Curr Obstet Gynecol Rep (2016) 5:20–29
DOI 10.1007/s13669-016-0142-6

Hormonal Contraceptive Effects on the Vaginal Milieu: Microbiota and Immunity

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Published online: 29 January 2016

Abstract Hormonal contraceptives may influence the immunological and microbiological milieu of the vagina and alter the risk of acquisition of sexually transmitted infections (STIs) and human immunodeficiency virus (HIV). Most studies demonstrate more normal vaginal flora and less bacterial vaginosis in hormonal contraceptive users compared to non-users, suggesting that contraceptive-induced alteration in vaginal microbiota is an unlikely mechanism for increased risk of STI/HIV acquisition. Measured impacts of hormonal contraceptive use on the presence and activity of vaginal immune cells and vaginal cytokine secretion varies depending on the experimental model, progestogen used, contraceptive delivery method, and length of use of the method, limiting cohesive conclusions. Further study is needed to evaluate the effects of specific progestogens, delivery methods, and long-term use of contraceptives, particularly intrauterine devices and implants, on innate and adaptive immune cells and function in order to ultimately understand impacts on susceptibility to sexually transmitted infections including HIV.

This article is part of the Topical Collection on *Family Planning*

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Keywords Hormonal contraception · HIV risk · Progestins · Vaginal microflora · Vaginal microbiota · Genital tract immune cells

Introduction

Endogenous, as well as exogenous progestogens and estrogens in the form of contraceptives, may influence the immunological and microbiological milieu of the vagina in a way that alters the risk of acquisition of sexually transmitted infections (STIs) [1–3]. While it has been known for at least 30 years that female reproductive hormones can alter immune function [4], more recently collective evidence from observational studies suggests that some progestin-containing contraceptives, particularly depot medroxyprogesterone acetate (DMPA), may increase acquisition risk of HIV [5–8]. A recent randomized controlled trial of South African women using injectable contraceptives (DMPA or norethisterone enanthate (NET-EN)) demonstrated significantly increased risk of HIV acquisition among women using DMPA compared to those using NET-EN (hazard ratio 1.53, 95 % CI 1.12–2.08; $p = 0.007$) [9•]. Are there differential impacts of these two injectable progestins that are responsible for differential HIV acquisition risk in similar women? In response to these concerns and questions, research interest focused on understanding various impacts of hormonal contraceptive use on vaginal and systemic immune function has intensified.

Hypotheses for biologic mechanisms that plausibly link increased risk of acquiring HIV/STIs and hormonal contraceptive use include changes in innate or adaptive immune function, alterations in protective or pathologic vaginal flora, changes in the mucosal barrier of the vagina, direct hormonal effects on STI pathogens, changes in number or density of HIV target cells present in the genital tract, and alterations in

function, presence, and/or expression of HIV co-receptors on immune cells in the genital tract. One long-standing observation appears consistent with the hypothesis that alterations in local and/or systemic immune populations may influence HIV acquisition risk: coexisting STIs, particularly ulcerative STIs, have been well documented to increase HIV acquisition risk [5, 10, 11]. Unfortunately, efforts to reduce risk by treating STIs have largely failed to reduce HIV incidence rates. Our goal is to review recent publications and current research gaps relating to hormonal contraception and STI/HIV risk, with a particular focus on the microbiologic and immunologic environment of the lower female genital tract. A deeper understanding of this topic is critically important to inform contraceptive recommendations for all women, especially those living in high HIV prevalence areas, and to guide future contraceptive and microbicide research and development.

What Effects Do Contraceptives Have on Vaginal Flora?

Mucosal surfaces of the gastrointestinal and reproductive systems support their own ecosystems of microorganisms. These "microbiomes" have been shown to affect both host health and disease susceptibility. Vaginal microorganisms have increasingly been implicated in adverse reproductive tract outcomes, including susceptibility to and transmission of STIs, risk of preterm birth [12], and implantation failure with assisted reproduction [13]. Women with vaginal flora dominated by *Lactobacillus* species, particularly *Lactobacillus crispatus*, have been shown to have decreased incidence of HIV, human papillomavirus (HPV), and trichomonas infection [14] as compared to women with bacterial vaginosis (BV), a polymicrobial anaerobic vaginal flora typically with low prevalence of *Lactobacilli* and elevated vaginal pH [15]. Historically, symptomatic BV has been primarily attributed to *Gardnerella vaginalis*, but molecular technologies, such as 16s gene microarray, have identified a host of other bacteria associated with dysbiotic, or imbalanced, vaginal microbial states including *Atopobium vaginae, Lactotrichia amnionii, Megasphaera*, and newly described members of the *Clostridiales* sometimes referred to as BV-associated bacteria (BVAB) [15–17].

Lactic acid is produced by *Lactobacilli* through glycogen metabolism [18] and has been shown to protect against pathogenic vaginal organisms by acidification of the vagina [16, 19]. High estrogenic states, including pregnancy, are associated with increased presence of vaginal glycogen and thus increased lactic acid production [14], while low estrogenic states such as menopause are associated with lower vaginal glycogen levels and decreased *Lactobacilli* [20]. While some studies suggest that vaginal flora in an individual are stable over time [21], most published data suggest that shifts between flora considered normal and flora considered intermediate or abnormal occur frequently, even from day to day [22, 23]. Given the frequent shifts in endogenous hormones and flora throughout the menstrual cycle, contraceptive hormone use would likely need to cause dramatic shifts in vaginal microbiome to impact STI/HIV acquisition risk.

Most studies demonstrate decreased prevalence of BV as well as more successful remission of BV after treatment in women using contraceptive hormones compared to those not using hormonal contraceptives [6, 24–27]. Women using DMPA have decreased BV incidence when compared to women using no contraception [6, 28]. Mitchell, et al., further showed that women using DMPA had a reduction in colonization with *Lactobacilli* compared to their baseline without any difference in incidence of BV over 2 years [29]. Although the IUD in general has not been found to increase risk of BV when compared to no contraception [30], it may increase risk in individuals with irregular bleeding [31] or appear to increase risk when compared to oral contraceptives and condoms, which are known to be protective [25]. The aforementioned studies do not specify or separate hormonal versus non-hormonal IUDs. A recent study focusing solely on levonorgestrel (LNG) IUD users showed no significant change in vaginal flora within 12 weeks of LNG-IUD placement [32]. Understanding long-term impacts of hormonal IUD and contraceptive implant use on vaginal flora is a key research gap.

The impact of monthly contraceptive vaginal rings, such as NuvaRing®, on vaginal microbiota is of particular interest given the interest in developing both vaginal microbicide and dual-use (microbicide and contraceptive) vaginal rings. Studies investigating monthly contraceptive vaginal rings on vaginal microbiota suggest an increase in lactobacilli colonization over time without a change in BV incidence [33, 34]. Longer-duration (90-day and 365-day) vaginal rings are also in development, and there is keen interest in understanding if long-term use of rings will have any significant impact on the vaginal microbiome, particularly since a study of vaginal rings done in non-human primates show that biofilms develop on rings during long-term use [35]. Huang, et al., showed that use of an ethinyl estradiol/Nestorone® contraceptive ring designed for use for 1 year had no significant effect on either *Lactobacillus* colonization or BV incidence over 1 year. There was a significant increase in low levels of anaerobic Gram-negative rods in participants using these contraceptive rings for 1 year; however, the authors concluded that was unlikely to be clinically significant [36].

Several recent meta-analyses that have addressed the influence of hormonal contraceptive use on BV have similarly noted protection from BV with use of hormonal contraceptives [37•, 38•]. In one meta-analysis, the three studies determined to have the highest quality demonstrated 10–20 % reduction in BV in combined oral contraceptive pill (COC) users and 18–30 % reduction in DMPA

users compared to non-hormonal contraceptive users, and all of the studies included in this meta-analysis showed either statistically significant decrease or no difference in BV prevalence in hormonal contraceptive users when compared to non-hormonal contraceptive users [37•]. Similarly, a meta-analysis including 55 studies showed approximately 25 % reduction in both incident and prevalent BV in hormonal contraceptive users compared with non-users, irrespective of whether the method of hormonal contraception was progestin-only or combined estrogen-progestin [38•]. In summary, the available body of evidence strongly suggests that use of hormonal contraception either does not alter or *decreases* BV incidence. Furthermore, since having BV has been associated with increased risk of HIV acquisition, it is therefore unlikely that alterations in vaginal microbiota affected by hormonal contraceptive use are responsible for changes in susceptibility to STIs/HIV.

What Effects Do Contraceptive Hormones Have on the Vaginal Mucosal Immune Environment?

Sex steroid hormones influence immune function [39, 40], theoretically to facilitate reproduction, including fertilization and subsequent fetal development, without immune interference [41]. Hypotheses such as the "window of vulnerability" [42] propose that hormones suppress normally protective immune activities during mid-cycle through the luteal phase of menses to allow fertilization and to prevent immune destruction of a new blastocyst. Immune function is dynamic throughout the menstrual cycle, with changes demonstrated in toll-like receptors (TLRs), inflammatory transcription factors such as NF-κB, cytokines, and secretion of antimicrobial proteins [1, 42]. Immune changes that increase immune tolerance may also increase vulnerability to STIs. For HIV transmission in particular, this relationship is likely complex, since elements of the immune system may either protect or enhance susceptibility to infection, given that local immune cells are both immune sentinels and targets for HIV infection. Biologically plausible mechanisms that could explain reproductive hormone-dependent increase in HIV susceptibility include alterations in the integrity or structure of the vaginal epithelium, changes in innate immune components in cervicovaginal fluid, increased density of target cells, increased expression of viral co-receptors CCR5 and/or CXCR4 on immune target cells, increased activation of immune cells, changes in secreted cytokines and chemokines, and changes in a myriad of secreted proteins with innate anti-viral activity.

DMPA is often used in laboratory animal models to render animals more susceptible to human STI pathogens including *Chlamydia trachomatis* [43], herpes simplex virus (HSV) [44], and HIV [45]. Significant thinning of the vaginal epithelium was observed in several of these animal models following administration of DMPA [46], which led to hypotheses that structural changes effected by DMPA were responsible for altering HIV susceptibility. In distinct contrast to animal models, mucosal thinning has not been observed in multiple human studies of both short-term and long-term DMPA use [47–51]. While other alterations in the integrity of the vaginal mucosal barrier could theoretically increase risk, no differences in epithelial tight junctions and adherin proteins have been demonstrated following DMPA injection [47].

Properties or components of cervicovaginal fluid (CVF) may provide inherent protection from vaginal infection with HIV [52, 53]. Recent work examining CVF in pre- and post-menopausal women showed that both postmenopausal women and premenopausal women using DMPA had significantly less viscous CVF than normally cycling premenopausal women in either phase of menses and than women using either LNG-IUDs or COCs. Increased CVF viscosity appears to inhibit movement of HIV virions in vitro, which suggests that increased viscosity may confer some protection from infection [54, 55]. Women using DMPA also had decreased CVF glycosylation by GlcNAc and terminal galactose [53], carbohydrate structures that, as components of glycoproteins, have been linked to antibody-dependent cellular cytotoxicity [56]. In this study, no changes were observed in a variety of other carbohydrate binding proteins examined by high-throughput lectin microarrays. Observed changes in viscosity and glycosylation patterns in these studies were subtle and were confounded by the presence of BV [52, 57]. Innate antiviral activity of CVF against HIV, HSV-1 and HSV-2 was not affected by use of COCs, LNG-IUD, or DMPA, but was significantly lower in postmenopausal women [58]. A myriad of other CVF-associated molecules have been evaluated, particularly in women with BV compared with those with normal flora, in search of a biomarker that may help link the higher HIV/STI acquisition risk with BV [59, 60].

One of the largest efforts to evaluate the impact of contraceptive methods on cytokines and innate immune protective molecules in CVF was a nested case control study comparing vaginal biomarkers of 199 users of DMPA, COCs, or no hormonal contraceptives who acquired HIV during a longitudinal study to 633 controls who remained HIV negative [61•]. Higher RANTES levels and lower secretory leukocyte protease inhibitor (SLPI) levels in cervical secretions were associated with higher risk of HIV seroconversion. RANTES, an acronym for regulated on activation, normal T cell expressed and secreted, is a secreted peptide released by T cells that may

competitively block the entry of HIV to a target cell by binding the HIV co-receptor and also attracts HIV target cells to mucosal surfaces [62], and SLPI is a protein with inherent anti-HIV activity that is associated with human mucosal surfaces [63]. RANTES has been significantly increased in DMPA users in several in vivo studies [61•, 64]. Conversely, RANTES mRNA was decreased in an in vitro model using medroxyprogesterone acetate (MPA) [65], highlighting that there can be inconsistencies between in vitro and in vivo models. Other studies evaluating vaginal cytokine and secreted antimicrobial peptides, including SLPI, in DMPA users compared to non-DMPA users have shown mixed results, likely due to both the non-specific nature of these markers of inflammation and the comparison group encompassing 'non-DMPA users' who may have been using a variety of other progestins for contraception [64, 66, 67].

In vitro and in vivo studies addressing hormone-dependent changes in number and co-receptor expression of target cells as well as secreted antimicrobial molecules published over the past 2 years are summarized in Table 1. Notably, studies are variable in contraceptive methods evaluated, outcome measures, and study methodology. The most relevant study methodologies are likely those that evaluate cells that are freshly harvested from the female genital tract with minimal manipulation that may itself impact the viability and/or surface protein receptor expression patterns. Due to the specific concern for DMPA use and increased susceptibility to HIV, DMPA has been the most commonly studied contraceptive to date. Some studies have examined other hormonal contraceptives, including NET-EN, COCs, and LNG-IUD. One recent study showed an increased density of target cells and HIV co-receptors 12 weeks after DMPA injection [47], while another found decreased density of T cells but stable proportion of T cells and dendritic cells that are CCR5+ after 12 months of DMPA use [29], suggesting that the short-term effects may differ from long-term effects. Both LNG and copper IUDs were associated with decreased CCR5 co-receptor expression in endocervical CD4+ T cells and endometrial CD8+ T cells, indicating that hormonally independent mechanisms may also have impacts on upper genital tract immune cells in the case of IUDs [69].

Contraceptive hormones may alter the function of circulating systemic immune cells. Studies tend to show an association between hormonal contraceptives and decreased secretion of pro-inflammatory cytokines, including the antiviral IFN-α, TNF-α, and IFN-γ, from peripheral blood mononuclear cells (PBMCs) [66, 68, 72]. PBMCs incubated in vitro with MPA but not estradiol (E2) or progesterone (P4) showed increased CXCR4 and CCR5 co-receptor expression [72]. The relevance of data on circulating immune cells to the immune activity of the genital tract is unclear but certainly important for control of and susceptibility to systemic infection.

Do the Various Contraceptive Progestins Affect the Immune System and Vaginal Flora Differently?

Fortunately, a great diversity of contraceptive hormones and delivery methods exist, providing women a variety of methods from which to choose. Although much is known about the impact of these contraceptives on fertility, little is known about other non-contraceptive impacts, including impacts on genital tract immune cells and function and on vaginal microbiota. Most published studies that have compared the effects of contraceptives on the genital tract have compared various contraceptive methods that differ significantly with respect to both delivery method and hormone content (e.g., contraceptive injections containing medroxyprogesterone vs. implant containing etonogestrel or levonorgestrel), making interpretation challenging. There are currently no published studies comparing the vaginal microbiological effects of progestins contained in different oral contraceptives, so it is unknown if different formulations of COCs cause differential effects. Further, there are no published studies that examine vaginal microbiota differences when the same hormone is delivered through different delivery systems (e.g., levonorgestrel contained in oral contraceptives vs. subcutaneous implants vs. IUDs).

MPA may behave quite differently from the other contraceptive progestins with respect to non-contraceptive effects, and this may be due to the binding properties of MPA to the immunosuppressive glucocorticoid receptor [65, 73]. In cervical cell lines, IL-6, IL-8, and the anti-HIV RANTES molecule are constitutively expressed, and this expression is suppressed by MPA binding to the glucocorticoid receptor, unlike NET or endogenous progesterone (P4), neither of which suppress RANTES. This suppression is likely mediated by recruitment of the glucocorticoid receptor to the IL-6 and IL-8 gene promoter region, similar to the effect of dexamethasone [65]. Additionally, MPA but not NET or P4 enhance peripheral CD4+ T cell apoptosis, likely also via the glucocorticoid receptor [73]. In summary, in vitro and in vivo studies have demonstrated some differences in effect of MPA compared to other contraceptive hormones and have proposed a mechanism for this difference; however, very little is known about non-contraceptive effects of other specific exogenous hormones on the lower genital tract. Future research is needed that specifically categorizes evaluated contraceptives by progestin type and by local concentration of progestins to evaluate alterations in immune cells, cell surface receptors, and secreted molecules.

Table 1 Recently published studies of contraceptives and genital tract immunity

	Publication	Contraceptive method (s)	Methodology and follow-up	Population	Results	Basic summary of effect
In vitro	Govender 2014 [65]	MPA, NET-EN	HeLa and HPV-immortalized endocervical cell lines incubated with MPA, NET-EN, E2, P4		Cells incubated with MPA but not NET-EN had decreased secretion of IL-6, IL-8, and expression of RANTES mRNA vs. E2, P4. MPA recruits glucocorticoid receptor to IL-6 and IL-8 promoter region and represses transcription of these genes.	MPA decreased secretion of pro-inflammatory cytokines and peptide with antimicrobial effects, in part by repressing transcription of pro-inflammatory genes via the glucocorticoid receptor. Strength: demonstrates biologically plausible explanation for DMPA modulation of genital tract immunity Limitation: in vitro
Ex vivo	Huijbregts 2013 [68]	MPA	VMMCs isolated from remnant vaginal tissue from pelvic reconstructive surgery incubated with MPA, E2, P4		MPA inhibits release of IFN-γ, IL-2, IL-5, GM-CSF, IL-13 from VMMCs vs. E2, P4. No significant difference in many other cytokines examined.	MPA decreased secretion of some pro-inflammatory cytokines. Strength: lymphocytes isolated from genital tract tissue Limitation: use of surgical tissue with heterogeneous pathology, relevance of vaginal tissue of postmenopausal women Challenge: No significant effect of DMPA on most cytokines examined
In vivo	Achilles 2014 [69]	LNG-IUD, Cu-IUD	Endocervical and endometrial biopsy at baseline and 2 months after IUD placement	34 healthy women randomized to LNG-IUD or Cu-IUD and 8 non-contraceptive users in United States	Decreased CCR5 expression on endometrial CD4+ and CD8+ T-cells of LNG-IUD users and on endometrial CD8+ T-cells on Cu-IUD users compared to baseline Decreased CCR5 expression in endocervical CD4+ T-cells of LNG- and Cu-IUD users compared to baseline	Both LNG- and Cu-IUD are associated with decreased HIV co-receptor expression in upper genital tract T-cell populations. Strengths: fresh non-manipulated tissue, healthy women, comparison to baseline Limitation: short follow-up
	Chandra 2013 [47]	DMPA	Vaginal biopsies at baseline and 12 weeks after DMPA injection	20 healthy women in United States	Increased density of CD8+ T-cells and increased expression of CCR5 on T-cells after DMPA compared to baseline	DMPA is associated with increased density of some target cells and increased HIV co-receptor expression in T-cells. Strengths: fresh, non-manipulated tissue, healthy women, comparison to baseline Limitation: follow-up sampling taken at time when DMPA is due for re-dosing and thus taken at relative trough concentration
	Coleman 2013 [70]	LNG-IUD	Cervicovaginal fluid at baseline and 6 months after IUD placement	25 HIV+ women in Kenya	Increased IL-1β after 6 months compared to baseline. No increase in IL-8, SLPI, or HIV RNA shedding	LNG-IUD use associated with increased levels of one pro-inflammatory cytokine but not another. No change in the measured antimicrobial peptide. Strength: comparison to baseline in the same individual, examines effects HIV+ participants Limitation: HIV+ participants relevance to general population, single follow-up point Challenge: mixed results

Table 1 (continued)

Publication	Contraceptive method (s)	Methodology and follow-up	Population	Results	Basic summary of effect
Deese 2015 [64]	DMPA, NET-EN	Cross-sectional study, cervicovaginal fluid	376 healthy women in South Africa and Kenya	Increased MIP-1α, MIP-1β, IL-6, IL-8, IL-10, RANTES in DMPA users vs. no HC. Increased IL-6, IL-8, RANTES in NET-EN users vs. no HC.	DMPA and NET-EN use increased measured pro-inflammatory cytokines and an HIV co-receptor blocking molecule compared to no HC Strengths: large numbers, healthy African women Limitation: cross-sectional Challenge: mixed results that suggest mechanisms for both increased and decreased HIV acquisition risk
Guthrie 2015 [67]	DMPA, COC, LNG implant	Cross-sectional study, cervicovaginal fluid	228 healthy women in Kenya	Increased HNP1-3 and lactoferrin in DMPA users vs. no HC. No change in SLPI, LL-37, or HBD-2. No change seen in COC or implant users.	DMPA use associated with increased levels of some peptides that have both inherent antiviral properties and ability to increase HIV target cells and HIV co-receptors. Strengths: large numbers, healthy African women Limitation: cross-sectional Challenge: mixed results
Michel 2015 [66]	COC, DMPA, etonogestrel/ EE ring	Cross-sectional study, vaginal biopsy, and cervicovaginal fluid	84 healthy women in United States	Decreased density of Langerhans cells in vaginal biopsy of COC and etonogestrel/ EE ring users compared to HC non-users. In DMPA users compared to HC non-users, Langerhans cells localized closer to apical surface of vaginal biopsies but no change in frequency of target cells. Decreased levels of IFN-α, CXCL10, MCP-1, and GM-CSF in CVF of DMPA but not etonogestrel/EE or COC users.	HC may influence the number and distribution of HIV target cells in vaginal biopsies. DMPA use associated with decreased levels of pro-inflammatory cytokines in CVF. Strengths: fresh, non-manipulated tissue, healthy women Limitation: cross-sectional; heterogeneous HC group Challenge: mixed results
Mitchell 2014 [29]	DMPA	Vaginal biopsies at baseline and every 3 months for 2 years of DMPA use	32* healthy women in the USA	Decreased density of T-cells in follow-up biopsies compared to pre-DMPA. Increased number of CD4 + CCR5+ and HLA-DR + CCR5+ cells but stable proportion of CD4+ and HLA-DR+ cells that are CCR5+ compared to pre-DMPA.	DMPA use associated with increased quantity of target cells expressing HIV co-receptors and decreased density of all T-cells in vaginal biopsy. Strengths: fresh, non-manipulated tissue, healthy women, comparison to baseline, long follow-up Limitation: no comparison group Challenge: mixed results
Morrison 2014 [61•]	COC, DMPA	Nested case-control study of endocervical swabs from the visit immediately prior to HIV seroconversion and matched	199 incident HIV seroconvertors and 633 matched uninfected controls in Uganda and Zimbabwe	COC users had increased IL-1β, IL-6, IL-8, MIP-3α, VEGF and LSP-1 and lower BD-2 levels vs. no HC. DMPA users had higher RANTES and ICAM-1 and lower IL-1RA vs. no HC.	COC use associated with increased level of some measured pro-inflammatory cytokines. DMPA use was not associated with higher levels of different pro-inflammatory molecules. Strengths: large numbers, African women

Table 1 (continued)

Publication	Contraceptive method (s)	Methodology and follow-up	Population	Results	Basic summary of effect
		controls who remained HIV negative			Limitation: cross-sectional Challenge: mixed results
Ngcapu 2015 [71]	DMPA, NET-EN	Case control of 42 cytokines cervicovaginal fluid at a single time point	64 healthy injectable HC users and 64 HC non-users in South Africa	Injectable HC users had lower levels of IL-12p40, MCP-1, MDC compared to HC non-users. No difference in TNF-β, IL-1β, IL-6, IL-8, MIP-1α, MIP-1β, and RANTES.	Neither DMPA nor NET-EN use associated with most of the studied pro-inflammatory cytokines. Strengths: examination of diverse immune modulators Limitations: cross-sectional, did not distinguish between DMPA and NET-EN users

*32 volunteers had 1 year of follow-up and were included in the analysis; 22 volunteers had 2 years of follow-up

(D)MPA (depot) medroxyprogesterone acetate, *NET-EN* norethisterone enthanthate, *E2* 17β-estradiol, *P4* progesterone, *IL* interleukin, *LNG* levonorgestrel, *Cu-IUD* copper intrauterine device, *VMMC* vaginal mucosal mononuclear cells, *IFN* interferon, *GM-CSF* Granulocyte-macrophage colony-stimulating factor, *HNP* human neutrophil peptide, *HBD* human β-defensin, *LL-37* antimicrobial peptide LL-37, *HC* hormonal contraception, *EE* ethinyl estradiol, *MIP* macrophage inflammatory protein, *(C)OC* (combined) oral contraceptives, *MCP* monocyte chemoattractant protein, *CVF* cervicovaginal fluid, *VEGF* vascular endothelial growth factor, *LSP* lymphocyte specific protein, *ICAM* intracellular adhesion molecule, *MMP* matrix metalloproteinase, *TIMP* tissue inhibitor of metalloproteinases, *MDC* macrophage-derived chemokine

Conclusions

There is clear evidence that use of hormonal contraceptives, primarily COCs and DMPA, confers a protective effect against abnormal vaginal flora. Future research is needed to evaluate potential changes to the vaginal flora with use of contraceptive implants and LNG-IUDs, and with long-term use of all contraceptive methods. While studies focusing on the effects of contraceptive use on the vaginal innate and adaptive immune environment continue to yield more information, consistent conclusions cannot yet be made because of lack of contraceptive progestin specificity along with widely varying experimental models, contraceptive delivery methods, and methodologic approaches. No conclusive evidence, either behavioral or biological, yet explains the observed approximately 1.5- to twofold increased risk of HIV acquisition seen in women using DMPA in large observational studies. A large randomized controlled trial (ECHO) in which women will be randomized to use DMPA, LNG contraceptive implants, or copper IUDs for contraception has recently opened to enrollment with the goal of understanding if there is real increased risk of HIV acquisition with DMPA use [74]. If the evidence continues to indicate increased risk of HIV/STI acquisition in for women using DMPA, further research will need to be directed toward elucidating the mechanism (s) for increasing risk, evaluating the comparable safety of alternative highly effective long-acting contraceptive methods, and increasing the acceptability of these alternative options in regions where use is low. Further, efforts should continue toward developing dual-use technologies where HIV and pregnancy prevention can be combined. While addressing family planning needs and enabling protection from sexually transmitted infections are paramount for all women's health, these needs are particularly urgent in areas with high HIV prevalence, many of which also have high rates of unintended pregnancy and, therefore, a great need for safe contraception.

Acknowledgments This study was supported by the US National Institutes of Health/National Institute of Allergy and Infectious Diseases grant support (NIH R01-AI102835)

Compliance with Ethical Standards

Conflict of Interest Jessica Tarleton declares that she has no competing interests.

Lisa Haddad declares personal fees for working as a consultant on a medical advisory panel for Pfizer Pharmaceutical.

Sharon L. Achilles declares personal fees for consultant work for Merck Sharp & Dohme Corp.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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