Effects of a vaginal contraceptive ring on vaginal microbiota and local immunity

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STATEMENT OF COMPLIANCE

This clinical trial will be conducted in accordance with the protocol and Good Clinical Practices (GCP) as required by the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR 46; 21 CFR Parts 50 and 56; 21 CFR Part 312).

- ICH E6; 62 Federal Register 25691 (1997)

All key personnel (all individuals responsible for the design and conduct of this study) have completed Human Subjects Protection Training.

SIGNATURE PAGE

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable US federal regulations and ICH guidelines.

Principal Investigator:

Signed: _____ Date: _____

CHRISTINE JOHNSTON, MD, MPH

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PROTOCOL SUMMARY

Title:	Effects of a vaginal contraceptive ring on vaginal microbiota and local immunity
Population:	Premenopausal women 18 years of age or older with BV or recent history of BV, desire to use CVR, and not intending to become pregnant over the course of participation
Number of Sites:	1
Study Duration:	5 years
Participation Duration:	7 months (plus optional 6 month extension)
Description of Agent or Intervention:	Contraceptive vaginal ring (CVR)
Objectives:	To assess potential benefits associated with both intermittent (use for 3 weeks, remove for 1 week, as defined in the package insert) and continuous (use for 4 weeks, then replace) CVR use among women either with BV or at high risk for BV. We will also recruit women who are HSV2-infected.
Time to Complete Enrollment:	18 months

1 BACKGROUND INFORMATION

1.1 Significance

Our objective is to study effects of a contraceptive vaginal ring (CVR) containing estrogen and progesterone (NuvaRing) on vaginal bacteria (the vaginal microbiome), local immunity, and lower genital tract infection in women. Emerging data suggest a favorable effect of this CVR on vaginal bacteria, specifically hydrogen peroxide-producing lactobacilli (LB), which are supported when the vagina is under the influence of estrogen.[1,2] These lactobacilli are critical for defense against infection with HIV and other sexually transmitted infections (STI). Bacterial vaginosis (BV) is characterized by loss of these LB and instead, overgrowth of a mix of anaerobic bacteria. BV significantly increases risk of STI/HIV, including genital herpes caused by HSV2.[3,4] BV is characterized by declines in protective immunologic mediators (certain defensins, secretory leukocyte protease inhibitor (SLPI)) and increases in inflammatory cytokines and chemokines,[5] alteration of cervical immune cell populations[6] and activation of local immune cells, including T cells.[7] Whether local delivery of hormones through a product like the CVR restores the lactobacilli and the protection they provide from BV and STI/HIV is unknown. In contrast, the contraceptive systemic depot progesterone—commonly used worldwide—may independently increase risk of HIV acquisition and transmission.[8] Our overarching hypothesis is that NuvaRing contributes to reduction in BV, healthy markers of cervicovaginal immune response and decreased rates of HSV2 shedding. Long-acting vaginal delivery of hormonal contraception as the foundation of "multicomponent prevention" – for example, delivery systems that can combine activity against unintended pregnancy, HIV, and other STI—has become a major focus for scientists, advocates, and funders alike,[9-11] but effects on the vaginal environment need careful definition before broad implementation.

The need for contraceptive options has taken on urgency with evidence indicating that systemic hormonal contraception (primarily injectable, long-acting progestin) may increase women's risk of HIV acquisition and transmission to male sex partners.[12] Lack of other contraceptive options is a critical barrier to progress towards optimizing women's reproductive health. Moreover, BV remains a major problem for women, causing symptomatic vaginitis and conferring increased risk for adverse outcomes of pregnancy and STI/HIV acquisition. We propose to explore the hypothesis—supported by limited data—that a contraceptive vaginal ring (CVR) that is commonly used in the U.S., the NuvaRing, will enhance women's genital and reproductive health. We propose that this CVR will increase the bacteria that help the vaginal environment protect against infection by HIV and other STIs, reduce rates of BV, and that in women who already have HSV2, use of the CVR will lower HSV2 shedding in the female genital tract. Importantly, CVR acceptability in general is excellent.[13] Moreover, the format of drug delivery through the form of a vaginal ring is also being intensely explored with the use of antiretroviral agents (dapivirine, tenofovir) as a means of topical, female-controlled HIV prevention, and formulation work with several antiretroviral drugs for sustained delivery with the NuvaRing format is underway.[9]

We believe the NuvaRing specifically will enhance vaginal health because we hypothesize that it will promote the growth of the *Lactobacillus* bacteria that protect women from infection with HIV and other STI. These bacteria, called *L. crispatus* and *L. jensenii*, are promoted by adequate levels of vaginal estrogen. These bacteria maintain low vaginal pH (<4.7) by producing lactic acid. BV occurs when these vaginal lactobacilli are replaced by overgrowth of other vaginal bacteria (primarily anaerobes, or bacteria that live in low-oxygen conditions). BV is the most common cause of vaginal complaints in reproductive-aged women. In pregnant women, BV is strongly associated with increased risk of preterm births and pregnancy-associated infections. In non-pregnant women, BV increases the risk of pelvic

infections, and acquisition of multiple STI, including HSV2 and HIV.[14] BV may elevate these risks in several ways, including activation of local immune cells. Finally, recent data indicate that onset of menstruation triggers key shifts in local vaginal bacteria, and in many women, may actually precipitate BV. Continuous use of the NuvaRing (use for four weeks, then replace) suppresses menstruation, and is increasingly used by many women with excellent reports of acceptability and safety. Whether this suppression of menstruation could suppress BV recurrence is not known.

Although short-term antibiotic therapy improves symptoms and restores *Lactobacillus* predominance in most women with BV, up to 20% of women fail routine therapy. In addition, BV recurrence after successful therapy is the rule. Recurrence is likely caused by failure to recolonize the vagina with desirable lactobacilli, which requires adequate vaginal estrogenization. In one study, women who used the NuvaRing for 3 consecutive 28-day cycles had higher quantities of desirable vaginal lactobacilli relative to women who used oral contraceptive pills containing 20 **μ**g ethinyl estradiol and 100 **μ**g levonorgestrel.[1] The NuvaRing releases 120 mcg/day of ENG (the active metabolite of desogestrel, a progesterone) and 15 mcg/day of ethinyl estradiol (the estrogen component). While generally well tolerated, incidence of local complaints (increased vaginal discharge or discomfort) with continuous CVR use is higher than those reported by OC users;[15] thus, the risk: benefit ratio of this approach needs careful assessment.

Pregnancy is also an independent risk for HIV acquisition and transmission, when acquisition confers high rates of perinatal transmission.[16,17] However, systemic depot progesterone, commonly used worldwide, may independently increase risk of HIV acquisition and transmission.[8] Increased HIV risk related to hormonal contraceptive use would be of global public health importance, given the large number of women using such methods. In a recent technical report, the World Health Organization noted that "expansion of contraceptive method mix and further research on the relationship between hormonal contraception and HIV infection is essential."[18]

For all of these reasons, then, a careful study of the microbiological, clinical, and immunologic benefits of the CVR is a high scientific and public health priority.

1.2 Study Hypothesis

- CVR use will be associated with favorable vaginal changes assessed by BVAB qPCR, and community definition by high-throughput sequencing

- Initiation of CVR use will be associated with an increase in desirable protective factors in the vagina, including increases in SLPI and reduction in proinflammatory cytokines.

- CVR use will be associated with lower rates of and longer time to incident BV assessed by clinical status (Amsel criteria and symptoms)[19] and Nugent score,[20] and among HSV-2-infected women, with a reduction in HSV-2 shedding.

2 STUDY OBJECTIVES

We propose to assess potential benefits associated with both intermittent (use for 3 weeks, remove for 1 week, as defined in the package insert) and continuous (use for 4 weeks, then replace) CVR use

among women either with BV or at high risk for BV. We will also recruit women who are HSV2-infected.

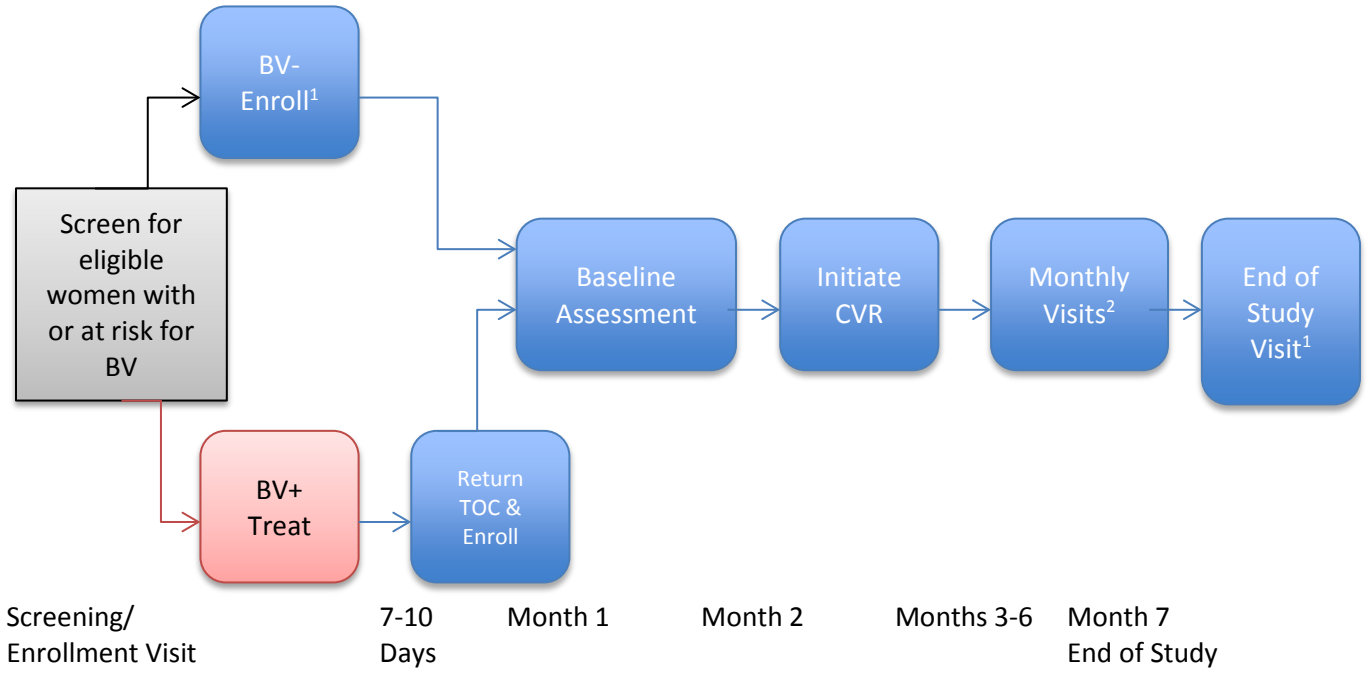
Primary outcomes will be as follows:

- The primary outcomes will be the quantity of *L. crispatus* determined by species-specific qPCR assay (Aim 1), local immune parameters, including cytokines and innate mediators (Aim 2), and incidence of and time to clinical BV episodes over the course participation (with the exploratory aim of measuring genital HSV-2 shedding among HSV-2 seropositive women (Aim 3).

- Secondary objectives will include a determination of Nugent score on Gram stain of vaginal fluid, vaginal colonization with bacteria associated with BV, the vaginal microbiome as determined with broad range 16S rRNA and pyrosequencing, and rates of adverse events with CVR use. At study end, all participants will be offered the choice of continuing NuvaRing use, or will be counseled on alternative (non-CVR) contraception methods. Women who opt to switch to an intrauterine device (IUD) at the end of the study will be offered participation in IUD an Extension Study, in which we will follow subjects for an additional 6 months to assess changes in immunity and the microbiome after IUD placement.

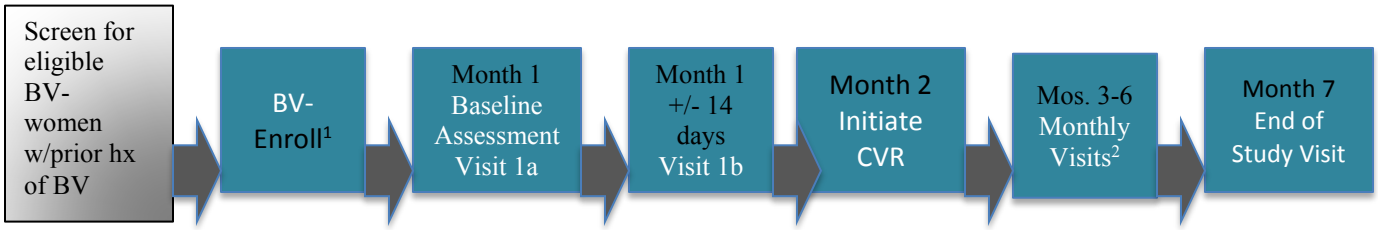
3 STUDY DESIGN

Figure 1. Study Design & Timeline



[1]At the Month 7 End of Study Visit, subjects who opt to have an IUD placed for contraception will be offered participation in optional IUD Extension Study.

Timeline for Optional Hormonal Sub-Study Participants



[1]Subjects who are BV- at screening may enroll at the same visit.
[2]At the Month 6 visit, subjects are given the opportunity to discontinue CVR use.

4 STUDY ENROLLMENT

4.1 Eligibility Criteria

- Women who are 18 years of age or older
- BV+ by Amsel criteria and Nugent score OR history of BV in the prior 12 months
- Premenopausal (as defined by having had at least 3 menstrual periods in the last 6 months, unless using hormonal contraception in which the investigators will use clinical discretion in determining eligibility).
- Willing to stop hormonal contraception
- Willing to use the NuvaRing as directed
- Not intending or wishing to become pregnant over the course of the study
- Daily access to a freezer for specimen storage
- Capable of providing written informed consent

4.1.1 Additional Eligibility Criteria for Enrollment into Hormonal Sub-Study

- BV negative at Screening/Enrollment
- Have regular menstrual cycles 25-35 days in length
- Have not used hormonal contraception within the past 2 months (no DMPA within the past 6 months)

4.1.2 Additional Eligibility Criteria for Enrollment into Optional IUD Extension Study

- Completion of Month 7 End of Study Visit
- Desiring IUD placement
- Willing to have IUD placed at Public Health STD clinic or by other health care provider
- No contraindications to IUD use (current pelvic inflammatory disease, distorted uterine cavity due to fibroids or other uterine pathology)

4.2 Exclusion Criteria

- Current pregnancy
- Women who are less than 6 weeks postpartum
- Contraindications to hormonal contraceptive use per package insert, including history of deep vein thrombosis, smoking in women older than 35 years
- IUD at study initiation
- Unable to comprehend consent material because of language barrier or psychological difficulty

4.3 Withdrawal from Study

Subjects are free to withdraw from the study at any time. Subjects who become pregnant during the course of the study will not continue the study. If a subject withdraws from the study due to CVR side effects or other issues and has used the CVR for at least 2 cycles, we will ask that they complete one final month of daily home swab collection.

4.4 Study Termination

The study may be terminated at any time by the principal investigator.

5 STUDY INTERVENTIONS

5.1 Study Overview

This study will be conducted at the University of Washington Virology Research Clinic (VRC) located at Harborview Medical Center in Seattle. Eligible women with BV will be invited to enroll as will women with a high likelihood of experiencing BV in the near term (12 months). We plan to enroll approximately 120 women.

Among those with BV at Enrollment, all will be treated with standard antibiotic therapy. After assessment for cure at 7-10 days, they will be asked to return for a visit at one month from enrollment to establish their baseline status. They will be offered non-hormonal methods for pregnancy prevention during that initial month.[21] After one month (Month 2 visit), they will then be asked to initiate NuvaRing use using one of the following two approaches:

- Continuous CVR use for four 28-day cycles, with replacement of the CVR after each cycle

- Non-continuous CVR use involving use for 21 days, then removal for 7 days, with replacement after the 7-day CVR-free period

Following initiation of CVR use, all participants will be evaluated monthly for BV with standard criteria (Amsel and Nugent criteria) and have specimens obtained for performance of bacterial quantitative PCR (qPCR) assays for BV-associated bacteria, L. crispatus and L. jenseni. Women will also be asked to seek care with the study team for interim vulvovaginal symptoms, with provision of diagnostic workup and appropriate treatment.

While participants will have the right to voluntarily stop using CVR at any point during the study, they will specifically be offered the option of discontinuing CVR use at six months (after 4 months of use) for the purpose of documenting whether the vaginal environment changes after CVR cessation. For this, they will return for one additional monthly visit.

5.1.1 Hormonal Sub-Study

In a sub-study of 40 women, we will compare cervicovaginal effects of the contraceptive ring to the normal changes occurring during the luteal phase of the menstrual cycle in response to endogenous estrogen and progesterone. Cervical cytobrush specimens will be collected to obtain an adequate sample of endocervical epithelial cells, which will be used for microarray-based gene expression analyses. Subjects who are otherwise eligible for the main study and who are BV negative, have regular menstrual cycles 25-35 days in length, and have had no use of hormonal contraception within the past 2 months (no DMPA within the past 6 months) will be eligible for the sub-study. Hormonal Sub-study participants will be asked to return for one additional visit prior to initiation of CVR for serum progesterone level testing and collection of cervical cytobrush samples in addition to regular visit specimens (same as Visit 1).

5.1.2 Intrauterine Device (IUD) Extension Study

During the Month 7 visit (or after completion of the study), we will offer women enrollment in an optional Extension Study, which aims to assess changes in cervicovaginal immunity, vaginal microbiome, and HSV-2 shedding among participants who opt to initiate an IUD. Subjects who have completed the Month 7 visit and daily vaginal swabs during the main study will be eligible to participate. All participants will receive counseling on contraceptive options at completion of the main study. Women who opt for an IUD will receive information regarding clinics that offer IUD placement, including the Public Health – Seattle & King County STD Clinic and other local family planning clinics. Subjects will receive IUD placement per standard of care at individual clinics; IUDs will not be placed as part of study procedures. IUD Extension Study participants will be asked to return approximately one month after IUD placement for their first Extension Study Visit. Women who previously participated in the Hormonal Sub-study will also have cytobrush specimens and progesterone levels collected at 2 time points during the IUD Extension Study.

5.2 Study samples

5.2.1 Vaginal Fluid

Swabs of vaginal fluid will be obtained from the posterior fornix for PCR assays. BV diagnosis will be made using any three of the following: (1) vaginal pH ≥4.7, (2) clue cells >20% of vaginal epithelial cells on microscopy, (3) fishy odor with KOH and (4) abnormal vaginal discharge (increased amount, differing from usual consistency, or malodorous). Diagnostic evaluation for C. trachomatis, N. gonorrhoeae, and Trichomonas vaginalis will be performed using nucleic acid amplification testing (APTIMA-Combo, GenProbe).

Women with any condition for which treatment or further testing is indicated will be managed

according to standard guidelines, and will remain eligible for enrollment. BV will be treated with standard treatment (either metronidazole gel .75%, one application vaginally nightly for 5 days, or oral metronidazole, 500 mg orally twice daily for 7 days, according to the participant's preference). All women treated for BV will be asked to return at 7-10 days for a test-of-cure visit, with BV cure defined by absence of >2 Amsel criteria.

5.2.2 Daily Home Swab Samples

For one month before CVR initiation, one month after, and one month following CVR discontinuation, all subjects will be asked to self-collect two vaginal swabs daily and to store in their freezer for return to the clinic at the next visit or beforehand at their convenience. These swabs will be analyzed for vaginal bacteria with bacterium-specific qPCR assays. Women with HSV-2 infection will be asked to collect one additional mixed anogenital swab to measure daily HSV-2 genital shedding. IUD Extension Study participants will be asked to complete two vaginal swabs daily (plus an additional swab for women with HSV-2) at two additional time points: one month after IUD placement, and 6 months after IUD placement. If a subject withdraws from the study due to CVR side effects or other issues and has used the CVR for at least 2 cycles, we will ask that they complete one final month of daily home swab collection.

5.2.3 Vaginal Biopsies

Vaginal biopsies are optional. Vaginal biopsies will be collected at two time points during the study: the Month 1 baseline visit (for women who agreed to the hormonal sub-study, the Month 1 vaginal biopsies will ideally be collected at the cycle day 6-10 (follicular phase) visit), and the Month 6 visit. Participation in this procedure is optional, and subjects willing to provide vaginal biopsy specimens will sign a separate consent form. ~~Subjects in the optional Hormonal Sub-Study will have one an additional vaginal biopsy procedure at Visit 1b.~~ Subjects in the IUD Extension Study will have one additional vaginal biopsy procedure at Month 13 (IUD 6). Subjects will be instructed to not begin collecting vaginal swabs until 5 days after the biopsy to ensure healing.

The procedure for the vaginal biopsy is as follows: A speculum will be placed into the vagina. After injection of 0.5 mL of 1% lidocaine with epinephrine, two biopsies approximately 2 x 2 mm in size will be collected from the upper posterior vaginal wall with baby Tischler forceps using standard technique. Hemostasis will be obtained with silver nitrate or Monsel's ferric subsulfate solution, if indicated. Biopsy samples will be analyzed for epithelial thickness, glycogen content of epithelial cells, immune cell populations, and gene expression.

5.3 Contraception

After the enrollment visit, women will be asked to maintain a one-month period of no hormonal contraceptive use. They will be counseled on non-hormonal methods of contraception (abstinence, barrier methods) and will be provided male and/or female condoms (as they prefer) at no cost.

5.4 CVR Use

After one month (Month 2 visit), women will be asked to initiate NuvaRing use using one of the

following two approaches. Participants will choose the approach they prefer, and will be provided standard counseling and health information about the risks and benefits associated with each:

- Continuous CVR use for four 28-day cycles, with replacement of the CVR after each cycle
- Non-continuous CVR use involving use for 21 days, then removal for 7 days, with replacement after the 7-day CVR-free period

Participants will initiate CVR use at any time in the cycle, with backup contraception (condoms) to cover the appropriate time period. We will follow standard guidelines from the U.S. Centers for Disease Control and Prevention (CDC), the Selected Practice Recommendations for Contraceptive Use, which state that women who initiate CVR within 5 days of the start of menstrual bleeding do not require back up contraception. Women initiating CVR >5 days after the start of menstrual bleeding require backup protection for the next 7 days.

While the package insert mentions initiation early in cycle, we aim to keep the number of pre-initiation visits to a minimum. After four months of CVR use (at Month 6 visit), women will be offered the option to discontinue use of the CVR, will be provided condoms and will then be assessed again after one month. Women may elect to continue CVR use at the Month 6 visit. CVR will be provided by the study.

For subjects who opt for continuous CVR use and prefer to have their CVR removed by the study clinician, the CVR will be saved for future analysis.

5.4.1 IUD Use

All subjects will receive comprehensive contraceptive counseling at the Month 6 and 7 visits. Women who choose to discontinue the CVR and initiate an IUD for contraception will be offered participation in an optional IUD Extension Study. Women will receive a list of clinics where they can schedule IUD placement, including the Public Health – Seattle & King County STD Clinic (located in the same building and floor as the research visits), Harborview Women's Clinic, University of Washington affiliated clinics, or other family planning clinics in the area. Subjects can choose the IUD brand that they prefer, including the levonorgestrel-releasing IUD (Mirena, Liletta, Skyla, Kyleena), or the nonhormonal copper IUD (Paragard). Screening, pre-procedure preparation, and the IUD insertion will be performed as part of primary care by health care providers and will not be part of study procedures. Subjects will be instructed to contact the study coordinator after IUD placement to schedule their Month 8 (IUD 1) visit.

5.5 Concomitant Medications/Treatments

Administration of any medication or therapies considered necessary for the subject's welfare will be documented in the subject's case report form in the concomitant medication section at each visit, including treatment for BV and STIs.

6 STUDY SCHEDULE

Specimens collected at each study visit and by home collection are summarized in Table 1.

6.1 Screening Visit and Enrollment Visit for BV- Women

Subjects will be consented prior to any study procedures. Screening procedures include a self-collected vaginal swab (for assessment of Amsel's critera), a brief medical history, and urine pregnancy test. Subjects who test positive for BV will be treated with standard treatment for BV and asked to return in 7-10 days for test of cure (TOC).

Women who are BV- will proceed with enrollment procedures including a pelvic examination and collection vaginal specimens for Gram stain, specimens for qPCR, broad range pyrosequencing and NAAT testing. CVF via Soft Cup will also be collected. Blood will be drawn for HIV and HSV antibody testing.

Information on symptoms (vaginal discharge, pruritis), types, frequency, and timing of sexual behavior (oral, vaginal, anal), menstrual history, antibiotic and hormone use, lubricants, condoms, and douching will be collected via study staff interview and computer questionnaire. Subjects will be counseled in use of non-hormonal contraception and offered condoms and will be asked to return in one month.

Women will be assessed and offered enrollment into the Hormonal Sub-Study and Vaginal Biopsy Sub-Study. For women enrolled in the *optional hormonal sub-study*, additional specimens may be collected (see Section 6.3.2 below), if within the cycle day window.

6.2 7-10 day/Enrollment Visit for BV+ Women

Subjects who are BV+ at screening will be asked to return in 7-10 days and then will proceed with enrollment procedures described for BV- women in Section 6.1 above. Subjects who remain BV+ at the 7-10 Day visit are eligible for enrollment.

6.3 Follow up visits

Women will be asked to return for up to follow up visits monthly at Months 1, 1b (for those women participating in the Hormonal Sub-Study), 2, 3, 4, 5, 6, and end of study visit at Month 7 for repeat interview/questionnaire, pelvic examination, and collection of study specimens. Between all study visits, women will be asked to avoid self-treatment with over-the-counter vaginal medications until evaluated by study clinicians if possible.

At each follow-up visit, information on symptoms (vaginal discharge, pruritus), types, frequency, and timing of sexual behavior (oral, vaginal, anal), menstrual history, antibiotic and hormone use, lubricants, condoms, and douching will be collected. Additionally, pH and Gram stain of vaginal fluid, vaginal swabs for BVAB-specific PCRs and cervical fluid will be performed.

6.3.1 Month 1 Visit

A pelvic exam will be performed and study specimens will be collected. Information on symptoms, sexual behavior, antibiotic and hormone use, condoms and douching will be collected via interview and questionnaire. Subjects will be provided with a daily home collection kit and instructions for collecting specimens over the 30 days prior to CVR initiation. Subjects will also receive their HSV and HIV test results at this visit.

For women enrolled in the *optional hormonal sub-study*, additional specimens may be collected (see Section 6.3.2 below), if within the cycle day window and if not already collected at Enrollment.

Vaginal biopsy will offered at this visit. Women who choose to participate will sign the vaginal biopsy consent form and the biopsies will be collected. For women enrolled in the *optional hormonal sub-study*, the Month 1 vaginal biopsies will ideally be collected at the cycle day 6-10 (follicular phase) visit.

6.3.2 Hormonal Sub-Study Visit 1b (Cycle day 6-10 or Cycle day 20-25)

For women enrolled in the hormonal sub-study, additional samples will be collected at two specific time points during their menstrual cycle prior to CVR initiation: follicular phase (cycle day 6-10) and luteal phase (cycle day 20-25).

- The first visit will be timed to occur at Enrollment or Month 1, depending on cycle day.
- The second (and additional) visit (Visit 1b) will occur outside their monthly study visits, timed to capture their other cycle phase; either before or after Month 1 depending on cycle timing. Procedures at Visit 1b will be identical to the monthly visits.

Additional Sub-Study Samples: At each sub-study visit, a cervical cytobrush specimen will be collected during the pelvic exam and blood will be drawn (1.0 mL) to measure progesterone levels.

6.3.3 Month 2

A pelvic exam will be performed and study specimens will be collected. Information on symptoms, sexual behavior, antibiotic and hormone use, condoms and douching will be collected via interview and questionnaire. In addition, CVR will be initiated at this visit. Daily home swab samples from the previous 30 days will be collected and reviewed. Subjects will be provided with a new daily home collection kit and instructions for collecting specimens over the next 30 days following CVR use.

6.3.4 Months 3, 4 and 5

A pelvic exam will be performed and study specimens will be collected. Information on symptoms, sexual behavior, antibiotic and hormone use, condoms and douching will be collected via interview and questionnaire at each visit. New CVRs will be dispensed. At the Month 3 visit, daily home swab samples from the previous 30 days will be collected and reviewed.

For women enrolled in the *optional hormonal sub-study*, at the Month 3 visit, a cervical cytobrush specimen will be collected during the pelvic exam and blood will be drawn (1.0 mL) to measure progesterone levels.

6.3.5 Month 6 - Stop CVR

A pelvic exam will be performed and study specimens will be collected. Information on symptoms, sexual behavior, antibiotic and hormone use, condoms and douching will be collected via interview and questionnaire. Participants will be asked if they wish to discontinue CVR at this visit. Those who elect to

stop will be provided with non-hormonal contraception (condoms). Subjects who wish to continue using CVR will be provided a new ring at this visit. All subjects will be asked to collect daily swabs at home over the next 30 days. Home collection kits and instructions will be provided. Vaginal biopsies will be collected at this visit, for women who choose to participate and had baseline biopsies at the Month 1 visit.

6.3.6 Month 7 - End of Study Visit

A pelvic exam will be performed and study specimens collected. Information on symptoms, sexual behavior, antibiotic and hormone use, condoms and douching will be collected via interview and questionnaire. Daily home swab samples from the previous 30 days will be collected and reviewed. Participants will receive comprehensive contraception counseling. Participants who were HIV and/or HSV-2 seronegative at baseline will have blood drawn for repeat testing.

Women who express interest in the IUD will be provided a referral to clinics that provide IUD placement and will be offered participation in optional IUD Extension Study. Women will be offered same-day placement at the Public Health – Seattle & King County STD Clinic (as available), or will be able to schedule visits at Harborview Women's Clinic or other family planning clinics in the area.

6.4 Optional IUD Extension Study

6.4.1 Month 8 (IUD 1) – First Extension Study Visit

Informed consent for the optional IUD Extension Study will be obtained prior to any study procedures. A pelvic exam will be performed and study specimens will be collected. Information on symptoms, sexual behavior, antibiotic and hormone use, condoms and douching will be collected via interview and questionnaire. Subjects will be asked to collect daily swabs at home over the next 30 days. Home collection kits and instructions will be provided.

6.4.2 Month 9 (IUD 2)

A pelvic exam will be performed and study specimens will be collected. Information on symptoms, sexual behavior, antibiotic and hormone use, condoms and douching will be collected via interview and questionnaire. Daily home swab samples from the previous 30 days will be collected and reviewed. For women enrolled in the Hormonal Sub-study, a cervical cytobrush specimen will be collected during the pelvic exam and blood will be drawn (1.0 mL) to measure progesterone level.

6.4.3 Months 10-12 (IUD 3-5)

Monthly phone calls for retention and questions about IUD use.

6.4.4 Month 13 (IUD 6)

A pelvic exam will be performed and study specimens will be collected. Information on symptoms, sexual behavior, antibiotic and hormone use, condoms and douching will be collected via interview and questionnaire. Subjects will be asked to collect daily swabs at home over the next 30 days. Home collection kits and instructions will be provided. Vaginal biopsy will be collected at this visit, for women who choose to participate and had previous biopsies at Month 1 and Month 6 visits.

6.4.5 Month 14 (IUD 7)

A pelvic exam will be performed and study specimens will be collected. Information on symptoms, sexual behavior, antibiotic and hormone use, condoms and douching will be collected via interview and questionnaire. Daily home swab samples from the previous 30 days will be collected and reviewed. For women enrolled in the Hormonal Sub-study, a cervical cytobrush specimen will be collected during the pelvic exam and blood will be drawn (1.0 mL) to measure progesterone level.

7 STUDY PROCEDURES

7.1 Clinical Examination

Pelvic examination: all findings will be recorded in the CRF Exam Form.

Pelvic Examination and Specimen Collection:
A non-lubricated speculum must be used during pelvic examination. After visual external examination and CVF (soft cup) collection, specimen collection/examination should take place in the following order. Table 1 summarizes the samples to be collected at various visits.

Vaginal Discharge Identification
Observe for presence of discharge and, if present, characterize by visual examination.

7.2 Laboratory Evaluations

7.2.1 Clinical Laboratory Evaluations

pH Measurement
Measure pH of vaginal discharge with pH indicator sticks. Samples for pH should be obtained from the lateral vaginal wall. Avoid contact with cervical mucus.

Vaginal & Cervical Swabs/Fluid
There will be a total of 6 vaginal swabs, collection of vaginal fluid using a soft cup, and 1 endocervical cytobrush for those participating in the optional study at Months 1a, 1b and 3:

1. Vaginal fluid collected using soft cup for measurement of cytokines and other immune mediators;
2. Saline and KOH microscopy (to asses for amine odor, clue cells, trichomonads, and yeast);
3. Gram stain of vaginal fluid (to confirm presence of BV, if present);

4. Nucleic acid amplification test (NAAT) for gonorrhea, chlamydia, and trichomoniasis; (lateral vaginal wall, p. 23; collected at Enrollment and then as indicated at subsequent visits)
5. Vaginal fluid swab for storage (Epi-Centre);
6. Quantitative PCR assays for vaginal bacteria (2 Epi-Centre swabs); (lateral vaginal wall, p. 23)
7. Endocervical cytobrush for immune cell characterization (collected at Enrollment (Month 1a), Month 1b and Month 3 visits) for those subjects enrolled in the Hormonal sub-study.

Test for Volatile Amines (Whiff Test), Yeast, Clue Cells and Motile Trichomonads
Swab lateral vaginal walls with a cotton-tipped applicator and place a liberal amount of discharge on each of two glass slides. Mix two drops of 10% potassium hydroxide (KOH) with the discharge on one slide and immediately determine if a fishy amine-like odor is present. Mix two drops of 0.9% saline solution with the discharge on the other slide and place a cover slip over both slides. Examine both slides under a light microscope at 10X and at 40X magnification. On the KOH slide, note the presence or absence of yeast (buds and hyphal elements). On the saline slide, note the presence or absence of motile trichomonads and clue cells. Clue cells will be identified as vaginal epithelial cells with such heavy coating of bacteria surrounding them that their peripheral borders are obscured. Three representative fields should be examined and the ratio of clue cells to vaginal epithelial cells determined. Clue cells should be $\geq$ 20% of vaginal epithelial cells to meet criteria for diagnosis of BV.

NAAT for STIs on Vaginal Swabs
The clinician will obtain a vaginal swab using the swabs supplied for the GenProbe Aptima test for trichomonas/chlamydia/gonorrhea). The swab will be sent to the HMC CT Laboratory.

Urine Sample
Collected to test for pregnancy at Screening and at additional visits, if indicated.

Serum Samples
For HSV testing, 10.0 ml of blood will be collected in a red top tube. For HIV testing, 2.0 ml of blood will be collected in a lavender-top tube. Specimens will be transported to the UW Virology Laboratory in Seattle.

For progesterone testing, collect 1.0 mL of blood in a red top tube for testing at RTS located at HMC.

7.2.2 Research Laboratory Evaluations

Collection of Endocervical Fluid Using SoftCup
Soft Cup: After all swabs are collected, a flexible menstrual soft cup will be inserted gently into the vagina. The participant will be encouraged to ambulate following insertion. The cup will then be removed, divided and placed into two tubes, and stored for eventual transport to the Fredricks' laboratory in Seattle.

Sample for Gram Stain
Swab posterior fornix with a cotton-tipped applicator. Place a moderate amount of discharge on slide and spread it as evenly as possible and allow the slide to air dry. With a pencil, label the slide with the subject's study ID, visit number and date. Place the slide in a plastic slide holder or slide box for transport to the lab for reading. All Gram stains will be read by microbiologists without knowledge of

any patient's clinical condition and interpreted in accordance with pre-determined criteria. Slides will be sent to the Fredricks' Laboratory in Seattle.

Vaginal Swabs for Molecular Analyses and Storage
Obtain three additional swabs of vaginal fluid, using the Epi Probe swabs supplied, from the lateral vaginal walls. Two are for performance of quantitative PCR assays, and the other is for storage for future analysis. Two swabs should be placed in the -70 freezer for later transport to Dr. David Fredricks' lab. The remaining (3rd) swab should be retained in the -70 freezer at UW VRC for future analysis.

Vaginal biopsies
Vaginal biopsies will be collected at 2 study visits (Month 1 and Month 6), and Month 13 (IUD 6) for IUD Extension Study participants.

Two biopsies will be collected at each visit. One specimen will be divided; one half will be analyzed by Dr. Dorothy Patton's lab for epithelial cell layer thickness and immunohistological staining for cellular glycogen content, and the other half will be analyzed for immune cell populations at the Fred Hutchinson Cancer Research Center (Dr. Florian Hladik and Dr. Jennifer Lund's lab). The second biopsy will be preserved in RNAlater (at room temperature for 24 hours to allow tissue penetration, followed by storage at -70C) and processed for RNA-Seq.

CVR Storage for Future Analysis
CVRs that are removed at the clinic will be saved for future analysis.

7.2.3 Laboratory Procedures and Specimen Processing

At all visits (listed in Table 1) vaginal material will be collected using polyester-tipped swabs or Epi-Centre swabs which will be stored locally at -70 C prior to shipping to the Fredricks Lab in Seattle. Refer to the SoftCup SOP for specimen processing.

Quantification of cytokines, chemokines, and antimicrobial soluble immune mediators in vaginal fluid*:* For measurement of local immune mediators, we will use a combination of transcriptional profiling of endocervical cells as well as ELISAs. We will use RNASeq and RT-PCR to measure transcriptional profiles of endocervical cells collected before, during, and after CVR initiation. We will measure concentrations of total protein (BCA Protein Kit) and the following: IL-1α, IL-1β, IL-2, IL-6, IL-7, IL-8, IL-12p70, IL-1ra, IFN-epsilon, RANTES, MIP-3α, MIP-1α and MIP-1β by multiplex proteome array with beads from Chemicon International, measured using Luminex and analyzed using StarStation (Applied Cytometry Systems). These mediators were selected because they are present in genital tract secretions and have been linked to modulation by exogenous sex hormones, and with STI/HIV risk. We will also measure the concentration of select anti-inflammatory mediators and antimicrobial proteins that may contribute to host defense using commercial ELISA kits: SLPI (R & D Systems), HNP1-3 (HyCult Biotechnology), HBD-1, HBD-2 and HBD-3 (Alpha Diagnostics), lactoferrin (Calbiochem) and lysozyme (Alpco Diagnostics).

Performance of vaginal proteomic analysis: We will perform proteomic analysis on saved samples from this study, at a laboratory to be determined. We anticipate that the Protein Profiling technique of Multiplexed Isobaric Tagging Technology for Relative Quantitation (iTRAQ)[22] will be used to determine protein expression changes in the vaginal proteome between 20 women, obtained at two visits: one

prior to CVR insertion, and one two months after CVR insertion. For the analysis, we will select visits at which BV (or other STI) are not present to maximize likelihood that proteomic changes will be less likely to be influenced by other processes. To maximize likelihood of identifying unique proteins that contribute, we will select samples with highest and lowest quantities of *L. crispatus* and lowest and highest Nugent scores, respectively. For this set of 40 samples, 6 x 8 plexes will be used. One tag in each iTRAQ set will contain a pooled standard of all of the samples and will be used in analysis to cross compare the 6 iTRAQ samples sets. LC-MS/MS is performed on an AB SCIEX TripleTOF® 5600 mass spectrometer equipped with a Waters nanoACQUITY UPLC system. Protein identification and quantitation is done with the AB ProteinPilot™ software. Data are deposited into the Yale Protein Expression Database (YPED)[23] which is an integrated web-accessible software system that addresses the storage, retrieval, and integrated analysis of high throughput proteomic and small molecule analyses. For biostatistical analysis, the ProteinPilot peptide summary is filtered to include only peptides with 1) no mis-cleavages, 2) high confidence peptide identifications, and 3) two or more iTRAQ reagent ratios for this peptide. A cyclic lowess normalization[24] is then performed to compensate for any differences between iTRAQ labels.

8 ASSESSMENT OF SAFETY

Side effects of CVR use: As noted above, the NuvaRing has an acceptable safety profile, is approved for use by the U.S. F.D.A., and is widely used by U.S. women for contraception. We will routinely query participants prior to use for any contraindications to CVR use, and also carefully assess at each visit for the development of any interim clinical processes of concern (for example, thrombosis) that might represent a side effect of the CVR as listed in the package insert. Participants will also be asked to contact us at any time during the study should they have concerns about new symptoms or signs.

Management of adverse events & counseling related to CVR**:** Participants will receive standard counseling about anticipated side effects of CVR initiation, and will be assessed for any adverse events reported during the course of the study as needed. The main adverse event we anticipate is irregular bleeding among women who use the CVR continuously; this is typically mild and resolves within first two cycles of use. Dr. Sarah Prager, a collaborator on this project, is highly trained in management of contraceptive use, and will provide expert guidance as needed.

Phlebotomy: May cause some pain or bruising. Some persons may feel light-headed or faint when blood is drawn.

Collection of swabs and cervicovaginal samples from genital areas: If samples are collected during herpes episodes, this may cause some irritation from the already sore lesion. Collection of swabs does not pose risk of additional infection.

IUD risk: There is a very small risk that the IUD could be pulled out at the time of pelvic exam or during sample collection.

Data collection: Participants may feel some stress or discomfort answering personal and sensitive questions about their health and sexual practices.

Pelvic/genital examination: Discomfort should be similar to that experienced for a PAP test. There may be a feeling of pressure from the speculum, which may be used to hold the vaginal walls open.

Participants may briefly feel pressure when the swab touches the cervix when samples are taken for bacterial or viral cultures.

Side effects of oral and intravaginal metrondiazole gel: These medications may be needed to treat BV during study participation. Interactions between oral metronidazole and alcohol can produce nausea, vomiting, and flushing. Alcohol should be avoided while taking oral metronidazole or using intravaginal metrondiazole gel. Yeast infections may be associated with metronidazole gel. Participants will be checked for a yeast infection if they develop symptoms during the study.

Risks of vaginal biopsy: Patients may experience pain associated with the speculum examination. They also may have pain associated with lidocaine injection and the biopsy. There are small risks of bleeding and infection. Risk of bleeding will be minimized by biopsy location along the upper anterior or posterior aspect of the vagina, use of lidocaine with epinephrine, observation and use of silver nitrate or Monsel's solution to ensure hemostasis. To minimize the risk of delayed bleeding, participants will be instructed to avoid sexual intercourse or placement of tampons for 5 days. Subjects will be instructed to not begin collecting vaginal swabs until 5 days after the biopsy to ensure healing. All procedures will be performed by a board-certified gynecologist or experienced nurse practitioner who has performed vaginal biopsies for other research studies.

8.1 Known Potential Benefits

Subjects may benefit from participation in this study by having a blood test for detection of infection with HSV2 and HIV, and by finding out more about the likelihood of their having BV or recurrent BV. They will not be charged for any of the diagnostic tests. Subjects may benefit from pre and post-test counseling, treatment and referrals, as necessary. We have found that subjects are empowered by knowing how frequently they shed HSV-2, for example, and by knowing more about what BV is and how it can be prevented. Otherwise, this study will not be of direct benefit to the subjects.

8.2 Specification of Safety Parameters

Safety will be assessed by the frequency, incidence and severity of AEs and SAEs solicited in-clinic and via phone call.

8.3 Methods and Timing for Assessing, Recording, and Analyzing Safety Parameters

8.4 Adverse Events, Serious Adverse Events

The investigator is responsible for ensuring all AEs that are observed or reported during the study are documented.

8.4.1 Definition of Adverse Event

Adverse Event: International Conference on Harmonisation (ICH) guideline E6 defines an AE as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product regardless of its causal relationship to the study treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease

temporally associated with the use of medicinal (investigational) product. The occurrence of an AE may come to the attention of study personnel during study visits and interviews or by a vaccine recipient presenting for medical care.

Severity of Event: All AEs will be assessed by the clinician using a standard grading system (NIH DAIDS Adverse Events Toxicity Tables). For events not included in the protocol defined grading system, the following guidelines will be used to quantify intensity:

> Mild: events require minimal or no treatment and do not interfere with the patient's daily activities.
>
> Moderate: events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
>
> Severe: events interrupt a patient's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually incapacitating.
>
> Life threatening: any adverse drug experience that places the patient or subject, in the view of the investigator, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that had it occurred in a more severe form, might have caused death.

Relationship to study procedures: The investigator's assessment of the relationship of an AE to study procedures is part of the documentation process. If there is any doubt as to whether a clinical observation is an AE, the event should be reported to the PI. AEs will be assessed according to the relationship to the study process: associated or not associated. To help assess, the following guidelines are used.

> **Associated –** The event is temporally related to the study procedures and no other etiology explains the event.
>
> **Not Associated –** The event is temporally independent of study the study procedures and/or the event appears to be explained by another etiology.

8.4.2 Definition of Serious Adverse Event

A SAE is defined as an AE meeting one of the following conditions:

- Results in death during the period of protocol defined surveillance.
- Is life-threatening (defined as a subject at immediate risk of death at the time of the event).
- Requires inpatient hospitalization or prolongation of existing hospitalization during the period of protocol-defined surveillance.
- Results in congenital anomaly or birth defect.
- Results in a persistent or significant disability/incapacity.
- Any other important medical event that may not result in death, be life threatening, or require hospitalization, may be considered an SAE when, based upon appropriate medical judgment, the event may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood

dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

8.5 Reporting Procedures

Adverse events including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate CRF. Information to be collected includes event description, date of onset, investigator assessment of severity, investigator assessment of relationship to study product, date of resolution of the event, seriousness, and outcome. The **intensity** of nonserious AEs can be assessed by a licensed clinician (i.e., physician, nurse, Nurse Practitioner, Physician Assistant). **Causality** of nonserious AEs can be assessed only by a clinician licensed to make medical diagnoses (ie, physician, Nurse Practitioner, Physician Assistant). All AEs occurring while on study will be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution or until considered stable.

Any medical condition that is present at screening will be considered as baseline and will not be reported as an AE. If the severity of any pre-existing medical condition increases during the study period, then it will be recorded as an AE.

All SAEs will be reported immediately to the Investigator.

SAE Contact: Christine Johnston, MD PI.
Phone: 206-520-4340, Fax 206-520-4371, Pager 206-540-6324, cell 206-370-2091

SAE Alternate: A physician from the Virology Research Clinic is available at any time by calling the clinic pager at 206-598-0924.

The SAE report will include the following information (as available)

- Patient ID
- Description of SAE (onset date, severity, causal relationship)
- Basic demographic information
- Outcomes attributed to the event
- Summary of relevant test results, laboratory data, and other relevant history
- The first and last dates of study drug administration
- Statement whether study drug was discontinued or schedule modified
- Statement whether the event abated after study drug was discontinued or schedule modified
- Statement whether the event recurred after reintroduction of the study drug if it had been discontinued.

8.5.1 Follow-up of SAE

All SAEs will be followed until satisfactory resolution or until the principal investigator or sub-investigator deems the event to be chronic or the subject to be stable.

8.5.2 Serious AE Detection and Reporting

All SAEs will be:

- Recorded on the appropriate SAE report form.
- Followed through resolution by a study physician.

Timelines for submission of an SAE form are as follows:

- All deaths and life-threatening events regardless of relationship, will be recorded on the SAE form and the PI will be notified within 24 hours of identification of the SAE.

All SAEs determined to be related to study procedures will be reported to the Human Subjects Division of the University of Washington within 10-days of becoming aware of the event.

8.5.3 Reporting of Pregnancy

Pregnancies occurring in study subjects will be recorded.

8.6 Type and Duration of Follow-up of Subjects after Adverse Events

Adverse events will be followed until resolved or considered stable.

9 STATISTICAL CONSIDERATIONS

9.1 Outcomes

Aim 1. The primary outcome will be quantity of *L. crispatus* measured by qPCR at all available follow-up visits and will be compared during five months of CVR use to one month prior to CVR use. Secondary outcomes will be BV defined clinically by Amsel and confirmed by Nugent criteria at any follow-up visit, quantities of other BVAB defined by qPCR assay, and reported genital adverse events. Quantity and presence of other lactobacilli and BVAB species detected by PCR will be assessed using the methods outlined above. All available follow-up observations will be used. Quantity of *L. crispatus* will be log transformed and analyzed using a linear mixed model with subject random effect to account for correlation within person. Binary outcomes will be analyzed using generalized estimating equations with log link and robust standard errors to estimate relative risks while accounting for correlation.

Aim 2. The outcomes of interest will be compared within participants, using each woman as her own control; observations from the time prior to CVR initiation will be compared to those collected during CVR use. We will use methods for Normal data and then use non-parametric approaches or dichotomize and use binomial link GEE if data are highly skewed and cannot be transformed into a Normal distribution. Some analyses will be exploratory; we will compare quantities of several immune mediators noted above by CVR use. These comparisons will not likely be independent, so we will adjust for multiple comparisons by permutation procedure. We will also correlate concentrations of immune mediators with qPCR results in Aim 1 to test the hypothesis that higher concentrations of protective lactobacilli will be associated with higher levels of protective mediators, and lower levels of inflammatory cytokines/chemokines. Continuous variables between comparison groups will be compared using t-tests or Wilcoxon rank-sum tests, and dichotomous variables will be compared by chi-square tests or Fisher's exact tests. Linear mixed effects models or generalized estimating equation (GEE) models will be used to examine whether outcomes (mediator concentrations, vaginal bacterial

quantities) differ significantly between comparison groups after adjustment for confounding factors identified through univariate analysis. All analyses will assume a two-sided significance level of 0.05.

Aim 3. The primary outcomes will be (1) incidence of BV at all available follow-up visits, and (2) time to BV during four months of CVR use relative to the two months prior to CVR use. For secondary analysis, we will measure the quantity of HSV-2 by PCR. This will be log transformed and association with CVR use examined using a linear mixed model as for Aim 1. *Sample size calculation:* Data from our prospective studies of BV were used to estimate the likelihood of these conditions occurring during follow-up. Over six months of observation, we have documented BV recurrence rates after cure of an initial BV episode of 28 / 100 person-years in absence of any hormonal contraception. A recent meta-analysis estimated that HC was associated with a 30% reduced likelihood of BV.[25] With these assumptions, we will have reasonable power to detect a 30% or greater difference in incidence of BV by CVR status (Table 5). However, BV incidence may be lower, and mechanisms for a favorable shift in vaginal microbiome, if affected by CVR, may be heterogeneous. If so, we will still be able to generate important preliminary data in support of the CVR's favorable effect on the vaginal environment through demonstration of improved microbiome maintenance and immune profile; this would support future study of the CVR in populations with higher incidence of BV and other cervicovaginal STI.

9.2 Sample Size

Aim 1: Total anticipated enrollment is 120 women. We will screen approximately 56 women per month over 18 months and estimate that about 14 women/month will be interested in participating. We anticipate that of the interested women, approximately 50% will have BV or a history of BV in the prior 6 months, for predicted enrollment of 6-7 women per month. Assuming at least 75% of women return for at least one pre- and one post-CVR visit, we will have at least 90 analyzable women.

Aim 2: Participants are as described in Aim 1.

Aim 3: Participants are as described in Aim 1. Moreover, we anticipate that approximately half of the total women enrolled will be HSV-2-infected at study entry (60 women).

10 QUALITY CONTROL AND QUALITY ASSURANCE

The principal investigator or designee will review the charts of the first 5 participants who enroll in the study to ensure high quality data are being collected. A 10% chart review will then be performed quarterly by the PI.

11 ETHICS/PROTECTION OF HUMAN SUBJECTS

11.1 Ethical Standard

The investigator will ensure that this study is conducted in full conformity with the principles set forth in The Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research of the US National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research (April 18, 1979) and codified in 45 CFR Part 46 and/or the ICH E6; 62 Federal Regulations 25691 (1997).

11.2 Ethical Approval

Prior to the initiation of the study, the principal investigator will obtain written approval to conduct the study via her Institutional Review Board (IRB). Approval is required for the study protocol plus amendments, informed consent form, participant information sheet and advertising materials.

11.3 Informed Consent

The investigator or study clinician/staff will explain the purpose and nature of the study, including potential benefits and risks to the participant, to each potential participant before enrollment in the study. The participant must sign an informed consent form approved by the IRB before entering the study. All original informed consent forms must be retained by the principal investigator with the participant's records. The participant will receive a copy of the signed informed consent form.

11.4 Exclusion of Women, Minorities, and Children (Special Populations)

This study will be inclusive of all healthy women who meet the inclusion/exclusion criteria, regardless of religion, sex, or ethnic background. Pregnant women, men, and children will not be eligible for this study.

11.5 Subject Confidentiality

Subjects will have coded study identification numbers and will not be identified by name. Subject confidentiality is strictly held in trust by the investigator, her staff, and the University of Washington. This confidentiality extends to genetic and biological sample tests, in addition to the clinical information relating to participating subjects.

The study protocol, documentation, data and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

11.6 Study Discontinuation

The participant may stop the study at any time. The investigator may terminate a participant's study participation. In the event of study discontinuation, reasons for discontinuation will be clearly documented.

12 DATA HANDLING AND RECORD KEEPING

12.1 Data collection

Clinic records for participants are the responsibility of the investigator.

12.2 Case Report Forms (CRFs)

Case report forms are to be completed and signed for each participant by the investigator or designated member of the study staff. CRFs will be completed neatly and legibly. Any modification of previously

entered data must be made by the investigator or study coordinator by striking through the original entry with a single line, initialing and dating changes, and entering the correct data nearby. A valid explanation must be given for any missing information. All data will be entered into a computer database and further quality control check will be made to produce a final database for analysis.

12.3 Data Management

Data will be entered and stored in RedCAP (Research Electronic Data Capture), a secure online application used for data collection and management. RedCAP provides audit trails for tracking user activity and data manipulation, as well as automated data export compatible with statistical software packages. [26]

12.4 Study Records Retention

Subject charts including CRFS, notes, and other source data will be retained according to the archival policy of the University of Washington.

12.5 Protocol Deviations and Violations

A protocol deviation is any noncompliance with the study protocol, or GCP requirements. The noncompliance may be either on the part of the subject, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed and implemented promptly.

These practices are consistent with ICH E6:
4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3
5.1 Quality Assurance and Quality Control, section 5.1.1
5.20 Noncompliance, sections 5.20.1 and 5.20.2.

All deviations from the protocol must be addressed in study subject source documents. Protocol deviations and violations will be sent to UW IRB per their guidelines. The site principal investigator/study staff is responsible for knowing and adhering to their IRB/Independent Ethics Committee requirements.

13 PUBLICATION POLICY

Following completion of the study, the investigator will publish the results of this study in a scientific journal.

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Table 1. Laboratory specimens to be obtained at each visit or by self-collection

Test	Screen	Enroll	Month 1 Baseline		Month 1b substudy[1]	Month 2 CVR Start		Month 3-5	Month 6[2]		Month 7	Month 8[12] (IUD 1)		Month 9 (IUD 2)	Month 10-12 (IUD 3-5)	Month 13 (IUD 6)		Month 14 (IUD 7)
Microbiome																		
pH measurement	X	X	X		X	X		X	X		X	X		X		X		X
Saline microscopy	X	X	X		X	X		X	X		X	X		X		X		X
Gram stain	X	X	X	DSC	X	X	DSC	X	X	DSC	X	X	DSC	X		X	DSC	X
Bacterial qPCR assays[3]		X	X	DSC	X	X	DSC	X	X	DSC	X	X	DSC	X		X	DSC	X
Broad-range 16S rRNA PCR pyrosequencing		X	X	DSC	X	X	DSC	X	X	DSC	X	X	DSC	X		X	DSC	X
Cervical cytobrush[4]			X		X			X[5]						X				X
Vaginal biopsy[13]			X						X							X		
Immune environment																		
CVF: mucosal immune mediators[6], proteomics		X	X		X	X		X	X		X	X		X		X		X
Progesterone level[7]			X		X			X[5]						X				X
STI acquisition/shedding																		
NAAT: *C. trachomatis, N. gonorrhoeae, T. vaginalis*		X	X[8]		X[8]	X[8]		X[8]	X[8]		X[8]							
HSV PCR vaginal swab[9]			DSC			DSC			DSC			DSC				DSC		
HSV serology		X									X[10]							
HIV serology		X									X[10]							
Urine Pregnancy Test	X	X[11]	X[11]		X[11]	X[11]		X[11]	X[11]		X[11]							
Clinical exam for LGTI		X	X						X			X				X		
IUD supplement																		
Monthly phone calls for retention															X			

Abbreviations: DSC = daily self-collection, BVAB = bacterial vaginosis-associated bacteria, CVF = cervicovaginal fluid, NAAT = nucleic acid amplified test, HSV = herpes simplex virus, LGTI = lower genital tract infection (BV, VVC, cervicitis, genital ulcer disease), PCR = polymerase chain reaction.

[1] Only women enrolling in the optional Hormonal sub-study will be asked to come to the clinic for visit 1b procedures.

[2] We anticipate that approximately ¼ of subjects will elect to discontinue CVR use at the end of the study; all participants will be invited to perform daily self-collection of vaginal swabs as noted during the month after CVR cessation.

[3] Vaginal bacterial assays include qPCR for G. vaginalis, Megasphaera phylotypes 1 and 2, L. crispatus, L. jensenii, and L. iners.

[4] Women enrolled in the optional Hormonal sub-study will undergo cervical cytobrush collection at Month 1,/Enrollment, Visit 1b and Month 3, and Month 9 and 14 if enrolled in Extension Study.

[5] Will be obtained at month 3 only.

[6] Includes SLPI, defensin, lactoferrin. These will be collected periodically during follow-up.
[7] Women enrolling in the optional Hormonal sub-study undergo serum progesterone testing at 3 time points in the study (Month 1/Enrollment, Visit 1b and Month 3), and Month 9 and 14 if enrolled in the Extension Study.
[8] NAAT will be performed as indicated at subsequent visits during study participation
[9] HSV-2 seropositive women will be asked to perform DSC of 1 additional vaginal swabs before and after insertion of CVR and after cessation.
[10] If HIV and/or HSV-2 seronegative at baseline.
[11] Urine pregnancy testing will be performed, as indicated at subsequent visits during study participation.
[12] IUD placement will be done prior to Month 8 visit as part of clinical care, not part of study procedures.
[13] Optional vaginal biopsy procedures. For women who agreed to the hormonal sub-study, the Month 1 vaginal biopsies will ideally be collected at the cycle day 6-10 (follicular phase) visit.

Table 2. Table of Study Procedures

	Main Study (& Hormonal Sub-Study)									Optional IUD Extension Study				
Study Month	**Screen**	**Enroll**	**1**	**1b[1]**	**2**	**3**	**4-5**	**6**	**7**	**8[2]**	**9**	**10-12**	**13**	**14**
Consent	X									X				
Medical and Interim History	X	X	X	X	X					X	X		X	XX
Urine Pregnancy Test	X	X[3]	X[3]		X[3]	X[3]	X[3]	X[3]	X[3]					
Self- Collected Vaginal Swab for BV Assessment	X													
Blood draw for HSV and HIV Testing		X							X[3]					
Pelvic Exam and Vaginal Sample Collection		X	X	X	X	X	X	X	X	X	X		X	
Cervical Cytobrush[1]			X	X		X					X			X
Blood for Progesterone Levels[2]			X	X		X					X			X
Vaginal Biopsy (optional)			X[4]					X					X	
STD testing		X	X[3]	X[3]	X[3]		X[3]	X[3]	X[3]					
NuvaRing					X[5]	X	X	X[6]						
Redcap Computer Questionnaire		X	X	X	X	X	X	X	X	X	X		X	X
Daily Home Swab Collection (30 days)			X → (two 30-day sessions)					X →		X →			X →	
Monthly Phone Calls												X		

[1] Hormonal Sub-study only. Samples may be collected at Enrollment or Month 1, depending on cycle timing. The additional visit (Visit 1b) will occur outside their monthly study visits, timed to capture their other cycle phase.
[2] Participants will have an IUD placed
[3] As needed
[4] For women participating in the Hormonal Sub-Study, vaginal biopsy will ideally occur at the Day 6-10 visit.
[5] CVR Initiation will occur at Month 2. A new CRV will be dispensed monthly
[6] Subjects will be asked if they wish to discontinue CVR at the Month 6 Visit. Subjects who wish to continue using CVR will be provided a new ring at this visit.