Examining the Impact of Tampon Use on the Vaginal Microbiota

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Tampon Study Proposal

Abstract

To date, a great deal of work has been done to characterize the composition of the vaginal microbiota (VMB) in women of reproductive age. While several different community types have been identified, it is widely accepted that communities dominated by *Lactobacillus* spp confer colonization resistance, and thus protect against bacterial vaginosis (BV), STI, and other urogenital infectious diseases [Hickey 2012]. Conversely, mixed communities that often include *Gardnerella vaginalis*, *Prevotella* spp., *Mycoplasma* spp., and/or Gram-positive cocci and relatively few *Lactobacillus* spp are considered unhealthy states and may contribute to BV and various sequelae including infertility, endometriosis, pelvic inflammatory disease, and increased risk of STI [Hickey 2012]. Considering both the relationship between VMB composition and reproductive health, and the high prevalence of tampon use, which, for many women, occurs at regular intervals for the duration of their reproductive life, it is surprising how few studies have examined the effect of tampon use on VMB composition. Moreover, the majority of these studies are limited by the use of culture-dependent methods focused on the presence/absence of a small number of species (*Lactobacillus* spp, *Staphylococcus aureus*, *E. coli*, group B *Streptococcus*, and/or *Gardnerella vaginalis*) [Onderdonk 1986, Onderdonk 1987, Chow 1989, Shehin 2003, Chase 2007, Chase 2010, Hochwalt 2010]. The one study to date that has used culture-independent VMB community analysis in response to tampon use was limited by its sample size of 7 women. Further, the investigators only collected vaginal swabs at two points during each menstrual cycle [Hickey 2013]. The conflicting findings of these studies make this an even more compelling research question. In order to address this striking knowledge gap, we propose a longitudinal, interventional, case-crossover study design to examine whether expanded tampon shape affects VMB composition during, and in between menses. We will recruit 20 women from the University of Michigan, Ann Arbor to use Tampax® Pearl® Regular Tampons (expand into a rectangular prism-like shape) and Playtex® Gentle Glide® 360°® Regular Tampons (expand into a conical shape) for one menstrual cycle each, in a randomly assigned order. We will obtain twice-weekly self-collected vaginal swabs for a 2-3 week baseline window before the first menstrual cycle with an assigned tampon, followed by the two menstrual cycles with assigned tampon use, and finishing with one menstrual cycle with menstrual product use according to each subject's normal habits. DNA will be isolated from the vaginal swabs, and the bacterial 16S rRNA encoding genes will be amplified by PCR. Sequencing will be performed using the MiSeq Illumina platform, and analyzed to answer the above questions. We hypothesize that VMB composition will vary between menses using the Tampax® Pearl® Regular Tampons and Playtex® Gentle Glide® 360°® Regular Tampons, and that persistence of these VMB changes will vary based on baseline VMB community type.

Background

To date, a great deal of work has been done to characterize the composition of the vaginal microbiota (VMB) in women of reproductive age. While many bacterial genuses have been identified in VMB samples, it is widely accepted that communities dominated by *Lactobacillus* spp promote a healthy vaginal environment and good reproductive health [reviewed in Hickey 2012]. The primary function of *Lactobacilli* in promoting vaginal health is the provision of

colonization resistance to the vaginal epithelium [Borovkova 2011, Lopes 2011, Borges 2014, reviewed in Hickey 2012]. Through the production of lactic acid, *Lactobacilli* create an acidic environment, which prevents the proliferation of nonindigenous organisms, including pathogens and those associated with BV [Cook 1990, Boskey 1999, Aroutcheva 2001, Valore 2002, Atassi 2006, Kaewsrichan 2006, O'Hanlon 2010, Borges 2014, reviewed in Hickey 2012]. However, it has been noted in multiple studies that a low abundance of *Lactobacilli* does not necessarily translate to poor vaginal health [Gajer 2012, Martin 2012]. This has lead to the recent hypothesis that it is not the presence of *Lactobacilli* in abundance that promotes vaginal health, but instead it is the capacity of the VMB to produce lactic acid, which can be accomplished by several taxa normally isolated from the VMB including *Atopobium*, *Streptococcus*, *Staphylococcus*, *Megasphaera*, and *Leptotrichia* [Hickey 2012]. Despite the fact that diverse VMB that are not dominated by *Lactobacilli* have been found in asymptomatic women with good vaginal health, this is not the norm. It is widely accepted that mixed VMB including *Gardnerella vaginalis*, *Prevotella* spp., *Mycoplasma* spp., and/or Gram-positive cocci and relatively few *Lactobacilli* are considered unhealthy states and are often present in women with BV [Spiegel 1980, Amsel 1983, both Spiegel 1983, Hill 1993, Falagas 2007, Cribby 2008, Lopes 2011, Hickey 2012]. BV imposes a large burden on the healthcare system both directly and indirectly. Directly, it is the most common vaginal disorder of reproductive age women and results in millions of healthcare visits each year in the US alone [Sobel 2005, Koumans 2007]. Indirectly, BV is associated with a great number of serious sequelae including, but not limited to, infertility [Sweet 1995], endometritis [Haggerty 2004], and pelvic inflammatory disease [Wiesenfeld 2002]; as well as an increased risk of acquiring HIV and other STIs [Hillier 1998, Taha 1998, Martin 1999, Schmid 2000, Wiesenfeld 2003].

A considerable volume of work has also been conducted to characterize the fluctuations of the VMB across the menstrual cycle. While some studies (both culture-dependent and culture-independent) have reported no change in community composition across the menstrual cycle [Sautter 1980, Wilks 1987, Gajer 2012, Hickey 2013], others have reported varying relative abundances of aerobic and anaerobic bacteria across the cycle [Bartlett 1977] or a greater proportion of non-*Lactobacillus* species during menses [Priestley 1997, Eschenbach 2000, Srinivasan 2010, Lopes 2011, Gajer 2012, Hickey 2013]. In light of these findings, in particular those of Gajer et al. (2012) and Hickey et al. (2013), it is currently thought that the degree and type of VMB fluctuation seen across the menstrual cycle varies between women [Lopes 2011, Chaban 2014]. Further, the increased abundance of non-*Lactobacilli* species seen during menses is of particular interest given the changes to vaginal innate immune cell presence [Hill 1992], temperature [Hill 2010], oxygen and carbon dioxide concentrations [Hill 2005], and volatile fatty acid concentrations [Preti 1975] that occur during menses. These changes alter the growth conditions present in the vagina, and as such, may promote the growth of certain bacterial species over others. Given that non-*Lactobacilli* species are often present in greater abundance during menses, it is possible that the conditions of the vagina during menses could promote their growth and potentially shift a *Lactobacilli*-dominated VMB to a diverse VMB and promote BV. Moreover, the changes to vaginal temperature, dissolved gas concentrations, and volatile fatty acid concentrations that occur during menses have been shown to be exacerbated by tampon use [Preti 1975, Hill 2005, Hill 2010], suggesting that tampon use may compound any VMB changes that occur during menses.

Considering the well-established link between VMB composition and vaginal health, the VMB fluctuations seen over the course of the menstrual cycle, the changes to the vaginal environment that occur during menses (which are exacerbated by tampon use), and the high prevalence of tampon use (it is estimated that as many as 81% of reproductive age women in the US use tampons [Omar 1998]); surprisingly few studies have examined the effects of tampon use during menses on VMB composition. The majority of these studies are limited by the use of culture-dependent methods focused on the presence/absence of a small number of species (*Lactobacillus* spp, *Staphylococcus aureus*, *E. coli*, group B *Streptococcus*, and/or *Gardnerella vaginalis*) [Onderdonk 1986, Onderdonk 1987, Chow 1989, Shehin 2003, Chase 2007, Chase 2010, Hochwalt 2010]. The one study to date that has used culture-independent VMB community analysis in response to tampon use was limited by its sample size of 7 women. Further, the investigators only collected vaginal swabs at two points during each menstrual cycle [Hickey 2013]. Not only are these studies severely limited by their designs, but they have also failed to produce consistent findings on the impact of tampon use on VMB composition. This knowledge gap represents a major shortcoming of public health research to identify and describe the effect of a near-ubiquitous modifiable risk factor on vaginal health. Addressing this gap has the potential to improve individuals' vaginal health through public health interventions, and decrease the societal burden of poor vaginal health, namely due to BV.

We propose a longitudinal, interventional, case-crossover study design to examine whether expanded tampon shape affects VMB composition during, and in between menses. We will recruit 20 women from the University of Michigan, Ann Arbor to use Tampax® Pearl® Regular Tampons (expand into a rectangular prism-like shape) and Playtex® Gentle Glide® 360°® Regular Tampons (expand into a conical shape) for one menstrual cycle each, in a randomly assigned order. We will obtain twice-weekly self-collected vaginal swabs for a 2-3 week baseline window before the first menstrual cycle with an assigned tampon, followed by the two menstrual cycles with assigned tampon use, and finishing with one menstrual cycle with menstrual product use according to each subject's normal habits. DNA will be isolated from the vaginal swabs, and the bacterial 16S rRNA encoding genes will be amplified by PCR. Sequencing will be performed using the MiSeq Illumina platform, and analyzed to answer the above questions.

Outcomes and aims

- Primary Outcome: Change in relative abundance of *Lactobacillus* species from: baseline to the end of first menstrual cycle, baseline to the end of second menstrual cycle, and baseline to the end of third menstrual cycle (approximately 14 weeks)
- Secondary Outcome: Change in relative abundance of *Gardnerella vaginalis* from: baseline to the end of first menstrual cycle, baseline to the end of second menstrual cycle, and baseline to the end of third menstrual cycle (approximately 14 weeks)
- Exploratory Outcomes
    - To characterize intra-woman, during-menses changes in VMB composition caused by (see Methods – Study Design and Methods – Whole Community Analysis & Statistics sections for notes on causal inference) changing the expanded tampon shape of tampon used.
        - Hypothesis 1: VMB composition will vary between menses with Tampax® Pearl® Regular Tampon use and Playtex® Gentle Glide® 360°® Regular

Tampon use because the different expanded shapes of the two tampons will alter the vaginal environment differently and thus promote growth of different taxonomic groups. The primary structural difference between Tampax® Pearl® Regular Tampons and Playtex® Gentle Glide® 360°® Regular Tampons is that, once inserted into the vagina, Tampax® Pearl® Regular Tampons expand axially into a rectangular prism-like shape, while Playtex® Gentle Glide® 360°® Regular Tampons expand radially into a conical shape. The radial expansion of the Playtex® Gentle Glide® 360°® Regular Tampons likely results in increased surface area of the tampon that is in contact with the vaginal epithelium as compared to the Tampax® Pearl® Regular Tampons. This may allow for increased absorption of menses by the Playtex® Gentle Glide® 360°® Regular Tampons as compared to the Tampax® Pearl® Regular Tampons, resulting in differing growth conditions in the vagina during Playtex® Gentle Glide® 360°® Regular Tampon use and Tampax® Pearl® Regular Tampon use.

- To characterize intra-woman, between-menses changes in VMB composition caused by (see Methods – Study Design and Methods – Whole Community Analysis & Statistics sections for notes on causal inference) changing the expanded shape of tampon used.
    - Hypothesis 2.1: Persistence of VMB changes observed during menses caused by changing the expanded shape of the tampon used will vary dependent on baseline VMB community type such that VMB changes will persist for greater time between menses in women with typically diverse VMB that confer reduced colonization resistance compared to a *Lactobacilli* dominated VMB [Borovkova 2011, Lopes 2011, Borges 2014, reviewed in Hickey 2012].
    - Hypothesis 2.2: Within women with *Lactobacilli* dominated baseline VMB, persistence of VMB changes observed during menses caused by changing expanded tampon shape will vary based on the degree of reduction of *Lactobacilli* abundance during menses such that changes resulting in greater reductions of *Lactobacilli* abundance will persist for greater time between menses.

Methods

Recruitment

- We will recruit 40 women from the University of Michigan, Ann Arbor during the Winter 2017 semester.
    - We will make an in-person announcement in Women's Studies 400: Women's Reproductive Health (taught by Dr. Tim Johnson, Dept. of OB/GYN) according to the following script:
        - Hello everyone, we are here to recruit forty healthy female students to participate in our study examining the impact of changing tampon use on the composition of the vaginal microbiota. The study period will consist of a baseline window after a subject's last period and three full menstrual cycles. For the first two menstrual cycles, each subject will use Tampax® Pearl® Regular

Tampons and Playtex® Gentle Glide® 360°® Regular Tampons for one menstrual cycle each, in a randomly assigned order. For the third menstrual cycle, each subject will use menstrual products according to their normal habits. For the course of the study, approximately 4 months, subjects will self-collect vaginal swabs twice a week. These samples will be used for bacterial community analysis to assess the composition of the vaginal microbiota at each time point, and compare composition during periods using different tampons and determine how long these changes persist between periods. For the first two menstrual cycles, the study tampons and Always® Infinity® Regular Pads will be provided to subjects free of charge. Additionally, each subject will receive $20. In order to be eligible to participate in this study, you must be at least 18 years old, have regular menstrual cycles that last 3-5 weeks with periods that last for at least 4 days, currently use tampons, and be in good general and vaginal health. You are ineligible to participate if you are pregnant or planning on becoming pregnant in the next 4 months, if you have difficulty using tampons, if you currently have or have had toxic shock syndrome (TSS), if you currently have a sexually transmitted infection (STI) or urinary tract infection (UTI), if are currently using antibiotics or antifungals or have used antibiotics or antifungals within the last month, or if you have an autoimmune condition. If you are interested in participating in this study, please email (kaycart@umich.edu) and then we will set up an enrollment meeting.

- We will also pass out flyers describing the objective of the study, the duration of subject participation, subject responsibilities, subject compensation, eligibility requirements, and exclusion criteria. It will also include the email address for setting up enrollment meetings. We will also post these flyers at the Michigan Union and other places around central campus.
- We will also post the study in UMHealthResearch.org.
- We will respond to interest emails according to the following script in order to set up enrollment meetings with each interested student:
    - Dear [name],

      Thank you for your interest in participating in this study. The next step is to set up an enrollment meeting at which you will be assigned a random study ID number, review the informed consent document for the study, give informed consent to participate in the study if you choose to, complete an online enrollment survey, and receive the tampons, pads, and vaginal swabs for the study. The following times are available for the enrollment meeting:

      Monday 9:30 AM – 4:15 PM

Tuesday 1:30 PM – 4:00 PM
Wednesday 11:00 AM – 4:15 PM
Thursday 1:30 PM – 4:15 PM

Please respond to this email with your preferred day and time for the enrollment meeting. The meeting will take approximately 30 minutes.

Thanks

  - At the enrollment meeting we will obtain informed consent from each student, assign a random ID number to the student, administer the baseline survey (assess eligibility, collects data on covariates (covered in Methods subsection Study Design)) on a lab computer using Qualtrics, review subject responsibilities with each student, and distribute study materials including 30 vaginal swabs, vaginal swab instructions, 30 collection day surveys, 24 Tampax® Pearl® Regular Tampons, 24 Playtex® Gentle Glide® 360°® Regular Tampons, 24 Always® Infinity® Regular Pads with wings to each subject. All research data will be kept in files that include the subject ID number, but not name or other information that is likely to allow someone other than the researchers to link the data to a subject. These files will be maintained in a password protected lab file manager. We will enroll the first 20 eligible students who schedule an enrollment meeting.
- Inclusion criteria: Subjects must be 18 years of age or older; have regular menstrual cycles lasting 21-35 days with menses for at least 4 days; be a current tampon user; be in self-reported good general health; and be in self-reported good vaginal health.
- Exclusion criteria: Respondents will be excluded if they are pregnant or are planning on becoming pregnant during the study; have difficulty using tampons; currently have, or have had TSS; currently have an STI; currently have a UTI; are currently using current antibiotics, or used antibiotics within 4 weeks prior to enrollment; are currently using current antifungals, or used antifungals within 4 weeks prior to enrollment; or have an autoimmune condition.

Study Design

- This study will be a longitudinal, interventional, case-crossover study with three exposure levels:
  - Experimental exposure levels: Each subject will use Tampax® Pearl® Regular Tampons and Playtex® Gentle Glide® 360°® Regular Tampons for one menstrual cycle each, in a randomly assigned order.
  - Control exposure level: Each subject will use menstrual products according to their normal habits for a third menstrual cycle.
  - A case-crossover design ensures exchangeability between treatment windows within each subject for all time invariant characteristics. Under exchangeability and the assumptions of an intent-to-treat analysis (covered in Methods – Whole Community Analysis &

Statistics), the actual outcome observed under one treatment for an individual equals the counterfactual outcome under that treatment for that individual, so association between expanded tampon shape and VMB composition represents a causal relationship. This study design and the intent-to-treat analytic strategy will allow us to make causal inference in reference to the effect of expanded tampon shape on VMB composition.

- Vaginal swabs will be self-collected by each subject twice weekly for four different exposure windows (Fig. 1):
    - A 2-3 week baseline window after the last menstrual period prior to enrollment; 4-6 baseline samples per subject.
    - One menstrual cycle using the Tampax® Pearl® Regular Tampons; 8 samples.
    - One menstrual cycle using Playtex® Gentle Glide® 360°® Regular Tampons; 8 samples.
    - The final menstrual cycle with menstrual product use according to the subject's normal habits; 8 samples for the subject's normal habits.

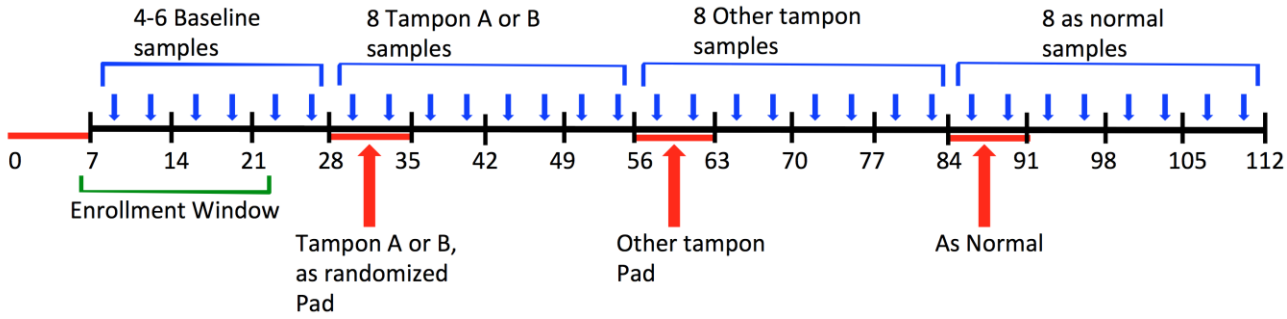

Figure 1: Study design.

- We will administer a baseline survey using the online survey system Qualtrics to obtain information on covariates including age; race; normal menstrual product use (types of products used; frequency of use of different products; specific tampon details including brand, perfume, absorbency); hygiene habits (douching, use of scented soaps); contraception use (current and past use of hormonal methods, current use of other methods); sexual activity (types of current and past, sex of partners, number of lifetime partners, frequency, use of lubricant); social history (smoking, drinking, yogurt); obstetric/gynecological history (medical problems, pregnancies and outcomes, first day of last period); medical history (medical problems, height, weight, surgical history, current medications).
    - We had 100% completion in our pilot and expect a similar completion rate in the proposed study (HUM 00098494).

Menstrual Products

- Tampon A: Tampax® Pearl® Regular Tampons (Fig. 2)
    - http://tampax.com/en-us/shop-products/pearl/pearl-regular
    - Applicator: plastic with pigments of color
    - Absorbent core: cotton and/or rayon

- Thin fabric around absorbent core: rayon and polyester or polyethylene and polypropylene
- String: cotton and/or polyester; polypropylene braid
- Thread: cotton-wrapped polyester or polyester
- $0.21 on amazon prime; 4.6 stars
    - 4 tampons/day * 6 days = 24 tampons per subject * 20 subjects = 480 tampons * $0.21/tampon = $100.80

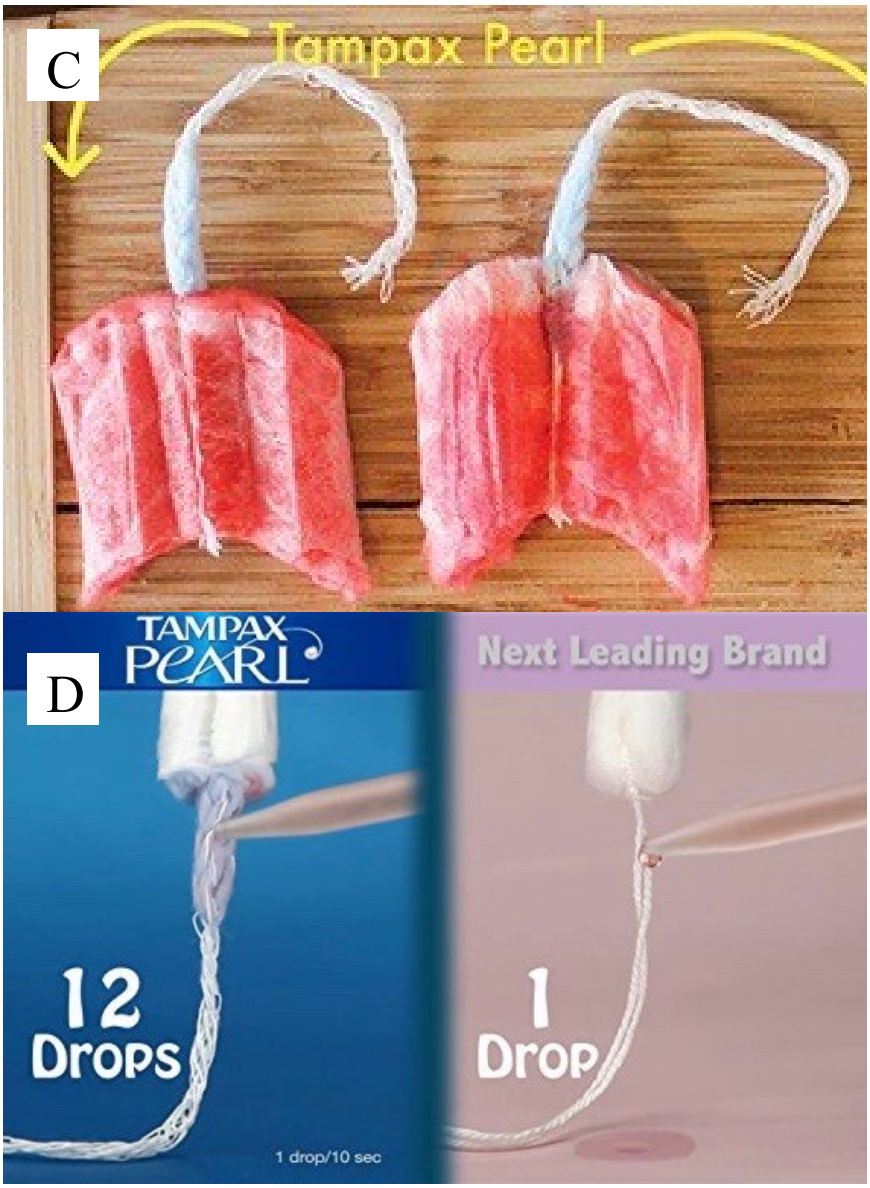

Figure 2: Tampax® Pearl® Regular Tampons. A) Tampon in applicator. B) Tampon. C) Expanded tampon. D) Leak-stopping braided string.

- Tampon B: Playtex® Gentle Glide® 360°® Regular Tampons (Fig. 3)
    - *http://www.playtexplayon.com/products/gentle-glide-tampons*
    - Applicator: plastic
    - Tampon: rayon fiber, polyester
    - String: cotton
    - $0.32 per tampon on amazon prime; 4.0 stars
        - 4 tampons/day * 6 days = 24 tampons per subject * 20 subjects = 480 tampons * $0.32/tampon = $153.60

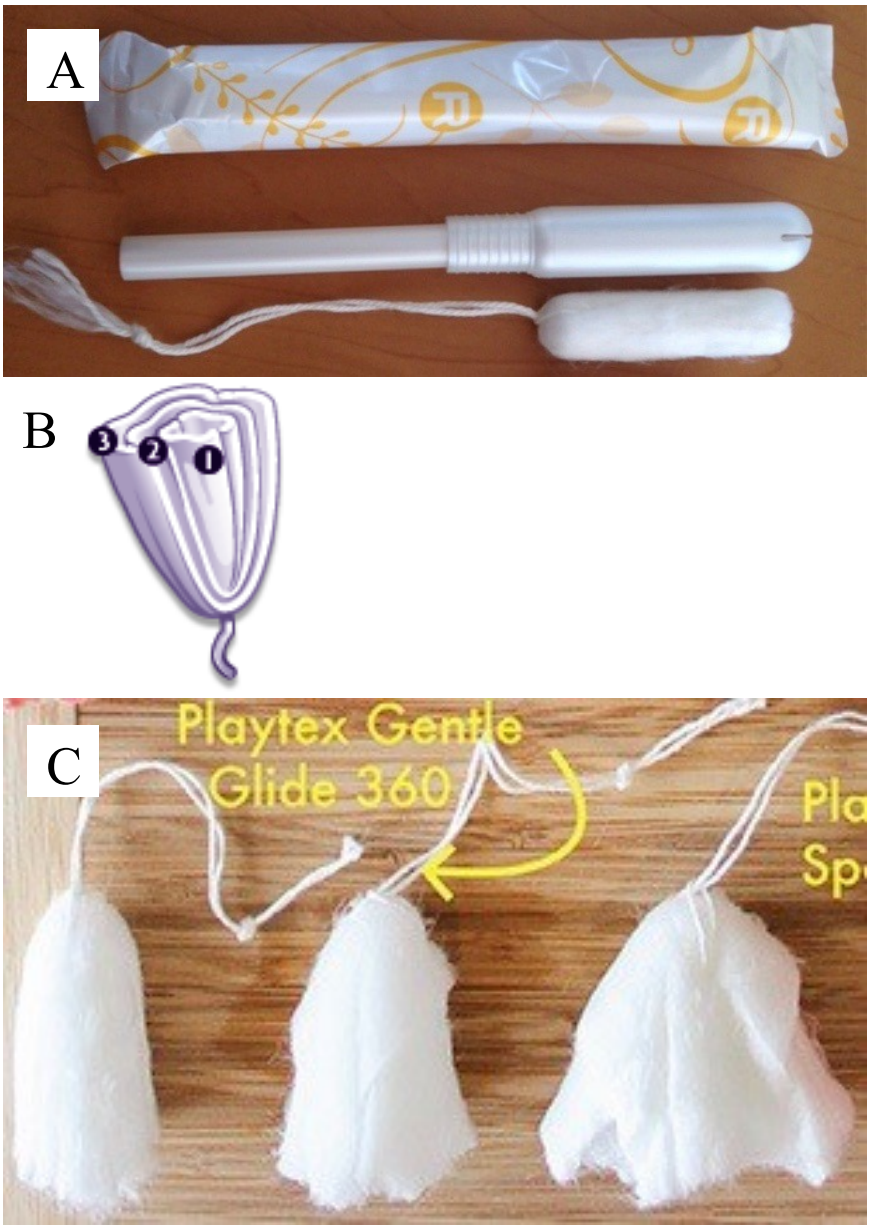

Figure 3: Playtex® Gentle Glide® 360°® Regular Tampons. A) Tampon in applicator and tampon. B) Triple layer design. C) Expanded tampon.

- Justification for choice of tampons A and B: The primary structural difference between Tampax® Pearl® Regular Tampons and Playtex® Gentle Glide® 360°® Regular Tampons is that, once inserted into the vagina, Tampax® Pearl® Regular Tampons expand axially into a rectangular prism-like shape (Fig. 2C), while Playtex® Gentle Glide® 360°® Regular Tampons expand radially into a cone-like shape (Fig. 3C). The radial expansion of the Playtex® Gentle Glide® 360°® Regular Tampons likely results in increased surface area of the tampon that is in contact with the vaginal epithelium as compared to the Tampax® Pearl® Regular Tampons. This may allow for increased absorption of menses by the Playtex® Gentle Glide® 360°® Regular Tampons as compared to the Tampax® Pearl® Regular Tampons, resulting in differing growth conditions in the vagina during

Playtex® Gentle Glide® 360°® Regular Tampon use and Tampax® Pearl® Regular Tampon use. Both tampons are inserted with a plastic applicator, and both are made of a cotton/rayon blend. The only other substantial structural difference between the two is that the Tampax® Pearl® Regular Tampons have a braided string (Fig. 2B) while the Playtex® Gentle Glide® 360°® Regular Tampons have a straight, 2 strand string (Fig 3A). Tampax® Pearl® reports that the braided string is designed to stop tampon leaks, and that it can hold up to 12 drops of fluid in addition to the volume held by the tampon (Fig. 2D). We do not anticipate this difference to substantially alter the growth conditions in the vagina because any contact between the string (braided or not) and the vaginal epithelium will be much less than that between the actual tampon and the vaginal epithelium. Another reason for choosing these tampons is that, for the past 6 years (2011-2016), Tampax® and Playtex® brand tampons have been the two most used tampon brands in the US [Experian 2016]. Examining how these tampon brands impact VMB composition will increase the generalizability of the study findings, as compared to if we selected other tampon brands.

- Pad: Always® Infinity® Regular Pads with wings (Fig. 4)
    - *http://always.com/en-us/shop-products/menstrual-pads/infinity-pads-with-flexfoam/always-infinity-regular-pads-with-wings*
    - $0.31 per pad on amazon prime; 4.4 stars
        - 2 pads/day * 12 days = 24 pads * 20 subjects = 480 pads * $0.31/pad = $148.80

Figure 4: Always® Infinity® Regular Pads with wings.

- Distribution: Subjects will receive their study tampons and pads during their enrollment meeting.
- We will instruct subjects to use the study tampons as they normally would, not wear a tampon for more than 8 hours, not wear more than one tampon at a time, contact a doctor if they experience any serious adverse effects related to tampon use including TSS symptoms, remove the tampon prior to collecting a swab during menses, and use the pads in addition to the tampons if they so choose.

- We will provide subjects with written instructions from the tampon manufacturer on tampon use and ways to minimize risk of TSS.

Self-Collected Vaginal Swabs

- As in our pilot study, a double-headed, dry, rayon swab (Starplex Scientific, Inc., S09D) will be used for sample collection (HUM00098494).
- Swab distribution: Subjects will receive their vaginal swabs for the duration of the study (assumed duration of 15 weeks) during their enrollment meeting. If additional swabs are needed, subjects will pick them up from the first floor of MSRB1.
- Swab collection: Subjects will drop off their self-collected vaginal swabs on the first floor of MSRB1 during designated time windows each week; swabs will be stored in -80°C until analysis.
- Swabbing procedure: All swabs will include the following written instructions for self-collecting the vaginal swab (HUM00098494, HUM00086661):
    1. Read these instructions before starting.
    2. Locate a sterile swab labeled with your study ID number
    3. Write today's date (the day you used the swab) in the space provided on the swab label. Complete the short survey included with the swab. It is OK to collect a sample if you are menstruating, but if you are currently using a tampon, remove it before using the swab.
    4. Wash your hands.
    5. Remove the sterile swabs from the tube. Do not let them touch your body or any surface.
    6. Gently part your labia and insert both swabs approximately 2 inches into your vagina, imagining that you are pointing it towards your lower back.
    7. Gently turn the swabs 4 times, rotating the swab about 90 degrees with each turn.
    8. Remove the swabs taking care to not touch anywhere on your body.
    9. Place both swabs back into the tube.
    10. Place the tube into the plastic bag. Wash your hands.
    11. Return the swab to the first floor of MSRB1 during one of the designated time windows this week.
- Covariate data collection with each swab: We will provide a short survey with each swab on which subjects will record whether the swab was collected during menses as well as any changes in sexual activity, hygiene, medication use, general health, vaginal health, contraception use, pregnancy, menses since the baseline study or prior swab.
- We will remind subjects to collect swabs with twice-weekly text messages, emails, or phone calls based on preference.

Whole Community Analysis & Statistics

- DNA will be isolated from vaginal swab samples using a PowerMag Soil DNA isolation kit (Mo Bio Laboratories, Inc.) and an epMotion robot (Eppendorf epMotion 5075). The DNA samples will submitted to the University of Michigan

Center for Microbial Systems Laboratory for amplification and sequencing of the V4 region of the 16S rRNA gene. The V4 region of the bacterial 16S rRNA gene will be amplified using dual-indexing sequencing strategy and sequenced on the Illumina MiSeq platform as described by Kozich et al. and Seekatz et al. After sequencing is completed, the 16S rRNA gene sequences will be processed and analyzed using the mothur software (version 1.34.3) and MiSeq SOP (http://www.mothur.org/wiki/MiSeq_SOP). Following sequence processing and alignment to the SILVA reference alignment [Preusse 2007], sequences will be binned into operational taxonomic units (OTUs) of 97% sequence identity. In our preliminary studies, we were able to detect and distinguish 6 different types of *Lactobacillus* among the top OTUs (>2% in at least 1 sample) from vaginal swab samples using an OTU definition of 3% sequence difference with sequences from the V4 region of the 16S rRNA gene. Relative abundances of OTUs will be calculated and we will test the dependence of within subject θ_{YC} distances on expanded tampon shape using Mann-Whitney tests or Kruskal-Wallis test, as dictated by the number of comparisons. Principal components analysis (PCoA) will be used to visualize θ_{YC} distances between samples within subjects. The same analysis protocol was used in our previous studies (HUM00098494, HUM00086661).

- Intent to treat analysis: This analytic strategy assumes each subject's actual exposure is exactly as it was assigned (complete compliance with assigned interventions, exposure fixed for each subject during each treatment window, complete follow-up). The implications of this analytic approach in conjunction with the within-subject exchangeability produced by the case-crossover design (covered in Method – Study Design section) is that the actual outcome observed under one treatment for a subject equals the counterfactual outcome under that treatment for that subject, so association between expanded tampon shape and VMB composition represents a causal relationship. This study design and analytic strategy will allow us to make causal inference in reference to the effect of expanded tampon shape on VMB composition.

Benefits

- This study will help to provide information on whether the use of tampons is associated with changes in the VMB over the course of a menstrual cycle. If there are associated changes in the VMB associated with tampon use then our research could help provide information on a potentially modifiable time point to help improve the vaginal health of women.

Risks

- No more than minimal risk
    - Discomfort with tampon use - rare - we are recruiting women that are currently tampon users.
    - Toxic shock syndrome (TSS) - rare- the rates of TSS with currently available tampons are extremely low. Each women will be given manufacturers instructions which include ways to decrease the risk of TSS.

    - Discomfort with vaginal swab collection - rare- the Starplex swabs have been used in numerous trials by our lab without any problems given that the swab is considerably smaller than the size of the tampon introducer. Additionally, detailed instructions on sample collection are provided.
    - Disclosure of subject information - rare- each subject will be provided a unique ID for the data collected (samples and surveys). All collected information will be maintained in a password protected lab file manager.
- All recruited women in this trial will be active tampon users and thus we do not foresee any likelihood of these risks interfering with the subjects day to day living.

Incentives

- Each subject will receive a $20 gift card for participating in this study.
- Each subject will receive: 24 Tampax® Pearl® Regular Tampons, valued at $5.04; 24 Playtex® Gentle Glide® 360°® Regular Tampons, valued at $7.68; and 24 Always® Infinity® Regular Pads with wings, valued at $7.44.

Literature Cited

1. Hickey, R. J., et al. (2012). "Understanding vaginal microbiome complexity from an ecological perspective." Translational Research **160**(4): 267-282.
2. Onderdonk, A. B., et al. (1986). "Methods for quantitative and qualitative evaluation of vaginal microflora during menstruation." Applied Environmental Microbiology **51**: 333-339.
3. Onderdonk, A. B., et al. (1987). "Quantitative assessment of vaginal microflora during use of tampons of various compositions." Applied Environmental Microbiology **53**(2774-2778).
4. Chow, A. W. and K. H. Bartlett (1989). "Sequential assessment of vaginal microflora in healthy women randomly assigned to tampon or napkin use." Review of Infectious Diseases **11**.
5. Shehin, S. E., et al. (2003). "Clinical safety-in-use study of a new tampon design." Infectious Disease in Obstetrics and Gynecology **11**: 89-99.
6. Chase, D. J., et al. (2007). "A prospective, randomized, double-blind study of vaginal microflora and epithelium in women using a tampon with an apertured film cover compared with those in women using a commercial tampon with a cover of nonwoven fleece." Journal of Clinical Microbiology **45**: 1219-1224.
7. Chase, D. J., et al. (2010). "Randomized, double-blind crossover study of vaginal microflora and epithelium in women using a tampon with a "winged" apertured film cover and a commercial tampon with a nonwoven fleece cover." Journal of Clinical Microbiology **48**: 1317-1322.
8. Hochwalt, A. E., et al. (2010). "Clinical Safety Assessment of an Ultra Absorbency Menstrual Tampon." Journal of Women's Health **19**(2).
9. Hickey, R. J., et al. (2013). "Effects of tampons and menses on the composition and diversity of vaginal microbial communities over time." BJOG: An International Journal of Obstetrics & Gynecology **120**(6): 695-706.
10. Borovkova, N., et al. (2011). "Influence of sexual intercourse on genital tract microbiota in infertile couples." Anaerobe **17**(6): 414-418.
11. Lopes dos Santos Santiago, G., et al. (2011). "Longitudinal Study of the Dynamics of Vaginal Microflora during Two Consecutive Menstrual Cycles." PLoS One **6**(11): e28180.
12. Borges, S., et al. (2014). "The role of lactobacilli and probiotics in maintaining vaginal health." Archives of Gynecology and Obstetrics **289**(3): 479-489.
13. Cook, R. L. and J. D. Sobel (1990). "Emerging role of lactobacilli in the control and maintenance of the vaginal bacterial microflora." Review of Infectious Diseases **12**(5): 856-872.
14. Boskey, E., et al. (1999). "Acid production by vaginal flora in vitro is consistent with the rate and extent of vaginal acidification." Infection and Immunity **67**(10): 5170-5175.
15. Aroutcheva, A., et al. (2001). "Defense factors of vaginal lactobacilli." American Journal of Obstetrics and Gynecology **185**(2): 375-379.
16. Valore, E. V., et al. (2002). "Antimicrobial components of vaginal fluid." American Journal of Obstetrics and Gynecology **187**(3): 561-568.

17. Atassi, F., et al. (2006). "Lactobacillus strains isolated from the vaginal microbiota of healthy women inhibit Prevotella bivia and Gardnerella vaginalis in coculture and cell culture." FEMS Immunology & Medical Microbiology **48**(3): 424-432.
18. Kaewsrichan, J., et al. (2006). "Selection and identification of anaerobic lactobacilli producing inhibitory compounds against vaginal pathogens." FEMS Immunology & Medical Microbiology **48**(1): 75-83.
19. O'Hanlon, D. E., et al. (2010). "Cervicovaginal fluid and semen block the microbicidal activity of hydrogen peroxide produced by vaginal lactobacilli." BMC infectious diseases **10**(1): 1.
20. Gajer, P., et al. (2012). "Temporal Dynamics of the Human Vaginal Microbiota." Science Translational Medicine **4**(132): 132ra152-132ra152.
21. Martin, D. H. (2012). "The Microbiota of the Vagina and Its Influence on Women's Health and Disease." The American journal of the medical sciences **343**(1): 2-9.
22. Spiegel, C. A., et al. (1980). "Anaerobic bacteria in nonspecific vaginitis." New England Journal of Medicine **303**(11): 601-607.
23. Amsel, R., et al. (1983). "Nonspecific vaginitis: diagnostic criteria and microbial and epidemiologic associations." The American journal of medicine **74**(1): 14-22.
24. Spiegel, C. A., et al. (1983). "Curved anaerobic bacteria in bacterial (nonspecific) vaginosis and their response to antimicrobial therapy." Journal of Infectious Diseases **148**(5): 817-822.
25. Spiegel, C., et al. (1983). "Diagnosis of bacterial vaginosis by direct gram stain of vaginal fluid." Journal of Clinical Microbiology **18**(1): 170-177.
26. Hill, G. B. (1993). "The microbiology of bacterial vaginosis." American Journal of Obstetrics and Gynecology **169**(2): 450-454.
27. Falagas, M. E., et al. (2008). "Probiotics for the Treatment of Women With Bacterial Vaginosis." Journal of Lower Genital Tract Disease **12**(1): 65.
28. Cribby, S., et al. (2008). "Vaginal Microbiota and the Use of Probiotics." Interdisciplinary Perspectives on Infectious Diseases **2008**: 9.
29. Sobel, J. D. (2005). "What's new in bacterial vaginosis and trichomoniasis?" Infectious Disease Clinics of North America **19**(2): 387-406.
30. Koumans, E. H., et al. (2007). "The prevalence of bacterial vaginosis in the United States, 2001–2004; associations with symptoms, sexual behaviors, and reproductive health." Sexually transmitted diseases **34**(11): 864-869.
31. Sweet, R. L. (1995). "Role of bacterial vaginosis in pelvic inflammatory disease." Clinical Infectious Diseases **20**(Supplement 2): S271-S275.
32. Haggerty, C. L., et al. (2004). "Bacterial vaginosis and anaerobic bacteria are associated with endometritis." Clinical Infectious Diseases **39**(7): 990-995.
33. Wiesenfeld, H. C., et al. (2002). "Lower genital tract infection and endometritis: insight into subclinical pelvic inflammatory disease." Obstetrics & Gynecology **100**(3): 456-463.
34. Hillier, S. (1998). "The vaginal microbial ecosystem and resistance to HIV." AIDS research and human retroviruses **14**: S. 17-S. 21.
35. Taha, T. E., et al. (1998). "Bacterial vaginosis and disturbances of vaginal flora: association with increased acquisition of HIV." Aids **12**(13): 1699-1706.
36. Martin, H. L., et al. (1999). "Vaginal lactobacilli, microbial flora, and risk of human immunodeficiency virus type 1 and sexually transmitted disease acquisition." Journal of Infectious Diseases **180**(6): 1863-1868.

37. Schmid, G., et al. (2000). "Bacterial vaginosis and HIV infection." Sexually Transmitted Infections **76**(1): 3-4.
38. Wiesenfeld, H. C., et al. (2003). "Bacterial vaginosis is a strong predictor of Neisseria gonorrhoeae and Chlamydia trachomatis infection." Clinical Infectious Diseases **36**(5): 663-668.
39. Sautter, R. L. and W. J. Brown (1980). "Sequential vaginal cultures from normal young women." Journal of Clinical Microbiology **11**(5): 479-484.
40. Wilks, M. and S. Tabaqchali (1987). "Quantitative bacteriology of the vaginal flora during the menstrual cycle." Journal of Medical Microbiology **24**(3): 241-245.
41. Bartlett, J. G., et al. (1977). "Quantitative Bacteriology of the Vaginal Flora." Journal of Infectious Diseases **136**(2): 271-277.
42. Priestley, C. J., et al. (1997). "What is normal vaginal flora?" Genitourinary Medicine **73**(1): 23-28.
43. Eschenbach, D. A., et al. (2000). "Influence of the Normal Menstrual Cycle on Vaginal Tissue, Discharge, and Microflora." Clinical Infectious Diseases **30**(6): 901-907.
44. Srinivasan, S., et al. (2010). "Temporal Variability of Human Vaginal Bacteria and Relationship with Bacterial Vaginosis." PLoS One **5**(4): e10197.
45. Chaban, B., et al. (2014). "Characterization of the vaginal microbiota of healthy Canadian women through the menstrual cycle." Microbiome **2**(1): 1-12.
46. Hill, J. A. and D. J. Anderson (1992). "Human vaginal leukocytes and the effects of vaginal fluid onlymphocyte and macrophage defense functions." American Journal of Obstetrics and Gynecology **166**(2): 720-726.
47. Hill, D. R., et al. (2010). "Intravaginal and in vitro temperature changes with tampons of differing composition and absorbency." Journal of Biomedical Materials Research Part B: Applied Biomaterials **92B**(2): 535-541.
48. Hill, D. R., et al. (2005). "In vivo assessment of human vaginal oxygen and carbon dioxide levels during and post menses." Journal of Applied Physiology **99**(4): 1582-1591.
49. Preti, G. and G. R. Huggins (1975). "Cyclical changes in volatile acidic metabolites of human vaginal secretions and their relation to ovulation." Journal of Chemical Ecology **1**(3): 361-376.
50. Omar, H. A., et al. (1998). "Tampon use in young women." Journal of pediatric and adolescent gynecology **11**(3): 143-146.
51. Experian (2016). 2016 Simmons Profile Report, Statista; US Census Bureau.
52. Kozich, J. J., et al. (2013). "Development of a Dual-Index Sequencing Strategy and Curation Pipeline for Analyzing Amplicon Sequence Data on the MiSeq Illumina Sequencing Platform." Applied and Environmental Microbiology **79**(17): 5112-5120.
53. Seekatz, A. M., et al. (2015). "Fecal Microbiota Transplantation Eliminates Clostridium difficile in a Murine Model of Relapsing Disease." Infection and Immunity **83**(10): 3838-3846.
54. Pruesse, E., et al. (2007). "SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB." Nucleic Acids Research **35**(21): 7188-7196.
55. Cole, J. R., et al. (2014). "Ribosomal Database Project: data and tools for high throughput rRNA analysis." Nucleic Acids Research **42**(Database issue): D633-D642.

56. Wang, Q., et al. (2007). "Naïve Bayesian Classifier for Rapid Assignment of rRNA Sequences into the New Bacterial Taxonomy." Applied and Environmental Microbiology **73**(16): 5261-5267.