Impact of Hormonal Contraception on HIV acquisition and transmission risk

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Abbreviations and acronyms

AE	Adverse event
AIDS	Acquired immunodeficiency syndrome
ART	Antiretroviral therapy
ARV	Antiretroviral
CRF	Clinical research form
CVF	Cervicovaginal Fluid
CVL	Cervicovaginal Lavage
DMPA	Depo Medroxyprogesterone Acetate, Depo Provera
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
HAART	Highly active antiretroviral therapy
Hb	Hemoglobin
HIPAA	Health Insurance Portability and Accounting Act
HIV	Human immunodeficiency virus
IUD	Intrauterine Device
IRB	Institutional review board
PI	Principle Investigator
PIN	Participant identification number
RA	Research Assistant
RNA	Ribonucleic acid
SAE	Serious adverse event
STI	Sexually transmitted infection

Project overview

1.1 Summary

This study is a *proof of concept prospective cohort study f*ocusing on HIV negative women. The 3 proposed aims will evaluate the effect of depot medroxyprogesterone acetate (DMPA), Etonogestrel impant (Eng-Implant, Nexplanon) and Levonorgestrel intrauterine device (Lng IUD, Mirena IUD or copper IUD) exposure on 1) HIV target immune cells within the female genital mucosa; 2) markers of T-cell activation and trafficking within the female genital mucosa; and 3) secreted cytokines and chemokines within the female genital mucosa. A prospectively recruited cohort of HIV negative women will allow the examination of the overarching hypothesis that alterations in HIV target immune cells and function within the female genital mucosa underlie the relatively higher HIV risk associated with the pharmacologic doses of exogenous sex hormones, Because the anticipated mucosal immune changes with progestin-only contraceptives are to a large extent mediated via estrogen suppression, the impact of Nexplanon and Mirena IUD (with milder anti-estrogen effect) is expected to be significantly less pronounced compared with that of DMPA. The outcomes of the proposed study could have significant clinical implications for the provision of family planning services for women worldwide.

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Role of the investigators

Dr. Haddad will provide general oversight for this study as part of her K23 training grant. Drs. Ofotokun and Jamieson will serve as advisors for the study. Dr. Hart will facilitate the laboratory component of the study while Dr. Amara will assist in interpretation and evaluation of immunologic findings.

The investigators have no conflicts of interest to report.

2 Introduction

2.1 Background

Interconnected goals of HIV and unintended pregnancy prevention: Globally, nearly 16 of the 33 million people living with HIV infection are women, most of whom are of childbearing age.(1) Beyond the direct morbidity and mortality of HIV, the majority of these women live in regions overwhelmed by high fertility and where the baseline rates of pregnancy associated morbidity and mortality are especially high. Pregnancy prevention can mitigate maternal morbidity and mortality, decrease unsafe abortion, reduce poverty, and improve the educational attainment and status of women. Additionally, for women with HIV, pregnancy prevention can prevent mother-to-child transmission of HIV and resultant "AIDS orphans". Of note, the World Health Organization, United Nations and global public health organizations recognize the prevention of new HIV infections and prevention of unintended pregnancy as critical overlapping public health strategies to prevent mother-to-child transmission, reduce child and maternal mortality and improve health.(2, 3)

Condoms, while effective to some degree in preventing sexually transmitted infections (STIs) including HIV, are not an ideal method of preventing unintended pregnancies when used alone. Condom use is associated with a high contraceptive failure rate of ~15% in the first year with typical use.(4) Furthermore, despite three decades of aggressive promotion of condom use for the prevention of HIV transmission, consistent use remains inadequate.(5) The most common forms of contraception used worldwide are progestin-containing hormonal contraceptive methods, either alone or in combination with estrogen, with over 150 million users(6). Hormonal contraceptives have higher pregnancy prevention efficacy rates than condoms, with contraceptive failure rate of ~3% following typical DMPA

use, and <1% following contraceptive implant and intrauterine device use.(4) Hormonal contraceptives have high acceptance rates in many communities around the world where condom use may be poor or where non-hormonal contraceptive options, such as the copper intrauterine device, are not acceptable or available.

Concerns regarding hormonal contraception*:* Despite its excellent contraceptive profile, there are growing concerns that contraception hormones, specifically DMPA, may influence susceptibility to HIV and contribute to the spread of HIV infection.(7-9)*If confirmed, this risk would represent a significant threat to women's health with far-reaching implications for public health policy.* Contraceptive approaches that increase the risk of HIV acquisition or transmission would have important implications for health at the individual and population levels, as well as on the overall socioeconomic impacts of the AIDS epidemic.

The significance of this topic is underscored by the ongoing debate it has generated among experts in the field and the increasing research focus in this area. The rationale for these intense research activities is anchored by animal data demonstrating several fold increases in the risk of SIV acquisition in rhesus macaques following DMPA exposure,(10) and from corroborative human epidemiologic studies. Notable among these studies is the Mombasa study in Kenya where DMPA use was associated with a 2-fold rise in the incidence of HIV infection among 1,500 female sex workers.(8) Another example, from the Demographic and Health Surveys of over 4,000 young women from four African countries, reported higher HIV sero-prevalence with DMPA use, with ~6% of new HIV infection attributable to DMPA.(11) Summarized in Table 2 are the outcomes of recent studies that

have evaluated the impact of DMPA on HIV acquisition risk among women.

It should be noted that the HIV risk of contraceptive hormones has not been consistently observed across all studies. Some studies have completely failed to show this association(12-15) and others report an increased risk of HIV infection only in a subgroup of individuals with differences such as age or HSV -2 status.(16-19) The wide discrepancies in study design, subjects' selection, sample sizes, contraceptive type, method discontinuation, dose, and method of administration also adds to the complexity of interpreting the results across these studies and limit conclusions that can be drawn from the existing data. To our knowledge no well-designed studies have evaluated the impact of progestin releasing implants or IUDs on the risk of HIV-infection. *While these data are far from conclusive, they are nevertheless worrisome, and provide a strong impetus to further assess the potential risk of HIV acquisition among existing hormonal contraceptive methods.*

Table 2: Impact of DMPA on HIV Acquisition/Transmission Risk among Women

Study	Country	Year	Study Design	"n"	Effect
Heffron R, et al(7)	Africa	2012	Prospective cohort study	3,790	Increased risk of HIV acquisition/transmission
Morrison CS, et al(17)	S. Africa	2012	Prospective cohort study	5,567	No significant risk of HIV infection observed
Wand H & Ramjee G(20)	Australia	2012	Prospective cohort study	2,236	Increased risk of HIV acquisition
Reid, SE, et al(21)	S. Africa Zambia Zimbabwe	2010	Prospective cohort study	1358	No significant risk of HIV infection observed
Kumwenda J, et al (22)	Malawi	2008	Prospective cohort study	842	Increased risk of HIV acquisition
Leclerc PM, et al(11)	Africa	2008	Demographic/health survey	4,549	Increased risk of HIV acquisition

Baeten JM, et al(23)	Kenya	2007	Prospective cohort study	1,206	Increased risk of HIV acquisition
Myer L, et al(24)	S. Africa	2007	Prospective cohort study	4,200	No significant risk of HIV infection observed
Morrison CS, et al(16)	Uganda Zimbabwe Thailand	2007	Prospective cohort study	6,109	Increased risk observed only with HSV-2 subjects
Lavreys L et al(8)	Kenya	2004	Prospective cohort study	1,498	Increased risk of HIV acquisition
Kiddugavu M, et al(15)	Uganda	2003	Population-based cohort study	5117	No significant risk of HIV infection observed

***Potential mechanisms of increased HIV acquisition risk with hormonal contraception**:* How might pharmacologic doses of sex hormones alter the risk of HIV transmission in women? It is understood that the male to female sexual transmission of HIV infection is a multifaceted process of virus-host interactions (Figure 1). Simply, following exposure, initial infection occurs at the genital mucosa and may involve complex interactions between a number of HIV target immune cells – CCR5 expressing $CD4^+$ T cells, macrophages, and dendritic cells (DCs) including myeloid DCs and Langerhans cells (LCs).(25-28) It is thought that CD4+ lymphocytes are the first cells to be infected, and that DCs subsequently spread the virus to regional lymph nodes.(29) DCs may further facilitate the transmission process through mucosal antigen processing necessary for activation of CD4+ and CD8+ lymphocytes, a step that is important for continuing propagation of the infection, as activated CD4+ T cells are more likely to be infected and to support viral replication *in vivo.(30)*

Viruses recovered during primary HIV infection almost always are of the non-syncytium-inducing, R5-tropic phenotype, presumably due to the abundance of the viral entry co-receptors, CCR5, on cells of the mucosal surface and/or because of R5 expressing cells' capacity to support viral replication and propagation of the infection.(31) In addition to the disease state and local viral load of the donor, factors that increase susceptibility to HIV infection and infectivity include STIs, mucosal micro-abrasions, secreted immune factors – all of which affect the local immune status, the protective quality of the epithelial barrier in the genital tract, and the population of HIV target cells involved in the early stages of infection(32). Natural resistance to HIV acquisition has been ascribed to a variety of factors including genetically acquired resistance (e.g., CCR5 receptor deletions), the presence of host proteins such as secretory leukocyte peptidase inhibitor (SLPI), normal vaginal flora with peroxide-producing lactobacilli (void of bacterial vaginosis), and acquired immunity mediated by vaginal secretory IgA or cytotoxic T-lymphocyte (CTL) responses in blood.

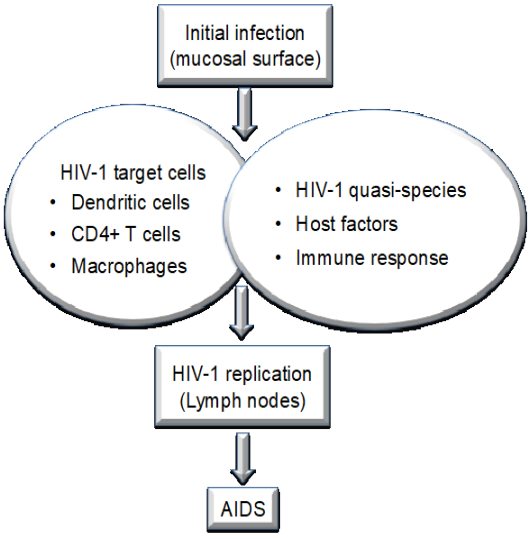


Figure 1. Schematic representation of HIV-1 mucosal transmission adapted from Wu L. Curr Opin HIV AIDS. 2008;3:534–40.

Clearly underappreciated in the discussion of HIV susceptibility of at-risk

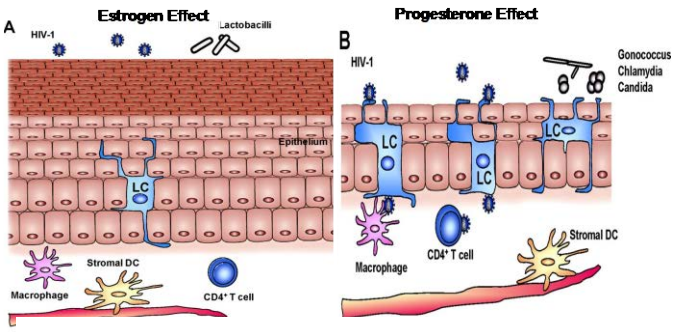


Figure 2. Potential effects of female sex hormones on the vaginal mucosal tissue. Adapted from Hel Z et al. Endocrine Reviews 2010;31:79-9

women is the emerging evidence that the robustness and functional capacity of the innate and adaptive immune responses are influenced by female sex hormones.(33-35) *The immuno-regulatory influence of estradiol and progesterone ensures a favorable environment for fertilization and pregnancy altering the local female genital tract (FGT) immunologic milieu and the composition of key HIV target immune cells.* For example, systemic and topical administration of estrogen thickens the vaginal epithelial layer in ovariectomized rhesus macaques as illustrated in Figure 2A.(35-37) By increasing the thickness of the vaginal mucosal epithelium, estrogen blocks access of SIV and other pathogens to target cells (DCs/LCs, $CD4^+$ T cells, and macrophages) within the epithelial and sub-epithelial layers. Furthermore, estrogen reduces the population of LCs within the vaginal epithelium, alters vaginal epithelial tight junctions and mucosal permeability(38, 39) and supports healthy genital tract flora making the mucosa hostile and less susceptible to the virus.(40)

Contrary to the effect of estrogen, progesterone exposure leads to a thinner epithelial layer in female macaques allowing easier access of SIV to LCs and other target cells (Figure 2B). These results in non-human primates have not consistently been shown to occur in humans thus alternative mechanisms deserve further exploration in light of the purported HIV risk of hormonal contraception. Emerging data suggest that progesterone treatment increases the population of LCs within the epithelial layer and induces changes in the vaginal microbiota, such as reduction in colonization with lactobacilli, increasing the predisposition to bacterial vaginosis. (41) Divergence from the healthy vaginal flora has been associated with increased HIV acquisition risk(42, 43). Furthermore, progestin contraceptives are associated with increased incidence cervical candidiasis

and of STIs such as gonorrhea and chlamydia(35) – factors that may enhance the risk of HIV acquisition.(44) It is unclear if these changes stem from direct biological effects of contraceptive exposure or behavioral changes, such as reduced condom use.

Other actions of progesterone pertinent to HIV acquisition risk include suppression of IgG and IgA production and trans-epithelial transport within the FGT,(45-47) inhibition of CTL responses and blockade of T cell perforin expression.(48, 49) Progesterone alters the expression of cytokines, favoring Th2-type over Th1-type responses.(50) Progesterone significantly alters infiltration of genital tissue with natural killer (NK) cells, lymphocytes, and macrophages, and inhibits NK cell activity and Fc-gamma receptors (FcγR) expression on monocytes, thus reducing the functional capacity of the antibody-dependent cell cytotoxicity.(51-53) The progestin contraceptive DMPA inhibits Toll-like receptor-9-induced interferon alpha (IFN)-α production by plasmacytoid DCs thereby impairing IFN-α induced antiviral state and the innate antiviral immunity.(54) Lastly, studies have indicated that expression/secretion of chemokines, cytokines and endogenous antimicrobials in FGT secretions alter over the course of the menstrual cycle, suggesting a role for hormones in regulating their release(55, 56). For example, studies have demonstrated significant luteal phase suppression of SLPI, HBD2, HNP1-3 and lactoferrin from FGT reduced SLPI secretion in postmenopausal compared to premenopausal women(57, 58) and reduced endometrial expression of SLPI among women using DMPA(59).

Taken together, these actions of progesterone may compromise the integrity of the protective epithelial layers against HIV infection, promote enhanced homing and thus the availability of HIV susceptible cells within the FGT, and dampen the robustness of the local innate antiviral and the

adaptive secretory immune responses. As hormonal contraception can alter endogenous hormone levels and have direct impact on the FGT, well-designed studies are needed to evaluate for changes in these factors that influence HIV susceptibility following different hormonal contraceptive exposures.

Different progestin-containing contraceptives and the potential risk of HIV acquisition:

Hormonal contraceptives employ different strategies to prevent pregnancy, including ovulation inhibition, altered endometrial structure and thickening of the cervical mucus barrier. Common progestin-containing contraceptives currently available in the United States differ by the type of progestin they contain, mode of delivery, typical-use efficacy, duration of effectiveness, and degree of endogenous hormone and ovulation

Table 3: Comparative properties of three progestin-only contraceptives method

	Depot Medroxyprogesterone Acetate (DMPA)	**Etonogestrel Implant**	**Levonorgestrel IUD**
Trade-name	DepoProvera	Nexplanon	Mirena
Progestin	Medroxyprogesterone acetate (MPA) 1st generation	Etonogestrel (ENG) 3rd generation	Levonorgestrel (LNG) 2nd generation
Delivery	Intramuscular Injection	Sub-dermal Implant	Intrauterine Device
Length of use	3 months	3 years	5 years
Ovulation inhibition	Yes	Yes/No	No
Endogenous estrogen inhibition	Yes	Yes/No	No
Typical use failure for 1st yr of use(4)	3%	0.05%	0.2%
Amenorrhea at 1 yr	50%	20%	20%
Continuation at 1 yr(60)	57%	83%	88%

inhibition(60) (Table 3). Each progestin thus has variable degrees of estrogenic, androgenic, anti-andogenic, glucocorticoid and anti-mineralocorticoid activity(61, 62). Medroxyprogesterone (MPA), for example, has androgenic activity, no anti-mineralicorticoid activity and potent glucocorticoid activity, higher than any other progestin or endogenous progesterone.

Different formulations of injectable contraceptives are currently licensed for use. Due to its lower cost and availability, **DMPA**, at a dose of 150 mg given intramuscularly (IM) every 3 months, is widely used globally. One factor limiting contraceptive effectiveness is poor adherence to the scheduled repeat injections. Thus, efforts have been made to promote longer-term contraceptive options, such as contraceptive implants and IUDs. The contraceptive implant prototype, Norplant was introduced in 1984,(63) and since then, over 10.5 million implants have been distributed worldwide. A major difference between the injectable contraceptives and the implants is in the kinetics of systemic progestin release. This difference leads to varying degrees of hypothalamic-pituitary-ovarian (HPO) axis modulation resulting in differential suppression of estrogen among the different progestin-only contraceptive formulations. (62) DMPA plasma concentrations, for example, quickly increase after intramuscular injection, peak within the first month followed by a plateau of serum MPA concentrations at 1.0-1.5ng/mL for about 3 months, after which blood concentrations decline slowly. Endogenous estradiol and progesterone levels are suppressed for several months after DMPA injection corresponding to suppression of ovulation.(64) The Etonogestrel Implant (Nexplanon), on the other hand, is a single sterile rod implant inserted sub-dermally. Each implant rod contains 68 mg of the synthetic progestin, etonogestrel (ENG). After a peak of 813 pg/ml at about day 4 following insertion, concentrations reach steady state (200

pg/ml) at about 4 – 6 months and remain sufficient to prevent pregnancy for 3 years. Although it initially suppresses follicular development and estradiol production, ovarian activity slowly increases after 6 months, and follicle stimulating hormone and estradiol levels are almost normal thus serum estrogen concentrations are significantly higher compared to that observed with DMPA use.(65) The **levonorgestrel releasing intrauterine device** (Mirena IUD) has a steady state plasma levonorgestrel concentration of 150-200 pg/mL that is reached in the first few weeks after placement of the IUD with levels maintained for up to 5 years after insertion. There is no change in serum estradiol and progesterone concentrations in Mirena IUD users compared to non-users; women with Mirena IUDs continue to have normal ovulatory function (66). There is a gradual reduction in endometrial thickness with the Mirena IUD with an associated gradual reduction in blood flow to spiral-arteries to the endometrium and menstrual blood flow.

Although systemic levels of Lng are low, it is unknown how the high concentrations localized to the FGT may impact local immune function. *Given the salutary effect of estrogen on the FGT, we speculate that Mirena IUD and Nexplanon , due to reduced suppression of endogenous estrogen compared with DMPA, will have lower impact on mucosal immune function and therefore be less likely to increase HIV acquisition risk.* **The copper IUD** is a non-hormonal method that provides effective contraception up to 12 years. Although the copper IUD does notn impact the normal hormonal mileau, different changes in the local immune environment are potential and may impact infection risk.

2.2 Significance of proposed study

This research is significant in that it will:

- It will test novel hypotheses related to sex hormone-induced homing of HIV target immune cells to the genital mucosal to explain the purported increased HIV acquisition risk of progestin-only contraceptives.
- It will generate novel data on the potential contributions of one non-hormonal IUD (copper) and 3 different progestin-only contraceptives (DMPA), Nexplanon and Mirena IUD on immunologic parameters that may increase the risk of HIV acquisition among at-risk women.
- It will employ the collection of cervicovaginal lavage (CVL)cervical biopsies, vaginal biopsies and two endocervical cytobrush samples to provide cellular markers representative of a susceptible population of cells from the lower FGT, free from circulatory contamination.

3.0 Study Objectives and Hypotheses

3.1 Research hypotheses and objectives

This research will evaluate the impact of one non-hormonal IUD and 3 different progestin-only contraceptive methods: 1) DMPA, 2) an Etonogestrel impant (Nexplanon), 3) the Levonorgestrel Intrauterine Device (Mirena IUD), and 4) Copper IUD, on known factors in the FGT that can modify HIV acquisition risk, including changes in the distribution and activation of HIV susceptible T-cells and mucosal chemokines and cytokines. One hundred and twenty (120) HIV-negative women seeking family planning will be recruited into a prospective study evaluating vaginal mucosal immunologic markers of HIV susceptibility before and after contraceptive initiation.

It is unclear how different progestin analogues and varying systemic and FGT hormonal concentrations associated with their different contraceptive delivery and pharmacokinetics may influence FGT immune function. Because many of the anticipated mucosal immune changes with

progestin-only contraceptives are mediated via estrogen suppression, we hypothesize that the impact of Nexplanon and Mirena IUD (milder anti-estrogen effect) will be significantly less pronounced compared with that of DMPA – a finding that would have significant clinical implications for family planning for women at risk for HIV.

Aim 1: To evaluate the effects of DMPA, Nexplanon and two IUDs (Mirena and copper) on the exposure on the frequency of HIV target immune cells within the female genital mucosa of HIV negative women.

Hypothesis*: Compared to a no contraception baseline, there will be an increase in HIV target CD4+ T cells in the female genital mucosa following initiation of hormonal contraception and these effects will be greater with DMPA because of its more pronounced anti-estrogen action relative to Nexplanon and Mirena IUD.*

Aim 2: To evaluate the effects of DMPA, Nexplanon and two IUDs (Mirena and copper) on the exposure on markers of T cell activation and trafficking in the female genital mucosa of HIV negative women desiring hormonal contraception.

Hypothesis: *Compared to a no contraception baseline, there will be an increase in T cell activation and trafficking following hormonal contraception and these effects will be greater with DMPA because of its more pronounced anti-estrogen action relative to Nexplanon and Mirena IUD.*

Aim 3: To evaluate the effects of DMPA, Nexplanon and two IUDs (Mirena and copper) on cytokines and chemokine profiles within the female genital mucosa.

Hypothesis*: Compared to a no contraception baseline, there will be an increase in proinflammatory and chemotaxic cytokines and chemokines, and a decrease in anti-inflammatory*

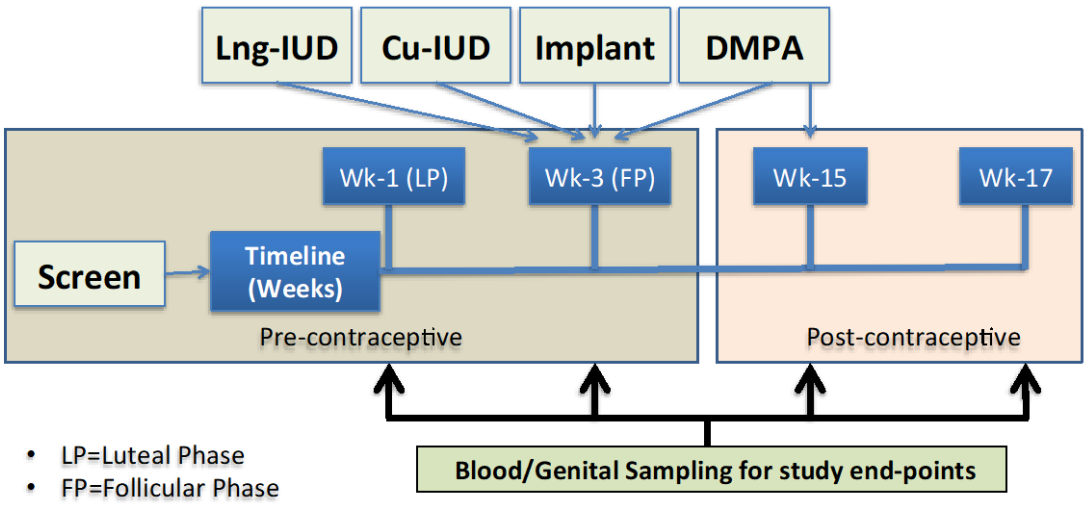
and inhibitory cytokines and chemokines following initiation of hormonal contraceptive use and these effects will be greater with DMPA because of its more pronounced anti-estrogen action relative to Nexplanon and Mirena IUD.

Overall, this evaluation of the immunologic correlates associated with HIV susceptibility following exposure to different progestin-only contraceptives will explore the relative impact of these contraceptives on HIV risk. This work will contribute to the growing literature on hormonal contraception and HIV acquisition risk, establish critical baseline data needed to explore the safety of hormonal contraception in high-risk women, and set the foundation for the candidate to evaluate the impact of exogenous hormonal exposure on mucosal immunology and reproductive infectious disease in future research.

4 Study Design

Overview and study design: To explore plausible mechanisms for the link between HIV acquisition risk and hormonal contraceptives, the research component of this proposal will examine changes in the FGT following exposure to three progestin only contraceptives, DMPA, Nexplanon , and Mirena IUD and the non hormonal copper IUD, in women who are seeking contraception.The research design allows the exploration of multiple aims concurrently (Figure 3).

Figure 3: Study Schema 4.1



Recruitment, screening and enrollment

4.1.1. Recruitment: Participants will be recruited from one of two potential sources

1) The Grady Family Planning Clinic within Grady Memorial Hospital: This site is where over 4600 healthy reproductive-aged women from across the state of Georgia seek family planning annually. Additionally, recruitment will take place at Emory Clinic Gynecology and Obstetrics practice and at the IDP/Ponce Clinic. Women who present for a family planning visit and who are interested in one of the 4 contraceptives under study will be approached for participation by study staff not involved with the individuals clinical care. Clinicians will alert the research staff of potential participants and the research staff will approach the potential participant. Research staff will read a standard recruitment script (Appendix A).

If interested, staff will complete a screening checklist to see if the individual is eligible for participation (Appendix A)

2) By self-referral: Individuals will contact study staff in response to a flyer. Flyers will be placed throughout the Atlanta community including but not limited to Grady Memorial hospital gynecology clinic, Emory Campus and Clinics. Women who contact study staff will complete a screening checklist to see if the individual is eligible for participation (Appendix A), and if interested they may be scheduled for a screening visit

Recruitment will continue until 30 individuals are enrolled into each contraceptive arm (120 total). We anticipate recruiting at the rate of ~30 participants per year and should complete accrual by year 3, all study visits should be completed by year 4. Although a randomized controlled study design ensures balance between arms, for feasibility reasons and because contraceptive choice in our population is a personal decision, the specific contraceptive type will be left to the preference of participants.

4.1.2. Screening for eligibility will include a complete history and physical examination, pelvic examination and point of care urine sampling for pregnancy test. In addition. The HIV test will be a point of care/rapid test using OraQuick® , an FDA-approved oral swab in-home test for HIV-1 and HIV-2. This is an oral swab test that doesn't require blood. Notably, screening visit may occur at the same time as visit one. Notably to avoid any false positives all screen, positive tests with the OraQuick® will be sent to a clinical lab for confirmatory HIV testing. Similarly, to prevent any false negatives, all women who screen negative and enroll in the study, will have HIV serum testing as part of their enrollment laboratory evaluation. Any positive test in the research lab, will trigger a clinical evaluation with confirmatory testing in a clinical labortatory and evaluation.

4.1.3 Eligibility

Participants who meet all of the following ***inclusion criteria*** will be eligible for the study (a) female (b) age 18-45 years, (c) normal menses (22-35 day intervals) for at least 3 cycles, (d) intact uterus and cervix, (e) interested in DMPA, Nexplanon, Mirena IUD, or Copper IUD (f) willing to delay initiation of hormonal contraception for up to 1 month to complete pre-contraceptive study visits, (g) willing to use condoms or abstain from sexual intercourse for at least 24 hours before each genital tract sampling (condoms will be made available), (h) able and willing to provide informed consent, and undergo serial blood and vaginal sampling, (i) negative HIV screening. Participants who meet any of the following ***exclusion criteria*** will not be eligible for the study: (a) pregnant within the last 3 months (b) breastfeeding, (c) history of loop electrosurgical excision procedure, conization, or cryosurgery within the past year, (d) use of hormonal contraception or IUD in the past 6 months or, (e) known history of medical condition that would interfere with the conduct of the study, (f) symptomatic vaginal infection or genital ulcer disease at screening, (g) taking medications that interact with selected contraceptive, and (h) contraindications to selected contraceptive per the CDC medical eligibility criteria(67) or judgment of clinician. For the copper IUD, exclusion criteria: allergy to copper. Participants who agree to the cervical and vaginal biopsy will have the addition exclusion criteria: allergy to lidocaine.

The cost of their selected contraceptive will be covered by research study funds. They will be informed of this during the consenting process.

4.1.4 Consent

Voluntary, informed written consent for participation will be administered prior to initiation of study activities (See Consent Forms in Appendix B). The consent form will describe the purpose of the study, the study participants' role in the study, the risks and benefits of the study, the duration of participation, the study procedures, contact persons for questions about the study (including the chair of the Institutional Review Board at Emory University and Grady Memorial Hospital), the voluntary nature of participation, and the right to decline specific questions or procedures or withdraw from the study at any time without penalty. An explanation will be provided about the use of protected health information (PHI) for research purposes and how confidentiality will be maintained as far as possible under the federal Health Insurance Portability and Accounting Act (HIPAA). Study participants will be asked to sign an authorization form for the use of PHI prior to enrollment in the study and will receive a copy for their records (Appendix B). Under no circumstances will the study participants be permitted to enroll in the study without their signed consent. All signed forms will be kept in a locked cabinet at all times.

4.1.5 Study completion

Study will be complete when 120 women, 30 in each contraceptive arm, complete the screening and the study visits. As this is a longitudinal study, we may have some loss to follow-up, and the study may be considered complete after three unanswered attempts to contact missing participants have been made. There is minimal risk, so we do not anticipate any study related adverse events or complications related to study activities that would require specific stopping rules and interim analyses.

4.1.6 Study visit schedule

Upon successful screening, participants will be scheduled for **study visit 1** three weeks

following the onset of their menses (luteal phase for women who cycle every 22-35 days). Participants will be instructed to refrain from vaginal intercourse or using intra-vaginal products for 24 hours before FGT sampling. This and subsequent study visits will occur according to the schedule outlined in Figure 3 and Table 4. At each visit, an interval assessment of menstruation and hormonal contraception use will be collected and used to determine the phase of the menstrual cycle. If a participant is experiencing menstrual bleeding during a follow-up visit, evaluations will be deferred for 1 week.

On the day preceding a study visit, participants will be asked to abstain from vaginal intercourse to minimize the risk of contamination of genital tract samples by semen.

Table 4: Schedule of study event activities

Study Activities	Screen	Visit 1	Visit 2	Visit 3	Visit 4
Week from study initiation		Wk-1	WK-3	WK-15	WK-17
Week from contraceptive initiation	Pre-contraceptive			WK-12	WK-14
Informed consent	X				
Complete history & physical examination	X				
Target history & physical examination		X	X	X	X
HIV test	X	X			
Urine Pregnancy test	X	X	X	X	X
Speculum pelvic examination	X	X	X	X	X
DMPA administration			X	X	
Vulvar/vaginal biopsy			X		X
Nexplanon or Mirena or copper IUD placement			X		
**Genital sampling for study endpoints (includes 2 cytobrush samples)		X	X	X	X
***Blood sampling for study endpoints		X	X	X	X

Swab for gut microbiome		X	X	X	X
Blood testing for RPR and HSV		X			
** Cervicovaginal lavage for HIV target inflammatory/immune cells, Swab for multiplex PCR for STIs and swab for Wetprep/Nugent Score and vaginal microbiome, vaginal PH, endocervical cytobrush samples of genital specimens for immune phenotyping *** Blood for HIV target inflammatory/immune cells, E2 and P4					

4.2 Study visits

A brief questionnaire to assess sexual behavior and bleeding or vaginal symptoms will be asked, urine will be collected for a pregnancy test, a pelvic exam will be performed with measurement of vaginal pH using a PH meter, a swab will be collected for a wet-mount, a swab for multiplex PCR of vaginal fluid, a swab for vaginal microbiome, a CVL collection (for immunological testing and prostatic specific antigen (PSA)), a swab for gut microbiome, and blood (for immunological testing and hormonal concentrations). At visit 1, additional blood will be collected for HSV serology, HIV and RPR. At visit 2 and visit 4, a cervical or vaginal biopsy will be performed for a subset of participants that consent (10 per contraceptive arm) so that one or more samples of tissue from the cervix or vagina are acquired for evaluating HIV susceptibility and inflamation markers.

At visits 1-4, two endocervical cytobrush samples of genital specimens will be collected for immune phenotyping.

Hormonal contraceptives Participants will be given the option to choose between DMPA, Nexplanon, copper IUD and Mirena IUD. DMPA will be dispensed from the Grady Pharmacy Service and will be administered every 12 weeks at the standard dose of 150 mg IM, beginning from week 3 of study enrollment and repeated at week 15. A standard Nexplanon rod Implant or the IUD (Mirena or copper) will be placed at study week 3 by Dr. Haddad or a trained clinician.

The cost of study contraceptives will be covered by this study.

Of note, study visits are scheduled to ensure that the collection of pre-contraceptive samples occur at both the luteal (week 1 visit) and the follicular (week 3 visit) phases of the menstrual cycle. Serum hormone levels will confirm cycle phase. Post-contraceptive samplings are strategically delayed to avoid sampling during the initial (first 3 months) phase when irregular bleeding associated use may occur. Furthermore, the proposed sampling schedule will allow us to capture data at the trough (week 15) and peak (week 17) of the concentration-time-curve for DMPA. If bleeding at the time of a scheduled visit, the visit will be postponed to occur within one week or at a subsequent point corresponding to their intended cycle phase (luteal/follicular) or drug exposure (DMPA peak or trough) of the missed visit. Because the study requires intensive and invasive sample collection, concerted efforts will be made to *enhance retention and study completion*.

4.3.1 Disenrollment

A study participant may elect to discontinue participation in the study at any time without disruptionof their clinical care. If during follow-up, any laboratory testing indicates the presence of an infection, participants will be referred clinical clinical providers for further evaluation. If pregnant or if the protocol chair thinks it is in the woman's best interest, she will no longer be followed by the study. The study may be discontinued at any time by a participating IRB, the Office for Human Research Protections, or other government agency as part of their duties to ensure that research subjects are protected.

4.3.2 Participant referral

If a participant is diagnosed with any clinical issues during the course of study participation, they will be referred to a clinician or the health department to receive further testing and/or clinical care. Specifically, this refers to anyone diagnosed with any infection, pregnancy, or contraceptive issue. Clinical followup within 1 week will be encouraged for all participants and a written record relating to the clinical issue (test results, clinical finding or impression) will be provided to the participant.. Should they have a specific provider, it will be recommended that they contact that provider for an appointment. If the individual does not have a provider, we will facilitate getting the participant into care at the Grady Title X clinic or their health department for evaluation and/or treament. We will follow-up with the participant within one week to confirm that they have sought appropriate care and if care has not been received, we will reinforce with the participant the importance of seeking treatment and actively assist in getting the participant into care.

5 Data and Biological Specimen Collection

Participants enrolled will undergo an interview, blood draw, and gynecologic exam with specimen collection at four study visits spanning 14 weeks.

5.1 Clinical evaluation

The clinical evaluation and all specimen collections will be performed by a study clinician or the trained study nurse using standard operating procedures determined prior to study initiation. All exam results will be documented on the biologic study clinical exam form (Appendix C). At the first visit, a urine HCG pregnancy test will be performed to document study eligibility after the consent form has been signed. Additionally a point-of-care HIV test will be administed to confirm

HIV negative status at baseline. At all visits, a pelvic exam will be performed. The clinician will examine for genital lesions and purulent vaginal discharge, and any abnormalities in the cervicovaginal mucosa will be documented on the physical exam data collection form. Wet-prep evaluations will be performed for bacterial vaginosis, yeast, and trichomoniasis during each pelvic exam. If during the course of study participation, any laboratory testing indicates the presence of infection, or if the participant's symptoms or physical examination raise suspicion of any other genital infection, a study clinician will refer for further care. There will be no extra information related to treatment or treatment outcomes recorded for study participants beyond what is standard of care. If during follow-up a woman is determined to be pregnant, she will be referred for care and no longer followed.

During the pelvic exam, vaginal pH will be measured with a pH meter, a vaginal swab will be collected for evaluation of vaginal microbiome, a swab for a multiplex PCR, a swab will be collected for a wet Prep, cervicovaginal fluid (CVF) will be collected by cervicovaginal lavage (CVL) for inflammatory marker quantification and two endocervical cytobrush samples of genital specimens for immune phenotyping. A swab will be collected into the anal opening to evaluate the gut microbiome. To enhance cellular yield from the posterior fornix of the FGT, CVL will be performed using 10 cc of phosphate buffered saline (PBS). Lavage will be repeated with a second aliquot of 10 cc of PBS. . Previous experience with this approach yielded 0.2-1X10^6 leukocytes per 10 cc of CVL, an amount of cells adequate for the proposed study endpoints and importantly avoided the need for the more invasive cervical cytobrush or vaginal biopsy sampling that also risks blood contamination of the sample. A second 10 cc of PBS will be collected in a similar

fashion to increase the potential yield for analysis. Obtained PBMC and CVL leukocytes will be processed and analyzed in real time for flow cytometry based phenotypic analysis. Additional CVL, vaginal swab, anal swab and plasma samples will be aliquoted and cryopreserved or frozen at -80^{o}C until analyzed for other endpoints. Dr. Haddad or a trained study nurse will perform all exams and sampling. Also at visit 2 and visit 4, a cervical or vaginal biopsy will be performed for a subset of participants that consent (10 per contraceptive arm) so that one or more samples of tissue from the vulva or vagina are acquired for evaluating HIV susceptibility and inflamation markers in the future.

In addition to the pelvic evaluation, blood will be collected at each visit in two 8 mL-vacutainer tubes (2 Cell Preparation Tubes (CPT)) for immunologic markers, and hormonal assessment (E2/P4 levels) at all four visits. Additional blood (1 Plasma Preparation Tube (PPT) will be collected at visit one for syphilis with rapid plasma reagin (RPR), HIV and HSV serology at the research lab. Table 4 lists the schedule of events and biologic specimen collection for the study. The collection of specimens at each visit will be noted on the specimen collection form (Appendix D). Positive test results from the research lab can not be relied upon for patient care. Any positive test results concerning for infection from the research lab will trigger further clinical investigation with referral for further testing and treatment.

5.2 Laboratory testing

All laboratory results from assays on blood or vaginal swabs, and CVL will be documented on the laboratory results forms (Appendix E).

Specimens collected will be transported within 4 hours the lab. Blood will will be separated and immediately processed for immunophenotyping and immune cell-activation assays, as described below for the vaginal samples. The separated plasma will be aliquoted and stored at -80^{o}C until batch testing commences, as described below for the vaginal samples. Progesterone (P4) and estrogen (E2) levels will be measured in plasma using the Milliplex kit for Luminex Assay (EMD Millipore, Billarica, MA).

A vaginal swab and anal swab will be collected and stored for future microbiome characterization via next-generation sequencing using the Ion Torrent platform to sequence 16S rDNA from bacterial species present in the vaginal compartment.

The CVL specimens will be tested for blood and leukocyte levels with a urine dipstick test and prostate specific antigen (PSA), a marker of semen exposure, using the Abacus ABAcard p30 test. The sample will be centrifuged, and the cell pellet will be re-suspended in cell culture media,

Table 5: Summary of Covariates and Analytic Methods for All Specific Aims

Demographics/Behaviors: Questionnaire/Exam: Age, BMI, Race, Parity, Coital frequency
STD Screen: Vaginal swab multiplex PCR for *N.gonorrhea, C. trachomatis, T. vaginalis, M. genitalium, T. pallidum,* HSV-1 & 2. Serum EIA for HIV & HSV. Serum RPR Kit for RPR
Candida vaginitis: Vaginal swab for KOH wet mount examination for hypae (yeast)
Bacterial Vaginosis: Vaginal swab for gram stain assessment for Nugent score BV diagnosis
Semen: CVL supernatant ABAcard® testing for qualitative prostatic specific antigen (PSA)
Serum sex hormones: Serum RIA for E2, P4
Microbiome: Next generation sequencing of 16s rDNA

and the leukocytes will be isolated from epithelial cells using Percoll gradient centrifugation. The resuspended CVL cell pellet (and PBMC pellet, separately) will be split for analysis. One half of the sample will be used for immune cell characterization (i.e., T cell subsets, HIV co-receptor expression) by antibody staining and multi-color flow cytometry using a LSR-2 platform. The other

half of the sample will be used to assess functional attributes of the T-cells. Cells will be stimulated with PMA and ionomycin (a polyclonal activator) in a tissue culture dish for 5 hours in the presence of brefeldin A and monensin and analyzed for the production of selected cytokines and chemokines by flow cytometry. *Multicolor flow cytometry* will be performed on matched PBMC and CVL samples utilizing the LSR-II platform (BD Biosciences) as previously described.(68) For assessment of HIV target cells, samples will be stained with a Live/Dead viability dye and fluorochrome labeled antibodies against CD3, CD4, CD27, CD45RA, CCR5 and CXCR4. Effector memory (CD45RA-/CD27-) CD3+CD4+ cells will be analyzed for surface expression of HIV co-receptors CCR5 and CXCR4 and reported as percent of total leukocytes and total CD4+ T cells.

We will examine a panel of proinflammatory, anti-inflammatory, inhibitory and chemotaxic cytokines and chemokines, focusing our analysis on those previously reported to influence recruitment of HIV target cells to the FRT such as IL1 and IP10(69). Using multiplex Luminex® assays combined with a customized multi-analytical panel of 22 human cytokines and chemokines, we will quantify concentrations of cytokines and chemokines in plasma and CVL supernatant at each study visit from participants enrolled

Recent semen exposure will be measured with a qualitative PSA ABAcard® p30 test (Abacus Diagnostics) (70). The wet-mount will be evaluated using saline and 10% KOH for evaluation of budding yeast or hyphae. A gram stain will be performed for Nugent scoring (71) to diagnose BV. A multiplex PCR assay, able to screen for multiple STIs concurrently without the need for multiple samples to be collected, will be used to evaluate for STIs including *N. gonorrhea, C. trachomatis, T. vaginalis, M. genitalium, T. pallidum*, and HSV-1/HSV-2. Serological tests for HIV

and HSV will be performed via enzyme immunoassay (EIA) using a Vitros® 3600 Immunodiagnostic system (Ortho Clinical Diagnostics) and syphilis screening determined via a commercially available rapid plasma reagin (RPR) kit. Positive test results from the research lab can not be relied upon for patient care. Any positive test results concerning for infection from the research lab will trigger further clinical investigation with referral for further testing performed by a CLIA-approved laboratory and treatment.

Table 6. Biological specimens and laboratory methods

Sample	Analytic Method	Outcomes of Interest
Cells from CVL and PBMC	Multi-parameter flow cytometry	• T cell subtypes: CD4, CD8, CCR7, CD25, CD27,CD28, CD45RA+, CD57, CD11a, PD1, HLA-DR. • HIV co-receptor use: CCR5, CXCR6, CXCR4. • T cell functional attributes:, IFNγ, TNFα, IL-2, MIP1α, MIP1β.
CVL Supernatant	Luminex panel	• **Proinflammatory:** IL-1β, IL-6, IL-12 (p70), G-CSF, M-CSF, IFN-α2, IFN-γ, IL-1α, IL-17, IL-2, TNF-α, TNF-β, IL-4, IL-5 • **Anti-inflammatory:** IL-10 • **Inhibitory (CCR5 agonists):** RANTES, MIP-1α, MIP-1β • **Chemotaxic:** IP10, IL-8, MCP-1, Eotaxin • PSA
Plasma	ABBA Card RIA RPR Kit EIA	• Plasma progesterone concentrations. • RPR • HIV/HSV
Vaginal, endocervical and Anal Swabs	Next-generation sequencing using	• 16S rDNA from bacterial species present in the vaginal compartment

	the Ion Torrent platform Mulitplex PCR	• N.gonorrhea, C. trachomatis, T. vaginalis, M. genitalium, T. pallidum, HSV-1 & 2.

5.3 Specimen management and storage

As the transmission of HIV and other blood-borne pathogens can occur through contact with contaminated needles, blood, and blood products, appropriate blood and secretion precautions will be employed by all personnel in the drawing of blood and handling of all specimens for this study, as currently recommended by the Centers for Disease Control and Prevention. All infectious specimens will be transported using packaging mandated in the Code of Federal Regulations, 42 CFR Part 72. Specimens, inclusive of the cervical or vaginal biopsies that are not used immediately will be processed and stored at CDC for future testing. Plasma and peripheral blood mononuclear cells (PBMC) will be stored at -70°C. Vaginal swabs, Anal swab and CVL supernatants will be stored at -70°C. Specimens collected will be used for the evaluations described within this protocol. Any remaining specimens will be stored for potential future use only if the patient has consented to storage of specimens. These specimens may be used in the future to further evaluate HIV, immunologic microbiologic factors of interest or as a source of DNA for future genetic analysis. A subject may request that any stored specimens are destroyed prior to completion of the study. Specimens will be stored with the study ID of participant only, and no patient identifiable information. At study completion any identifiers that link the subject to the study ID will be destroyed. Specimen storage will be for an indefinite amount of time if consent for storage had been signed. During the consent process, specimen storage will be discussed and participants will be consented to allow for storage and future testing of specimens.

5.4 Specimen collection considerations

Swabs and cervicovaginal lavage collections are minimal risk procedures. Specimens contaminated with blood may need to be excluded from our analyses. Every effort will be taken to reduce such risks by starting with the least invasive collections (swabs and tear-flo strips), then proceed with cervicovaginal lavage collection which introduces specimen dilution. We do not anticipate that collecting multiple samples in this order will influence any of the subsequent data collection endpoints, given the large vaginal surface. By maintaining a consistent order of specimens collected at each visit we will minimize experimental variability.

6 Ethics and Risks

The study will not commence until the study protocol has been reviewed and approved by the Emory University institutional review board (IRB) and the Grady Health System Research Oversight Committee.

6.1 Potential risks

The risks involved in study participation are those related to providing personal information about sensitive topics such as sex, contraception, antiretroviral medication use, and pregnancy. Participants' privacy and the confidentiality of data will be protected through training of interviewers and other study staff, conducting all interviewing and physical examinations in private, storing study materials in a locked room, and securing computer files that include identifiers. Only the study identification number will identify participant research records. Linkages between the ID number and participants' identifying information will be maintained in a computer database that is password protected and only accessible to study staff. These linkages will be destroyed after completion of study activities. Breaches of confidentiality are possible, though safeguards are in place to

protect the confidentiality of participants. Thus, the potential for experiencing an adverse event is minimal.

There is a risk that the participant may get pregnant while enrolled in the study. This risk is the greatest during the first few weeks of the study before the participant starts their contraceptive. To reduce this risk we will ask the participant to either abstain from sex or use condoms during this time. If pregnancy occurs during the study, we will refer the patient for further care.

Each of the four study visits consists of a brief interview, blood collection, pelvic examination, and vaginal sample (swab and lavage) collection. The potential additional risks to participating include discomfort from the blood draw and gynecologic exam with specimen collections, as outlined below:

1. The standard risks of phlebotomy: Skin irritation at the collection site, some bleeding, bruising, fainting, and pain are risks associated with blood collection. There is also a very small risk of infection at the collection site.
2. Discomfort from physical and gynecologic examination: Gynecologic examinations may cause both physical and emotional discomfort.
3. The risks associated with suspicion of a STI or vaginitis: Individuals who test positive or have any suspicious examination finds concerning for any infections during the research evaluation, will be referred for clinical evaluation and/or treatment. Individuals diagnosed with an STI may experience emotional discomfort upon receiving their diagnosis or upon notifying sexual partners, which would be recommended by their provider during

counseling if a test result is positive.

4. Risks associated with their selected contraceptive – including routine risks during insertion and side effects.

Risks associated with cervical or vaginal biopsy collection is bleeding and infection. Subjects will be informed on how to care for the area and to contact the PI or their medical care provider if there is a persistent smell, bleeding or discomfort.These procedures are not anticipated to be associated with any deleterious impact on participants' health or well-being.

6.2 Protection against risks

In implementing this protocol, we will be following HHS guidelines for research practices outlined on 45 CFR 46 (also known as the common rule). The investigators will adhere to the basic principles of "Good Clinical Practice" as outlined in Code of Federal Regulations (CFR) 312, subpart D, "Responsibilities of Sponsors and Investigators," CFR 21, part 50, and CFR 21, part 56 and Section 4 of International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use **(**ICH) Harmonized Tripartite Guideline for Good Clinical Practice. Specifically, we will provide careful training of study personnel who interview or collect participant samples. Training will include a component on careful patient counseling to enable the counselor to recognize complications, and when indicated, provide immediate referral for evaluation. A study participant will be informed of the voluntary nature of this study and that they may elect to not answer certain questions or to discontinue participation in the study at any time. Additionally, comprehension of study procedures will be assessed by study staff prior to

signing the informed consent form. Study staff will first review the informed consent document. After reviewing the consent document, the research staff member will ask the participant the appropriate comprehension questions (see Appendix B). If a participant incorrectly responds to one of the comprehension questions, the staff member will further clarify study procedures. Those participants who incorrectly respond on a second attempt, will be ineligible to participate in the study. Risks from receiving the contraceptive will be minimized by ensuring that a qualified trained clinician performs all insertions and a comprehensive consent for that contraceptive is reviewed prior to their receipt of the contraceptive as part of routine clinical practice at the clinic.

All laboratory specimens, evaluation forms, reports, and other records that leave the site to collaborating laboratories will be identified by a coded number only, to maintain subject confidentiality. All study records will be kept in a locked cabinet or on password-protected computers that will be stored in locked cabinets, accessible only to Emory based study research staff. The clinical data associated with the pelvic examination and placement of the contraception that would correspond to routine clinical care data will be shared with the patients clinical care provider, and included in their Grady electronic medical record,. This information in the electronic medical record is available to all members of the participant's clinical care team. We will encourage patients to share their results with any health care providers they have outside of the Emory or Grady setting, if they wish; however we will not attempt to contact these providers directly. For sexually transmitted diseases that are identified, a clinician on the research team will contact the individual directly via phone to discuss results and clinical referral. As study samples will be processed in a research lab, these results can not independently be acted on for clinical

management, thus patients will be referred for clinical care. For any positive results identified during their research visit, such as a positive pregnancy test or vaginitis, a clinician from the research team will be available to counsel the patient at that visit on their results and discuss referral for care.

Any other clinical information will not be released without written permission of the subject, except as necessary for monitoring by IRBs at participating institutions or the Office for Human Research Protection. The study may be discontinued at any time by an IRB at a participating institution or the Office for Human Research Protection.

6.3 Benefits to participation

The benefits of this research extend to potentially leading to improvements in the reproductive health care provided at the facility. Moreover, the results could potentially impact individuals globally as we aim to better understand transmission of infections such as HIV, which can improve or better target our methods of prevention. Participants will receive a $50 compensation for each study visit, including their screening visit with a maximum of $250 received if she completes all study activities.

6.4 Importance of the knowledge gained

The current study could help us to better understand the biologic effects of hormonal contraception on HIV transmission risk. If hormonal contraception increases the risk of transmission, dual protection methods with non-hormonal methods, such as the copper IUD and condoms, may be an important alternative strategy. The knowledge gained from this research may be used on multiple levels to help prevent unintended pregnancy, HIV transmission, and STI

infection.. Study findings will be disseminated further via abstracts submitted to scientific meetings and manuscripts submitted to peer-reviewed journals.

6.5 Limitations and Future Directions

By design the study does not directly address the risk of HIV acquisition from hormonal contraceptives; rather, it explores surrogates that might provide a mechanistic explanation to this purported impact of contraceptive hormone use. Being exploratory in nature, the sample size is small, and we may not be able to detect small differences in some of the outcomes. We have carefully selected our markers to focus on our study aims, however we acknowledge that additional markers may be of interest and can be explored in future evaluations. Additionally, the design is limited by all the inherent biases and confounding associated with a non-randomized, non-blinded study design. Attempts to control for covariates will be performed in the analysis. Further, as our follow-up is limited to 3 months, we cannot evaluate the impact of long-term contraceptive use nor the effect of irregular bleeding on HIV risk. Furthermore, we acknowledge that many other factors that influence HIV susceptibility warrant further investigation, such as epithelial integrity and endogenous microbicides. We plan to use these banked samples to explore some of these other potential mechanisms in future research. The data generated from the successful completion of this study will be instrumental in the design of a future prospective contraceptive trial to explore HIV acquisition risk and to establish safe family planning options for high-risk women.

6.6 Inclusion of women, minorities, and children

Pursuant to HHS policy, women (females over the age of 21), members of minority groups, and children should be included in biomedical and behavioral clinical research projects involving human subjects. The proposed study conforms to this policy.

7 Monitoring and Oversight

7.1 Site monitoring

Site monitoring may be performed by the Emory University's IRB and Office of Research Compliance, OHRP, FDA, or other government regulatory authorities. Clinical research site monitoring may include the review of the individual participant records, including consent forms, CRFs, supporting data, laboratory specimen records, and medical records (physicians' progress notes, nurses' notes, individuals' hospital charts) to ensure protection of study participants, compliance with the protocol, and accuracy and completeness of records. The monitors may also inspect sites' regulatory files to ensure that regulatory requirements are being followed. The investigator will make study documents (e.g., consent forms, CRFs) and pertinent hospital or clinic records readily available for inspection.

7.2 Data quality assurance and protection

There will be study protocol and procedure training for all study personnel prior to beginning of the study. A standard operations manual will be available to staff to refer to for operational details in running the study.

Each woman screened will be assigned a participant identification number (PIN) at the time of study entry. The PIN will be used on all data forms, specimens and communications related to the study.

Samples will be labeled at the time of collection and processing using study labels. Clinical research forms (CRFs) will be used to collect all study related data, including demographic, clinical, and laboratory data at study entry, and additional clinical data at subsequent study visits. Subjects will be identified only by the PIN on the CRFs. To maintain subject confidentiality, only a coded number will identify all stored laboratory specimens, forms, reports and other records. All records will be kept in locked file cabinets, accessible only by local study staff. Electronic files will be password-protected, with access only by authorized study personnel. Clinical information will not be released without written permission of the subject, except as necessary for monitoring from funding agencies and designees or except as mandated by law.

The study investigators will provide instructions concerning the recording of study data on CRFs. It will be the responsibility of the study investigator to assure the quality of computerized data. This role extends from protocol development to generation of the final study databases. Data from screening forms and study visit will be entered into Microsoft Access 2007. Electronic data will be secured in a password protected database that is accessible to members of the Emory investigative team. The Emory study investigators will maintain the link in a separate file that associates subjects with their PIN.

Staff will double-enter data using appropriate software. The data entry screens will be prepared with built-in quality control checks. To safeguard against loss of data, backups of the data will be made. Study staff will train the data entry staff in all data management procedures. We will conduct all statistical analyses using the SPSS System for Windows version 18.0, SAS Proc Mixed (version 9) or similar analysis software program. The database and the laboratory data

generated by this study will be maintained at Emory and will be accessible to collaborators. The principal investigators will be responsible for documentation of these data.

The initial data entry and maintenance of laboratory data, identified only by PIN, generated will be the responsibility of the laboratory investigator generating and entering the data and of study investigators who will oversee data quality.

7.3 Protocol violation

A protocol violation is any intentional or unintentional change from the IRB-approved protocol that adversely affects (1) the risk/benefit ratio of the study, (2) the rights, safety, or welfare of the participants or others, or (3) the integrity of the study. Examples include breach of confidentiality, inclusion of ineligible participants, or initiation of study procedures before participant has signed the consent form. If a protocol violation were to occur, it would be documented on the appropriate protocol deviation report form, and if the violation were to meet reporting requirements, it would promptly be reported to the participating IRBs.

7.4 Adverse events

An adverse event (AE) is defined as any health-related reaction, effect, toxicity or abnormal laboratory result that a study participant experiences during the course of the study, irrespective of relationship to the study intervention. This includes changes in a participant's condition or laboratory results that have or could have a deleterious effect on a participant's health or well-being. A serious adverse event (SAE) is defined as any experience that is fatal or life-threatening, requires in-patient hospitalization or prolongation of an existing hospitalization, or results in a persistent or significant disability or incapacity.

This study includes interviews, blood draws, pelvic examinations, and vaginal sample (swab and lavage) collections. These activities are not anticipated to be associated with any deleterious impact on participants' health or well-being. AEs will be managed according to good clinical practice and the judgment of the on-site physician. All clinical and laboratory AEs will be followed-up closely by study staff. Any SAEs will be expeditiously reported to the principal investigators, via a designated SAE form. Notification and submission of SAE forms should occur within 48 hours of the site awareness of the SAE. The PI will then make the final independent judgment as to the severity, relatedness, and anticipated or unanticipated nature of the SAE and finalize the Human Subjects Adverse Event Report Form. If the event is determined to be serious, unanticipated, and the relationship is anything other than probably or definitely not related, then the PI will report the event within 48 hours and submit the required forms to the IRB at Emory and Grady will be simultaneously notified. Thus, all anticipated SAEs that are at least possibly related to study intervention and all deaths that are at least possibly related to study intervention will be submitted to Emory IRB, and Grady ROC.

8 Statistical Considerations and Analysis

Sample Size/Statistical Considerations: We used the preliminary data from CVL of healthy women (unpublished) for our power analysis (CCR5: n=17, mean = 17.6%, Standard deviation (SD) = 17.4%, CD38 n=6, mean =41.9%, SD=11%), using the SD from the cross sectional data as an estimate for the SD for change. Due to the expected correlation between repeated-measures, this is a conservative estimate as the standard deviation for change should be smaller than the observed between-subject standard deviation. For evaluating change following exposure to any

contraceptive method (compared to no method), a sample size of 90 women achieves statistical power exceeding 95% to detect a **CCR5** increase of 10% (or larger) (two-sided one-sample t-test on change, 5% α-level). Similarly, with a sample size of 90 women, we achieve statistical power exceeding 95% to detect an increase in **CD38** of 10% (or larger) from before to after any contraceptive exposure. Comparing the different contraceptives, assuming an increase of 25% in CCR5 in the DMPA group and an increase of 10% in Nexplanon group and Mirena IUD group (pre to post exposure), a sample size of 30 women per contraceptive group (Nexplanon vs. DMPA or Mirena IUD vs. DMPA) will achieve 90% statistical power to detect a contraceptive group difference of 15% (2-sided two-sample t-test on change, 5% α-level). Similarly, assuming an increase of 20% in CD38 expressing T-cells in the DMPA group and an increase on average of 10% in Nexplanon group and Mirena IUD group, a sample size of 30 women per contraceptive group will achieve 93% statistical power to detect a contraceptive group difference of 10%.

Data Management and Statistical Analysis: Study data will be recorded on case report forms and entered into a web-based password-protected relational database (REDCap). Repeated-measures analyses using mixed linear models will be performed with the assistance of Emory CFAR biostatistical core for study endpoints and analyzed with SAS Proc Mixed (version 9) providing separate estimates of the means by contraceptive method and time on study before (weeks 1 and 3) and after (15 and 17 weeks) contraceptive administration. An unstructured variance-covariance form among the repeated measurements will be assumed for each outcome and estimates of the standard errors of parameters will be used to perform statistical tests and construct 95% confidence intervals. The model-based means are unbiased with unbalanced and missing data,

assuming missing data are non-informative and random. Mean changes over time for each contraceptive method will be tested for linear trend. Data will be summarized using adjusted means and observed differences plus 95% confidence intervals. Statistical tests will be 2-sided. A P value ≤ 0.05 will be considered statistically significant.

For multivariable repeated-measures analyses, a mixed linear model will be fit including age, body mass index, race and parity as potential baseline confounders in addition to contraceptive method, time on study and the interaction between time on study and contraceptive method. The multivariable results will be summarized with adjusted means and 95% confidence intervals. The adjusted mean for each contraceptive subgroup (DMPA, Nexplanon, copper IUD and Mirena IUD) will be defined as the mean response obtained by fitting the statistical model at the mean age and the mean BMI of the three subgroups and averaged across levels of the other risk factors. To integrate time-dependent variables, such as BV and PSA, into the longitudinal analyses, each participant at each of the 4 scheduled visits will be classified as positive or negative for each variable. Outcome change will be compared between incident cases and the other women (baseline positive and those that remain negative) after adjusting for the other covariates.

9 Dissemination of Results

The results will be disseminated in the form of abstracts submitted to scientific meetings and manuscripts submitted to peer-reviewed journals.

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