA-HIV-PT123 and AIDSVAX® B/E HIV vaccines at weeks 0,4,24 and 48 (Group A), or a combination of DNA-HIV-PT123 vaccine and CN54gp140 followed by boosting with Modified Vaccinia Ankara-Chiang Mai Double Recombinant (MVA-CMDR) and CN54gp140 adjuvanted with Monophosphoryl lipid A (MPLA-L) (Group B) or a saline placebo (Group C). The second and concurrent two arm 1:1 randomisation will compare two regimes of pre-exposure prophylaxis, either daily Descovy or daily Truvada in the first 26 weeks of immunization. **Figure 8** provides the study schema for the PrEPVacc vaccine trial.

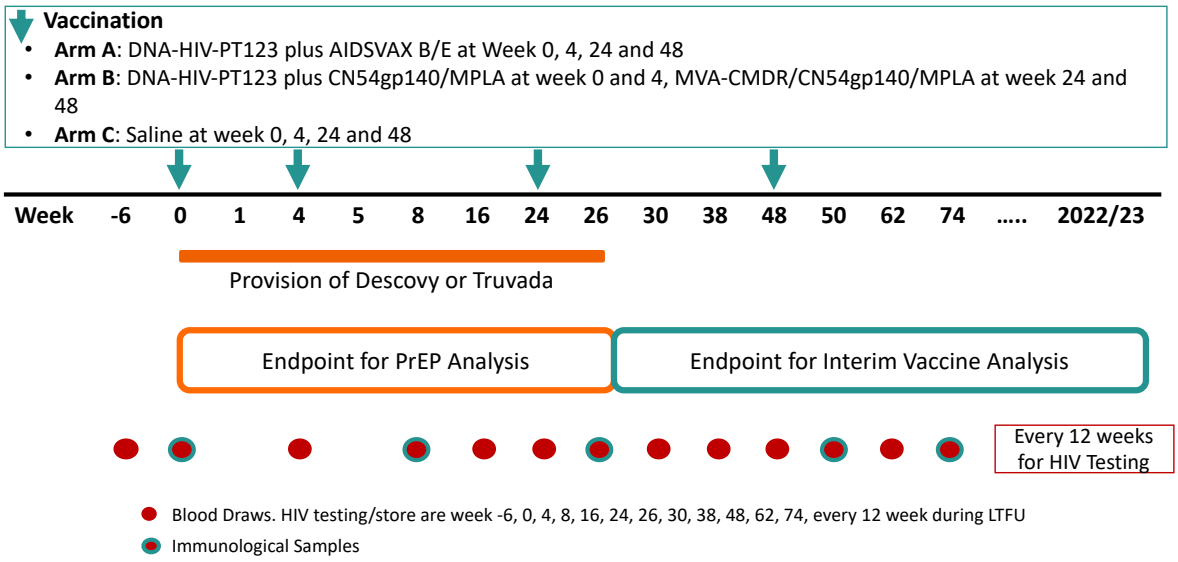


Figure 8 Study schema for the PrEPVacc vaccine Trial

At each of the PrEPVacc vaccine trial sites, a registration cohort of HIV negative individuals at high risk for HIV infection were established. The main objectives of these cohorts were to estimate the HIV incidence and facilitate enrolment into the PrEPVacc vaccine trial. At the Dar es Salaam site, secondary objectives for the cohort included: estimating the prevalence of Syphilis, Hepatitis B virus (HBV) and Hepatitis C virus (HCV) infections, ascertaining the knowledge, perceptions, uptake of and adherence to PrEP, ascertaining the knowledge, perceptions, and willingness to participate in HIV vaccine trials, developing and refining key messages about HIV vaccines and PrEP as well as tools for conveying these messages and educate participants about, PrEP, HIV vaccine research in general and the PrEPVacc vaccine trial in particular.

The PrEPVacc Registration cohort in Dar es-Salaam is conducted at the MUHAS HIV Vaccine Clinical Trial Unit. The study site is located within the National referral hospital, Muhimbili National Hospital. The site offers linkage and referral of participants to HIV care and treatment clinic, family planning clinic, Hepatitis clinic as well as Methadone assisted therapy located within the Muhimbili National Hospital. A total of five phase I/II HIV vaccine trials have been conducted at the study site (135-142) as described in the section 2.5 of this thesis. The study site is easily accessible through public transport. The study clinic has quiet and private rooms to ensure participants' confidentiality and limited distraction. The clinic has designated rooms which have restricted access for storage of completed case report forms. Biological specimens are collected at the site and transferred to be stored at the MUHAS's Microbiology and Immunology laboratory.

5.2.2 The microbicide and vaccine cohorts in northern Tanzania

The microbicide and vaccine preparedness cohort studies were established between 2008-2010 in northern Tanzania. The vaccine feasibility study was conducted in Moshi town while the microbicide feasibility study in the northern lake zone towns of Geita, Shinyanga, and Kahama. Geita and Kahama towns have large-scale gold mines while Shinyanga has a diamond mine. The mines are a large attraction for men seeking jobs and consequently sex workers migrate to these areas. Moshi town is along a major highway connecting northern Tanzania with Kenya. Moshi town is also along the slopes of Mountain Kilimanjaro, a key tourist destination in Tanzania and hence the town has large number of hotels and guesthouses. Together, the cohort studies recruited women who worked in bars, guesthouses, hotels and other food and recreational facilities in the selected towns. Women working in these facilities are known to supplement their income by selling sex to clients and hence highly vulnerable to HIV and other sexually transmitted infections (47, 51, 70, 151, 152).

The two cohorts were established as additional sites for recruiting larger study populations needed to participate in vaginal microbicides trials and HIV vaccine trials. These studies were part of the Microbicides Development Programme collaboration whose aim was to develop and evaluate effective vaginal microbicides. At the time, a phase III microbicide study of the PRO-2000 vaginal gel was ongoing however, this trial was later stopped for lack of efficacy (153). It was anticipated that future trials within the Microbicides Development Programme collaboration may need to compare new products or combinations of earlier products which had shown limited efficacy i.e using active control group. Based on the anticipated need for larger sample size, the European and Developing Countries Clinical Trials Partnership (EDCTP) had funded the establishment of new cohorts that might be used in future microbicide or HIV vaccine trials.

The microbicide and vaccine preparedness cohort studies from northern Tanzania did not proceed into efficacy trials as initially intended. Participants in these cohorts were later on enrolled into clinical trials evaluating the effect of treatment of sexually transmitted infections in reducing HIV risk (154). The cohorts have also been evaluated to assess suitability of this population for participation in other HIV prevention trials (52, 92, 155). Information on recruitment, follow-up and retention of women in these cohorts has helped inform the design and conduct of PhD studies included in this thesis.

5.3 STUDY POPULATIONS AND RECRUITMENT OF PARTICIPANTS

The research studies in this PhD thesis utilized two study populations to answer the research questions. Study I, III and IV included female sex workers enrolled in the PrEPVacc registration cohort in the Dar es Salaam region. Study II included women enrolled in the microbicide and vaccine feasibility studies in northern Tanzania (**Figure 6 and 7**). Section **5.3.1** describes the study population and recruitment for participants included in studies I and III, section **5.3.2** describes the study population and recruitment of participants in study II whereas section **5.3.3** describes recruitment of participants for study IV.

5.3.1 Study population and recruitment of participants for Study I & III.

Study populations for study I &III were female sex workers enrolled in the PrEPVacc registration cohort in Dar es Salaam. The cohort included women self-identifying as street, home or brothel-based sex workers living in Dar es Salaam. They were eligible for inclusion if they met the following criteria: aged 18 to 45 years, reported to have exchanged sexual intercourse for money within the past month, considered by the investigator to be at increased risk for HIV infection, willing to undergo pregnancy testing, HIV pre and post-test counselling, resided within Dar es Salaam, able and willing to provide written informed consent. **Table 2** below provides a list of the PrEPVacc registration cohort inclusion and exclusion criteria.

Table 2 Inclusion and exclusion criteria for cohort enrolment into the PrEPVacc registration cohort

Inclusion criteria • Women aged 18-45 years at enrolment • Willing and able to give informed consent • Willing to undergo HIV testing at screening, and receive their HIV test result • Willing to have regular urine pregnancy tests • Not planning to move away from Dar es Salaam for the next 12 months • Willing to provide adequate locator information for tracking and willing to be contacted by the study staff **Exclusion criteria** • HIV-positive • Current participation in another HIV prevention study that may reduce HIV incidence • Any condition(s) that in the opinion of the investigator, might make it difficult for an individual to participate in the study or for the study to achieve its objectives

5.3.1.1 *Respondent driven sampling for recruitment of participants for Study I & III*

Between October and December 2018, a total of 775 female sex workers were recruited from whom 700 were eligible and consented to enrol into the PrEPVacc registration cohort. Female sex workers were recruited using Respondent Driven Sampling (RDS). This is a special sampling method used to recruit marginalised and hard-to reach population such as the female sex workers. The respondent driven sampling methodology has been previously described in literature (156-159). This method has been used successfully to recruit female sex workers for participation in surveys within Tanzania (36, 160, 161) and in other settings (162, 163).

Respondent driven sampling began with selection of "seeds" who are members of the female sex workers community in Dar es salaam and known by the study investigator. The seeds were selected purposively to represent the diversity of female sex workers in Dar-es- Salaam. The diversity of the seed included the following parameters; district of the work area, age and HIV status i.e one known HIV positive female sex worker was included as a seed. Seeds were provided with three study coupons and instructed to recruit peers who were aged 18 to 45 years and lived in Dar es Salaam. Participants recruited by the seeds (wave one) were also provided with three coupons to further recruit other peers (wave two). This process was continued until the number of eligible participants consenting to enrol into the PrEPVacc registration cohort reached the estimated sample size. Recruiters and their recruits were linked by unique identifiers written on their recruitment coupon. **Figure 9** shows an illustration of recruitment chains from a single seed in respondent driven sampling.

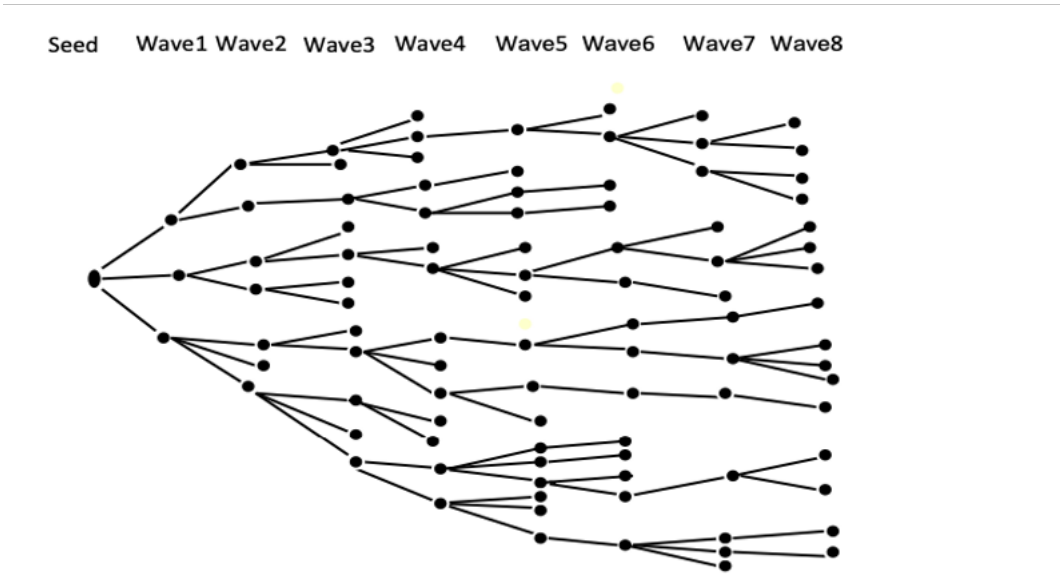


Figure 9 Illustration of recruitment chain in respondent driven sampling

Source: Johnston LG, Sabin K (2010): Sampling hard-to-reach populations with respondent driven sampling.

A research assistant who was a female sex worker vetted recruits to confirm that they were sex workers by asking questions about number of sex acts, client meeting points and specific idioms used by the female sex worker community. Potential participants with valid recruitment coupons were provided with study information and procedures in Kiswahili. Thereafter, willing participants were required to sign an informed consent form. The study nurses and doctors performed the eligibility screening procedures using eligibility assessment case report forms based on criteria in **Table 2.**

A total of 8,000 Tanzanian Shillings (Equivalent to 3.50 US dollars) and 4000 Tanzanian Shillings (Equivalent to 1.7 US dollars) were paid to each participant for transport reimbursement and as incentive for successful recruitment of a peer.

5.3.2 Study population and recruitment of participants for study II.

The study population for study II was women enrolled in the microbicide and vaccine preparedness cohort studies in northern Tanzania. Both cohorts included HIV negative women aged 18 to 44 years working in food and recreational facilities. These facilities included, bars, restaurants, guesthouses, hotels, entertainment venues, shops selling traditionally brewed beer and food vendors.

Recruitment was done in stages, with a separate screening and enrolment visit. First, mapping of recreation facilities was done to obtain an approximate count of women working at these facilities. Meetings were held at facilities to provide information on the research and to invite women to participate in the study. Following the mapping, clusters that would be able to recruit 250-330 women were created and a study clinic was established in each cluster. Women working in the recreational facilities were invited to attend a screening visit at the study clinic. If they satisfied a number of eligibility criteria, they were invited to return to the clinic one month later for study enrolment. Eligibility criteria included: willingness to undergo HIV testing and to receive the result, a HIV negative result, and likely to be resident in the study community for one year. Further eligibility check and written informed consent were performed for women returning for the enrolment visit. They were interviewed and data on socio-demographic and behavioural characteristics were collected. Participants also underwent genital examination and collection of genital samples. Women enrolled in the study were asked to attend follow-up visits at the study clinics every 3 months for HIV testing and follow-up interviews

5.3.3 Study population, recruitment of participants and sample size for Study IV (qualitative study)

Study IV included female sex workers who had a positive urine pregnancy test at the time of enrolment into the PrEPVacc registration cohort (between October and December 2018). A pre-determined sample size of 18 diverse female sex workers was considered sufficient to answer the research question. The 18 female sex workers were purposively sampled out of 32 (sampling frame) who had a positive urine pregnancy test during study enrolment. The sampling of study participants considered demographic and phenomenal considerations so as to purposefully maximize diversity of the informants (164). Young and older sex workers were selected to reflect variations in their years of experience in sex work as well as their sexual and reproductive health history. Parity and past contraceptive use were the phenomenal considerations used during sampling. Participants were invited for an interview by a female research assistant who was part of the PrEPVacc study team, and well known in the Dar-es-Salam sex workers' community. In-depth interviews were initially conducted with nine of the 18 sampled women who were willing to participate in the study. Three sampled participants could not be reached and six declined to be interviewed (2 were out of town, 1 had a new born baby and 3 reported to be busy). Those agreeing to participate were provided with a daytime interview appointment that was convenient for them. During data collection two additional informants were included in the study making a total of 11 study informants. The two informants were considered as "deviant cases" from which, emerging themes could be tested to achieve saturation (165). One of the deviant cases was a younger sex worker who had never been pregnant. She provided perspective on contraception and abortions from female sex workers with no pregnancy experience. The other deviant case, a HIV positive woman was included to explore the opinions from preceding informants who had suggested that HIV positive sex workers are less likely to use condoms, (therefore more likely to get pregnant) and that they were more likely to abort the pregnancy in fear of mother-to-child HIV transmission.

5.4 SAMPLE SIZE AND POWER ESTIMATION FOR QUANTITATIVE STUDIES

5.4.1 Sample size estimation for Study I

The sample size estimates for Study I were based on achieving a desired HIV incidence of 4 cases per 100 person-years after 12 months of follow-up in the PrEPVacc registration cohort—and also the primary outcome for Study I. The desired HIV incidence of 4/100 per-years-at risk is recommended for trials so as to allow sufficient statistical power in estimating efficacy of HIV prevention products (23, 92, 93, 166-169). This desired HIV incidence rate is within the observed HIV incidence rate range of 2 to 13 cases per 100 person years in similar cohorts of high-risk women in Tanzania and other parts of sub-Saharan Africa (50, 71, 170-175). The estimated sample size was adjusted for a 25% expected loss to follow-up rate as female sex workers are a highly mobile population. The sample size was also corrected for an expected design effect of 2, based on a similar design effect used in respondent- driven sampling studies conducted among female-sex workers in Dar es Salaam (36, 160, 161). As shown in **Table 3** below, at power of 80% and 5% significance level, a minimum sample size of 650 was deemed sufficient to detect an HIV incidence of 4 cases per 100 person-years after 12 months of follow up. The estimated sample size was rounded to 700.

Table 3 Sample size estimation assumptions for Study I

Desired HIV incidence (per 100)	Delta (Incidence 100)	Precision (HIV incidence per 100)	Sample size (N)	Adjusting for DEFF of RDS studies (Nx2)	Assuming 25% LTFUP (N x (1/(1-0.25)))
4	+/- 3.5	0.5-7.5	161	322	430
4	+/-3	1-7	244	488	650
4	+/-2	2-6	630	1260	1680
4	+/-1	3-5	3221	6442	8589
Assumptions used to achieve a 12-month HIV incidence of 4% at risk at power 80% and 5% significance level. DEFF-Design effect; RDS -Respondent driven sampling; LTFUP-Loss to follow up					

5.4.2 Power estimations for Study II

At the study design stage, the primary outcome for study II was HIV incidence at 12 months with the main exposure being changes in sexual behaviour after 12 months of follow up. Power of 86% was estimated to achieve statistical significance at $p<0.05$. These estimations were based on the assumption that, out of the 1,378 women in the combined dataset, one-third of the women would have high risky sexual behaviours. The HIV incidence at 12 month in the group of women with high-risk behaviours was anticipated to be 6% compared to 2% in the group of women with less-risky sexual behaviours. Due to the small number of HIV cases in the dataset (44 cases of HIV seroconversion), the study was deemed to have low statistical power to detect the adjusted estimates for the association between changes in sexual behaviours and HIV incidence at 12 month (the intended primary outcome). Therefore, preliminary decision was made to regard this analysis as exploratory. The results of these exploratory analysis are presented in this thesis (**in Table 9**) but these results are not included in the published paper for study II. **Table 4** below shows the assumptions for power estimation in Study II.

Table 4 Power estimation assumptions for Study II

HIV incidence among individuals with decreased risky behaviours	HIV incidence among individuals with Increased/no change in risk behaviours	Sample size (N1+N2)	Sample size ratio (N1/N2)	Significance level (1-α)	Estimated Power (1-β)
2%	6%	1378	1	95	95
2%	**6%**	**1378**	**3**	**95**	**86**
2.5%	5.5%	1378	1	95	77
3%	5%	1000	1	95	42
N1/N2 sample size ratio – women with risk behaviours and women with less-risky behaviours. Assumptions were made that one-third of women in the cohort would have high-risk behaviour (exposure of interest)					

5.4.3 Power estimations for Study III

The primary outcome for study III at the study design stage was the proportion of female sex workers aware of PrEP at cohort enrolment—that is, out of the 700 HIV negative female sex workers enrolled in the PrEPVacc registration cohort. Power to detect this outcome was estimated because this was a fixed population i.e analysis performed within the cohort of 700 HIV negative female sex workers. A power of 100% was estimated to achieve a statistical significance at $p<0.05$ for the proportion of PrEP awareness. These estimations were based on the assumption that out of the 700 HIV negative female sex workers enrolled in the cohort, two-third of the women would be aware of PrEP. This assumption was based on findings from the survey conducted among female sex workers in Dar es Salaam in late 2017(36). In this survey, 31% of the female sex workers reported to have ever heard of PrEP *(personal communication with author)*. Given the fact that recruitment and enrolment in to the PrEPVacc registration cohort took place nearly a year after data collection in the mentioned survey had been concluded, it was assumed that the number of female sex workers aware of PrEP would have doubled. It was also expected that the number of PrEP users in the cohort would be low due to the intermittent supply of PrEP in Dar es Salaam *(personal communication with the Ministry of health Tanzania)*. **Table 5** below shows the assumptions for power estimations in study III. Preliminary analysis of the PrEPVacc registration cohort's baseline data also showed that there were very few female sex workers using PrEP. Therefore, a decision was made to estimate power using expected proportion of female sex workers aware of PrEP. Analysis of determinants of PrEP use would be regarded as exploratory to avoid statistic errors due to low power. Results for determinants of PrEP awareness are presented in this thesis (in **Table 10**) but not included in the manuscript for study III.

Table 5 Assumptions for Study III power estimation

PrEP use Awareness in the population	PrEP use Awareness in the PrEPVacc cohort	Sample size	Significance level (1-α)	Estimated power (1-β)
0.66	0.76	700	95	100%
0.66	0.46	700	95	100%
0.31	0.66	700	95	100%

5.5 DATA COLLECTION

5.5.1 Data collection for I & III

This section describes the data collection process in the PrEPVacc registration cohort from which data for Study I and III were obtained.

5.5.1.1 *Data collection procedures in the PrEPVacc registration cohort.*

Eligible participants underwent face-to-face baseline interviews in private rooms at the study site. Information on sexual risky behaviour practices, HIV risk perception, reproductive history, drug and alcohol use were collected. Each interview lasted about 30 to 45 minutes. Participants were required to provide 10 mL of whole blood for rapid HIV, Syphilis, HBV and HCV virus infection detection and 5ml of urine for pregnancy testing. Pre- and post-test counselling were performed by trained Nurse Counsellors. Follow-up interviews and HIV testing were performed every three months. A summary of the study visit procedures is provided in **Table 6** below.

5.5.1.2 *Laboratory procedures.*

Rapid tests were performed at the study site by a trained Laboratory Scientist and verified in the Microbiology and Immunology Laboratory at the Muhimbili University of Health and Allied Sciences. The Tanzanian HIV rapid testing algorithm (176) was applied which uses two sequential rapid diagnostic tests; SD Bioline HIV1/2 (Standard Diagnostics Inc., Republic of Korea) for screening and Uni-Gold HIV-1/2 (Trinity Biotech, Ireland). Participants who were reactive to both rapid HIV tests were regarded as HIV infected. ELISA tests; Murex HIV Ag/Ab Combination (DiaSorin S.p.A. UK Branch) and Enzygnost. HIV Integral 4 ELISA® (Siemens Healthcare, Germany) were used to resolve discordant results between two the HIV rapid tests.

Syphilis testing was performed using a Laborex rapid Treponemal assay (Orient Gene Biotech Co Ltd, Zhejiang, China) and all reactive samples were confirmed on Treponema Pallidum Haemagglutination Assay. Blood samples were tested for Hepatitis BsAg, HBsAb, HBc IgM Ab, HBeAg, Hepatitis C virus IgM antibodies. All reactive rapid Hepatitis B and C test samples were confirmed using Murex Hepatitis B and Hepatitis C ELISAs (Diasorin S.P.A., Italy), respectively. A urine pregnancy testing was performed by detection of β-Human Chorionic Gonadotrophin (β-HCG) using (Orient Gene Biotech Co Ltd, Zhejiang, China). HIV-infected participants were provided with a referral note and escorted to their preferred health care facility to receive free HIV care and treatment. Participants who tested positive for Hepatitis B or C were referred to the Hepatitis clinic at Muhimbili National Hospital for further management and follow-up.

Table 6 Summary of study visits procedures in the PrEPVacc registration cohort (Study I &III)

Procedures	BASELINE Visit	SHORT visit	LONG visit	FINAL visit	PhD STUDY Data
Visit Month	**0**	**3, 9**	**6, 12**	**exit**	
Provision of study information and informed Consent	x				I, III
Assessment of eligibility	x				I, III
Baseline Demographic Questionnaire	x				I, III
HIV Risk and Risk Reduction Questionnaire	x		x	x	I, III
Information on PrEP	x		x	x	III
HIV Counselling and Testing	x	x	x	x	I
Testing for Hepatitis B, C virus infection	x				I
Contraceptive counselling	x	x	x	x	I
Urine pregnancy test	x		x	x	I

5.5.2 Data collection for study II

Study II was a secondary analysis of data from the combined cohorts of the vaccine and microbicide feasibility studies conducted in northern Tanzania. While I was not involved in the data collection of these studies, below I provide a brief description on the data collection procedures. An account of my involvement in data cleaning and preparation of the datasets for analysis is also provided.

5.5.2.1 *Data collection procedures in the microbicide and vaccine feasibility studies.*

Eligible HIV negative women consenting to enrol into the cohorts underwent structured face-to- face interviews. During the interviews, information on their socio-demographic characteristics, employment, reproductive history and work mobility was collected. Information about alcohol use was obtained using the four CAGE questions which are used to determine alcoholism(177). Alcohol use information was also obtained from the Alcohol Use Disorders Identification Test (AUDIT) based on responses from 10 questions (178). Blood sample collection was performed for laboratory testing of HIV, syphilis and HSV-2. Clinical examination and genital samples were collected for detection of genital infections (*Neisseria gonorrhoeae, Chlamydia trachomatis and Trichomonas vaginalis*). Bacterial vaginosis was diagnosed by Nugent's criteria (179). Participants were followed every three months for 12 months with similar interviews, clinical examination, and collection of samples performed at each visit. Syphilis tests were not performed in the vaccine preparedness cohort (Moshi) while *N.gonorrhoeae*, *C.trachomatis* and *T.vaginalis* tests were not done at the 3-month and 9-month visits in both cohorts. Participants with positive laboratory tests for curable sexually transmitted infections (*N.gonorrhoeae, C.trachomatis*, T. *vaginalis* or Syphilis) received free treatment as per Tanzanian ministry of health guidelines.

5.5.2.2 *Data cleaning and management for study II datasets*

I was provided with two datasets which had variables related to the study II research question. Each dataset contained combined data from the two cohorts. The first dataset contained baseline information and the second dataset contained information collected at the 3-, 6-, 9- and 12-months follow-up visits. In the baseline dataset, socio-demographic variables included: age at enrolment, religion, educational status and marital status. Employment history and work mobility variables included: type of work place, average monthly income, history of travel in the last 12 months and permanence of residence in the current town. Reproductive history variables included: number of lifetime pregnancies and contraceptive methods. Sexual behaviour variables included: age at sex debut, number of lifetime partners, number of sex partners in the last 1, 3, and 12 months, history of condom use in last sex act, history of condom use with regular and/or non-regular partners in the last 3 months, history of transactional sex in the last 3 and 12 months, history of anal sex, rape or sex during menstruation in the last 3 months and perception of HIV acquisition risk. Information on alcohol intake in the last 12 months was available from both CAGE and AUDIT scores.

The follow-up dataset contained time-varying exposures measured at 3 monthly follow-up visits including: number of partners in the past 1 and 3 months, frequency of condom use with regular partners and/or non-regular partners in the last three months (categorized as always, inconsistently or never) as well as history of transactional sex, rape, sex during menses and anal sex in the past three months.

Minimal data cleaning was performed because the datasets had previously been cleaned for use by the principal study investigators. Data checking was performed by summarizing, describing, cross tabulating and inspecting the frequency distribution for missing values, extreme data values and other inconsistencies. Thereafter, variables were categorized based on the distribution so as to minimize data sparsity. History of travel in the last 12 months preceding baseline clinic visit was categorised in two groups: No travel (no nights away from home) and at least once (continuous week away from home on one or more occasions). Alcohol drinking from the 10- item AUDIT score was dichotomized as score <8 points versus ≥8 points (Non-drinker or low-risk versus Harmful or hazardous drinking) while CAGE score (Cutting down, Annoyance by criticism, guilty feeling and Eye-openers) was categorized into three categories: 0-1 as non-drinker or no problem drinking, 2=possible problem drinking and ≥3 probable problem drinking. A “high-risk sex” variable was generated by combining women reporting a history of rape, sex during menses or anal sex in the past 3 months.

5.5.3 Data collection for study IV

Study IV used in-depth interviews to collect qualitative data. The choice for using in-depth interview instead of focus-group discussion was made because in-depth interviews offer an opportunity for the informant to provide a personal account of the unintended pregnancy experience as well as an account on the decision-making considerations for contraceptive use. Additionally, individual interviews are more ethically accepted for collection of sensitive data (165) because both sex-work and abortions are illegal in Tanzania (55).

5.5.3.1 *Development of the interview guide for Study IV*

During the development of the interview guide, the *Health Believe Model* (180) was used to conceptualize sex work and its relation to unintended pregnancy. The decision to use the *Health Believe Model* in developing the interview guide was based on the theoretical papers in behaviour research by Nathason et al (181) and Hall et al (182). In these papers, the authors used the *Health Believe Model* to explain contraceptive behaviour among unmarried young women (181) and to explore the determinants of uptake of modern contraceptive behaviour (182). Taking these papers together, the *Health Believe Model* was deemed applicable in the context of contraceptive use among female sex workers because; i) pregnancy among female sex workers is an undesired condition and therefore avoided— arguably more than in women in the general population (ii) decision to use contraceptives e.g. condom for prevention of unwanted pregnancy or performing an abortion are directly influenced by multiple sexual partners and (iii) contraception behaviour is complex because it includes different action points i.e initiation, consistent/correct use and continuation. These action points are influenced by perceived benefits and barriers operating at community (structural) and individual levels. The components of the *Health Believe Model* were applied to conceptualize how female sex workers use a multidimensional approach to make decisions on using contraceptives and when dealing with unintended pregnancy (94). For instance, the interview guide included probes on how access to contraceptives versus side effects (*perceived barriers*) influence decisions to initiate or continue contraceptive use. The guide also probed on how sex workers perceived their susceptibility to unintended pregnancy given that the decision to use condom was influenced by their partners, and if this *perceived susceptibility* influenced their decision on using dual contraceptives (*perceived benefit*).

The interview guide evolved with subsequent interviews accommodating emerging themes based on responses provided by preceding interviews thereby increasing theoretical sensitivity (165). The interview guide developed for Study IV include the following thematic areas: facilitators and barriers of contraceptive use; experiences related to unintended pregnancies and attitudes towards induced abortion.

5.5.3.2 *In-depth Interviews for study IV*

I conducted all the in-depth interviews for the Study IV. All interviews were conducted in the local language of Kiswahili. Each interview lasted for about 45 to 60 minutes. They were conducted in an isolated room at the PrEPVacc study site. The venue was familiar to the informants because they had visited the site for at least three occasions prior to the interview. Informants were also familiar with me (the interviewer) because I was part of the PrEPVacc study team and was involved in the PrEPVacc enrolment and follow-up interviews.

The In-depth interviews began with a brief personal introduction, explanation of the study aims and objectives, signing of consent form and followed by background demographic questions. Thereafter, respondents were asked to narrate their experiences and reactions upon receiving a positive pregnancy test during enrolment into the PrEPVacc registration cohort. Participants were asked to describe their thoughts on how pregnancy affected their sex work. The influence of peers, families and partners on unintended pregnancy were also explored. Follow-up questions (probes) were asked in order to give a chance to the informants to explain if the recent pregnancy had influenced their intentions to use contraceptives in the future. Attitudes towards induced abortions were explored among women who had reported to have had an abortion and those who had not.

The interview guide was used in a flexible manner so as to encourage informants to speak in their own words and therefore allow for new emerging ideas and unexpected information (165). Because the interview guide evolved with subsequent interviews, later interviews were more focused and covered more concepts albeit risking more narrow and specific responses (165, 183). For example, the interview with the first informant (in-depth interview conducted in January 2019), covered fewer concepts but provided a long and detailed narration of the issues whereas the interview with the tenth informant (in-depth interview conducted in June 2019) covered more concepts but elicited narrower responses.

All interviews were transcribed and then translated to English. They were all read through to obtain the sense of the whole. English translated transcripts were compared with audio files several times to identify and revise any unclear areas.

5.6 DATA ANALYSIS

This section provides a description of the study outcomes and the analysis approach for each study outcome in the respective PhD study.

5.6.1 Study I

5.6.1.1 *Study outcomes:*

a) Prevalence of HIV, HBV, HCV and Syphilis among *all* female sex workers screened for enrolment into the PrEPVacc registration cohort.
b) Determinants of the baseline HIV prevalence.
c) HIV incidence after 12 months of follow up among the *HIV negative* female sex workers enrolled into the PrEPVacc registration cohort.
d) Determinants of HIV incidence at 12 months.

5.6.1.2 *Statistical analysis*

- *Outcome(a):* Prevalence (proportion) of HIV, HBV, HCV, and syphilis infections among all female sex workers screened during recruitment into the PrEPvacc registration cohort were estimated. The respective confidence intervals for the prevalence were estimated.
- *Outcome (b):* Frequency and percentages were used to describe baseline sexual and demographic characteristics. Chi-squared test was used to compare sexual behaviours and demographic characteristics between women who tested HIV negative and HIV positive at baseline screening. Logistic regression was used to estimate the odds ratios for the factors associated with the baseline prevalence of HIV. Using a forward stepwise approach, a multivariable logistic regression model was built beginning with the set of significant ($P<0.20$) univariate predictors. Adjusted odds ratios for the factors associated with prevalent HIV infection and their respective 95% confidence intervals were summarized.
- *Outcome (c):* HIV incidence after 12 months of follow up was calculated as number of HIV seroconversions divided by the total persons years of observation. For each infection, the date of seroconversion was assumed to be halfway between the last negative and first positive results. Data was censored at the earliest seroconversion date for those who became HIV infected. For those remaining HIV negative, data was censored at date last seen or at 12 months.
- *Outcome (d):* Factors associated with HIV seroconversion were determined using Poisson regression models. Incidences of HIV seroconversion were summarized by rate ratios, 95% CI and likelihood ratio test significance value. Due to the few cases of HIV seroconversion, only univariate Poisson regression analysis was performed. Nelson-Aalen cumulative hazard curves were plotted to compare HIV cumulative hazards between participants with different risk characteristics.

5.6.2 Study II:

5.6.2.1 *Study Outcomes*

a) Baseline prevalence of risky sexual behaviours among women enrolled in the microbicide and vaccine feasibility cohorts.
b) Changes in sexual behaviours over time among women enrolled in the microbicide and vaccine feasibility cohorts.
c) Association between baseline characteristics and changes in sexual behaviours among women enrolled in the microbicide and vaccine feasibility cohorts.
d) Association between changes in sexual behaviours and the incidence of HIV infection at 12 months among women enrolled in the microbicide and vaccine feasibility cohorts

5.6.2.2 *Statistical analysis*

- *Outcome(a):* Descriptive statistics including frequencies and proportions were performed to determine the distribution of socio-demographic variables and baseline self-reported sexual behaviours. Chi-squared tests were used to compare the distribution of socio-demographic characteristics and sexual behaviours at baseline between younger (aged <25 years) and older (≥25 years) women.
- *Outcome (b):* Changes in sexual behaviours over time in the cohort was determined in two ways, changes at cohort level and changes at individual levels. At cohort level, proportions of risky sexual behaviours were summarized for the baseline visit, 3,6, 9 and 12 month visits. For each variable, using a random-effects logistic regression model with a random intercept, odds for the change in sexual behaviours over time were estimated. For this analysis, each sexual behaviour (outcome) was dichotomized and coded 0 for no and 1 for yes and the model was adjusted for visit as a linear term (coded as 1,2,3,4,5) to obtained odds ratio per study visit. Trends in prevalence over time for each sexual behaviour outcome were assessed using likelihood ratio tests, and the p-values were presented. At the individual level, changes in risky sexual behaviours was determined using a risk score. The risk score was generated from regression coefficients from a Poisson regression model which had HIV seroconversion as an outcome. In this analysis, the date of seroconversion was assumed to take place midway between the last negative and first positive HIV serology results. Women were censored at the earliest date of HIV seroconversion, date last seen, or end of follow up. Baseline sexual behaviour factors whose univariate association reached statistical significance at $p<0.05$ were entered into the multivariable model. Variables that remained statistically significant in this model were considered for inclusion in the development of the risk score. All multivariable Poisson regression models were adjusted for age, study area, and marital status as a priori confounders. Selection of the "best" multivariable model was based on one that provided negative regression coefficients for behaviours known to be protective against HIV infection and positive regression coefficients for risky behaviours that increase HIV risk. For instance, a model that had a negative coefficient for transactional sex, i.e. indicating transactional sex was

protective against HIV acquisition, was considered implausible. This selective approach to model building was chosen for two main reasons: (i) so as to generate predicted values for the sexual behaviour risk scores in their appropriate category (protective behaviours versus harmful behaviours); (ii) with such a small number of HIV seroconversion events (44 HIV cases), stringent criteria were needed to select few "key" parameters for the model i.e a model with only four co-variates in accordance with the "rule of ten". The obtained risk coefficients were then used to predict and create new variables for sexual behaviour risk scores at baseline and 12 months visit, labelled as "*Baseline risk scores*" and "*12 month risk scores*" respectively. Histograms were used to display the distribution of the values of the predicted risk scores and their respective means were summarized. A paired t-test was used to compare the means of the baseline and 12- month risk scores. Thereafter, "Baseline risk score" and "12 month risk score" variables were dichotomized to: "*less-risky sexual behaviour practices*" (risk score values ≤ -5) and "*risky sexual behaviour practices*" (risk score values > -5). McNemar's test was then used to compare proportions of risky versus less-risky practices among participants who attended both visits. A "*risk score change*" variable was then generated by subtracting the baseline risk scores from 12 month risk scores. The, "risk score change" variable was grouped into three categories;(i) negative change in risk score =Decreased risky sexual behaviour practices (signifying that less-risky behaviour practices observed at 12 months as compared to baseline e.g. -4 score at baseline versus -6 score at 12 month), (ii) positive change in risk score =Increased risky behaviour practices (signifying that riskier behaviours practices were observed at 12 months compared to baseline e.g. -6 score at baseline versus -4 score at 12 month) and (iii) 0=No change in risk scores (signifying that same behaviour practices were observed in both visits e.g. -6 score at baseline versus -6 score at 12 month). Frequencies and proportions of these categories were summarized.

- <u>*Outcome (c):*</u> To examine the association between baseline characteristics and changes in sexual behaviours in the cohort, logistic regression was performed. The "risk score change" variable from above was dichotomized with the first category coded 1, combining those who had no change in sexual behaviour and those who had increase in risky sexual behaviours. The second category coded 0 included those with decreased risky sexual behaviours. Thus, odds ratio of >1.0 estimated from the models would represent higher odds of increase or no change in sexual behaviours. A univariate analysis was performed to estimate the unadjusted odds ratio for each baseline characteristics, including the predicted "baseline risk score" as a binary variable. Factors whose univariate analysis reached a statistical significance of $p<0.20$ were fitted in the multivariate model using the backward approach. Likelihood ratio test was used to assess statistical significance.
- <u>*Outcome (d):*</u> Poisson regression analysis was performed to determine the association between changes in sexual behaviours and the incidence of HIV infection. Incidence rates for HIV were calculated by dividing the number of new infections by the total person-years-at risk of follow-up. In the univariate analysis, rate ratios and their associated 95% confidence interval were estimated for the baseline covariates as well as the predicted "baseline risk score" variable. Because of the few HIV seroconversion cases (44 cases), a

comprehensive multivariate analysis of general risks factors for HIV incidence was not performed. Preliminary decision was made to focus the multivariate analysis on the effect of changes in sexual behaviour on HIV incidence. Therefore, the multivariate model was first adjusted for the baseline sexual behaviour risks score as a binary variable, and variables that had achieved a statistical evidence at P <0.20 in the univariate analysis. Statistical significance of all models was assessed using likelihood ratio tests.

Generating a sexual behaviour risk scores for predicting and estimating behaviour change.

Table 7 presents the four sexual behaviour variables used to generate sexual behaviour risk scores for each woman in the Poisson regression analysis —outcome (b) describe above. The four variables were selected by backward elimination using P<0.20 significance level at univariate analysis. All models were adjusted for age, town and marital status as priori confounders. Positive regression coefficients indicate an increased risk of HIV while negative coefficients represent protection against HIV infection. Model 2 and 3 had negative regression coefficients for the variable transactional sex and were therefore considered implausible. The sexual behaviour risk score values for baseline and at 12 months were obtained from Model 1 and generated using the following equation:

$$\begin{aligned}\textbf{\textit{Risk score}}\ (= -5.90 &+ \big(0.89 \times (No.\,partners\ in\ past\ 3months = 1)\big)\\ &+ \big(0.70 \times (No.\,partners\ in\ past\ 3months = 2)\big)\\ &+ \big(1.93 \times (No.\,partners\ in\ past\ 3\ months = 3\ or\ more)\big)\\ &- \big(0.16 \times (condom\ use\ in\ last\ sex = yes)\big)\\ &+ \big(0.56 \times (Highrisk\ sex\ in\ past\ 3months = yes)\big)\end{aligned}$$

Table 7 Results of Poisson Regression model of HIV incidence used to develop sexual behaviour risk score

Predictor	HIV infected/person-years	Rate per 100pyr	Model 1 N=1,361 Coef (SE)	Model 2 N=1,355 Coef (SE)	Model 3 N=1,355 Coef (SE)
No. of partners in the last 3 months					
0	1/69	1.4	0	0	0
1	28/859	3.3	0.89(1.03)	0.92(1.03)	0.94(1.03)
2	5/161	3.1	0.70(1.11)	0.77(1.11)	0.82(1.12)
3+	10/81	12.3	1.93(1.07)	2.05(1.09)	2.10(1.109)
Condom use at last sex					
No	22/670	3.3		N/A	0
Yes	22/511	4.3	-0.16(0.32)		-0.15(0.32)
High-risk sex, past 3m					
No	36/1082	3.3	0	0	0
Yes	8/98	8.2	0.56(0.43)	0.61(0.43)	0.60(0.43)
Transactional sex, past 3m					
No	30/871	3.4	N/A	0	0
Yes	14/304	4.6		-0.28(0.39)	-0.27(0.39)
Intercept	--	--	-5.90(1.19)	-5.91(1.19)	-5.90(1.19)

Coef= Poisson regression coefficient; SE= standard error. All models were adjusted for age, town, and marital status. Model 3 adjusted for all variables i.e. number of partners in the last 3 months, condom use at last sex, high risk sex in past 3 months and transactional sex in the last 3 months. Model 1 adjusted for all variable except transactional sex; Model 2 adjusted for all variables except condom use at last sex.

5.6.3 Study III:

5.6.3.1 *Study outcomes*

a) Proportion of female sex workers aware of PrEP and proportion of female sex workers willing to use PrEP at the time of enrolment into the PrEPVacc registration cohort and the changes in these proportions after 12 months of follow-up.
b) Association between PrEP awareness at baseline and socio-demographic characteristics.
c) Proportion of female sex workers in the PrEPVacc registration cohort who have ever initiated the use of PrEP and the socio-demographic determinants of PrEP use.

5.6.3.2 *Definition of measures of outcomes in Study III*

PrEP Awareness: Binary responses (Yes/No) from the question: "Have you heard about Pre-exposure prophylaxis (PrEP) i.e the use of anti-HIV drugs by HIV negative persons to protect themselves from catching HIV?"

PrEP Willingness: Assessed with three responses; Yes/No/Not sure to the question- "Would you be willing to use PrEP if it were offered to you?". Responses of "No" and "Not sure" were combined to "Not willing to use PrEP" and those remaining were categorized as "Willing to use PrEP".

PrEP Use: Binary response (Yes/No) to the question: "Are you currently using PrEP?". Participants responding "Yes" to this question during any of the three study visits (enrolment visit, 6 months or 12 months visits) were categorized as "Ever used PrEP" and the category was coded "1". Those remaining were categorized as "Never used PrEP" and coded "0".

5.6.3.3 *Statistical Analysis*

- *Outcome (a):* Descriptive statistics were performed to estimate proportions of female sex workers who were aware of PrEP and proportions of sex workers willing to use PrEP. The proportions were estimated separately for participants attending the enrolment visit and those who attended the 12-month visit. McNemar's Chi-squared test for paired proportion was used to compare the proportions at enrolment and at the 12 month visit. The paired analysis was restricted to participants who attended both visits.

- *Outcome (b):* Univariate Logistic regression was used to determine demographic and sexual behavioural characteristics associated with PrEP awareness. Likelihood ratio test were used to assess statistical significance.

- *Outcome (c):* The distribution of socio-demographic and behavioural variables was summarized as frequencies and proportions. This was done for the overall study population and separately for those reporting to have ever used PrEP and those who have never used PrEP. Chi-squared test for difference in proportions was used to compare proportions across the two groups. Variables with differences in PrEP use at $p<0.05$ were included in a multivariable logistic regression model to characterize associations. In building the multivariable logistic regression model, the "rule of ten" was used so as to ensure that covariate in the model did not exceed 10% of the primary outcome i.e 10% of the number of PrEP users in the cohort. Colinear variables i.e factors having the same meaning were excluded from the model. Adjusted odds ratio estimates from the final model, their 95% CI and the likelihood ratio test for statistical significance were reported.

5.6.4 Study IV:

5.6.4.1 *Study outcomes*

Study IV sought to explore the following research questions (1) What are the existing facilitators and barriers for contraceptive use among female sex workers? (2) How do female sex workers experience unintended pregnancy and how do these experiences influence their decisions regarding contraceptive use?

5.6.4.2 *Grounded theory approach to data analysis*

Analysis began with line-by-line open coding of interview transcripts in accordance with the grounded theory approach (165, 184, 185). Line-by-line coding allowed many codes to be generated on everything shared by the participants regardless of the relevance to the study questions (165). Coding was performed using the *Nvivo* software (Version 12). Codes were developed using keywords that summarized participants' information. Memos were written to describe the code, and its relevance to the research questions. In some transcripts, text was coded twice or more if the sentences contained more than one relevant aspect. Selective coding was performed by first clustering open codes into categories and then going through the transcripts again and deciding which initial codes made the most analytic sense when grouped together (165). Codes with similar meaning were merged and repetitive codes were deleted. For example, four codes; "*a child brings prosperity*", "*children bring many blessings*", "*children bring luck*", "*every child brings his/her own blessing*" were aggregated to one code "*a child is a blessing*". Collating and codifying of the initial codes was done and some codes were clustered and a label attached to the category.

Transcripts were re-read and in certain instances codes that did not fit in a category were dropped to maintain focus on the research question. An example of moving from text, open codes and selective codes to the category are given in **Figure 10.** In the later stage of the analysis, theoretical coding was performed to explain how the codes linked with each other in a particular category and between categories. An overall core-category (sex work impedes good contraceptive behaviour) that captured most important findings of the analysis was selected and linked with the other categories. A model grounded in the data was constructed to illustrate how the codes, the categories, and the core-category related with each other and with respect to the study research question (**Figure 11**).

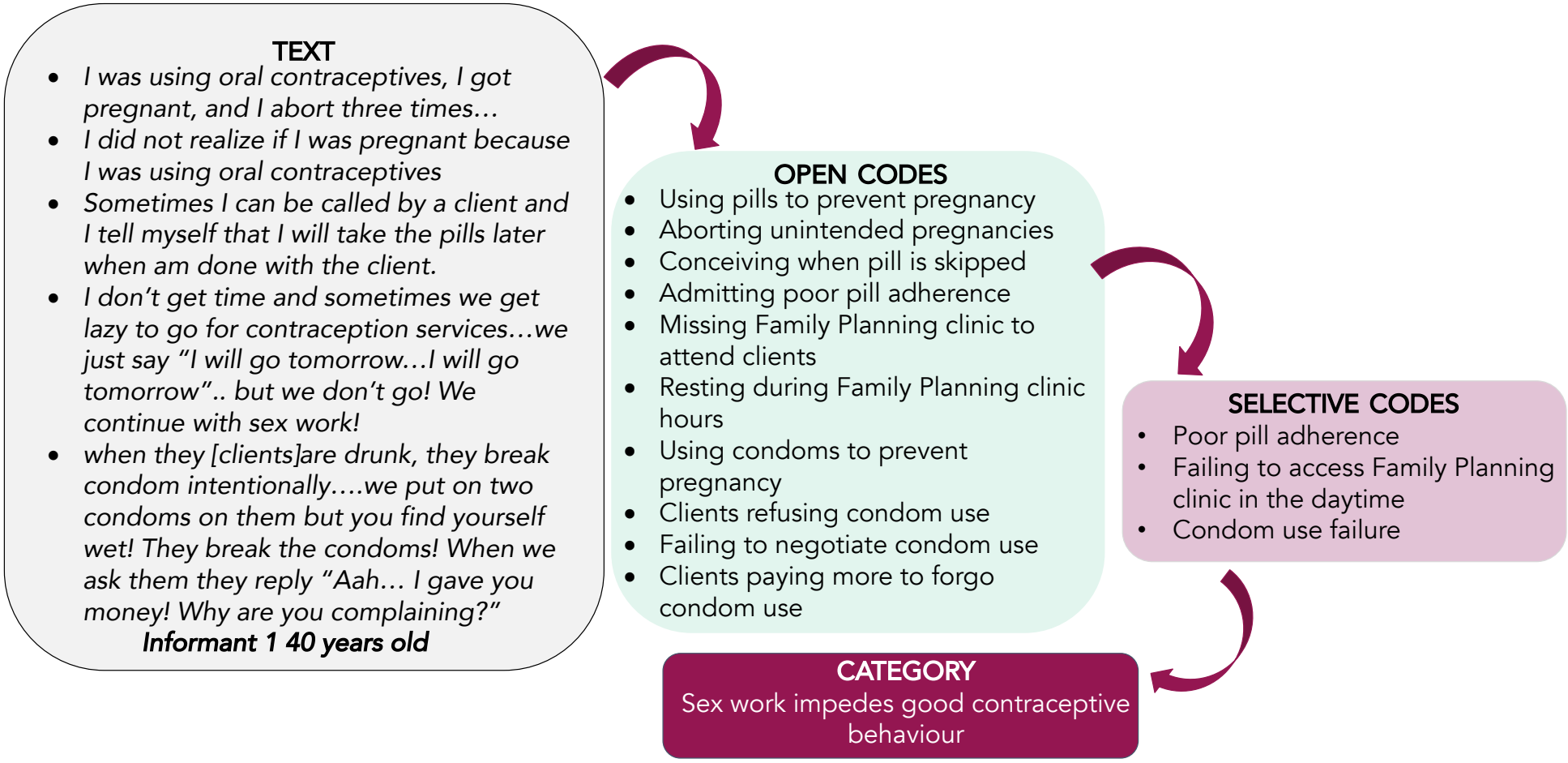


Figure 10 Illustration of Grounded Theory analysis, moving from text to category.

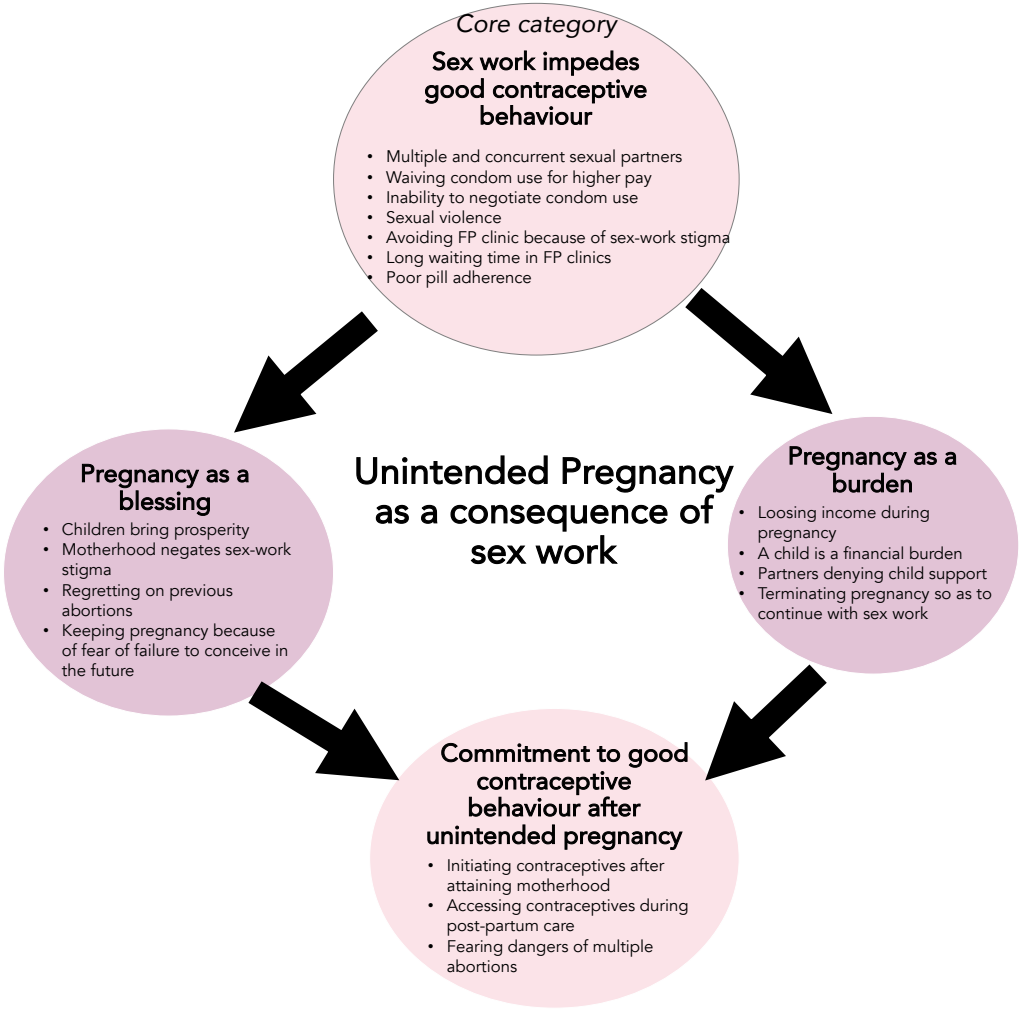


Figure 11 Conceptual categories and core category emerging from Grounded Theory analysis of barriers to contraceptive use among sex workers and experience of unintended pregnancy.

Unintended pregnancy was expressed to be an outcome of the poor contraceptive behavior, because good contraceptive behaviour was impeded by sex work. There was an ambivalence attitude toward unintended pregnancy, which was perceived to be either a burden or a blessing. This perception determined the outcome of the pregnancy i.e. to undergo an induced abortion or keeping the pregnancy. The experience of an unintended pregnancy, and the effect of abortion or child rearing on the sex worker's livelihood and wellbeing, greatly influenced their use or commitment to use contraceptive in the future.

6 ETHICAL CONSIDERATIONS

All the research studies included in this thesis involved human subjects and therefore, ethical clearance was sought from relevant authorities. The initial ethical approval for the PhD research studies was granted by the MUHAS institutional review board on April 4th 2018; Ref No. 2018-04-04/AEC/Vol. XII/87. Application for an extension of the ethical clearance was applied to and received on 22nd May 2020 Ref: IRB No. MUHAS-REC-4-2020-200. The PrEPVacc registration cohort in which the Study I, III and IV were nested was approved by both the MUHAS institutional review board and National Ethics Committee at the National Institute of Medical Research NIMR/HQ/R.8c/Vol.I/715. Ethical approvals for the microbicide and vaccine feasibility studies, were granted by the Ethics Committees of the National Institute for Medical Research, Kilimanjaro Christian Medical Centre and London School of Hygiene and Tropical Medicine.

Ethical concerns relating to participants in the PhD studies are discussed below. In the first part, description of the overall ethical issues related to the research subject is presented. Thereafter, an additional discussion is presented on ethical considerations relating to HIV standard prevention packages recommended for HIV prevention trials and other studies that have HIV seroconversion as the main outcome (as for the PrEPVacc registration cohort in Study I). Lastly ethical considerations in the qualitative study (Study IV) are described.

Informed consent process; At study enrolment an informed consent process was performed by first explaining the study to eligible participants. Participants were given a chance to read the patient information sheet or, if necessary, this was read to them by a member of the study. All questions arising on the study objectives and process were addressed. All participants verbally stated that they understood and agreed to all items contained in the patient information sheet before being enrolled in the study. Once a verbal consent was granted, participants were asked to sign the informed consent form. A thumbprint was taken from participants who did not know how to write their name or sign the consent form. A witness was asked to sign on behalf of the participant who was not able to read. A study staff obtaining participant consent also signed the consent form in the appropriate space and affix the participant's study identification number on the patient information sheet.

Risk to participants: There was a psychological risk in participating in the studies due to the sensitive nature of the questions asked e.g sexual behaviour practices and drug use. To minimize this risk, the questionnaires were administered by study personnel in a private, confidential setting. During the interviews, participants were reminded that they could refuse to answer any specific question. There was also a risk of minor injury, bruising and pain due to the collection of blood samples. In order to minimize these risks, samples were collected by

trained, laboratory technician who also provided counselling and reassurance before sample collection. Study participants were given the name and telephone number of the study principal investigator and clinical coordinator should they have any question about the study or believe they have been injured or not well treated.

Benefits to participants. The principal benefits to participants were knowing their HIV serostatus. Those testing HIV positives were escorted (with consent) to enrol into HIV care as soon as they were diagnosed to be HIV infected. Free condoms were provided as well as information on behaviour change and HIV prevention. Weekly participants engagement sessions were held to improve comprehension of the study objective. During these meetings, HIV risk reduction education was provided including condom use and PrEP. A Question & Answer session in these meetings provided participants with education which helped to demystify incorrect information related to HIV risk behaviour. Participants also benefited from screening and treatment of sexually transmitted infections as well as counselling on contraceptive methods.

Voluntary participation. All participants were informed that their participation in the study was strictly voluntary and that they were free to withdraw from the study at any time. During the course of follow-up some participants opted to discontinue follow-up. Some because of relocation while some out of personal choice.

Protection of privacy of individual and of confidential information. All interviews, counselling and laboratory rapid tests were conducted in private rooms. Privacy was maintained in these sessions by making sure that no other person was permitted in the room other than the interviewer and the study participant. Identification numbers were assigned to each of the participants and used for linking them to behavioural and laboratory data. Only aggregated behavioural and lab results were shared during weekly study meetings. All case report forms and test results were kept in locked cabinets and could only be accessed by authorized research member.

In the incidence study (Study I), termination of participants who seroconvert to HIV posed a risk of compromising confidentiality. This is because, other participants continuing with follow-up could speculate on the HIV status of those not continuing with follow up. To maintain confidentiality, data of participants who seroconverted were censored but participants were not refrained from attending the clinic every three months, and at any time as they wanted, just as they would have done if they were still enrolled in the cohort.

6.1 HIV PREVENTION ETHICAL CONSIDERATIONS IN STUDY I

The HIV incidence study (Study I) intended to demonstrate that the study population in the PrEPVacc registration cohort was suitable for participating in the PrEPVacc vaccine trial by detecting a "high" number of new HIV infections. Ethically, the objective of the study may have resulted to a perceived conflict of interest between the research team, who hope to demonstrate a high HIV incidence and study participants whose interest is to protect themselves from HIV infection. Because of this potential ethical dilemma, the guidelines for ethical consideration in HIV prevention trials recommends access to a package of prevention methods to study participants (186-192). The ethical guidelines stipulate that participants should have access to intervention so as to maximize benefits of HIV prevention and minimize risks of HIV seroconversion. Below is an outline of HIV prevention package provided in the PrEPVacc registration cohort.

- *Risk-reduction counselling* was provided to study participants on their three-monthly visits. The counselling was provided by trained research nurses and doctors who have experience working in HIV vaccine trials and therefore have skills to probe for risky behaviour practices. Counselling sessions covered safer sex practices and reproductive health. The message content of the counselling was included in the standard operating procedures so as to ensure that the sessions were comprehensive and consistent information was given to all study participants.
- *Condoms.* Male condoms were provided to all participants free of charge at every study visit.
- *Treatment of other sexually transmitted infections.* Screening of sexually transmitted infection was performed at recruitment. Rapid blood tests for syphilis, HBV and HCV were performed at enrolment. Participants who tested positive for HBV or HCV were referred to the Hepatitis clinic at Muhimbili National Hospital for further management and follow-up. Participants who were confirmed to be infected with syphilis, were provided with a drug prescription for treatment of the infection. Genital examination of vaginal ulcers and/or discharge was provided on demand basis. Syndromic diagnosis, drug prescription and/or referral to STI clinic for further testing or treatment was provided.
- *HIV pre-exposure prophylaxis (PrEP):* PrEP was not provided at the study site. This is because PrEP was not widely available in the country and plans for implementing the PrEP program among female sex workers in Tanzania were ongoing. Participants were informed that PrEP would be not offered at the study site as part of this preliminary study. However, education on PrEP (what it is, how it works, efficacy and side effects) was provided to participants so as to improve PrEP use willingness and ensure that uptake will be high once it becomes available. In the PrEPVacc vaccine trial, PrEP will be provided as a study drug to all participants in the first 26 weeks. Thereafter, participants will be referred to access PrEP through the national PrEP program which is anticipated to be in place by then. Reflections on ethical obligations of researchers to ensure availability and access to PrEP among study participant is discussed in the later sections of this thesis.

6.2 ETHICAL CONSIDERATIONS IN STUDY IV

An additional informed consent process was undertaken for participants included in the qualitative study (Study IV). Prior to the qualitative interviews, women were told of the objective of the study and provided with information on potential risk and benefits. A verbal consent was obtained from individuals willing to participate in the study and for their voice to be recorded. This was followed by a date, written name and signature of the researcher verifying that the consent was indeed taken. Participant identification numbers were used to label interview transcripts so as to ensure anonymity. Verbatim quotes used in reporting qualitative findings in this thesis as well as in the published paper do not bear respondent's names so as to maintain anonymity. Throughout the interviews, participants were reminded and assured that the information they disclosed was confidential. After the interviews, participants received one-to-one counselling on contraceptive methods and were referred to appropriate clinics for specialized family planning services. Participants who were pregnant were referred to ante-natal clinics of their choice. All respondents were provided with a transport refund of 20,000 TShs (~ 10 USD).

7 RESULTS

A summary of the main results in this thesis is presented in **Table 8** below.

Table 8 Summary of main findings from each PhD paper

Study	Research Question	Major findings
I	*What is the one-year HIV incidence among female sex workers in the PrEPVacc registration cohort? What determines their risk of HIV acquisition?*	❖ A high HIV incidence of 3.5 per 100 person-years at risk (95% CI 2.3-5.3/100 pyr) was observed after one year of follow-up. ❖ The risk of HIV infection was higher among younger female sex workers, those using illicit drugs and those diagnosed with either Syphilis, HBV or HCV infection. However, the study was not powered to ascertain these associations. ❖ There was good retention rate of 80% at 12 months even though early attrition was observed in the second visit amounting to 36%. ❖ The high HIV incidence demonstrates that the population in the PrEPVacc registration cohort is suitable for participating in HIV vaccine trials.
II	*Do HIV-risky behaviours change over time among high-risk women enrolled in HIV prevention cohorts? Do these changes predict their HIV incidence?*	❖ Nearly half of the women (49%) did not change their sexual behaviour practices after 12 months of follow-up, while 25% had higher risk practices after 12 months. ❖ There was insufficient statistical evidence to conclude if increases in higher-risk sexual behaviours was associated with a higher HIV incidence.
III	*What proportion of female sex workers are aware of PrEP and what is the proportion of those willing to use it? What are the determinants of PrEP use among female sex workers?*	❖ More than half of the female sex workers were aware of PrEP (67%). Nearly all (98%) were willing to use PrEP if it were offered. ❖ PrEP use was low (8%) mainly due to limited PrEP availability at the time of data collection. Most of the PrEP users were married and those who reported to engage in sex with a HIV infected partner.
IV	*What are the existing facilitators and barriers for contraceptive use among female sex workers? Do experiences of unintended pregnancy influence the use of contraceptives?*	❖ Female sex workers face barriers in initiating and adhering to contraceptive use because of sex work stigma, inability to negotiate condoms and failure to access medical services at their convenience. ❖ Financial hardships related to child rearing and painful abortion experiences influenced female sex worker's commitment to good contraceptive practices.

7.1 RECRUITMENT OF PARTICIPANTS IN THE PREPVACC REGISTRATION COHORT

Four seeds-initiated the respondent driven sampling and a total of 2,202 recruitment coupons were issued over three months. A total of 775 of the coupons returned met the recruitment criteria and were screened for study eligibility. The waves of recruitment per seed ranged from 6 to 16 with the majority of the recruits (82%) emanating from the social networks of one seed (seed 2). **Figure 12** and **13** provides a summary of the entire recruitment network of the four seeds. Socio-demographic characteristics, behavioural characteristics and the baseline prevalence for the HIV, HBV, HCV and Syphilis infections among female sex workers screened for enrolment into the PrEPVacc registration cohort is provided in the Paper I.

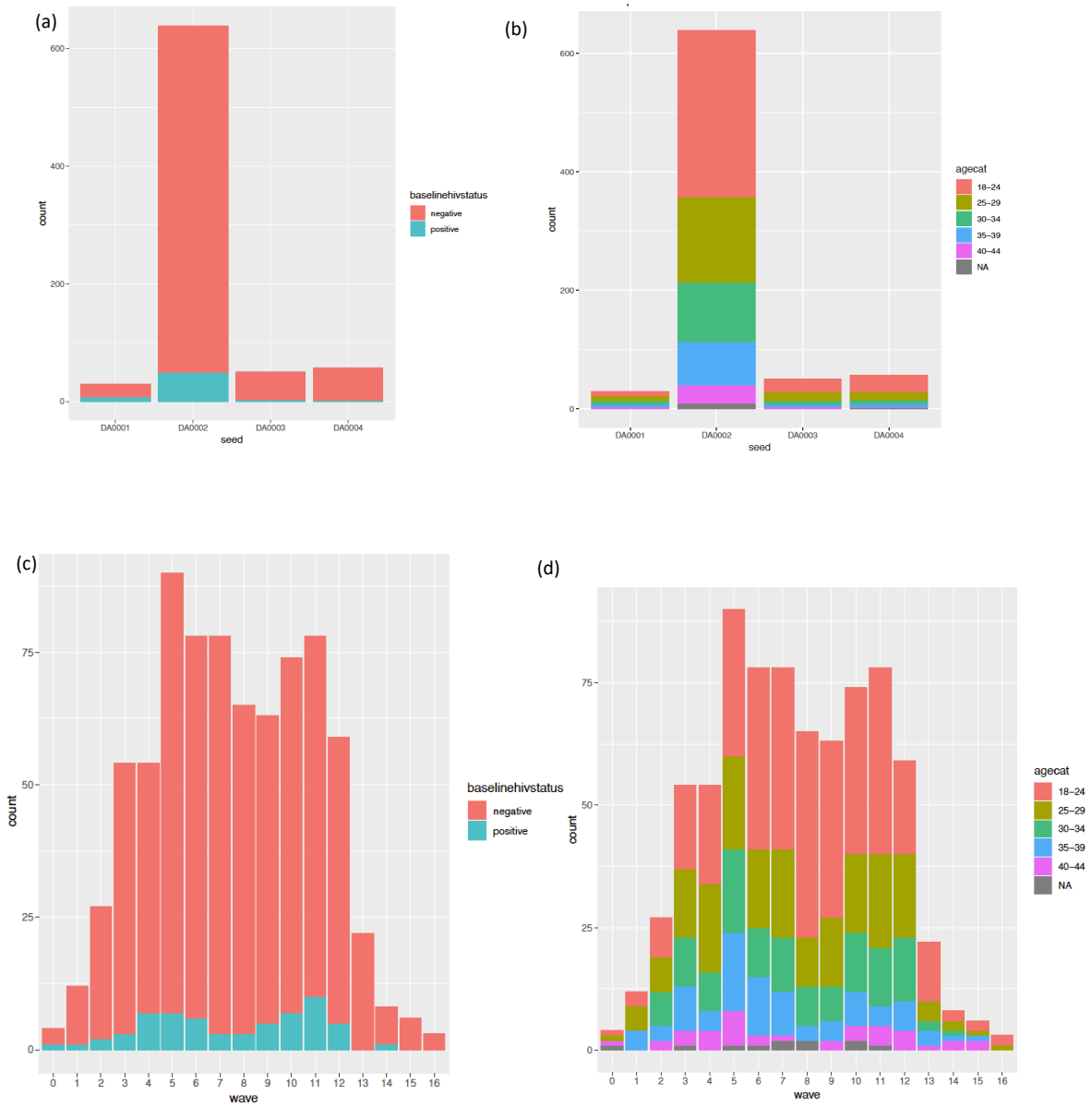


Figure 12 Respondent driven sampling showing recruitment by seeds and recruitment waves

Recruitment of each seed by (a)Baseline HIV status (b)Baseline age group
Recruitment at each wave by (c) Baseline HIV status (d) Baseline age group

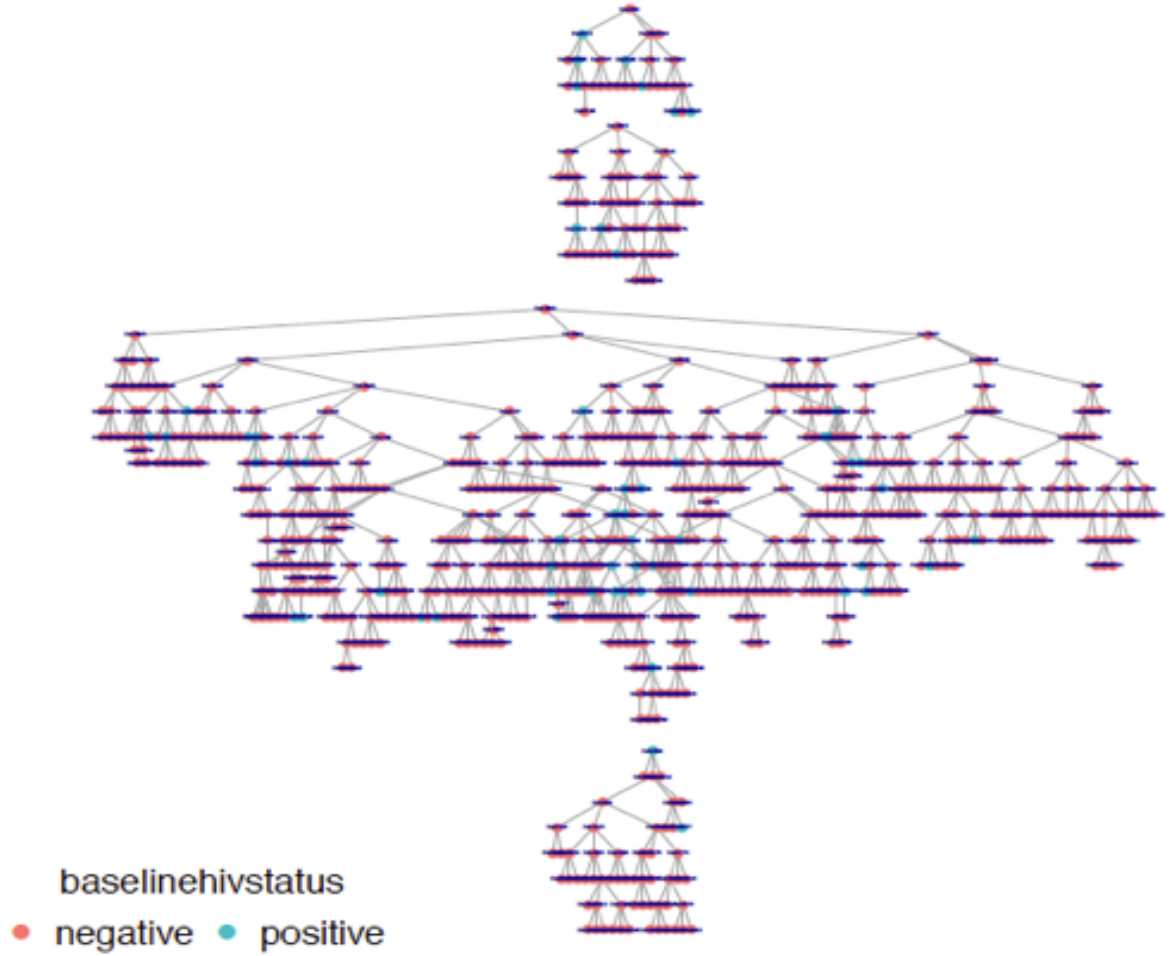


Figure 13 Respondent driven sampling recruitment trees showing networks of each seed by HIV status

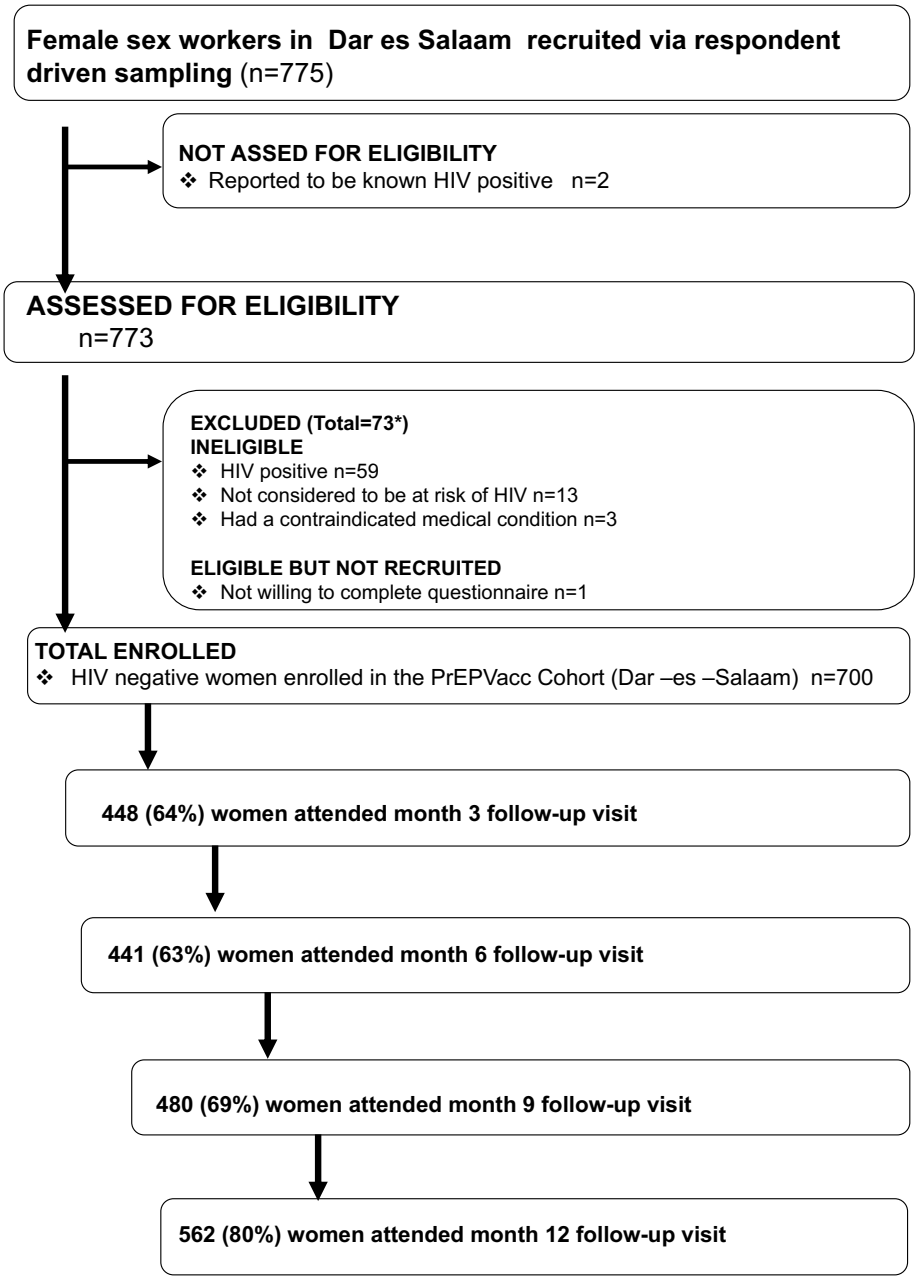


Figure 14 Screening, enrollment and follow-up of female sex workers in the PrEPVacc cohort, Dar es Salaam Tanzania.

*****_Some women met more than one ineligibility criteria; all reasons are reported; therefore, the sum is greater than the number of women not enrolled. Attendance excludes women who missed their study visit as well as those terminating cohort participation due to HIV seroconversion, death or travel. A window of +/- 1.5 months was allowed for each visit._

7.2 HIV INCIDENCE AND RISK FACTORS - PAPER 1

A total of 700 female sex workers were enrolled into the PrEPVacc registration cohort (**Figure 14).** During the 12 months follow up period, they contributed a total of 609 person-years-at risk. The total person-years at risk was defined as time from enrolling into the cohort until when censored after 12 months of follow up or exit from the study owing to HIV seroconversion. The mean follow-up duration was 10 months and the median follow-up duration was 12 months. There were a total of 21 HIV seroconversions within the 12-month follow up giving an overall incidence rate of 3.5 per 100 person-years-at risk (95% CI 2.3-5.3/100 person-years-at risk).

HIV incidence rate was high among the youngest (18-24 years) and oldest (35-45 years) age groups (4.31/100 person-years-at risk and 4.13/100 person-years-at risk, respectively **Figure 15**), however, there was no evidence of an age effect on HIV incidence (p=0.36). We also found that HIV incidence was high among participants with high education levels (5.03/100 person-years-at risk) and those who were separated/divorced/widowed (3.90/100 person-years-at risk). There was no statistical evidence on the effect of education or marital status on HIV acquisition among female sex workers in the cohort (p=0.54 and p=0.47 respectively).

Participants who reported not to have used condoms at their last sexual encounter had a 41% higher HIV incidence compared to those who did, however this difference was not statistically significant (RR 1.41, 95% CI 0.57-3.50, p=0.45, **Figure 15**). We also found that, participants who were pregnant at baseline screening (which may also imply inconsistent condom use), had nearly twice the HIV incidence compared to their counterparts although this difference was not statistically significant (RR 1.83, 95% CI 0.43-7.84, p=0.45).

Participants who had tested positive for either syphilis, HBV or HCV infection at baseline had a three times higher HIV incidence compared to those who were uninfected, though this difference was not statistically significant (RR 3.11, 95% CI 0.72-13.37, p=0.19). Participants reporting to have used illicit drugs had 27% higher HIV incidence compared to their counterparts, although this difference was not statistically significant (RR 1.27, 95% CI 0.37-4.32 p=0.71, **Figure 15**).

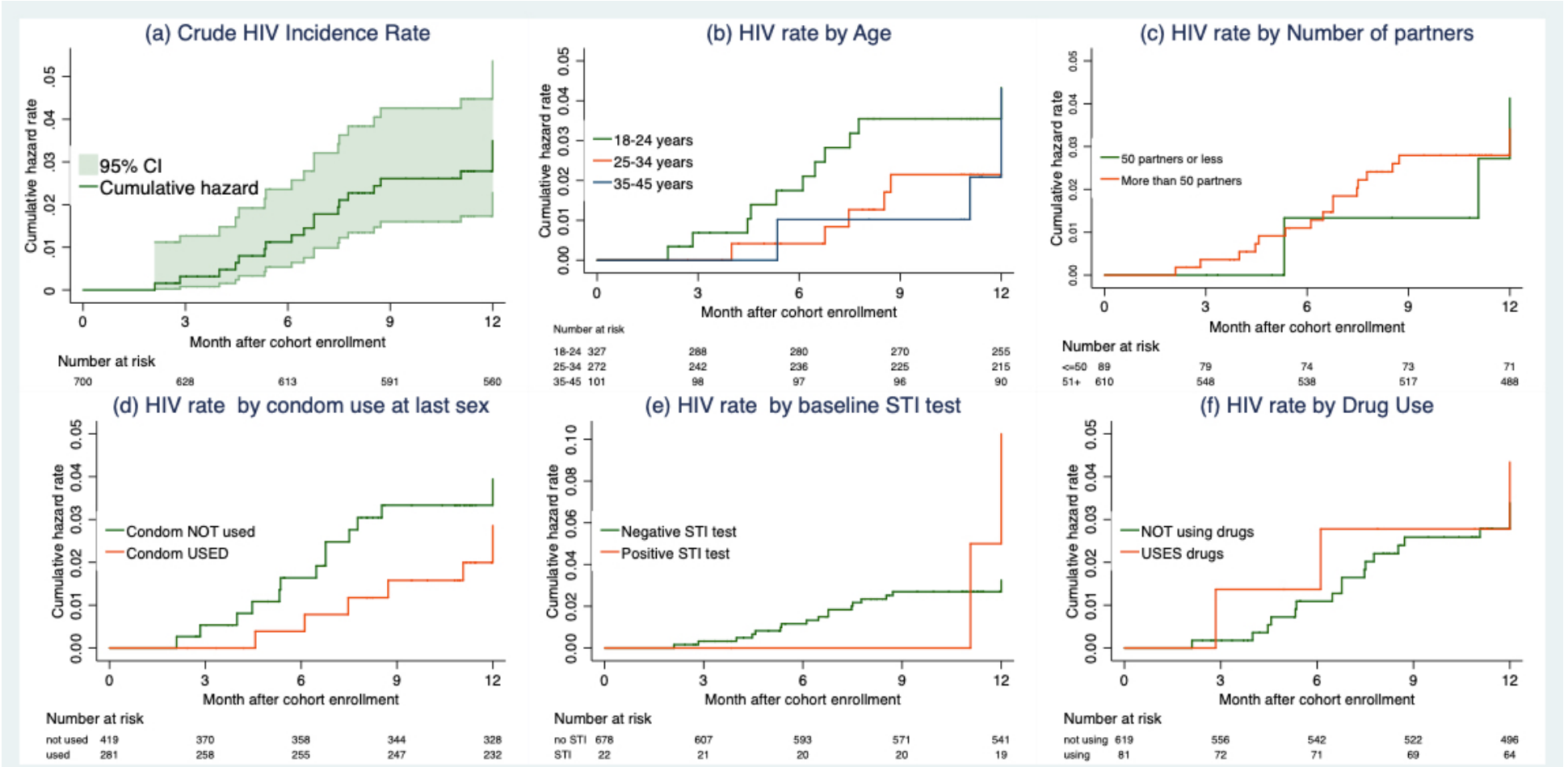


Figure 15 Cumulative hazard curves showing HIV incidence among female sex workers enrolled in the PrEPVacc registration cohort, Dar es Salaam by social demographic and behavioural characteristics

(Log-rank test for all variables >0.05)

7.3 CHANGES IN HIV RISKY-BEHAVIOURS AND ASSOCIATION WITH HIV INCIDENCE-PAPER II

7.3.1 Changes in risky behaviour among women enrolled in the microbicide and vaccine feasibility studies

In the analysis of the cohort of high-risk women in northern Tanzania; there was a substantial reduction in proportion of women reporting two or more partners in each follow up visit. In each study visit, there was a 33% reduction in the odds for having two or more partners (OR 0.67 95% CI 0.63-0.73, p for linear trend<0.001 **Figure 16).** There was also a 43% reduction in the odds of women engaging in high-risk sex (OR 0.57 95%CI 0.50-0.64, p for linear trend<0.001 **Figure 16**) and 47% reduction in the odds of transactional sex at each visit (OR 0.53 95%CI 0.49-0.57, p for linear trend<0.001 **Figure 16**).On the other hand, there was a 23% reductions in the odds of consistent condom use with regular partner at each visit, (OR 0.77 95% CI 0.72-0.82, p for trend <0.001 **Figure 16**) but there was no evidence for a change in the odds of consistent condom use with non-regular partners at each visit (0.95 95% CI 0.87-1.03, p=0.22 **Figure 16**).Together with the changes observed in the decrease in number of partners and condom use, there was an 13% increase in the odds of higher HIV risk perception at each visit (OR 1.13 95%CI 1.20-1.33 p=0.01 **Figure 16**).

Overall, the results indicate an increase in HIV risk perception with each subsequent study visit and a decrease in risky behaviour practices among women enrolled in the cohort.

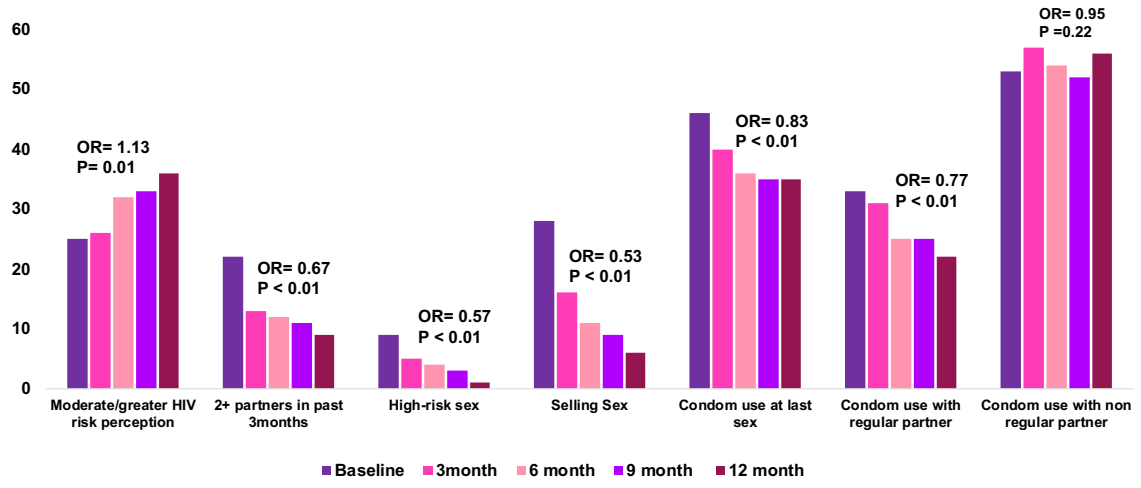
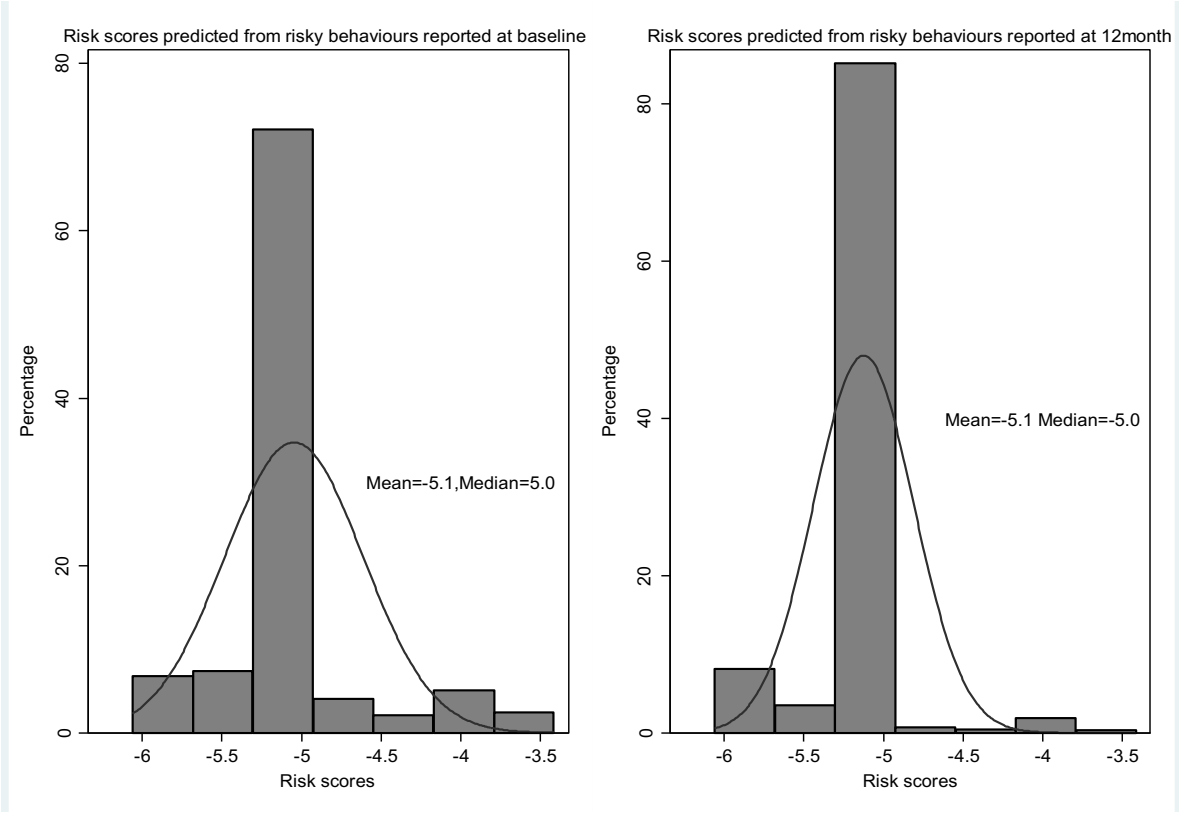


Figure 16 Changes in sexual behaviours at each visit among women enrolled in the microbicide and vaccine feasibility studies.

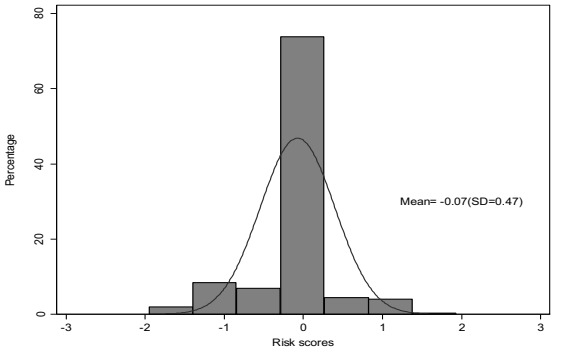
Sexual behaviour risk scores at baseline ranged from -6.06 (less-risky behaviour) to -3.41(risky behaviours) with a mean risk score of -5.05(95% CI -5.08 to -5.03). On the other hand, risk scores at 12 months had the same range of values but a lower mean, -5.12(95% CI -5.14 to -5.11). This lower mean indicates that on average, there were less-risky behaviour practices at the 12 months visit (**Figure 17).** Among the 1180 women who attended both the baseline and the 12 month visit, the proportion of women with less-risky behaviour practices at 12 months (defined as risk score ≤-5), was significantly higher than the proportion at baseline (97% vs 87%, McNemar P<0.001, **Figure 17**).



	12-MONTH VISIT		
BASELINE VISIT	*Less-risky behaviour practices (≤ -5)*	*Risky behaviour practices (>-5)*	**TOTAL**
Less-risky behaviour practices (≤ -5)	1013	16	**1029(87%)**
Risky behaviour practices (>-5)	128	23	**151(13%)**
TOTAL	**1141(97%)**	**39(3%)**	**1180**

Figure 17 Distribution of sexual behaviour risky scores at baseline and 12 month visit

The mean change in sexual behaviour risk score from baseline to 12 month was -0.07 (95%CI -0.10 to -0.05) further signifying strong evidence of less-risky sexual practices at 12 months compared to baseline (paired t-test for mean difference<0.001). The observed values for the changes in sexual behaviour risk score ranged from -1.95(safer behaviour) to 1.93(less safe behaviour). Nearly half of the women (49%, 573/1180) had no change in sexual behaviour risk score, 25% (300 women) had an increase in the risk score and 26% (307 women) had a decrease in risk score at the 12 months visit **Figure 18**.



CHANGES IN RISKSCORE N=1180 (col%)

No change in sexual behaviour practice (risk score ≤-0.001)	573(49%)
Decrease in risky behaviour practices (risk score=0)	307(26%)
Increase in risky behaviour practices (risk score >0	300(25%)

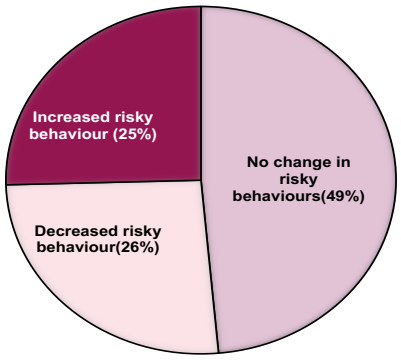


Figure 18 Distribution of change in risky sexual behaviours.

7.3.2 HIV incidence and changes in sexual risky behaviour

The 1378 women enrolled in the microbicide and vaccine feasibility studies contributed a total of 1181 person-years-at -risk, defined as, time from enrolling into the cohort until when censored after 12 months of follow up or exit from the study owing to death or seroconversion. There was a total of 44 women who seroconverted to HIV positive with an overall HIV incidence rate in the cohort of 3.7 per 100 person years at risk (95% CI 2.8-5.0/100 person years-at risk). **Table 9** summarizes the factors associated with HIV incidence among women enrolled in the two cohorts. The HIV incidence ranged from 8.6/100 person years-at risk among women with risky behaviour practices at baseline to 3.0/100 person years-at risk among those who had less-risky behaviour practices. Women who had risky sexual behaviour risks score at baseline had nearly three times the risk of HIV (RR 2.86 95%CI 1.49-5.46 p=0.001). HIV incidence was also higher among unmarried compared to married women (RR= 5.19, 95%CI 1.61-16.77, p=0.01) and in those who had two or more partners in the month preceding baseline visit (RR=2.62, 95%CI 1.28-5.38, p=0.01 **Table 9**).

Due to the few cases of HIV seroconversion, a fully comprehensive analysis of the general risk factors for HIV acquisition was not performed. The final model on the association of change in risk score with HIV acquisition was adjusted for the baseline risk scores and marital status. We found that HIV risk was lower among women who had increased or no change in risk score, compared to those with reduction in risky sexual behaviour risk score, however there was no statistical evidence for the effect of changes in risky behaviour on HIV incidence (aRR= 0.88 95%CI 0.39-2.01, p=0.76). Women who had risky sexual behaviour risks score at baseline had twice the risk of HIV after adjusting for other factors (aRR 1.99 95%CI 0.80-4.94 p=0.14). On the other hand, marital status was independently associated with HIV risk with unmarried women having nearly five times the risk of HIV compared to married women (aRR 4.60 95%CI 1.42-14.95).

Table 9 Crude and adjusted rate ratio estimates for HIV incidence by changes in sexual behaviours risk scores and baseline risk factors

Variable	HIV cases/ pyrs[1]	Rate /100 pyr	Unadjusted HR(95%CI)	Adjusted HR(95%CI) [2]
Change in sexual behaviour			**P=0.07**	**P=0.76**
Decreased risky behaviour	16/294	5.4	1	1
No change/Increased risky behaviour	26/844	3.1	0.57(0.30-1.06)	0.88(0.39-2.01)
Baseline sexual behaviour risk score			**P=0.001**	**P=0.14**
Less-risky behaviours (≤ -5)	31/1000	3.0	1	1
Risky behaviours (>-5)	13/151	8.6	2.86(1.49-5.46)	1.99(0.80-4.94)
Age at enrolment(years)			**P=0.74**	N/A
<25	17/428	4.0	1	
≥25	27/754	3.6	0.90(0.49-1.65)	
Town of residence			**P=0.94**	
Moshi	14/370	3.8	1	N/A
Lake zone	30/812	3.7	1.102(0.54-1.93)	
Marital status			**P=0.01**	**P=0.01**
Married	3/325	0.9	1	1
Single/separated/divorced/widowed	41/856	4.8	5.19(1.61-16.77)	4.60(1.42-14.95)
Education level			**P=0.35**	
None-Primary	39/984	4.0	1	N/A
Secondary-Tertiary	5/196	2.6	0.64(0.25-1.63)	
Contraceptive Methods			**P=0.59**	N/A
Condom only	14/463	3.0	1	
None/Traditional/safe period	15/350	4.3	0.70(0.34-1.46)	
Pill/injection/other hormonal+/-condom	15/368	4.1	0.95(0.46-1.94)	
Transactional sex past 3m			**P=0.37**	N/A
No	30/871	3.4	1	
Yes	14/304	4.6	1.34(0.71-2.52)	
Condom use (regular) partners past 3m			**P=0.69**	
Always/No partner	16/461	3.5	1	N/A
Never/Inconsistently	28/712	3.9	1.13(0.61-2.10)	
Condom use (non-regular) partners past 3m			**P=0.72**	
Always/No non-partner	36/934	3.9	1	N/A
Never/Inconsistently	8/239	3.3	0.87(0.40-1.87)	
Number of partners past 1m			**P=0.01**	N/A
0-1	29/935	3.1	1	
2+	10/123	8.1	2.62(1.28-5.38)	
HIV risk perception			**P=0.99**	
Small/no risk/ Doesn't know	33/884	3.7	1	N/A
Moderate/great risk	11/294	3.7	1.00(0.51-1.99)	
Baseline N. gonorrhoea			**P=0.24**	
Negative	40/1000	3.6	1	N/A
Positive	3/41.3	7.3	2.01(0.62-6.50)	
Baseline C.trachomatis			**P=0.17**	
Negative	41/1000	4.0	1	N/A
Positive	2/135	1.5	0.37(0.09-1.51)	
Baseline T. vaginalis			**P=0.75**	
Negative	25/643	4.0	1	N/A
Positive	5/151	3.3	0.85(0.33-2.23)	
Baseline Syphilis			**P=0.26**	
Negative	27/655	4.1	1	N/A
Positive(Active/past)	3/145	2.1	0.50(0.15-1.65)	
Baseline HSV-2			**P=0.11**	N/A
Negative	9/375	2.4	1	
Positive	35/805	4.3	1.81(0.87-3.77)	
Baseline B.vaginosis			**P=0.63**	
Negative	15/442	3.4	1	N/A
Positive	15/370	4.1	1.19(0.58-2.44)	

HR=Hazard ratio, CI=Confidence interval [1] Numbers may not add up to total due to missing data. [2] Estimated HR adjusted for change in risky behaviour score, baseline sexual behaviour risk score and marital status.

7.4 AWARENESS, WILLINGNESS AND USE OF PREP AMONG FEMALE SEX WORKERS - PAPER III

7.4.1 Awareness of PrEP and willingness to use PrEP

At the time of enrolment into the PrEPVacc registration cohort, more than half of the female sex workers had ever heard of PrEP (67%,469/700). Those reporting to have more than 100 partners in the past three months were 58% less likely to be aware of PrEP compared to those reporting to have had less than 50 partners in the same period (Unadjusted OR 0.42; 95% CI 0.23-0.76, p<0.001, **Table 10**). Sex workers who reported to have been raped in the past three months were 78% less likely to be aware of PrEP (Unadjusted OR 0.22; 95% C I 0.15-0.33, p<0.001). Sex workers who were using contraceptives (which may imply access to health services) were twice more likely to be aware of PrEP as compared to those not using contraceptives (OR 2.15, 95% CI 1.55-2.98, p<0.001).

Overall, there was high willingness to use PrEP among female sex workers in the PrEPVacc registration cohort. At enrolment, (98%, 684/700) of the participants said were willing to use PrEP if it was offered. Of the remaining 16 participants: four reported not to be willing to use PrEP and 12 female sex workers were not sure if they would use it. There was no change in PrEP willingness after 12 months follow-up in the cohort (98% vs 96% McNemar test p=0.84).

Table 10 Determinants of PrEP awareness

Characteristic	HIV Uninfected (col%)	PrEP Unaware (row%)	PrEP Aware (row%)	Unadjusted OR (95%CI)	P-value (LRT)
Overall	**700**	**231**	**469**	**--**	
Age(years)					
<25	328(47)	120(37)	208(63)	1	0.1
25-34	271(39)	77(28)	194(72)	1.45(1.03-2.06)	
35-45	101(14)	34(34)	67(66)	1.14(0.71-1.82)	
Education					
None/Incomplete Primary	83(12)	36(43)	47(57)	1	0.09
Complete Primary/Incomplete secondary education	480(66)	155(32)	325(68)	1.61(1.0-2.58)	
Complete secondary or higher	137(20)	40(29)	97(71)	1.86(1.05-3.28)	
Condom use at last sex					
Yes	281(40)	98(35)	183(65)	1	0.39
No	419(60)	133(32)	286(68)	1.15(0.84-1.59)	
Number of partners in the past 3 month					
≤50	89 (13)	15(17)	74(83)	1	<0.001
51-100	233(33)	93(40)	140(60)	0.31(0.16-56)	
>100	377(54)	123(33)	254(67)	0.42(0.23-0.76)	
Rape in the past 3 months					
Yes	120(17)	75(63)	45(38)	0.22(0.15-0.33)	<0.001
No	579(83)	156(27)	423(73)	1	
Contraceptive use					
Yes	448(64)	120(27)	328(73)	2.15(1.55-2.98)	<0.001
No	252(36)	111(44)	141(56)	1	

7.4.2 Use of PrEP

A total of 57 HIV negative female sex workers in the PrEPVacc registration cohort (8%, 57/700), reported to have ever initiated the use of PrEP at any point during the 12 months follow-up period. Nearly all of the female sex workers who had ever used PrEP (86%,49/57) reported to have received PrEP from a Non-governmental organization clinic while 11% (6/57) reported to have had received PrEP from a friend – possibly a peer educator within the female sex workers community. We found that marital status was strongly associated with PrEP use whereby; female sex workers who were married/co-habiting were four time more likely to use PrEP (aOR 4.19, 95%CI 1.44-12.18 **Table 11**), while those separated /divorced /widowed were twice more likely to use PrEP (aOR 2.38, 95%CI 1.17-4.83, **Table 11**) as compared to female sex workers who had never been married. We also found that female sex workers who reported to have had sex with a HIV infected partner were associated with four times the odds of using PrEP (aOR 3.98, 95% CI 1.20- 13.15, p=0.04, **Table 11**). However, we did not find any statistical association between PrEP use with number of partners, consistent condom use or with experiences of rape.

Table 11 Multivariable analysis showing factors associated with PrEP use

Characteristic	EVER used PrEP/Subtotal (row %)	Adjusted OR	P-value
Relationship status			
Never Married	40/594(7)	1	
Married /Cohabiting	5/22(23)	4.19(1.44-12.18)	
Separated/Divorced/Widowed	12/84(14)	2.38(1.17-4.83)	0.01
Number of partners in the past 3 months			
≤120	31/426(7)	1	
121+	25/273(9)	1.20(0.68-2.13)	0.52
Sex with HIV infected partner in the past 3 months			
No/Don't Know	52/683(8)	1	
Yes	4/16(25)	3.98(1.20-13.15)	0.04
Condom usage during transactional sex			
Always use	6/42(14)	1	
Sometimes use	47/622(8)	2.49(0.45-13.66)	
Never uses	2/34(6)	1.19(0.27-5.26)	0.34
Rape in the past 3 months			
No	42/579(7)	1	
Yes	14/120(12)	1.65(0.85-3.21)	0.15

OR adjusted for marital status, number of partners in the last three months, sex with a HIV infected partner, condom usage during transactional sex and rape

7.5 FACILITATORS AND BARRIERS FOR CONTRACEPTIVE USE – PAPER IV

7.5.1 How sex work impedes good contraceptive behaviour

Unintended pregnancy was expressed to be an expected outcome of sex work, because sex work impeded good contraceptive behaviour. On one hand, sex workers expressed awareness of contraceptives and had knowledge on how contraceptives worked, but on the other hand, they expressed frustration over how consistent contraceptive use was hindered by the nature of sex work. They reported failure of contraceptive use because of poor adherence leading to some women conceiving when using contraceptives. Failure to initiate, continue or appropriately use contraceptives was expressed as a fault at an individual level, but it seemed to be embedded in contextual factors that influenced contraceptive use e.g. gender power dynamics in negotiating condom use, sex work stigma in family planning clinics.

Waiving condom use for a higher pay from clients. Although condom use was generally desired by the sex workers who wished to protect themselves from not only pregnancy but also from sexually transmitted infections including HIV, women expressed that, it's use risked low payment from clients. With casual partners, sex workers waived condom use so as to get a higher pay. With non-commercial partners or long-term regular partners condoms were waived to maintain good relations for financial and emotional security.

> *"There some clients with whom I want to use protection, but he may force you to have sex without it (condom)..... This is your work and you need the money, so you do it. And this, (unintended pregnancy) is the result of it (unprotected sex)...."[Informant 8, 29 years].*

Sex work stigma from health workers, impedes initiation and continuation of non-barrier contraceptives. Sex workers tried to mitigate inconsistent condom use, by opting to use non-barrier contraceptives (e.g., birth control pills, Depo-Provera, implants and intrauterine devices). However, sex workers expressed frustration on the stigma they received when visiting the family planning clinic. This stigma resulted in avoidance of health services.

> *"...you can go to the hospital to get implants and they may speak to you harshly. You can't stop the person from talking negatively because they are doctors and you might not get the services. They can tell you do this or do that or you are not seated properly... Instead of telling you politely they just provoke you... But it's not just to me, even to my friends, some of my friends wanted to insert implants but they stopped.... There is a certain elderly woman there who talks a lot!" [Informant 4, 19 years].*

Sex work routine impedes adherence to non-barrier contraceptives. Women explained that contraceptives were readily available in health centres but long waiting time and inconvenient clinic hours made them not to seek services. Contraceptive services are offered in the clinics during daytime and often closing at 3pm, when sex workers are likely to be sleeping/resting in preparation for working at night. Consequently, they fail to adhere to monthly clinic visits for refills or injections and therefore miss or skip contraceptive use for a month or more. Pill adherence was hampered by the tendency to forget taking pills when intoxicated after drinking alcohol, oversleeping because of working late hours, or skipping the pill when called by a client. On the other hand, sex workers expressed that the side effects of contraceptives such as nausea, heavy menses etc. interfered with their work — risking loss of income. One would discontinue contraceptive or skip the pill to avoid side effects, and thus risking pregnancy.

> *"I don't get time and sometimes we get lazy to go for contraception services, that's why we get unexpected pregnancies!.....We just say I will go tomorrow....I will go tomorrow, but we don't go. We continue with our sex work….Sometimes a client might come at your working place and you may forget [taking the pill]because you are in hurry so you forget to take them." [Informant 1 40 years].*

7.5.2 How experiences of unintended pregnancy influence commitment to good contraceptive behaviour.

Having experienced the hurdles of unintended pregnancy, sex workers initiated contraceptive use and committed to consistent use and good adherence. Irrespective of the pregnancy outcome i.e. having an abortion or keeping the pregnancy, these women perceived unintended pregnancy experience as a wakeup call to contraceptive use. Postpartum initiation of contraceptive use was common among sex workers who reported to have learnt about contraceptives and being offered to initiate contraceptive use after delivery.

Female sex workers were also more likely to initiate contraceptive use post-delivery due to the myth of infertility associated with contraceptive use before childbirth. Sex workers expressed concerns that initiating contraceptive use at younger age resulted in infertility in the future and therefore it was safer to use contraceptives after having a child. This misconception was a reason why most women had not previously used hormonal contraceptives, even though they knew the risk of unintended pregnancy in sex work.

> *"When I had my first child, I didn't use contraceptives, I started after having the second child because they were close (spaced). I was not aware that there is such a thing as family planning.., and I didn't know if I could conceive again within such a short time! ... When I conceived for a second time that is when my mind was opened! Therefore, when I attended clinic they advised me to start using family planning immediately after*

delivery. Given the challenges I went through, the difficulties of raising those children, after delivery I wasted no time, honestly speaking! The same day when I started post-natal clinic, I also started using contraceptives! Nurses should promote (contraceptives), provide education to emphasize people on using it especially soon after delivery! They should not keep quiet! They should educate someone until she understands" [Informant 5,28 years].

Sex workers expressed painful abortion experience following an unintended pregnancy. They also expressed worries of having to go through another abortion in the future. Some expressed fear that abortion could result to infertility in the future. With this, they expressed commitment to use and adhere to contraceptives so as to prevent unintended pregnancy.

" We do not look further and consider what will happen later. So, you just do it (abort) because it's important to you at that moment. But at the end of the day you can decide to stop and when you want to have a child, you don't get one. It might be because of the multiple abortions in the past… may be the cervix has experienced some problem like getting loose… or perhaps you were only meant to have one or two children and later you fail to conceive again. You could even die! [Informant 9,27 years].

8 DISCUSSION

The findings in this thesis demonstrated the HIV incidence, and described the sexual and reproductive health behaviours among high-risk females recruited for participation in HIV prevention trials in Tanzania. Specifically, the results described the recruitment, screening, enrolment and retention of female sex workers living in Dar es Salaam into the PrEPVacc registration cohort. The discussion below provides an account of the suitability of the female sex worker enrolled in the PrEPVacc registration cohort for participation in HIV vaccine trials, in particular the PrEPVacc vaccine trial. Where appropriate, findings on HIV incidence and changes in sexual risky behaviour from the cohorts in the northern Tanzania will be used to discuss expected changes in sexual risky behaviour in the PrEPVacc registration cohort.

This discussion section includes a reflection on the following issues (a) the estimated one-year HIV incidence and its suitability for HIV efficacy trials (b) changes in HIV risky behaviour during cohort participation and if these changes influence the HIV incidence in the cohort (c) awareness, willingness and use of HIV pre-exposure prophylaxis among female sex workers and how this may affect evaluation of efficacy in HIV vaccine trials (d) barriers to contraceptive use among female sex workers and a reflection on ways to minimize unintended pregnancy during participation in HIV vaccine trials.

8.1 DETECTING A HIGH HIV INCIDENCE IN HIV PREVENTION TRIALS

The analysis in Study I found that female sex workers living in Dar es Salam have a high HIV incidence of 3.5 per 100 person- years at risk (95% CI 2.3-5.3/100 person-years at risk). The HIV incidence was not precise or higher than the expected incidence of 4.0/100 persons-years-at risk but it was within the expected margin from the sample size estimation (in **Table 3**). Importantly, this HIV incidence is sufficiently high to confirm that female sex workers living in Dar es Salaam are indeed a HIV high-risk population suitable for participation in the PrEPVacc HIV vaccine trial.

The HIV incidence of 3.5 per 100 person-years at risk estimated in the Study I was similar to the HIV incidence of 3.7 per 100 person-years-at risk (95% CI 2.8-5.0/100 person-years at risk) estimated in the cohorts from northern Tanzania (Study II) even though there was a 10 years difference between recruitment into the cohorts (2008 for cohorts in northern Tanzania versus 2018 for the PrEPVacc registration cohort). This finding indicates that women who sell sex, either as direct sex workers (Study I participants) or in-direct sex workers (Study II participants) are (unfortunately) still a HIV high-risk population in Tanzania and therefore a suitable population for HIV efficacy trials.

The estimated HIV incidence in the PrEPVacc registration cohort was similar to the incidence of 3.8 per 100 person-years at risk (3.5-4.2/100 persons-years-at risk) estimated among women

living in areas of high HIV incidence in Kenya, South Africa and Zambia (90). The HIV incidence in the PrEPVacc registration cohort was lower than the 5 per 100 person-years at risk HIV incidence estimated in the placebo arm of a PrEP study among African women (193). However, in this study there were no HIV incident cases among participants from Tanzania—possibly because the trial at the Tanzanian site was initiated just a few months before the trial unexpectedly ended due to lack of efficacy. The HIV incidence in the PrEPVacc registration cohort was also lower than the 10.4% estimated in a cohort of female sex workers in Iringa Tanzania (only incidence proportion was reported in the Iringa study and information on person years at risk was not provided). The higher HIV incidence among female sex workers in Iringa can be explained by the high HIV burden in the general population in that region (HIV prevalence of 11.2% in Iringa vs 4.3% in Dar es Salaam (25)).

The PrEPVacc registration cohort sample size was powered to detect a HIV incidence with an expected 95% confidence interval extending from 1.0 to 7.0 per 100 person years at risk (**Table 3**). Although the observed incidence was within the margin, it is also possible that this incidence is lower than the actual HIV incidence in the wider female sex worker community in Dar es Salaam. This is because cohort participants have better access to HIV prevention interventions unlike female sex workers in the community. For the same reason, the HIV incidence in the PrEPVacc registration cohort may decline over time as participants become better engaged with the cohort and receive access to condoms, risk reduction counselling, treatment of sexually transmitted infection and PrEP upon its availability. The HIV incidence in the cohort will also decline as most at-risk participants become HIV infected early and exit the cohort, leaving those at lower risk continuing with cohort follow-up. As of March 1st 2021, the HIV incidence in the PrEPVacc registration cohort had decline to 2.7 per 100 person years at risk (95% CI 1.9-3.8/100 persons-years-at risk). This observation of a declining HIV incidence is not uncommon in HIV prevention trials and has been reported in other vaccine preparedness cohorts (92, 93, 171, 194, 195). Because of this, open cohorts instead of closed cohorts are recommended for HIV vaccine preparedness cohorts. Additionally, it is worth mentioning here that the HIV incidence in the cohort could have been higher if the cohort did not suffer from early attrition by younger female sex workers who are known to have a higher HIV incidence. The impact of the loss-to-follow up bias on the HIV incidence and the preference for open cohorts are discussed in the later section of the thesis.

8.2 CHANGES IN SEXUAL RISKY BEHAVIOURS IN HIV PREVENTION TRIALS

In the analysis of the cohort of high-risk women in northern Tanzania (Study II), the mean change in sexual behaviour risk score from baseline to 12 months was -0.07 (95% CI -0.10 to -0.05, paired t-test for mean difference<0.001). These results provide strong evidence of less-risky sexual practices at 12 months compared to baseline. Enrolment into the cohort was associated with reduction in the number of sexual partners, selling sex and having high risk sex at each follow up visit. There was an increased HIV risk perception among participants at each visit. Consistent condom use with regular partners was significantly lower at each follow-up visit but there were no changes in consistent condom use practices with non-regular partners

The analysis in Study II did not confirm the hypothesis that risky sexual behaviour in the cohort increases over time and were associated with higher HIV incidence. There was no statistical evidence of an increased HIV incidence rate among women with no change/increased sexual behaviour risk scores at 12 months after adjusting for their baseline sexual behaviour (p=0.79). Contrary to subjective expectation and biological plausibility, women with no change/increased in risk score at 12 months, had a lower point estimate of HIV incidence compared to those with reduced sexual behaviour risk score although this finding could have been due to chance (aRR 0.89 95% CI 0.37-2.10). This finding could also partly be explained by the fact that the multivariate model suffered from low power due to the few HIV seroconversion cases; and that, the composite risk score used to quantify risky behaviours was based on self-reported behaviour and had left out a substantial number of risky sexual behaviour variables.

Taken together, the implications of findings from Study II to the PrEPVacc registration cohort are that: (i) Risky behaviour practices decrease after 12 months of follow up especially among those who had more risky practices at recruitment; and (ii) The reduction in risky behaviour may not affect the HIV incidence estimated after 12 months of follow up. It is possible that this association may be observed with longer follow up periods.

8.3 THE USE OF PRE-EXPOSURE PROPHYLAXIS (PREP) IN HIV PREVENTION TRIALS

Findings from Study III indicate that, majority of female sex workers in Dar es Salaam are aware and willing to use PrEP to protect themselves from HIV acquisition. However, there were very few female sex workers who had ever initiated the use of PrEP, possibly because it was not widely available in the country at the time of data collection. The use of PrEP as a biomedical HIV prevention intervention is appealing to female sex workers because it offers them control on its use (98, 196-198).

High PrEP awareness and willingness was sustained among participants possibly because of the repeated PrEP education provided in the weekly cohort engagement meetings. This finding could suggest the need for repeated PrEP education among PrEP users in the future PrEPVacc vaccine trial so as to sustain PrEP adherence. This is important because PrEP studies among female sex workers as well as among other high-risk groups have shown lower PrEP continuation even when high PrEP willingness was expressed (100, 104, 105, 199-201). One reason cited for PrEP discontinuation among individuals was not being in the "risk season" or taking a "PrEP break" (199, 202, 203). This is expected among female sex workers because they are known to occasionally take breaks from commercial sex. In support of this, evidence from PrEP implementation studies have suggested that PrEP should not be a lifelong intervention, only to be used during high-risk period and that adherence should be insisted during the high-risk period (200, 203-205). Therefore, continued education on PrEP will offer opportunities for re-assessment of HIV risk so as to promote willingness and adherence to PrEP during high-risk periods.

We found that PrEP use was higher among sex workers engaging in sex with an HIV infected partner. This finding is consistent with others that have reported good PrEP adherence among sero-discordant couples (206-208). The findings further show the helpfulness of PrEP as a woman-controlled HIV prevention intervention in instances where negotiating condom use with a partner (especially a regular or intimate partner) is not possible or fails (82, 209-211). Another plausible explanation for this observation would be behaviour disinhibition as a female sex worker may knowingly engage in sex with a HIV positive partner because of the assured protective effectiveness of PrEP. Encouragingly studies have reported that risk compensation is of limited concern among PrEP users and that the use of PrEP may actually increase risk perception and encourage safer sexual behaviour practices (98, 100, 102, 193, 196, 197).

This observation also serves as a reminder that in the future PrEPVacc vaccine trial, risk reduction counselling sessions should remind participants that PrEP (and the vaccine products) should not replace condom use or other behaviour modification. Participants should also be reminded of the persistent HIV risk, sexually transmitted infections and unintended pregnancy associated with unprotected sex.

8.4 PREGNANCY AND CONTRACEPTIVE USE AMONG PARTICIPANTS IN HIV PREVENTION TRIALS

There were a total of 46 pregnancies over the year of follow-up in the PrEPVacc registration cohort. Pregnancy is of concern because in most efficacy trials, pregnancy is an exclusion criteria and study product is usually discontinued once pregnancy is confirmed because of the unknown unintended effects of the product on pregnancy or foetus. Preventing unintended pregnancy among female sex workers is also important because it reduces their HIV vulnerability perpetuated by financial burdens of child rearing (77). The pregnancy incidence observed in the registration cohort may provide a guess of participant attrition attributable to pregnancy in the future PrEPVacc vaccine trial.

The risk of unintended pregnancy can be minimized by advocating for the use of dual contraceptives especially the use of long-acting reversible contraceptives. The analysis in Study I showed low use of long-acting reversible contraceptives such as implant and inter-uterine devices. Additionally, qualitative findings in study IV indicated that there was inconsistent use and regular switching of Depo Injections and oral contraceptive pills. For instance, some sex workers reported to have conceived when they had skipped/missed an oral contraceptive pill or Depo Injections. This indicate that even though contraceptive use may have been reported in Study I, discontinuation or skipping a pill is very common. For this reason, use of long-acting reversible contraceptives is recommended among female sex workers because these methods are less prone to incorrect use, discontinuation and frequent switching (80, 85, 90).

A notable finding from the analysis in Study I was that, the very group that is at risk of HIV acquisition (18 to 25 years old) was also the group who had the highest pregnancy incidence. Therefore, long-acting contraceptives in this group should be advocated in the trial so as to minimize study attrition. The findings from the qualitative study (Study IV) are disconcerting as it was noted that younger female sex workers (who are also likely to be null parous) may not agree to use contraceptive in fear of a popular myth that initiation of contraceptive at an early age causes infertility. There were also some indications from Study IV that the desire for motherhood could be a barrier to contraceptive use since having a child was highly regarded as a “blessing”. Because of the desire for motherhood reported among participants it may be problematic for the participants to opt for HIV vaccine product instead of motherhood. In other HIV vaccine trials that involve females, researchers suggested the need to involve male partners so as to reduce pregnancy (92). However, this may be challenging in the PrEPVacc registration cohort as sex workers have multiple sexual partners.

Findings from Study IV highlighted the need for integrating contraceptive services within the same location that offers HIV services to female sex workers e.g within the PrEPVacc study

site. This is because female sex workers face barriers at an individual or structural level that impede access to contraceptive services. These factors include sex work stigma in medical facilities as well as failure to attend family planning clinics during their clinic operating hours. Contraceptive services at the PrEPVacc study site will have to offer multiple contraceptive options as this has been shown to increase demand and uptake of contraceptives (81, 88, 90, 175, 212-214).

8.5 METHODOLOGICAL CONSIDERATIONS

This section discusses the key methodological limitations and consideration in the PhD studies.

8.5.1 Epidemiological considerations in quantitative studies (Study I, II, III)

8.5.1.1 *The Role of chance (random error)*

- In both study I and II the number of HIV seroconversion were few. This limited the statistical power of the studies to determine factors associated with HIV seroconversion. This can be evident with wide confidence intervals in the univariate and multivariate analyses for HIV risk factors (**Table 9** and in Paper I). This limitation was more prominent in Study II since the study was a secondary analysis of data. The sample size in study II was powered for the aims of the parent cohorts and therefore underpowered to detect significance level for the research questions in this thesis.

8.5.1.2 *Selection Bias*

- *The use of respondent driven sampling:* Participants included in studies I, III and IV were recruited using a peer-chain referral sampling technique which is a non-probability sampling technique. Respondent driven sampling was intentionally used so as to penetrate networks of hidden female sex worker populations. Respondent driven sampling employs the use of incentive system whereby recruits are reimbursed for their time and transport upon completion of interviews and also for successfully recruiting eligible peers. Consequently, recruits may have been coached on responses so as to meet eligibility criteria or participants may have preferentially invited peer sex workers whom they knew to be HIV negative. This may explain the high eligibility rate (700 eligible out of 775 screened) and the low HIV prevalence observed relative to the expected (8% HIV prevalence observed compared to the 15% HIV prevalence in a recent survey). In attempts to minimize this risk, a reasonable amount of incentive money was set in consultation with research teams at MUHAS who had conducted respondent driven sampling among female sex workers in Dar es Salaam around the same period. The team had conducted a formative study ahead of recruitment to approximate travel cost of participants to the research site at MUHAS. The incentive amount was also approved by the MUHAS institutional Review Board. Additionally, recruits arriving at the site were further vetted by a research assistant

who was a peer female sex worker and had experience of screening sex workers for participation in survey using a respondent driven sampling method.

- *Lost-to-follow up bias:* There was evidence of differential follow-up in both study I and II. In study I, we found that, compared to those remaining in the study, participants who did not attend the 12 month visit (n=138), were younger and reported more unprotected sex with two or more partners (Table included in Paper II). It is possible that HIV incidence in the study was underestimated because those loss-to-follow up were arguably at a higher HIV acquisition risk. To minimize attrition in the PrEPVacc registration cohort, a selected group of "tracers" are used to trace peers who miss visit. Tracers were selected based on whether they lived or work in the same neighbourhood as that of a participant who was lost-to-follow-up or missed a visit. Together with this, contact and locator information of the participants were constantly updated to facilitate tracing. In study II, women who did not attend the 12-month visit (n=194) were younger, unmarried and arguably "higher-risk". It is likely that reduction in sexual behaviours was underestimated as a result of early lost to follow up by higher-risk women because the proportion of women with higher number of partners, those involved in transactional sex and high-risk sex was significantly higher among women dropping out than those remaining in the cohorts. A sensitivity analysis using multiple imputation to determine the effects of missing data in both study I and II might have been performed, but are beyond the scope of this thesis.

8.5.1.3 *Information Bias &Measurement errors*

- *Social desirability bias* is of concern since sexual behaviours in Study I, II and III were based on self-reports. Social desirability bias is common in HIV research and has been extensively documented in literature (215-218). Participants may have falsely under-reported or over-reported behaviours so as to please the research staff. For example, in Study II, under-reporting of sexual partners or over-reporting condom use in the 12 months visits as compared to the baseline visit may have resulted in an apparent reduction in risky sexual behaviours. This may have consequently biased the observed odds ratios and over-estimated the reduction in risky behaviour. During data collection the study made attempts to minimize reporting bias by making the questions neutral, assuring participants of confidentiality and having frequent follow-up visits.

- *Measurement error in estimating behaviour change (Study II):* The predicted sexual behaviour risks scores in Study II were based on only four sexual behaviour variables and may have been inadequate in providing reliable estimates for the values of the individual's risk. Because of the few HIV seroconversion cases in the dataset, the development of the risk score used to quantify behaviours was difficult. Selection of the best model was based on a decision to ensure that the model coefficients provided the most plausible estimates albeit the model already suffered from low power. It is possible that the Models B and C which were not used because they had "implausible" coefficients (e.g., coefficients that predict condom use to be harmful instead of protective against HIV acquisition) could have

been different if they were generated in a larger dataset which has more HIV events e.g., at least 100 HIV events.

- *Limitations of midpoint imputation for HIV seroconversion date:* In both Study I and II, midpoint between latest-negative and earliest positive test date was assumed to be the HIV seroconversion date. Although the mid-point assumption is commonly used in prospective cohort and can give reasonable approximation of the incidence date (219, 220), it can also be subjected to errors once participants start to miss scheduled HIV test dates (221). This is because the missed test dates are likely to extend the width of the censoring interval. The use of the midpoint assumption is expected to be accurate when testing rate is more than 80% (221). This limitation is more likely to be pronounced in Study I than in Study II because of moderate testing rate in follow-up visit in Study I (~60%) compared to study II (~80%). The effect of the midpoint imputation for HIV seroconversion date can be noted in **Figure 15** as HIV infection events are concentrated in the middle of the observation period (around the 6 month visit) and a sharp decrease towards the end of the 12 month observation period. An alternative approach to minimize this would be to use the Monte Carlo methodology to impute a single random infection date within the censored interval (221). However, this type of analysis was beyond the scope of this thesis.

8.5.1.4 *Confounding*

- In Study II, follow-up period was relatively short (one year) to effectively observe any actual changes in behaviours. The study was also unable to assess if the changes in sexual risky behaviour observed were due to changes in the women's life circumstance or induced by the risk reduction counselling and other interventions provided in the cohorts.

- There was no information collected on the male partners in study I & II and therefore the analyses are not able to explore why unprotected sex, high-risk sex and transactional sex were not associated with HIV acquisition as expected.

- *Residual confounding:* In study II, residual confounding may have been introduced because "increased risky behaviour" and "no change in behaviours" were categorized together as the outcome. This was done with an assumption that, women who had no change in behaviour included those who retained high risk behaviours at 12 months. However, it is likely that this group consisted of women who retained low-risk behaviours. In study II, residual confounding may have been introduced by adjusting for the composite effect of the baseline sexual behaviour risk score in the multivariate analysis. For instance, previous studies have reported that number of partners, anal sex, sex during menstruation and rape to be individually associated with a higher HIV risk among female sex workers (172, 222). However, this was not observed in the study because these variables were included in the composite variable. This may have consequently biased the observed associations between the baseline sexual behaviour risk score and HIV incidence towards the null value.

8.5.2 Trustworthiness considerations in the qualitative study (Study IV)

This section provides an evaluation of Study IV trustworthiness based on criteria by Lincoln and Guba (223). In each criterion, a brief description of the criteria is provided and a discussion given on how the criteria were achieved in the study.

8.5.2.1 *Truth/Credibility:*

Credibility in qualitative research seeks to discern the truth-value of the study findings by ensuring that the multiple and subjective realities of the study informants is captured (165). In Study IV, the following strategies were used to achieve credibility.

- *Prolong engagement:* I had a chance of meeting the informants in two or more occasions before commencement of data collection (interviews). First during the scheduled baseline and follow-up interviews as part of the parent cohort study procedure (PrEPVacc registration cohort) and later during participant engagement meetings (weekly educative sessions held as part of improving comprehension on HIV vaccine trials). Engagement with the informants was also possible after the qualitative interviews as the participants attended subsequent follow up visit in the PrEPVacc registration cohort. I was also part of the study team who conducted the quantitative interviews for the PrEPVacc registration cohort. Data collected in these interviews included questions on the use and preference of contraceptive methods. This provided me with a chance to explore contraceptive use among sex workers other than those already participating in the qualitative interviews. I also took part in delivering pregnancy test results to the participants as part of the PrEPVacc registration cohort study visit procedures. This provided me with first-hand experience on how participants reacted to a positive pregnancy test results because the majority had not intended to become pregnant. In all these encounters, I took personal notes on observations relating to the study question.

- *Triangulation:* Sampling of study participants i.e women who were pregnant was done by obtaining a pregnancy test results from the PrEPVacc registration cohort laboratory records and counterchecked with the PrEPVacc registration cohort database. Socio-demographic characteristics, contraceptive use and number of children reported by the informants were confirmed from the cohort study database. Because the participants continued to attend the study site even after their qualitative interviews, it was obvious that not all positive pregnancies reached full-term during the course of follow up whether due to spontaneous or elective abortions.

- *Peer - debriefing:* By engaging the PrEPVacc registration cohort research team on the objective of the qualitative study, provided an opportunity for study nurses to share experiences on how women would react to the pregnancy positive results. For example, one of the study nurses recounted on how women who received a positive pregnancy test would not believe the results. Others would cry, seeking consolation and advise from the nurses on what to do. This was consistent with what was narrated by some informants in

the in-depth interviews. The informants reported on how they got advice from the study nurses which had an influence in their decision to initiate or adhere to contraceptives in the future.

Presentation of preliminary findings from the analysis was done to fellow qualitative researchers and experts in the Grounded Theory Approach. This was done as part of course work in Qualitative Data analysis at Umea University. Codes, categories, choice of core category and the emerging model were discussed. This provided me a chance to receive inputs and comments from those outside the research process.

- *Use of "deviant cases":* While the qualitative study aimed only at participants enrolled in the PrEPVacc registration cohort who had positive pregnancy test, two "deviant cases" were included to further explore theories raised in the interviews. One of the cases was a younger woman who had never been pregnant. She provided views on contraceptive and abortions from younger women with no pregnancy experience. The other case was of a HIV positive woman, mother of three children who had unintended pregnancy and was unsure of the paternity of the child. This participant was included to further explore the theory that, HIV positive sex workers are less likely to use condoms, (therefore more likely to get pregnant) and that they are more likely to abort the pregnancy in fear of mother-to-child HIV transmission. However, this theory was not supported by the subsequent interviews and therefore not further pursued.

8.5.2.2 *Applicability/Transferability*

Applicability refers to how relatable findings in the study are to other settings so that theories developed can be used to explain similar phenomenon in other setting or when designing interventions. Applicability can either be achieved through theoretical/analytical generalization or naturalistic generalization. Analytical generalization is achieved by purposively sampling which ensures that, selected study participants provide a comprehensive, complete and saturated accounts to the theory developed. On the other hand, naturalistic generalization is achieved if a study context (time and place) are detailed enough so that the reader is able to judge the suitability of generalizing the theories developed into his/her context (165) .

In Study IV, informants were selected purposively to ensure that their diversity i.e age, education, number of children and duration in sex work, allowed for richer variation of the theory developed (183). Inclusion of deviant cases (one HIV positive sex worker and a sex worker who had never been pregnant) allowed to saturate theories developed. Additionally, since the interviews dates were spaced from the date of the pregnancy test, by the time participants were interviewed, some had already undergone an abortion, some were still pregnant and some had already given birth. This diversity allowed to understand how experiences and decision around unintended pregnancy differed ensuring that theories developed were comprehensive.

Because Study IV was nested within the PrEPVacc registration cohort which had a different sampling technique, naturalistic generalization of the theories developed to another context may be challenging. The PrEPVacc registration cohort included sex workers who were recruited by peer chain-referral sampling through members in their social network using the respondent-driven sampling technique. It is likely that, sex workers included in the PrEPVacc registration cohort (the sampling population for Study IV) were more networked, and possibly had better health seeking behaviour (including contraceptive use) compared to those in their wider community. Therefore, this may limit the applicability of the study findings to female sex workers in other contexts.

8.5.2.3 *Consistency/Dependability*

Consistency in qualitative research refers to the ability of the researcher to recognize the epistemological nature of the qualitative research and therefore be able to account for the evolution of theory developed (165). The epistemological feature of qualitative research means that, the relationship between the researcher and informant is interactive, inseparable and greatly influences the knowledge created (165). With this, repeatability of findings from qualitative research is not possible as it is the case in quantitative research. The qualitative researcher is bound to adopt the research process based on his/her interaction with participants and during the analysis process, hence an emergent design (183). For this reason, keeping a trail of the evolution of the research process is recommended so as to achieve consistency/dependability.

Throughout the course of Study IV, a number of changes were made. These include, revision of research question, designing of the interview guide and its modification over time, changes in data collection period — a decision was made to conduct the interviews further away from the pregnancy test result so as to minimize the influence of the interview on the participant's decision-making process. Throughout this process, correspondence with co-investigators through emails, meeting minutes and revised drafts were maintained so as to provide a trail on why decisions were made.

During data collection, the interview guide evolved with subsequent interviews so as to allow covering a range of concepts and also accommodate new theories based on the responses provided by the preceding interviews. With this, the theoretical sensitivity increased as more data were collected (165). As a result, later interviews were more focused, covering more concepts but at a risk of narrow and specific responses (165, 183). For example, as noted in the earlier chapters of this thesis, the interview with the first informant (In-depth interview conducted in January 2019), covered fewer concepts but provided a long and detailed narration of the issues whereas the interview with the tenth informant (In-depth interview conducted in

June 2019) covered more concepts but provided narrower responses. However, there is also a risk that this extensive period of data collection may have resulted to inconsistency meaning and that questions may have not covered same areas for all participants (183).

During data analysis, memos were made documenting different stages of the Grounded Theory analysis process. Sketches of models developed were kept and revisited when changes were made so as to retain perspective of data from the transcript in relation to the research question.

8.5.2.4 *Neutrality/Confirmability*

Confirmability of qualitative research refers to the ability of the conclusions presented being grounded in the data when observed by a third party (inquiry auditor), i.e findings are observed to be neutral, independent of the researcher's subjectivity (165). Although this criteria conflicts with the epistemological assumption of qualitative research i.e the researcher and informant both make the data, neutrality in qualitative research is sought from the standpoint of the data presented and not the researcher.

Study IV used the Grounded Theory analysis approach. Line-by-line coding that was performed ensured that theory was developed from narrations of the informants. Minimal abstraction was used during labelling of codes so as to maximize neutrality of the data. Importantly, while a conceptual framework (*Health Belief Model)* had been used at the study design phase and to develop the interview guide, this framework was note used in theoretical coding and developing of the theories during analysis. This allowed me to maintain neutrality to the data and ensure an inductive process was used for theory development. Quotations used in the writing of the study results reflect how findings had emerged from the data and show that there was minimal abstraction.

Information on my gender, academic qualification, role in the PrEPVacc registration cohort, data collection process and analysis were submitted to the journal for the Study IV publication so as to allow readers determine neutrality of the data theory (183).

9 CONCLUSIONS

- The PrEPVacc registration cohort has demonstrated that it is possible to identify, enrol and follow up female sex workers living in Dar es Salaam for participation in HIV vaccine trials. We found that participants in the PrEPVacc registration cohort had a high HIV incidence rate, low pregnancy rate and high retention. This is an important step for site preparedness in anticipation of the future PrEPVacc vaccine trial. (**Study I**)

- There was an overall reduction in individual risky behaviours over time, and that HIV risk was higher among women who had riskier behaviours at during recruitment. There was insufficient evidence to ascertain the association between changes in sexual behaviour and HIV incidence at 12 months. (**Study II**)

- Female sex workers living in Dar es Salaam reported to be largely aware of PrEP and were willing to initiate PrEP. However, PrEP use was low reflecting limited access to PrEP in the country. (**Study III**)

- Initiation and adherence to contraceptive use among female sex workers is impeded by a number of contextual factors such as gender-power inequality, poverty as well as sex work stigma. The results show the need to integrate contraceptive services within HIV programs servicing female sex workers in their work areas. In such programs, providers should initiate contraceptive counselling and offer wide range of non-barrier contraceptives that meets the fertility desires of these women. (**Study IV**)

10 POINTS OF PERSPECTIVE

This section includes a reflection of the findings from this thesis to the wider field of HIV epidemiology, HIV preventive trials and HIV prevention programs in Tanzania.

Section **10.1** includes a reflection on the trend of HIV epidemiology in Tanzania. Section **10.2** discusses the considerations in the designs and conduct of HIV biomedical prevention trials. Specifically, and with reference to the PrEPVacc vaccine trial, I discuss on the complexity of designs in HIV trials in an era where a highly effective prevention intervention (antiretroviral based PrEP) is increasingly becoming available. Lastly, in section **10.3**, I highlight on how the available evidence on contraceptive use among female sex workers can be used to inform delivery of the PrEP program in Tanzania and also inform the need for long-acting PrEP agents which offer an adherence advantage.

10.1 REFLECTIONS ON THE TRENDS OF HIV EPIDEMIOLOGY IN TANZANIA

The high HIV incidence estimated among high-risk females in Study I and II indicate that there is persistence high HIV incidence in this population despite the decline of the HIV burden at population level in Tanzania. As noted earlier, the HIV incidence of 3.5 per 100 person-years-at risk estimated in the Study I is fairly similar to the HIV incidence of 3.7 per 100 person-years-at risk estimated in Study II despite the two cohorts having a 10 years difference in time. Contrary to this, the HIV prevalence in the general population at the national level has decline from 5.7% in 2007/2008 to 4.8% in 2016/2017 (25, 26). A similar declining trend in the HIV prevalence has also been observed in Dar es Salaam region where Study I was conducted (6.9% to 4.3%), and two of the three regions where cohorts for Study II were located (Shinyanga 7.4% to 5.5%; Kilimanjaro 3.8% to 2.2). However, this was not the case for Geita where there was an increase in the HIV prevalence from 4.7% to 5.2%. Notwithstanding the changes in demographic and the increased longevity of people living with HIV (due to the wide availability of anti-retroviral therapy) which can partly explain the HIV prevalence; the sustained high HIV incidence among high-risk females in Tanzania could be an indication of the HIV epidemic transition to an endemic phase. In a HIV endemic phase, HIV is concentrated in core groups e.g. Key and vulnerable populations including female-sex workers who have limited access to or engagement with services (4, 224).

The persistent high HIV incidence also highlights vulnerability of female sex workers and that in the past decade, the existing HIV prevention programs have not been tailored to the needs of this population. Poor access and/or uptake of available interventions may have been impeded because of sex work stigma, inconvenient of operating hours in the health care facilities or possibly because the available interventions are not under the control of females (e.g condoms).

This underlines the need for a targeted HIV service delivery model to female sex workers and the need for female controlled HIV prevention interventions such as PrEP so as to achieve HIV epidemic control in the country.

10.2 REFLECTIONS ON EPIDEMIOLOGICAL AND ETHICAL CONSIDERATIONS IN HIV PREVENTION TRIALS

10.2.1 Preference for open cohorts over closed cohorts in HIV prevention trials.

As stated earlier in the discussion section, decline in HIV incidence is usually expected in a cohort of high-HIV risk individuals. This is partially due to the standard prevention package offered to participants which includes risky reduction counselling. This was observed in results of Study II whereby, sexual risky behaviours decline over time— even though the study was not powered to detect the association between the decline and HIV incidence. HIV incidence is also expected to decline because those at greatest HIV risk get infected early and exit the cohort. Early attrition of those at higher risk of HIV infection also contributes to the decline of HIV incidence in the cohort—as was observed in Study I with loss-to-follow up significantly higher among younger female sex workers.

The decline of HIV incidence over time in the cohort may have implication in the recruitment and conduct of the PrEPVacc vaccine trial. This is because, progressively over time a pool of low-risk study participants remains in the cohort. If this low-risk group is enrolled into the vaccine trial, it may result in lack of efficacy results because the overall HIV incidence will be low. Thus, incidence studies are only useful to identify and prepare groups at risk for trials rather than to serve as trial populations

In order to minimize the declining HIV incidence in the cohort, continuous enrolment into the preparedness cohort i.e an open cohorts approach will allow to maintain a relative stable HIV incidence over time. As of 2020, the PrEPVacc registration cohort is open, with recruitment of new participants ongoing as others exit the cohort for various reasons. Participants in the PrEPVacc registration cohort have exited the cohort because of; personal choice, permanent relocation out of Dar es Salaam, death and study termination due to loss to follow-up after multiple attempts of tracing. Continuous recruitment of new participants from different catchment areas will enable accrual of high-risk individuals into the PrEPVacc registration cohort and subsequently the PrEPVacc vaccine trial. Another strategy to maintain high HIV incidence in the cohort would be to allow participants who remain seronegative to exit the cohort after a specified period e.g., two years as they may be considered “low risk. This strategy is currently not included in the PrEPVacc registration cohort but could be adopted in the future.

10.2.2 Use of PrEP as a standard of prevention in HIV prevention trials

The recently revised UNAIDS ethical considerations in HIV prevention trials published in January 2021, oblige researchers to ensure access to PrEP as part of the standard prevention package (187). In the PrEPVacc vaccine trial, oral PrEP (Truvada or Descovy) will be provided as a study drug up to week 26 when the vaccine induced immune response is expected to have peaked (refer PrEPVacc study schema **Figure 8**). Thereafter, participants will be referred to access PrEP via the national PrEP programme. Because availability of PrEP via the national programs differs among countries participating in the PrEPVacc vaccine trial, use of PrEP may vary among participants across trial sites. This may raise an ethical concern that standard of care package varies among participants in the same trial. For the MUHAS site, plans are underway to enact the MUHAS Clinical Trial Unit as a PrEP dispensing site under the Ministry of health's national PrEP program. Once this is established, trial participants and even non trial participants will have access to PrEP. This would help avoid any conflict of interest that staff may have in referring participants to access PrEP elsewhere as promotion of PrEP may be perceived to underpower the vaccine efficacy results. It is likely that having access to PrEP at the trial site would improve PrEP uptake as Study III showed that there was high willingness to use PrEP among participants.

There is an ethical concern in conducting trial among those not adherent to PrEP because effectiveness of oral PrEP is directly influenced by adherence. We noted from Study IV that sex work impedes use of daily oral contraceptive pill. This finding may indicate that adherence to daily oral PrEP in the trial will be sub optimal. Contrariwise, vaccine efficacy results somewhat relies on participants non-adhering to PrEP so as to increase the number of incidence cases powered to detect efficacy. This may pose an ethical predicament for researchers who are also required to advocate for good PrEP adherence. Fortunately, PrEP adherence in the PrEPVacc vaccine trial will be monitored by a urine test for the presence of Tenofovir. This adherence monitoring would allow the study team to objectively monitor and document PrEP adherence so as to offer adherence support.

Another potential ethical concern related to PrEP in the PrEPVacc vaccine trial is on providing and insisting participants to use daily oral PrEP while there is growing evidence on long-acting PrEP agents that offer adherence advantage and efficacy over oral daily PrEP. This has been shown in a recent concluded HPTN 084 trial which compared daily oral PrEP (Truvada) to 8 weekly injectable PrEP (Cabotegravir) among females at high risk of HIV infection. In the HPTN 084 trial there were 36 new HIV infection in the Truvada arm compared to only four new HIV infection among those receiving the injectable PrEP (113). This may pose an ethical concern that participants in PrEPVacc vaccine trial are not receiving the "state-of the art" HIV prevention modality as mandated by the ethical guidelines for HIV prevention trials. Cabotegravir injectable PrEP has been shown to be more effective than oral daily Truvada,

because it minimizes some of the challenges associated with oral daily PrEP pill use. It is administered less frequently, more discrete therefore not associated with judgment and stigma for being seen with PrEP pills. At the moment the PrEPVacc vaccine trial may be ethically justified to offer oral daily PrEP since the adherence benefit of Cabotegravir injection is a recent discovery, the drug is not licensed in Tanzania and commercial production is not yet been widely scaled. However, this rationale may probably not be viable over the course of the trial and the study may have to be flexible to adopt new PrEP agents or other HIV prevention tools has they become widely available.

10.2.3 Complexity of HIV prevention trial study designs in the era of PrEP

The availability of PrEP which can be up to 100% effective if taken correctly, raise the concern that individuals who are successfully using PrEP may not be considered a high HIV risk population. On the other hand, current HIV biomedical prevention trials (including vaccines, monoclonal antibodies and new antiretroviral based PrEP) use active control designs instead of placebo i.e all trial participants receive an anti-retroviral based PrEP as an ethically obligated standard package for HIV prevention. Consequently, HIV incidence in HIV prevention trials is bound to be very low rendering the study statistically underpowered to detect efficacy of the vaccine products. Large samples size or longer follow up periods would be needed in vaccine trials and therefore more resources. Because of this, design of HIV prevention trials has become complex in order to account for the epidemiological and ethical considerations needed to prove efficaciousness of new products in the context of known high effectiveness of PrEP.

Currently three strategies known as "*Compare*" "*Layering*" and "*Combine*" are being used in randomized clinical trials for new HIV prevention product while taking into account PrEP as an active control (169). In the first strategy "*Compare*" participants are randomised to receive either a new PrEP product or an existing PrEP product (Truvada). In this case, the new drug under research is intended to be used as an alternative to Truvada. For example in the HPTN 083 and HPTN 084 trials a new Injectable cabotegravir was compared to existing oral Truvada (113, 114) whereas in the DISCOVER trial daily oral Descovy as a new PrEP formulation was compared to the existing daily oral Truvada (108, 225) . In all these studies participants received a PrEP agent and no one received a placebo so that the ethical consideration of ensuring everyone receive PrEP as standard package of prevention was adhered (187). The second strategy "*Layering*" whereby everyone in the trial has access to PrEP and on top of that participants are randomized to receive either the experimental product or placebo. PrEP is not offered as a study drug but is made available through the study clinic. For example, in the *Mosaico Trial* (HVTN 706) (119) and *The AMP studies* (HVTN 703/HPTN 081 (133) and HVTN 704/HPTN 085 (132)) participants have/had access to PrEP but not mandated to use it. This strategy is intended where the new HIV prevention products that will be used together with PrEP in case the PrEP agent is not sufficiently effective e.g., because of poor adherence

to oral pill intake. Lastly the third strategy is "*Combine*" whereby participants are randomized to receive the new product together with PrEP or PrEP alone. This strategy is intended for products planned to be used in combination with PrEP.

The PrEPVacc vaccine trial is complex with all the strategies described above used at different time points. In essence the "*combine strategy*" will be used to asses efficacy of vaccine products given in combination with PrEP. However, in the first 26 weeks while the body has yet to generate immunity from the vaccine products, efficacy of Descovy will be "compared" to Truvada in reducing HIV incidence i.e "*compare strategy*" will be used. After the 26th week PrEP will be withdrawn (as a study drug) but participants will access it through the study clinic i.e like in the "*layering strategy*". The challenge with the combine/layering strategy in the PrEPVacc vaccine trial, is that evaluating efficacy of the vaccine products on top of the PrEP efficacy will be difficult if uptake and adherence to PrEP is high among participants.

Future trial designs will also have to use long-acting PrEP agent such as injectable or implantable PrEP as a HIV prevention standard because of their adherence advantage over oral PrEP. The use of long-acting PrEP agents in the future trials will results in more ethical and epidemiological challenges because of the high effectiveness and compliance associated with these products. First, trials will require large sample size of more than 100,000 participants because of the low expected HIV seroconversion rate (169). Second, it may ethically be deemed futile to conduct research for new HIV prevention products in settings where HIV prevention success has been achieved by the long-acting PrEP agents (6, 169). Nevertheless, HIV prevention trials may have scientific merit in risk populations where long-acting PrEP may be ineffective because of safety, intolerance or other reasons. Development and testing of long lasting, preventive HIV vaccines is still needed and essential to end the HIV/AIDS pandemic(10).

10.3 REFLECTIONS ON FINDINGS FROM CONTRACEPTIVE USE AND HOW THEY CAN INFORM PREP USE AMONG FEMALE SEX WORKERS

Some of the findings on contraceptives use practices among sex workers in Study IV can be used to inform PrEP uptake in the PrEPVacc vaccine trial and in the PrEP program at large. First, it is important that PrEP should be provided in a sex-worker friendly clinic and preferably integrated with contraceptive and/or HIV testing services. This is so as to avoid experiences of sex-work stigma as those reported from sex workers accessing family planning clinics in Study IV. The use of PrEP is also associated with the stigma of being labelled HIV infected or promiscuous (226-228) therefore there is need to ensure that PrEP is provided in a sex-worker friendly environment so as not to discourage potential users. The integration of PrEP, contraceptive use and HIV services is widely recommended and has been shown to improve demand and uptake of PrEP (229-232). Secondly, long-acting PrEP delivery methods would be ideal for sex workers so as to avoid problems of daily pill taking such as incorrect use,

skipping pills, taking pills when intoxicated with alcohol or discontinuation as observed in the case for oral contraceptive pills in Study IV. Already such PrEP delivery models are underway (6, 233, 234) including monthly oral Islatravir pill (117) , monthly Dapivirine vaginal ring (109, 112)and monthly Cabotegravir injectable PrEP(113). Having these long-acting PrEP tools will improve the uptake, use and adherence therefore reduce new HIV infections among in this population.

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