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REVIEW ARTICLE

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An updated review on the effects of depot medroxyprogesterone acetate on the mucosal biology of the female genital tract

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Abstract

Background: Access to safe, effective, and affordable contraception is important for women's health and essential to mitigate maternal and fetal mortality rates. The progestin-based contraceptive depot medroxyprogesterone acetate (DMPA) is a popular contraceptive choice with a low failure rate and convenient administration schedule.

Aim: In this review, we compiled observational data from human cohorts that examine how DMPA influences the mucosal biology of the female genital tract (FGT) that are essential in maintaining vaginal health, including resident immune cells, pro-inflammatory cytokines, epithelial barrier function, and the vaginal microbiome

Materials and Methods: This review focused on the recent published literature published in 2019 and 2020.

Results: Recent longitudinal studies show that DMPA use associates with an immunosuppressive phenotype, increase in CD4+CCR5+ T cells, and alterations to growth factors. In agreement with previous meta-analyses, DMPA use is associated with minimal effects of the composition of the vaginal microbiome. Cross-sectional studies associate a more pro-inflammatory relationship with DMPA, but these studies are confounded by inherent weaknesses of cross-sectional studies, including differences in study group sizes, behaviors, and other variables that may affect genital inflammation.

Discussion & Conclusion: These recent results indicate that the interactions between DMPA and the vaginal mucosa are complex emphasizing the need for comprehensive longitudinal studies that take into consideration the measurement of multiple biological parameters.

KEYWORDS
depot medroxyprogesterone acetate, DMPA, vaginal epithelium, immune cells, cytokines, chemokines, vaginal microbiome

Hossaena Ayele and Michelle Perner contributed equally.

1 | INTRODUCTION

Family planning methods are essential for women, empowering them in the prevention of unintended pregnancies, pregnancy-related sequelae, and vertical transmission of sexually transmitted infections (STIs) (including human immunodeficiency virus [HIV]).[1] In sub-Saharan Africa, the long-acting reversible contraceptive depot medroxyprogesterone acetate (DMPA) was the preferred method of contraception for roughly half of contraceptive users in 2017.[2] DMPA is a progestin-based injectable with the active component being medroxyprogesterone acetate (MPA). MPA is a synthetic progestin structurally related to the sex hormone progesterone. Each DMPA injection contains a dosage of 150 mg of MPA and is administered every 3 months.[3] The most common form of DMPA is the intramuscular injectable which is highly effective with a very low failure rate when used properly.[4-7] In addition to its effectiveness at preventing unwanted pregnancies, DMPA use comes with a convenient administration schedule and inconspicuousness with its use.

Concerns have been raised regarding a potential relationship between DMPA and STIs (ie, *Chlamydia trachomatis*, *Neisseria gonorrhea*, herpes simplex virus-2, and HIV), bacterial vaginosis, vaginal candidiasis, and cervical ectopy.[8-11] According to a recent review published by Hapgood et al, DMPA is likely to be associated with the following effects: compromised mucosal epithelial barrier, immune suppression of peripheral dendritic cells and T cells, increasing the presence of HIV-1 target cells expressing the CCR5 co-receptor, and inconsistent but significant changes to certain cytokines, chemokines, and other soluble factors.[12] Many of these changes have been speculated to be a contributing factor in observational studies linking DMPA use with increased risk of HIV acquisition. A meta-analysis by Morrison et al in 2015 evaluated risk of HIV infection with use of norethisterone enanthate (NET-EN), combined oral contraceptives (COC), and DMPA. They observed DMPA use to have the highest incidence rate of HIV infection at 5.1/100 woman-years with COC use having the lowest incidence rate at 3.4/100 woman-years.[13] However, the Evidence for Contraceptive Options and HIV Outcomes (ECHO) clinical trial, which randomized women to three different forms of contraceptives (copper intrauterine device (CIUD), levonorgestrel (LNG) implant, and DMPA), found no substantial difference in HIV infection between the three contraceptive arms to support its safety profile for women.[14] However, limitations of the ECHO study included a sensitivity to only detect a 50% increase in HIV incidence between study arms and the absence of oral contraceptives as a study arm comparator. Understanding the off-target biological impacts of DMPA is therefore an important area of study.

Many features of the mucosal environment of the female genital tract (FGT) are important for homeostasis, including its protective mucosal fluid, immune cells, epithelial barrier, and resident microbiome. Advances in flow cytometry, cytokine analysis, microbiome sequencing, and 'omics techniques such as transcriptomics and proteomics have allowed for a better characterization of the mucosal biology of the FGT. The recent review by Hapgood et al provided an in-depth evaluation of the literature on the effects of DMPA and HC on FGT biology. In this review, building upon this previous work we provide a review of the recent published literature in 2019 and 2020 that examine the effects of DMPA on FGT mucosal biology.

2 | HOST AND BACTERIAL COMPONENTS OF THE FEMALE GENITAL TRACT CONTRIBUTE TO OVERALL VAGINAL HEALTH

The FGT can be divided into upper and lower compartments. The upper FGT, which includes the endocervix, uterus, fallopian tubes, and ovaries, consists of a single layer of columnar epithelial cells with tight junctions preventing the movement of pathogens into the sub-epithelia and lamina propria.[15] The lower FGT, which includes the vaginal tract and ectocervix, is lined by multi-layered squamous epithelial cells. The basal layer of this epithelium is linked with tight junctions, while the apical layer is terminally differentiated creating a semi-permeable barrier that allows water, small molecules, and soluble proteins to cross while preventing the entry of pathogens.[16,17] A layer of mucus which coats the surface of the epithelium hinders the movement and penetration of pathogens to the epithelia below.[18] The epithelium contributes to the recognition and defense against invading pathogens (both bacterial and viral) through the expression of pathogen recognition receptors, which recognize pathogen-associated molecular patterns and induce the secretion of cytokines, chemokines, and other soluble factors to engage the innate and adaptive immune system.[17,19]

Immunological components of the FGT include soluble cytokines/chemokines, which aid in the activation, recruitment, and differentiation of immune cells that are either tissue resident or in transit. Cytokines commonly measured in the reviewed studies include inflammatory (interleukin-1α [IL-1α], IL-1β, IL-6, tumour necrosis factor-α [TNFα], IL-1RA, IL-10, IL-12 [p40], IL-18, macrophage migration inhibitory factor (MIF), TNF-β, TNF-related apoptosis-inducing ligand (TRAIL)) and adaptive (IL-2, IL-4, IL-16, interferon gamma (IFNγ), IFN-α2, IL-15, IL-2RA) cytokines. Chemokines commonly measured include macrophage inflammatory proteins-1α (MIP-1α), MIP-1β, monocyte chemoattractant protein-2 (MCP-2), interferon gamma-induced protein 10 (IP-10), stromal cell-derived factor-1β (SDF-1β), monokine induced by gamma interferon (MIG), IL-8, regulated upon activation normal T cell expressed and secreted (RANTES), chemokine cutaneous T-cell-attracting chemokine (CTACK), MCP-3, MIP-3, and MCP-1. The FGT also consists of resident and circulating immune cells such as Langerhans cells, dendritic cells, macrophages, neutrophils, and lymphocytes (CD4+ and CD8+ T cells, natural killer cells, and B cells). For viral infections, the presence of specific receptors on leukocytes within the vaginal compartment facilitates virus penetration and replication. In the case of HIV transmission through sexual contact, viral particles bind to the CD4 receptor and co-receptor CCR5 initiating viral attachment for cell entry.[20] Activation of T cells, categorized by expression of the following markers, HLA-DR, CD25, CD38, and CD69, has been

associated with heightened susceptibility to HIV infection and disease progression, and Th17 cells are among the first cells infected at mucosal layers.[21,22]

Commensal microbes can greatly influence the vaginal environment in ways that are either beneficial or detrimental to the host. In contrast to the other body sites, high bacterial diversity of the commensal vaginal microbiome is not associated with health. Instead, dominance of *Lactobacillus* (*L. crispatus* and *L. jensenii*) is considered optimal due to their production of lactic acid, H_2O_2, and anti-microbial factors (bacteriocins).[23] Dominance of the vaginal microbiome with non-*Lactobacillus* organisms can often be associated with clinical diagnosis of bacterial vaginosis (BV), though this is not always the case. Organisms commonly observed among women with BV include *Gardnerella*, *Prevotella*, *Mobiluncus*, *Atopobium*, and *Sneathia*.[24] In addition, *L. iners*, though a *Lactobacillus* species, has frequently been detected in the microbiome of women diagnosed with BV, often co-existing with BV-associated organisms.[25,26] Dominance of these BV-associated organisms has been shown to associate with vaginal inflammation and risk of STI acquisition.[27,28] Therefore, both host and bacterial components of the vaginal environment function to collectively maintain an environment that is favorable for overall mucosal health.

3 | DMPA USE AND ON THE IMMUNOLOGY OF THE VAGINAL ENVIRONMENT

The majority of studies that assessed how DMPA use may alter cellular immunity in the FGT have focused on immune cell phenotypes or functions related to HIV acquisition. A compilation of the observational data from human cohorts is shown in Tables 1 and 2 detailing cohort location, size, study design, sample type, and sample collection in terms of time since DMPA injection as well as their reported observations. Many of these studies reported changes to levels of cytokine, chemokine, and other soluble factors with DMPA use which are comprehensively reviewed elsewhere.[12] Our primary focus was HIV relevant cellular phenotypes, such as CCR5-expressing CD4+ T cells, which are essential targets for HIV infection, and soluble factors (ie, cytokines and chemokines) that affect inflammation and target cells in the FGT. In the past 2 years, eight papers have been published examining the relationship between DMPA and immunology of the FGT.

From the eight recently published articles, four explored the longitudinal use of DMPA. All of these studies included self-selection of contraceptive method instead of randomization to contraception arm. Achilles et al enrolled women that were STI/HIV negative with regular menstrual cycles seeking contraception counseling in Harare, Zimbabwe, excluding those with recent hormonal contraceptive use.[29] Use of DMPA (*N* = 38) associated with decreased cervical CD4+ T cells (percent and total cell numbers at days 30 and 180 after DMPA initiation, respectively) and CD11c+ antigen-presenting cells after 180 days, but no changes in CD4+ and CD8+ T cells expressing CCR5. In terms of soluble factors, they observed a significant increase in IL-10 at day 30, and a significant decrease in IL-1β, and a trending decrease in IL-8 at days 30 and 180 after DMPA initiation. No significant changes were observed for IFN-γ, IL-6, and RANTES at all visit timepoints. Interestingly, the authors' *in vitro* experiments showed anti-HIV activity of cervicovaginal fluid collected at day 30 to be significantly decreased. Overall, DMPA was associated with decreased HIV target cells, immunosuppressive phenotype, and anti-HIV activity in this study. An important component of this study which adds considerable value to their findings was the measurement of serum MPA concentrations (pg/mL), which peaked at day 30. However, women in the DMPA group reported having significantly increased frequency of sexual intercourse by 180 days post-DMPA initiation compared to copper IUD and implant users[29] which could have significantly impacted these observations.

Tasker et al reported on a longitudinal cohort of 27 women from Newark, New Jersey who were not pregnant, tested negative for HIV, *C. trachomatis*, *N. gonorrhea*, HSV-2, and syphilis, and identified as being black or Hispanic.[30] These women did not have any immunosuppressive conditions, were not using hormonal contraception for the previous 10 months, and self-reported no sexual intercourse three days before the enrollment visit. A strength of this study was their inclusion of three different sample types: cytobrush supernatants, endocervical and vaginal swabs capturing the potential effect of DMPA at different compartments of the FGT. Cervical mononuclear cells (CMCs) were isolated from endocervical cytobrushes collected at the following timepoints: pre-DMPA initiation, one month, and three months post-DMPA injection. The study reported an increase in the frequency of CD45+CD4+CCR5+ and a decrease in CD4+CCR7+CD45RA- (central memory) T cells at three months post-DMPA injection, but it is unclear if any of these results passed multiple comparison correction as this was not addressed. Interestingly, following multiple comparison correction DMPA associated with a decrease in pro-inflammatory cytokines (IL-15, IL-6), chemokines (MIP-1β), and growth factors (G-CSF, GM-CSF, vascular endothelial growth factor (VEGF)) at one month post-injection. Some of these changes were sustained or additionally observed at three months including decreases in IL-6, IL-15, GM-CSF, and IFNα2. Some of these observations were consistent in endocervical and vaginal secretions. Overall, this study suggests an immunosuppressive effect of DMPA while potentially increasing the proportion of CD45+CD4+CCR5+ T cells. However, a limitation of this study is that it did not include serum MPA measurements.

Li et al reported on a study that recruited 59 non-pregnant, HIV-negative American (*N* = 31) and Chinese (*N* = 28) women with regular menstrual cycles seeking contraception counseling who reported no-HC use in the last 60 days and had no signs or symptoms of an active genital tract infection.[31] Fifteen women on DMPA were included in this analysis. Endocervical cytobrush samples were collected at study enrollment and then 3–4 weeks following DMPA initiation. DMPA users had increased proportions of CD4+CCR5+ and CD8+CCR5+ T cells post-DMPA initiation in the endocervix.

TABLE 1 Effect of DMPA use on immune cell populations observed in human cohorts

Ref	Study	Cohort location and size	Cohort type	Study design	Sample type	Factors measured	Sample collection in terms of DMPA injection	Observations for DMPA use	Comparison group
[29]	Achilles et al 2020	Harare, Zimbabwe *N* = 250: *N* = 38 DMPA, *N* = 41 NET-EN, *N* = 36 MPA/EC, *N* = 43 LNG-I, *N* = 47 ENG-I, and *N* = 45 copper IUD	Healthy women attending the Spilhaus Family Planning Centre	Longitudinal	PBMCs and Endocervical Cytobrush	CD3, CD8, CD4, CD195 (CCR5), CD196 (CCR6), CD69, CD11c	Enrollment was timed for the follicular phase of menses (self-reported) with subsequent timepoints taken at 30, 90, and 180 days post-enrollment	Cervix: ↓ In the percent of CD3+CD4+ at 30 days and the number of CD3+CD4+ at 180 days ↓ In the number of CD11c+ APC at 180 days ↑ In the percent of CD3+CD8+ at 30 and 180 days	Pre-contraception initiation
[34]	Edfeldt et al 2020	Nairobi, Kenya *N* = 30 DMPA and *N* = 40 no-HC	Women from the Pumwani sex worker study who have practiced sex work for 3 years or less and were HIV-, NG-, CT-, syphilis-	Cross-sectional	Ectocervical biopsy	CD4, CCR5, Langerin, CD3	Enrollment was facilitated to occur approximately 2–6 weeks following their last DMPA injection with sampling taking place ever 2 weeks (for a total of 2 additional sample timepoints)	Comparing cell frequency of DMPA users with respect to controls: ↓ CD4+Langerin+, and no significant difference in CD4+CCR5+ and CD4+CD3+ T cells Comparing to total CD4+ T cell population of DMPA users with respect to controls: ↑ CD4+CCR5+, ↓ CD4+Langerin+, and ↑ CD4+CD3+	No-HC
[30]	Tasker et al 2020	Newark, New Jersey *N* = 27 women who returned for all follow-up visits	Women from the Rutgers New Jersey Medical School clinics and were HIV-, CT-, NG-, syphilis-, genital herpes-	Longitudinal	Cytobrush for cervical cells	CD3+ and CD4+ T cells, within the CD4+ population the following markers were measured: α4β7, CCR5, CD38, CCR7, CD45RA	Sample was collected at enrollment (pre-DMPA initiation; visit 1), and 1 month (visit 2) and 3 months (visit 3) post-DMPA injection	↑ CD4+CCR5+ frequency between visits 1 to 3, no significant increase in MFI No significant change in frequency or MFI for α4β7+CD4+ T cells at all visits No significant change in frequency or MFI CD4+CD38+ T cells at all visits ↓ CD4+CCR7+CD45RA- (central memory) T cells from visits 1 to 3, no significant change at all visits for CD4+CCR7+CD45RA+, CD4+CCR7-CD45RA-, CD4+CCR7-CD45RA+	Pre-contraception initiation

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TABLE 1 (Continued)

Ref	Study	Cohort location and size	Cohort type	Study design	Sample type	Factors measured	Sample collection in terms of DMPA injection	Observations for DMPA use	Comparison group
36	Dabee et al 2019	Cape Town and Johannesburg, South Africa (*N* = 37) Long-acting injectable contraceptives (*N* = 28 DMPA and *N* = 6 NET-EN), *N* = 3 COC/ NuvaRing	Recruited HIV- women aged 18–22 who were sexually active	Cross-sectional	CMC	CCR5, CD38, HLA-DR, and Ki67+ markers on CD4+/CD8+ T cells	2 weeks after injection and during the luteal phase (days 14–28) for COC/ NuvaRing users	CD38, HLA-DR, CCR5 and Ki67 expression frequencies on CD4+ T cells did not differ between groups	NuvaRing/COC
35	Lajoie et al 2019	Nairobi, Kenya *N* = 15 DMPA, *N* = 20 Controls (no-HC)	Pumwani sex workers (women were HIV-, CT-, NG-)	Cross-sectional	PBMC, CMC, and ectocervical tissues	HIV co-receptors, activation markers, and Langerin	4–8 weeks post-DMPA injection. Control samples were collected at day 21 of the menstrual cycle	[a]Results after controlling for douching and duration of sex work PBMC: ↓ % CCR5+CD4+, CD69+CD4+, CD95+CD4+, HLA-DR+CD4+ ↓CCR5 expression[b] on CD4+ T cells CMC: ↑ CD69+CD4+ proportion, and expression of CD69 and CCR5 Ectocervical tissue: ↑CCR5+CD4+	No-HC
31	Li et al 2019	USA and China (*N* = 59) *N* = 31 from USA and *N* = 28 from China *N* = 15 DMPA, *N* = 28 LNG-IUD, *N* = 16 ETG vaginal ring	Recruited HIV- women seeking contraception	Longitudinal	PBMC, endocervical cytobrush	Expression of CD4, CD8, CXCR4 and CCR5 on CD3+ T cells	3–4 weeks after contraception initiation for DMPA and LNG-IUD and 3 weeks after ETG ring insertion	PBMC: No differences in % of CCR5+CD4+, CXCR4+CD4+, CCR5+CD8+, CXCR4+CD8+ CMC: ↑ % of CCR5+CD4+ and CCR5+CD8+ T cells No difference in CXCR4+CD4+ and CXCR4+CD8+ T cells No differences in immune populations observed between American and Chinese women	Pre-contraceptive initiation

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TABLE 1 (Continued)

Ref	Study	Cohort location and size	Cohort type	Study design	Sample type	Factors measured	Sample collection in terms of DMPA injection	Observations for DMPA use	Comparison group
32	Thurman et al 2019	Virginia and Pennsylvania, USA Santo Domingo, Dominican Republic *N* = 62: *N* = 32 COC and *N* = 30 DMPA	Recruited HIV- women who had not used HC for >1 month (DMPA for >6 months)	Longitudinal	Vaginal biopsies	CD45, CD3, CD4, CD8, CD1a, CCR5, HLA-DR	Baseline samples taken at days 20–25 of menstrual cycle (luteal phase) and post-contraception initiation samples taken 6 weeks (±1 week) later (for DMPA and COC)	Vaginal epithelium: ↑ CD45+, CD3+, CD8+ T cells No significant change in HLA-DR+ and CD4+ T cells and CD1a+ DCs Lamina propria: ↑CD1a+ dendritic cells and CD45+, CD3+, CD8+, CD4+ T cells No significant change in HLA-DR+ and CCR5+ T cells	Pre-contraceptive initiation
44	Tasker et al 2017	Newark, New Jersey *N* = 29 women who returned for all follow-up visits	Women from the Rutgers New Jersey Medical School clinics	Longitudinal	PBMCs	CD3+ and CD4+ T cells, within the CD4+ population the following markers were measured: α4β7, CCR5, CD38, CCR7, CD45RA, and intracellular p24	Sample was collected at enrollment (pre-DMPA initiation; visit 1), and 1 month (visit 2) and 3 months (visit 3) post-DMPA injection	↑ α4β7+CD4+ frequency from visits 1 to 2, no significant change between visits 1 and 3, and 2 and 3 ↑ α4β7+ expression on CD4+ T cells from visits 1 to 2, from visits 2 to 3 a significant ↓, and no significant change between visits 1 and 3 ↓ CCR5 and CD38 MFI on CD4+ T cells from visits 2 to 3 (no significant change in the frequencies of CD4+ T cells expressing these markers)	Pre-contraception initiation
45	Birse et al 2017	Nairobi, Kenya (*N* = 86) *N* = 23 DMPA users *N* = 63 Controls (no-HC use: condom only, tubal ligation, copper IUD, no contraception, natural/rhythm)	Recruited HIV- women that were part of serodiscordant couples	Cross-sectional	Vaginal swab	Host/bacterial proteome	Not specified	Overabundant proteins in DMPA users included components of inflammation (IL36G, HMGB1, PPBP) and T-cell activation (GRB2, LCP1)	No-HC

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TABLE 1 (Continued)

Ref	Study	Cohort location and size	Cohort type	Study design	Sample type	Factors measured	Sample collection in terms of DMPA injection	Observations for DMPA use	Comparison group
46	Smith-McCune et al 2017	San Francisco, USA (*N* = 42) *N* = 15 DMPA users, = 27 Controls (no-HC or IUD)	Recruited HIV-, NG-, CT- women who were not pregnant or breastfeeding	Cross-sectional	Endocervical cytobrush (DMPA = 14, controls = 25), endometrial biopsy (DMPA = 10, controls = 16)	Immune cell types, activation markers, HIV co-receptors	DMPA: samples take median of 28.5 days after injection Controls: day 23 of menses (luteal phase)	Endocervix: No significant change in (T_{EM}): CCR7-CD45RA- CD4+, CCR7-CD45RA- CD8+ ↑CCR7+CD45RA+ CD8+ Endometrium: ↑CCR7+CD45RA+CD4+, CCR7+CD45RA-CD4+, CD38+HLA-DR+CD4/CD8+, CD38-HLA-DR+CD4/CD8+, CXCR4+CCR5+ CD4/CD8+, CXCR4+CCR5- CD4/CD8+ ↓ CD38+HLA-DR- CD4/CD8+, CXCR4-CCR5+ CD4/CD8+ No significant difference in NK and CD8+ T cells ↑macrophages ↓T-reg	No-HC or IUD users
47	Quispe Calla et al 2016	*N* = 7 women who agreed to use DMPA	Healthy women seeking contraceptive counseling	Longitudinal	Ectocervical biopsy	RNA for qRT-PCR	30–45 days following DMPA injection	↑ expression of CD14, CD177	Pre-contraception initiation
48	Byrne et al 2016	Umlazi, South Africa Total *N* = 432: *N* = 152 DMPA/NET-EN (*n* = 116 DMPA and *n* = 36 NET-EN), *N* = 43 other contraception, *N* = 222 no long-term contraception	Recruited HIV- women aged 18–23	Cross-sectional	Endocervical cytobrush and PBMC	HIV co-receptors and activation markers	Sampling was taken every 3-month. However, in terms of DMPA injection not specified	DMPA and NET-EN grouped together for analysis PBMC: No difference in CCR5+CD4, CCR5 proportions/expression and CD25+ Endocervical cells: ↑ CCR5+CD4+, CCR5 proportions on CD4+ and expression[b], CD25+, and no change in co-expression of HLA-DR and CD38	No long-term contraception

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TABLE 1 (Continued)

Ref	Study	Cohort location and size	Cohort type	Study design	Sample type	Factors measured	Sample collection in terms of DMPA injection	Observations for DMPA use	Comparison group
49	Weinberg et al 2016	Unspecified cities, USA *N* = 24 DMPA	Recruited HIV + women on cART treatment not on HC	Longitudinal	PBMC	Expression of CD25, CD38 HLA-DR on CD4/CD8 cells, FOXP3, CD25 FOXP3, CD39, IL10, IL35, and TGFB	enrollment, and week 4 and 12 after DMPA injection	↓ CD25+CD4+, CD25+CD8+ (not significant), and CD38+HLA-DR+CD8+ (not significant) at week 12 post-DMPA ↑ FOXP3+CD8+ at week 4, IL35+CD4+ at week 12, and CD39+CD4+ (not significant) at week 12 post-DMPA	Pre-contraceptive initiation
50	Goldfien et al 2015	San Francisco, California *N* = 15 DMPA users, *N* = 18 LNG-IUS, and *N* = 23 Controls (no-HC)	Recruited HIV-, NG-, CT-women with no clinically evident vaginal conditions	Cross-sectional	Endometrial and cervical TZ tissue biopsies	RNA microarray	Min. 6 months of DMPA/LNG-IUS use before sample collected and during mid-secretory phase of menstrual cycle	Endometrium: ↑ signatures predicting movement of myeloid cells, adhesion of immune cells, and inflammatory response (IPA) Cervical TZ: ↑ signatures predicting necrosis, ↓ signatures predicting proliferation of cells (IPA)	No-HC
51	Michel et al 2015	Birmingham, USA *N* = 84: *N* = 22 DMPA, *N* = 17 NuvaRing, *N* = 17 COC, *N* = 25 no-HC	Recruited healthy STI-women with no evident vaginal conditions who used DMPA, COC, NuvaRing, or no-HC	Cross-sectional	Vaginal biopsy	Langerin+, CD3+, CD3+CD4+, or CD3+CD8+	Not specified	No significant difference in Langerin+cell density, CD4+CD3+, and CD8+CD3+ T cells between DMPA and control groups ↑ CD3+ density with DMPA use (not significant)	No-HC
52	Sciaranghella et al 2015	New York, Los Angeles, San Francisco, Chicago, and Washington, USA (*N* = 126) *N* = 32 DMPA, *N* = 28 LNG-IUD, *N* = 32 COC, *N* = 34 Controls (regular menstrual cycle)	Recruited HIV-women engaging in high-risk behaviors[a]	Cross-sectional	PBMC	Proportion and expression of surface markers CD4, CXCR4, and CCR5 on monocytes, DC, and T-cell subsets	Not specified	No significant change in CCR5+CD4+ and CCR5+CD8+ T cells, CCR5+ monocytes or dendritic cells, CCR5+ T_{TD} and T_N No significant change in CCR5 expression[b] on monocytes and DCs No significant change in CXCR4 expression ↑CCR5 expression on CD4+ T cells and CD4+ T_{CM} and T_{EM}	Other methods of contraception (LNG-IUD, OCP, and no contraception)

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TABLE 1 (Continued)

Ref	Study	Cohort location and size	Cohort type	Study design	Sample type	Factors measured	Sample collection in terms of DMPA injection	Observations for DMPA use	Comparison group
53	Bahamondes et al 2014	São Paulo, Brazil *N* = 46: *N* = 23 "long-term" DMPA users and *N* = 23 copper IUD (TCu380A)	Recruited healthy women with no evident vaginal conditions	Cross-sectional	Vaginal biopsy	S-100-positive Langerhans stain (cells/mm)	DMPA: 90± 7 days following prior injection Controls: days 8–11 of menses	No significant difference between Langerhans` cell counts of women using DMPA and their matched IUD using controls	IUD users
54	Mitchell et al 2014	Washington, USA *N* = 32 women who initiated DMPA use	Recruited healthy women seeking contraception	Longitudinal	Vaginal biopsy	Cells expressing the following markers: HLA-DR, CD4, CCR5, CD3, and CD1a	Women used DMPA for 12 months, samples taken every 3 months	↓ Median CD1a+ Langerhans and CD4+ cells 12 months after DMPA initiation Trending ↓ Median vaginal CD3+, CCR5+, HLA-DR+, CD3+ CCR5+ and HLA-DR+CCR5+ cells 12 months after DMPA initiation (*N* = 15)	Pre-contraceptive initiation
55	Chandra et al 2013	Virginia, USA *N* = 15 from original study (Mauck et al 1999[56]) used	Recruited women with no STIs, BV	Longitudinal	Vaginal biopsy	CD1a, CD3, CD4, CD8, CD45, CD68, CCL5, and HLA-DR cell density (cells/mm^2)	Baseline samples: days 22–26 of menstrual cycle (luteal phase) and days 8–12 of menstrual cycle (follicular phase) 12 weeks following DMPA injection	↑ median counts of CD45, CD3, CD8, CD68, CCR5+, and HLA-DR+cells with DMPA use compared to controls (follicular and luteal phases)	No-HC
56	Ildgruben et al 2003	Umea, Sweden (*N* = 30) *N* = 15 HC (COCs, DMPA, and levonorgestrel subdermal implant) and *N* = 15 no-HC controls	Recruited healthy women using same contraceptive method for >1 year (control group no-HC for >8 weeks)	Cross-sectional	Vaginal biopsy	CD45, CD3, CD4, CD8, CD14, CD15, CD19, CD20, CD22, CD57, CD68, CD1a	Sampling during follicular and luteal phases of the menstrual cycle in controls. DMPA and LNG users were sampled at matched intervals	↑ CD45+ controls in the HC users (DMPA and LNG implant) compared to controls ↑ mean frequencies of CD8+ and CD4+ cells in DMPA and LNG implant users compared to controls, respectively CD14+ cells detected in DMPA users only No significant difference in the mean frequencies of CD1a+, CD57+, CD19+, CD20+, CD22+, and CD15+ cells between the 3 HC groups compared to the controls	No-HC

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TABLE 1 (Continued)

Ref	Study	Cohort location and size	Cohort type	Study design	Sample type	Factors measured	Sample collection in terms of DMPA injection	Observations for DMPA use	Comparison group
[57]	Vincent et al 2002	DMPA: *N* = 19 women from Jakarta, Indonesia and *N* = 4 women from Victoria, Australia Controls: *N* = ~13 women at days 1–3 and 26–28 of menstrual cycle	Women seeking family planning services	Cross-sectional	Endometrial biopsy	Immunohistochemistry for visualization of endometrial epithelium (stained for CD56, CD3, CD68)	3 weeks–12 months after DMPA injection	↑ CD3+ T cells in DMPA users compared to controls No significant difference in uterine NK cells and macrophages with DMPA use	No-HC
[58]	Bahamondes et al 2000	São Paulo, Brazil *N* = 40: *N* = 20 DMPA and *N* = 20 no-HC controls	Recruited healthy women with no evident vaginal conditions	Cross-sectional	Vaginal biopsy	S-100-positive Langerhans cells/mm	DMPA: 90 ± 7 days following prior injection Controls: days 20–25 of menses (luteal phase)	No significant difference in the mean Langerhans cells per mm between DMPA users and their matched controls.	No-HC
[10]	Miller et al 2000	Washington, USA *N* = 38 (all women using DMPA compared to pre-contraceptive initiation)	Recruited women who wanted to use DMPA, no evident vaginal conditions	Longitudinal	Vaginal biopsies (*N* = 10), vaginal swab (Gram stain)	Neutrophils	Baseline samples collected 19–24 days following last menstrual cycle. Post-contraceptive samples collected 3 and 6 months post-initiation.	No difference in counts of vaginal or cervical neutrophils 6 months after DMPA initiation ↓ vaginal subepithelial associated neutrophils at 6 months post-DMPA injection compared to baseline	Pre-contraceptive initiation
[59]	Mauck et al 1999	Virginia, USA *N* = 16 women all taking DMPA serving as their own control (pre-DMPA initiation)	Recruited women with no STIs, BV	Longitudinal	Vaginal biopsy	S100-Langerhan positive stain	1 and 3 months (±1 week) after first DMPA injection	No significant change in the presence of Langerhans cells comparing pre- and post-DMPA initiation (either luteal or follicular phase)	Pre-contraceptive initiation

Acronyms used: T_{TD} (terminally differentiated T cell), T_N (naive T cells), T_{CM} (central memory T cell), T_{EM} (effector memory T cell), DC (dendritic cells), NK (natural killer cells), CMC (cervical mononuclear cells from cytobrush), PBMC (peripheral blood mononuclear cells), TZ (transformation zone), NG- (tested negative for *Neisseria gonorrhea*), CT- (tested negative for *Chlamydia trachomatis*), syphilis- (tested negative for syphilis), TV- (tested negative for *Trichomonas vaginalis*), and ETG (etonogestrel delivering vaginal ring).

[a]Defined high-risk behaviors to include reporting at least one of the following: injection drug use, having an STI, having unprotected sex with >3 men, or having exchanged sex for drugs or money.

[b]Expression differences based on MFI (mean, or median, intensity fluorescence).

Important limitations of this study include variability in menstrual cycle phase at enrollment and the small sample size.

Finally, Thurman et al analyzed vaginal biopsies (*N* = 30) collected from both American women in Virginia and Pennsylvania and Dominican women from Santo Domingo, Dominican Republic.[32] The use of two diverse populations, similar to the Li et al study, is a strength of their study. Thurman et al focused on non-pregnant, HIV-negative women who reported regular menstrual cycles with no prior use of HCs in the past 30 days or DMPA in the last six months. All participants included in the study were also negative for hepatitis B, *Trichomonas vaginalis*, bacterial vaginosis, *N. gonorrhoeae*, and *C. trachomatis*. Collection of baseline vaginal biopsies occurred during the luteal phase of the menstrual cycle and compared to biopsies collected approximately 6 weeks after initiation of DMPA. In the vaginal epithelium and lamina propria, the study observed an increase in CD45+, CD3+, and CD8+ T cells, while no significant changes were observed for HLA-DR+, CCR5+, and CD4+ T cells, or CD1a+ dendritic cells. They also report certain soluble markers of inflammation and immunity to change within the cervicovaginal fluid post-DMPA initiation including an increase in regulatory cytokine IL-10 and pro-inflammatory cytokine TNF-α, a decrease in pro-inflammatory cytokines IL-1α and IL-1β, and anti-inflammatory protein SLPI. Multiple comparison correction was not performed in this study making it challenging to evaluate the strength of these findings.

Two papers explored the relationship of DMPA and mucosal inflammation in cross-sectional studies of women belonging to the Pumwani sex worker cohort in Nairobi, Kenya.[33] Inclusion of women from this cohort consisted of a negative status for HIV, *N. gonorrhea*, *C. trachomatis*, *T. vaginalis*, and syphilis. An important distinction that these studies share is that they studied women who were using DMPA for more than six months. The study conducted by Edfeldt et al focused on women who had practiced sex work for at least 3 years with self-reported use of DMPA (*N* = 30) or no-HC (*N* = 40) with comparable clinical and demographic variables.[34] Enrollment occurred between 2–6 weeks following their last DMPA injection. Two ectocervical biopsies were collected at two timepoints 2 weeks apart for all study participants, which was designed to capture both phases of the menstrual cycle for women in the no-HC control group. Interestingly, the distribution and proximity of CD4+ T cells within the vaginal epithelium was different in DMPA users compared to the no-HC group. This included a lower proportion of CD4+ T cells in the lower intermediate layer and higher in the upper intermediate layer in the DMPA group compared to controls in the luteal and follicular phases of their menstrual cycle. They also observed an increase in CD4+ and CD4+CCR5+ T cells at both the apical surface and the upper intramuscular layer with a decrease in CD4+Langerin+cells in the superficial and upper intramuscular layers compared to no-HC controls in the luteal phase. A weakness of this study is that MPA levels were not measured making it challenging to associate hormonal levels with these biological differences. The second study from the Pumwani sex worker cohort (Lajoie et al) examined CMCs and ectocervical tissue data from DMPA users (*N* = 15) and no-HC controls (*N* = 20).[35] Samples were collected 4–8 weeks following DMPA injection and at approximately day 21 of the menstrual cycle for women in the no-HC control group. This study observed increased proportions of activated cervical CD4+ T cells (CD4+CD69+ and CD4+CCR5+CD69+) in DMPA but decreased levels of CCR5 on a per cell basis compared to no-HC controls. However, ectocervical tissue biopsies showed the proportion of CD4+CCR5+ cells to be significantly increased compared to no-HC controls. Furthermore, MIP-3α was significantly increased with DMPA. A limitation of this study was that duration of sex work and the practice of douching were significantly different between study groups, despite the consideration in an adjusted analysis.

Another cross-sectional study by Dabee et al focused on young South African women from two sites, Cape Town and Johannesburg. Women included in this study were sexually active using DMPA, NET-EN, COC/NuvaRing, or no hormonal contraceptive.[36] Mucosal samples were collected 2 weeks following DMPA injection. Women were excluded if they were menstruating, pregnant, practicing douching, using spermicides two days prior, had used antibiotics in last two weeks, tested positive for STIs (*C. trachomatis*, *N. gonorrhea*, *T. vaginalis*, *Mycoplasma genitalium*, Herpes Simplex Virus (HSV)-1, HSV-2, *Haemophilus ducreyi*, and *Treponema pallidum*), candidiasis, or were diagnosed with BV (Nugent score between 7-10) at the time of enrollment. In a subset analysis of study participants (DMPA (*N* = 14), condoms only (*N* = 28), and no method of contraception (*N* = 5)), they observed alterations to soluble factors including an increase in pro-inflammatory cytokines (IL-1α, IL-1β, IL-12p40, MIF, TNF-β, TRAIL) several chemokines (CTACK, IL-8, MCP-3, IFN-α2), growth factors (β-NGF, HGF, IL-3, IL-9, LIF, PDGF-BB, SCF, SCGF-β, SDF-1α), and adaptive and regulatory cytokines (IFN-γ, IL-2RA, and IL-1RA) with DMPA compared to the non-HC group. Frequency of immune cell activation markers in CMCs was assessed in a sub-analysis which found no significant differences in expression of CD38, HLA-DR, CCR5, and Ki67 on CD4+ T cells between all contraceptive groups. Weaknesses of this study included differences in age of participants, where young women were more likely to use non-HC methods, and sample size between contraceptive groups.

Molatlhegi et al also reported data from young South African women apart of the CAPRISA-004 trial, a cross-sectional study.[37] Their cohort included women with self-reported use of DMPA (*N* = 448), NET-EN (*N* = 112), and other birth control methods (*N* = 104; COC, IUD, hysterectomy, and tubal ligation). Findings for this study were strengthened by their measurement of serum MPA concentrations for matched plasma samples. Observations included a decrease in SDR-1α, SCGF-B, M-CSF, LIF, G-CSF, IL-6, CTACK, and IL-1α in cervicovaginal lavage samples with matching high MPA concentrations in serum. Following adjustment for HSV-2, age, study arm, visit timepoint, number of sex acts per month, condom usage, and microbiome, they observed a decrease in IL-12p70 and VEGF at high MPA concentrations and an increase in IL-1RA, indicating that interactions with other behavioral, viral, and microbiome factors are important contributors to initial observations between DMPA and genital inflammation.

TABLE 2 Effect of DMPA on cytokine, chemokine, or secreted soluble factors observed in human cohorts

Ref	Study	Cohort location and size	Cohort type	Study design	Sample type	Factors measured
29	Achilles et al 2020	Harare, Zimbabwe *N* = 250: *N* = 38 DMPA, *N* = 41 NET-EN, *N* = 36 MPA/EC, *N* = 43 LNG-I, *N* = 47 ENG-I, and *N* = 45 copper IUD	Healthy women attending the Spilhaus Family Planning Centre	Longitudinal	Vaginal fluid	IFN-γ, IL-1β, IL-6, IL-8, IL-10, RANTES
30	Tasker et al 2020	Newark, New Jersey *N* = 27 women who returned for all follow-up visits	Women from the Rutgers New Jersey Medical School clinics	Longitudinal	Cytobrush for cervical cells and vaginal, endocervical, and rectal swabs*	IL-1β, IL-4, IL-6, IL-7, IL-8, IL-10, IL12p40, IL12p70, IL-13, IL-15, IL-17, TNFα, MCP-1, G-CSF, GM-CSF, MIP-1α, MIP-1β, IP-10, IFNα2, IFN-γ, IL-1α, RANTES, Eotaxin, VEGF, and EGF
37	Molatlhegi et al 2020	KwaZulu-Natal, South Africa *N* = 664: *N* = 448 DMPA, *N* = 112 NET-EN, and *N* = 104 other (COC, IUD, hysterectomy, tubal ligation)	CAPRISA-004 cohort, women were STI- (ie, HIV) excluding HSV-2	Cross-sectional	CVL	48 cytokines
36	Dabee et al 2019	Cape Town and Johannesburg, South Africa (*N* = 89) *N* = 14 DMPA, *N* = 37 NET-EN, *N* = 4 COC, *N* = 1 NuvaRing, *N* = 28 condom only, *N* = 5 none	Recruited HIV-, NG-, CT-, BV- women aged 18–22 who were sexually active	Cross-sectional	Soft-cup collection of cervicovaginal mucus	44 Cytokines
35	Lajoie et al 2019	Nairobi, Kenya *N* = 15 DMPA and *N* = 20 no-HC	Women from the Pumwani sex worker cohort (HIV-, NG-, CT-)	Cross-sectional	Plasma, CVL	19 Cytokines/ Chemokines

Sample collection in terms of DMPA injection	Observations with DMPA use	Comparison group
Enrollment was timed for the follicular phase of menses (self-reported) with subsequent timepoints taken at 30, 90, and 180 days post-enrollment	↓ IL-1β and IL-8 (not significant) at days 30 and 180 ↑ IL-10 at day 30 No significant change in IFN- γ, IL-6, RANTES	Pre-contraception initiation
Sample was collected at enrollment (pre-DMPA initiation; visit 1), and 1 month (visit 2) and 3 months (visit 3) post-DMPA injection	Cytobrush: ↓ IL-1β, IL-4, IL-6, IL-7, IL-8, IL-10, IL12p40, IL12p70, IL-15, IL-17, TNFα, MCP-1, G-CSF, GM-CSF, MIP-1α, MIP-1β, IP-10, IFNα2, IFN-γ, Eotaxin, VEGF, and EGF from visits 1 to 2 ↓ IL-1β, IL-4, IL-6, IL-7, IL-8, IL-10, IL-15, IL-17, TNFα, MCP-1, G-CSF, GM-CSF, MIP-1α, MIP-1β, IFNα2, RANTES, VEGF at visit 3 compared to visit 1 ↓ IL-1α at visit 2 and ↑ at visit 3 compared to visit 1 ↓ RANTES from visits 2 to 3 Endocervical swabs: ↓ G-CSF and ↑ IL-17 from visits 1 to 2 ↓ RANTES and IL-7 from visits 2 to 3 ↓ GM-CSF, MIP-1α, IL-15 from visits 1 to 3 Vaginal swabs: ↓ MCP-1, G-CSF, IP-10 from visits 1 to 2 ↓ IL-6, IL-7, IL-10, MCP-1, G-CSF, GM-CSF from visits 1 to 3 ↑ EGF from visits 2 to 3, and visits 1 to 3 Observations that also passed multiple comparison correction: Cytobrush: ↓IL-6, IL-15, and GM-CSF from visits 1 to 2 and 3, G-CSF, MIP-1β, and VEGF from visits 1 to 2 only, and IFNα2 from visits 1 to 3 only Endocervical swabs: ↓G-CSF from visits 1 to 2 Vaginal swabs: ↓IL-10 from visits 1 to 3 and ↑EFG from visits 2 to 3 and 1 to 3	Pre-contraception initiation
Self-reported DMPA use	↓SDR-1a, SCGF-B, M-CSF, LIF, G-CSF with high MPA concentrations. ↓ G-CSF and M-CSF in low and medium MPA concentrations as well. ↓ IL-15 with low and medium MPA concentrations ↓ IL-6 and CTACK with high concentrations and ↑ RANTES with low MPA concentrations. ↑ MIF, MIP-1B, IL-18, IL8 with medium MPA concentrations and ↓ IL-1a with high MPA concentrations Multivariate analysis controlling for HSV-2, age, study arm, study visit, number of sex acts per month, condom use, and microbial grouping (*Lactobacillus* dominant vs. non-dominant) ↑ IL-1RA with medium MPA concentrations, ↓ IL-12p70 and VEGF with high MPA concentrations	Associations made with matched plasma quantified for MPA
2 weeks after injection and during the luteal phase (days 14–28) for COC/NuvaRing users	↑IL-1α, IL-1β, IL12p40, IL-8, MIF, TNF- β, TRAIL, CTACK, MCP-3, IFN-α2, β-NGF, HGF, IL-3, IL-9, LIF, PDGF-BB, SCF, SCGF-β, SDF-1α, IFN-γ, IL-2RA, IL-1RA compared to women using no-HC	No-HC
Controls: samples taken during luteal phase of menses DMPA: samples taken 4–8 weeks post-injection	PBMC: ↑ MIP-1α CVL ↑MIP-3α No significant difference was observed for the other cytokines of interest	No-HC

(Continues)

TABLE 2 (Continued)

Ref	Study	Cohort location and size	Cohort type	Study design	Sample type	Factors measured
32	Thurman et al 2019	America and Dominican Republic (*N* = 27 DMPA analyzed in cytokine analysis)	Recruited HIV- women who had not used HC for >1 month (DMPA for >6 months)	Longitudinal	CVL	IL-1α, IL-1β, IL-6, IL-8, IL-10, IL-1RA, TNF-α, MIP-1α, RANTES, SLPI
60	Morrison et al 2018	Kampala, Uganda & Chitungwiza, Harare Zimbabwe *N* = 943: *N* = 233 DMPA, *N* = 273 COC, and *N* = 219 no-HC	Women recruited from family planning clinics who were HIV- at enrollment (HC-HIV cohort)	Longitudinal	Endocervical swabs	IL-1β, IL-1RA, IL-6, IL-8, RANTES, MIP-3α, VEGF, ICAM-1
46	Smith-McCune et al 2017	San Francisco, USA (*N* = 39) *N* = 15 DMPA and *N* = 24 Controls (no-HC or IUD)	Recruited HIV-, NG-, CT- women who were not pregnant or breastfeeding	Cross-sectional	Endocervical wick samples	13 Cytokines/ Chemokines
61	Jespers et al 2017	Kenya, Rwanda, and South Africa *N* = 80: *N* = 29 using DMPA or NET-EN, *N* = 10 COC, *N* = 4 Sterilization, and *N* = 21 Condoms only	Recruited healthy HIV- women (*n* = 32), adolescents (*n* = 6), and HIV- sex workers (*n* = 2)	Mixed effects model (both cross-sectional and longitudinal)	CVL	IL-1α, IL-1β, IL-6, IL-12(p70), MIP-1β, IP-10, IL-8, GM-CSF/ G-CSF, Elafin, SLPI, IL-1RA
45	Birse et al 2017	Nairobi, Kenya (*N* = 86) *N* = 23 DMPA users *N* = 63 Controls (no-HC use: condom only, tubal ligation, copper IUD, no contraception, natural/rhythm)	Recruited HIV- women that were part of serodiscordant couples	Cross-sectional	Cervical os and posterior vaginal fornix swab	Host proteome
47	Quispe Calla et al 2016	*N* = 7 women who agreed to use DMPA	Healthy women seeking contraceptive counseling	Longitudinal	Ectocervical biopsy	RNA for qRT-PCR
62	Francis et al 2016	Geita, Shinyanga, and Kahama Tanzania *N* = 67: *N* = 13 DMPA, *N* = 6 COC, *N* = 19 no contraception, *N* = 23 condoms only, *N* = 3 sterilization, *N* = 1 IUD, and *N* = 2 other	Recruited HIV- women at high risk for HIV who practiced vaginal cleansing who were STI-, BV-	Cross-sectional	CVL	23 soluble immune proteins
63	Roxby et al 2016	Mombasa, Kenyan *N* = 15 DMPA users	Recruited HIV- women engaging in transactional sex, high risk of HIV acquisition	Longitudinal	Vaginal swabs	IL-8, IL-6, IP-10, IL-1RA, RANTES, and SLPI
49	Weinberg et al 2016	Unspecified cities, USA *N* = 24 DMPA	Recruited HIV+women on cART treatment not on HC	Longitudinal	PBMC	IL-6, IL-8, IL-10, IFN-γ, TNF-α, TGF-β

Sample collection in terms of DMPA injection	Observations with DMPA use	Comparison group
Baseline: days 20–25 of menses (luteal phase) post-initiation: 6 weeks (±1 week) initiation	↑IL-10, TNF-α ↓IL-1α, IL-1β, and SLPI No significant changes in IL-1RA, RANTES, MIP-1α observed	Pre-contraceptive initiation
Every 12 weeks up to 24 weeks while HIV negative. Thereafter, at 4, 8, and 12 weeks once seroconverted	High RANTES across all timepoints measured ↓ β-Defensin-2 (BD-2) at timepoints prior to seroconversion	No-HC
DMPA: samples take median of 28.5 days after injection Controls: day 23 of menses (luteal phase)	↑MCP-1, IFN-α2 ↓IL-6, IL-1β No changes in IL-8, IL-1 α, MIP-1α, MIP-1β, RANTES, IFN-γ, IL-12, TNF-α, IL-10	No-HC or IUD use
Visit 1: enrollment Visits 2 and 4: day 23 (±2 days) Visits 3 and 5: day 9(±2 days) of menses	↑ IL-8, IL-12p70, MIP-1β in who have reached amenorrhea	Women with menstrual cycle
Not specified	↑ Inflammatory factors (IL36G, HMGB1, PPBP)	No-HC
30–45 days following DMPA injection	↑ expression of IL-1β	Pre-contraception initiation
Samples collected 3 times a week for 4 weeks. DMPA injection date not specified	↑IL-1α, IL-1β, IL-6, TNF-α, IL-2, IL-4, IL-16, IFN-γ, MIP-1α, MIP-1β, MCP-2, IP-10, SDF-β, MIG, IL-8, TGF-β, IFN-β, HBD4, IgA, IgG1, IgG2 compared to women who reported no-HC use	No-HC
Baseline sample taken 3 months before DMPA initiation and samples taken monthly after initiation for up to 1 year	↓IL-6, IL-1RA No change in IL-8 after adjusting for vaginal washing practices	Pre-contraceptive initiation
enrollment, and week 4 and 12 after DMPA injection	↓ TGF-β at week 12 No changes in IL-6, IL-8, IL-10, IFN-γ, TNF-α observed	Pre-contraceptive initiation

(Continues)

TABLE 2 (Continued)

Ref	Study	Cohort location and size	Cohort type	Study design	Sample type	Factors measured
[48]	Byrne et al 2016	Umlazi, South Africa (*N* = 432) *N* = 152 DMPA/NET-EN (*N* = 116 DMPA and *N* = 36 NET-EN), *N* = 43 other contraception, *N* = 222 no contraception	Recruited HIV- women aged 18–23 (FRESH cohort)	Cross-sectional	CVL	17 cytokines
[64]	Deese et al 2015	Bondo, Kenya and Pretoria, South Africa (*N* = 376: 75 DMPA, *N* = 37 NET-EN, *N* = 264 no-HC)	Recruited HIV- women who could have STIs, BV, were not using HC, or used DMPA or NET-EN for ≥3 months	Cross-sectional	Vaginal swabs	MIP-1α, MIP-1β, IL-6, IL-8, IL-1α, IL-1β, IP-10, RANTES, GM-CSF, SLPI
[51]	Michel et al 2015	Birmingham, USA *N* = 84: *N* = 22 DMPA, *N* = 17 NuvaRing, *N* = 17 COC, *N* = 26 no-HC	Recruited healthy STI- women with no evident vaginal conditions who used DMPA, COC, NuvaRing, or no-HC	Cross-sectional	Plasma, CVL	26 Cytokines
[65]	Ngcapu et al 2015	Durban, South Africa *N* = 64 DMPA & NET-EN and *N* = 64 no-HC (1 IUD)	Recruited HIV- women who used DMPA/NET-EN or no-HC, 80% sex worker, 51% BV+	Cross-sectional	CVL	42 cytokines
[66]	Guthrie et al 2015	Nairobi, Kenya (*N* = 228) *N* = 165 no-HC, *N* = 41 DMPA, *N* = 16 oral contraceptive, *N* = 6 implantable HC	Recruited HIV- women from HIV counseling and testing centers	Cross-sectional	Cervical and vaginal swabs	HNP1-3, LL-37, lactoferrin, HBD-2, SLPI

In summary, in recent longitudinal studies (Achilles et al, Tasker et al, Thurman et al and Li et al) DMPA injection is followed by an immunosuppressive phenotype in the vaginal mucosa illustrated by decreased pro-inflammatory cytokines, altered growth factor levels, and in two cases increased proportions of CD4+CCR5+ cells, while one study saw no significant change. However, data from recent cross-sectional studies (Dabee et al, Lajoie et al, Edfeldt et al) saw DMPA was associated with increased pro-inflammatory cytokines, proportion of CD4+CCR5+ T cells, and changes to T-cell proximity in the vaginal epithelium when compared to no hormonal contraceptives. However, observations of a decrease or no significant change in CCR5+ T cells were also reported. A disadvantage of many of these cross-sectional studies, for the most part, is the lack of consideration for vaginal microbiome, when DMPA was injected with relation to sample collection, an imbalance of study groups, and differences in sex behaviors which may contribute to differences in mucosal inflammation. The analysis by Molatlhegi et al exemplified the confounding effect of these variables by showing after adjustment for sex behaviors, STIs, and the microbiome the majority of their initial observations of mucosal cytokine changes with DMPA were no longer significant. Nevertheless, these papers support previous observations that DMPA is associated with an immunosuppressive phenotype and potential inflammatory differences. Future longitudinal studies which account for parameters that can affect genital inflammation, including the microbiome, sex behaviors, and other factors, would help to further strengthen these observations.

4 | DMPA USE AND THE COMPOSITION OF THE VAGINAL MICROBIOME

The use of hormonal contraceptives has been shown to impact the vaginal microbiota. Table 3 outlines papers over the last 20 years that have looked at the relationship between DMPA and vaginal microbiome in human cohorts, with inclusion of study location, size,

Sample collection in terms of DMPA injection	Observations with DMPA use	Comparison group
Not specified	No significant changes in the concentration of cytokines in the DMPA/ NET-EN contraceptive users compared to the no contraceptive controls	No long-term contraception
Not specified	↑MIP-1α, MIP-1β, IL-6, IL-8, IP-10, RANTES compared to reference group	No-HC
Not specified	Plasma ↓IFN-α, IL-8, IL-6 no significant change in: IL-1β, IL-2, IL-10, IL-12, TNF-α, IFN-γ, G-CSF, CXCL10, MCP-1 CVL ↓IFN-α, CXCL10, MCP-1 and G-CSF No significant change in IL-1β, IL-8, IL-6, MIP-1β	No-HC
At enrollment	↓ Eotaxin, MCP-1, MDC, PGDF-AA, IL-15, IL-12p40, fractalkine in women who used either DMPA or NET-EN	No-HC
Not specified	↑ HNP1-3, LL-37, Lactoferrin (LF) compared to no-HC	No-HC

study design, sample type, and sample collection details, much of which has been reviewed elsewhere.[12] For example, a meta-analysis by Vodstrcil et al which focused on studies published before January 2013 reported a decreased incidence of BV, defined by Nugent or Amsel scores, with the use of both progestin-based contraceptives and combined hormonal contraceptives.[8] A more recent review by Haddad et al noted similar observations.[38] Recently, six publications evaluated the vaginal microbiome with DMPA use.

Whitney et al performed a longitudinal analysis of vaginal microbiota in women from Nairobi, Kenya.[39] Inclusion criteria consisted of testing negative for HIV and other STIs, lacking cervicitis, and no current use of hormonal contraception. Vaginal samples were collected from women who chose to use DMPA (N = 33) at baseline, 9–14 days, and three months post-injection and compared to no-HC users (N = 21; condoms, lactational amenorrhea, rhythm method). The method of measurement for present bacterial species in the vaginal microbiota was quantitative-PCR. There was a significant decrease in mean Nugent score after three months post-DMPA initiation although this was not different from the no-HC group. In terms of specific bacterial taxa, DMPA use did not associate with significant changes in concentration of BV-associated bacteria *Sneathia* spp., *Mycoplasma hominis*, and *Parvimonas* sp. Type 1, though *M. hominis* was higher in DMPA users compared to the no-HC group. Interestingly, there was a significant decrease in bacterial load at days 9–14 during peak MPA levels. A strength of their study was their consideration of age, marital status, and whether intercourse had resumed post-partum for analyses that included all women, accounting for potential confounders in their cohort. However, their focus on post-partum women may contribute to unique results given the changes that happen to the vaginal microbiome following pregnancy.[40] Additionally, targeted PCR approaches do not provide comprehensive compositional information on the vaginal microbiome. Thurman et al utilized culturing techniques to determine longitudinal changes in *Lactobacillus* and several less optimal anaerobic taxa in American women. Although their observations cannot speak to long-term effects of DMPA use,

TABLE 3 Effect of DMPA use on endogenous bacteria and microbial communities observed in human cohorts

Ref	Study	Cohort location and size	Cohort type	Study design	Sample type	Factors measured
43	Noël-Romas et al 2020	KwaZulu-Natal, South Africa *N* = 449 DMPA, *N* = 123 NET-EN, *N* = 97 COC, and *N* = 16 no-HC	Women from the CAPRISA-004 cohort with contraceptive use data	Cross-sectional	CVL	Bacterial abundances by proteomics
39	Whitney et al 2020	Nairobi, Kenya *N* = 54; *N* = 33 DMPA use, *N* = 21 no-HC (condoms, lactational amenorrhea, rhythm)	Women that were 6–14 weeks post-partum and breastfeeding, seeking contraception	Longitudinal and cross-sectional	Vaginal swabs	*Gardnerella vaginalis*, *Mycoplasma hominis*, *Sneathia species*, *G. asaccharolytica*, *Eggerthella* sp. Type 1, *Megasphaera* spp. Types 1 and 2 (combined assay), *Parvimonas* sp. Type 1, and *Parvimonas* sp. Type 2
42	Wessels et al 2019	Nairobi, Kenya *N* = 58: *N* = 22 DMPA, *N* = 14 OCP, *N* = 22 No-HC	Pumwani sex worker cohort and were HIV-, CT-, NG-, syphilis-, TV-	Cross-sectional	CVL	16S rRNA
32	Thurman et al 2019	Virginia and Pennsylvania, USA Santo Domingo, Dominican Republic (*N* = 30 at baseline and *N* = 29 at visit post-DMPA injection in samples tested for bacterial growth)	Recruited HIV- women who had not used HC for >1 month (DMPA for >6 months)	Longitudinal	Vaginal swabs	Culturing of H_2O_2-*Lactobacillus*, *Escherichia coli*, *Candida*, *Staphylococcus aureus*, *Enterococcus*, *Ureaplasma*, *Mycoplasma*, Group B *Streptococcus* or pigmented anaerobic gram-negative rods
36	Dabee et al 2019	Cape Town and Johannesburg, South Africa *N* = 59	Recruited HIV-, NG-CT-, BV- women aged 18–22 who were sexually active	Cross-sectional	Vaginal swabs	Bacterial abundances by 16S rRNA (V4 region)
41	Yang et al 2019	New Jersey, USA *N* = 25 DMPA users (9 White and 16 Black women), only 16 women provided samples for all timepoints	Recruited women who wanted to use DMPA no history of STIs, no-HC >2 months	Longitudinal	Vaginal swabs	Bacterial abundances by 16S rRNA (V3-V4 region)

Sample collection in terms of DMPA injection	Observations with DMPA use	Comparison group
Not specified	↓ alpha diversity of microbiome for DMPA users compared to COC and no-HC ↓ *Gardnerella* and *Megasphaera* for DMPA users compared to COC users, however compared to NET-EN users no significant difference was observed No significant difference in *L. crispatus* and *L. iners* abundances between contraceptive groups	NET-EN, COC, and no-HC
Following enrollment (DMPA injection), post-enrollment vaginal swabs were taken 3 months later. For women in the DMPA use arm samples were also taken 9–14 days following injection (peak MPA serum levels)	↓ mean Nugent score among DMPA users No significant difference in change in Nugent score between DMPA and no-HC users No significant difference in total bacterial load with DMPA users, though compared to no-HC users change in bacterial load was significantly different No significant change in Sneathia spp., M. hominis and Parvimonas sp. Type 1 with DMPA use *M. hominis* was significantly different between contraceptive groups No significant change Nugent score and in the 8 bacterial taxa of interest with DMPA use at peak MPA timepoint (9–14 days post-enrollment) ↓ in bacterial load was observed at the timepoint when MPA concentrations peak within DMPA users	Pre-contraceptive initiation and No-HC
3–4 weeks +1 week following last DMPA injection	↑ diversity of microbiome of DMPA users Significantly less women using DMPA had *Lactobacillus* dominant microbiomes compared to OCP and no-HC Microbiome communities did not cluster by contraceptive group	No-HC and OCP Controls
6 weeks (±1 week) after initiation of contraceptive	No significant change in the microbiota between DMPA initiation and baseline in Nugent scores, *Lactobacillus* H_2O_2 producing species, *G. vaginalis*, or anaerobic gram-negative rods	Pre-contraceptive initiation
2 weeks after injection and during the luteal phase (days 14–28) for COC/NuvaRing users	HC choice did not associate with vaginal bacterial composition	Other methods of contraception
1 and 3 months post-DMPA injection	No significant effect on alpha or beta diversity in all women at all timepoints No significant effect on specific bacterial genera abundance between month 1 and 3 timepoints	Pre-contraceptive initiation

(Continues)

TABLE 3 (Continued)

Ref	Study	Cohort location and size	Cohort type	Study design	Sample type	Factors measured
67	Achilles et al 2018	Harare, Zimbabwe *N* = 266: *N* = 41 DMPA, *N* = 44 NET-EN, *N* = 40 MPA/EE, *N* = 45 LNG-I, *N* = 48 ENG-I, and *N* = 48 Cu-IUD	Recruited HIV- NG-, CT- non-pregnant women who did not use DMPA >10 months	Longitudinal	Vaginal swabs	Abundances of *L. crispatus*, *L. gasseri*, *L. jensenii*, *L. iners*, *G. vaginalis*, *A. vaginae*, *Megasphaera* phylotype by qPCR
68	Brooks et al 2017	Virginia, USA (*N* = 682) *N* = 94 DMPA, *N* = 186 Condom use, *N* = 206 COC, and *N* = 196 LNG-IUS	Healthy women no evident vaginal conditions (Human vaginal microbiome project)	Cross-sectional	Vaginal swabs	Bacterial abundances by 16S rRNA (V1-V3 region)
45	Birse et al 2017	Nairobi, Kenya (*N* = 86) *N* = 23 DMPA users *N* = 63 Controls (no-HC use: condom only, tubal ligation, copper IUD, no contraception, natural/rhythm)	Recruited HIV- women that were part of serodiscordant couples	Cross-sectional	Vaginal swab	Bacterial abundances by proteomics
69	Gosmann et al 2017	Umlazi, South Africa (*N* = 232) *N* = 102 DMPA or NET-EN, *N* = 121 no family planning, *N* = 6 COC, *N* = 3 other	Recruited HIV- women aged 18–23 (FRESH cohort)	Cross-sectional	Cervical swabs	Bacterial abundances by 16S rRNA (V4 region)
63	Roxby et al 2016	Mombasa, Kenya *N* = 15 initiating DMPA	HIV- Women engaging in transactional sex, high risk of HIV acquisition	Longitudinal	Vaginal swabs	Abundances of *L. crispatus*, *L. jensenii*, *L. iners*, and *G. vaginalis* by qPCR
48	Byrne et al 2016	Umlazi, South Africa Total *N* = 432: *N* = 152 DMPA/NET-EN, *N* = 58 other contraception or switched contraception methods	Recruited HIV- women aged 18–23 (FRESH cohort)	Cross-sectional	Cervical swabs	Bacterial abundances by 16S rRNA (V4 region)
70	Borgdorff et al 2015	Kigali, Rwanda (*N* = 174) *N* = 96 Controls, *N* = 14 COC, *N* = 38 Injectable contraceptive[a], *N* = 21 pregnant, *N* = 5 implant/IUD	Female sex workers included women with STIs	Longitudinal	Cervicovaginal sampling	Bacterial abundances by phylogenetic microarray

Sample collection in terms of DMPA injection	Observations with DMPA use	Comparison group
1,3, and 6 months post-DMPA injection	↓ concentration of *L. iners* compared to baseline No change in BV-associated bacteria and other *Lactobacillus* species compared to baseline	Pre-contraceptive initiation
Not specified	↑ abundance of *Atopobium vaginae*, *Dialister microaerophilus*, *Prevotella bivia*, *P. amnii*, *Aerococcus christensenii* in DMPA users ↑ abundance of *L. iners* within DMPA users compared to condom users Colonization by BV-associated bacteria (*Atopobium*, *Mobiluncus*, *Megasphaera*, *Prevotella*, *Ureaplasma*, *Mycoplasma*, *Fusobacterium*, *Leptotrichia*, *Gardnerella*, *Sneathia*, and BVAB1-3) were less common with DMPA use compared to condom users	Condom use
Not specified	No relationship observed between microbiome type and DMPA use	No-HC
Samples collected every 3 months. Relation to DMPA injection not specified	Use of injectable progestin-based contraceptives (DMPA or NET-EN) did not differ between identified CT groups	Other methods of contraception
Samples were collected monthly for up to 12 months	↓ of total bacterial load and *G. vaginalis* abundance No change in *L. iners* (highly prevalent at baseline) or in *L. crispatus* and *L. jensenii* (less prevalent at baseline)	Pre-contraceptive initiation
Not specified	Use of injectable progestin-based contraceptives (DMPA or NET-EN) did not correlate with specific bacterial communities	Other methods of contraception
Month 6 and year 2 after enrollment	No association between identified vaginal clusters and hormonal contraceptive use	No-HC and oral contraceptive use

(Continues)

TABLE 3 (Continued)

Ref	Study	Cohort location and size	Cohort type	Study design	Sample type	Factors measured
54	Mitchell et al 2014	Washington, USA *N* = 32 women all using DMPA	Recruited healthy women seeking contraception	Longitudinal	Vaginal swab	Bacterial culturing
10	Miller et al 2000	Washington, USA *N* = 38 (all women using DMPA compared to pre-contraceptive initiation)	Recruited women who wanted to use DMPA, no evident vaginal conditions	Longitudinal	Vaginal swab	Bacterial culturing

Abbreviation: CT, cervicotype.

[a]Type of injectable was not specified; however, Rwandan family planning clinics mostly offer DMPA and rarely offer NET-EN.

due to their comparison of pre-initiation timepoints to 6 weeks post-DMPA injection, they do show no overall change in their specific bacterial taxa of interest at 6 weeks (±1 week) post-injection. Similarly, Yang et al were another longitudinal study enrolling American women seeking contraception. In this study, women enrolled had no active genital infections and did not report using any form of HC in the last two months.[41] This study collected vaginal swabs from women using DMPA at enrollment before DMPA initiation and at one and three months post-injections. Overall, they observed DMPA users (*N* = 16) to exhibit no significant changes in alpha and beta diversity of their vaginal microbiome at both post-DMPA timepoints. An interesting component of their study was the inclusion of bacterial taxa changes among different racial groups (Table 3). However, their observations did not pass a false discovery *p*-value of <.05.

Wessels et al report on data collected from women enrolled in the Pumwani sex worker cohort following a recent DMPA injection.[42] This included data from cervical cytobrush and cervicovaginal lavage samples from women who were using DMPA (*n* = 22), COC (*n* = 14), and no-HC (*n* = 22) and were no longer engaging in sex work. Women were excluded if diagnosed with any STIs or BV. Sampling for this study occurred between 3–4 weeks following last DMPA injection and included measurements of plasma concentrations of MPA, progesterone and estradiol. Interestingly, they observed significantly higher alpha diversity among DMPA users in comparison with no-HC and COC controls and were less likely to have Lactobacillus dominant (>98% relative abundance) communities, but not when lower thresholds were used (50–95% abundance). DMPA use also associated with a decrease in estradiol, progesterone, and decreased abundance of vaginal glycogen and α-amylase, which may explain the decreased likelihood of *Lactobacilli* species. A significant weakness of this study was the lack of baseline vaginal microbiome measurements, which makes changes associated with DMPA difficult to ascertain, as the vaginal microbiome is considerably variable between individuals.

A study by Noël-Romas et al evaluated the vaginal microbiome in young South African women who were enrolled in the CAPRISA 004 trial.[43] While the primary endpoint in this study was HIV infection, the paper evaluated microbiome differences in women belonging to three contraceptive groups, including DMPA (*N* = 449), NET-EN (*N* = 123), oral contraceptives (*N* = 97), and non-HC users (*N* = 14). A unique aspect of this study is the utilization of a mass spectrometry approach to characterize the microbiome providing both compositional and functional information for the bacterial species present. Women using DMPA showed lower alpha diversity compared to oral contraceptive and no-HC users, as well as lower abundances of *Gardnerella* and *Megasphaera*. Functional analysis of bacterial pathways showed no differences between metabolism of vaginal bacteria in women using DMPA compared to the other groups. Interestingly, when stratified by *Lactobacillus* dominance (>50% abundance), higher MPA levels in women on DMPA associated with increased inflammatory pathways in vaginal mucosa related to gluconeogenesis/glycolysis, immune activation and tissue development, but this was not observed in women with BV-associated microbiomes. While this analysis adjusted for potential confounding variables such as age, condom use, and study arm, baseline differences in demographic information commonly observed between contraceptive groups are potential confounders. Dabee et al, however, observed alpha diversity of the microbiome in their South African young women cohort to not differ by contraceptive type.[36]

These recent papers would suggest that DMPA has a small effect on vaginal microbiome composition. Studies which reported increased vaginal microbial diversity or lower levels of *Lactobacillus* in women using DMPA compared to other contraceptive groups were confounded by small sample sizes or were cross-sectional in nature. The longitudinal studies and larger cross-sectional studies by Dabee and Noel-Romas et al indicate that differences in the vaginal microbiome with DMPA are mild and associated with

Sample collection in terms of DMPA injection	Observations with DMPA use	Comparison group
Women used DMPA for 12 months, samples taken every 3 months	↓ proportion of women with H_2O_2-positive *Lactobacillus* over 12 months trending ↑ in culture positive *G. vaginalis* over 12 months	Pre-contraceptive initiation
Baseline and 3 and 6 months post-DMPA initiation	↓ H_2O_2-positive *Lactobacillus* with 6 months of DMPA use ↑ H_2O_2-negative *Lactobacillus* with 6 months of DMPA use (not significant) No change in *Lactobacillus* recovery ↑ of women with *Ureaplasma urealyticum* No change in other non-*Lactobacillus* species were observed	Pre-contraceptive initiation

a reduction in BV-associated bacteria, as reported in previous meta-analyses.[8,38] An important observation, discussed in the Noel-Romas et al paper, is the influence of the microbiome in the context of examining DMPA-associated inflammation making it an important consideration.

5 | DMPA USE AND THE VAGINAL EPITHELIAL BARRIER

The integrity of the FGT epithelium with DMPA use has also garnered considerable interest due to previous observations that DMPA can impact epithelial thickness. Table 4 outlines papers from human cohorts that have examined the relationship of DMPA and the vaginal epithelium, with details on the cohort location and study design. Recently, there have been two studies that examined these relationships.

Edfeldt et al, as discussed above in the immunology section, also explored the impact of long-term DMPA use on the integrity of the epithelial barrier of the FGT from women in the Pumwani sex worker cohort.[34] They measured E-cadherin and thickness properties of the epithelium from ectocervical biopsies in women using DMPA, including the superficial, upper and lower intermediate, and parabasal layers. They observed that E-cadherin was decreased with DMPA compared to control women in the follicular phase, but not the luteal phase of non-HC controls. E-cadherin density correlated with plasma estradiol levels. While there were no differences in the overall thickness of the epithelium, the superficial layer of the epithelium of DMPA users was considerably thinner compared to no-HC controls, but only in comparison with the follicular phase. The findings were similar to those of Thurman et al for vaginal biopsies collected from American and Dominican women.[32] Thurman et al compared vaginal biopsies collected during the luteal phase (days 20–25 of menstrual cycle) to those that were collected approximately 6 weeks following their first DMPA injection and found no significant change in the overall thickness of the vaginal epithelium nor the density of E-cadherin with DMPA use. Overall, these studies suggest that while there are changes to epithelial integrity with DMPA, these differences are more pronounced when compared to the follicular phase of the menstrual cycle, and there is little change to that of the luteal phase when progesterone levels are high.

6 | DISCUSSION

In the past 2 years, there have been several well-designed longitudinal and improved cross-sectional studies that have evaluated the effect of DMPA on vaginal mucosal biology. Recent longitudinal studies generally support the observation of an immunosuppressive effect of DMPA, alterations to growth factors, and changes in immune cell phenotype, including an increase in CCR5+CD4+ T cells. Cross-sectional studies have primarily reported a more pro-inflammatory relationship with DMPA, but these studies are confounded by inherent weaknesses of cross-sectional studies, including differences in study group sizes, behaviors, and other variables that may affect genital inflammation. In agreement with previous meta-analyses, DMPA use is associated with minimal effects of the composition of the vaginal microbiome or a reduction in the number of BV-associated bacteria. Studies which examined the vaginal epithelium did not observe any significant difference with DMPA when compared to women in the luteal phase of the menstrual cycle, although differences in thickness and cell-cell adhesion markers were decreased in comparison with controls in the follicular phase. These results indicate that the interactions between DMPA and the vaginal mucosa are complex and highlight the need for better longitudinal studies that are more comprehensive, which take into consideration the inflammatory, immunological, microbiome, and epithelial effects with DMPA use. Understanding the effects of DMPA use, as well as other HCs, on mucosal biology of the vaginal

TABLE 4 Effect of DMPA use on the integrity of the epithelial barrier in various compartments of the FGT in human cohorts

Ref	Study	Cohort location and size	Cohort type	Study design	Sample type	Factors measured
34	Edfeldt et al 2020	Nairobi, Kenya *N* = 30 DMPA and *N* = 40 no-HC	Women from the Pumwani sex worker study who have practiced sex work for 3 years or less and were HIV-, NG-, CT-, syphilis-	Cross-sectional	Ectocervical biopsy	E-cadherin and thickness of epithelium
32	Thurman et al 2019	Virginia and Pennsylvania, USA Santo Domingo, Dominican Republic *N* = 62: *N* = 32 COC and *N* = 30 DMPA	Recruited HIV- women who had not used HC for >1 month (DMPA for >6 months)	Longitudinal	Vaginal biopsy	Epithelial thickness, number of cell layers, and E-cadherin density
71	Zalenskaya et al 2018	Virginia and Pennsylvania, USA Santo Domingo, Dominican Republic (*N* = 63) *N* = 31 DMPA and *N* = 32 COC	Recruited women who were STI-, BV- who were not on HC	Longitudinal	Ectocervical and Vaginal biopsy	RNA microarray
45	Birse et al 2017	Nairobi, Kenya (*N* = 86) *N* = 23 DMPA users *N* = 63 Controls (no-HC use: condom only, tubal ligation, copper IUD, no contraception, natural/rhythm)	Recruited HIV- women that were part of serodiscordant couples	Cross-sectional	Vaginal swab	Host/bacterial proteome
47	Quispe Calla et al 2016	*N* = 7 women who agreed to use DMPA	Healthy women seeking contraceptive counseling	Longitudinal	Ectocervical biopsy	Mucosal permeability using fluorescent 457 and 70 Da molecules, and splenocytes immunohistochemistry RNA for qRT-PCR
50	Goldfien et al 2015	San Francisco, California *N* = 15 DMPA users, *N* = 18 LNG-IUS, and *N* = 23 Controls (no-HC)	Recruited HIV-, NG-, CT- women with no clinically evident vaginal conditions	Cross-sectional	Endometrial and cervical TZ tissue biopsies	RNA microarray
54	Mitchell et al 2014	Washington, USA *N* = 32 women all using DMPA	Recruited healthy women seeking contraception	Longitudinal	Vaginal biopsy	Epithelial layers and glycogen-positive cells

Sample collection in terms of DMPA injection	Observations with DMPA use	Comparison group
Enrollment was facilitated to occur approximately 2–6 weeks following their last DMPA injection with sampling taking place ever 2 weeks (for a total of 2 additional sample timepoints)	Compared to no-HC controls in the luteal phase E-cadherin was similar, though compared to no-HC controls in the follicular phase lower E-cadherin was observed, specifically in the lower IM[a] and parabasal layers of the epithelium. Though E-cadherin expression measured as mean fluorescence intensity was similar between groups for all layers of the epithelium. No significant difference in total epithelial thickness between DMPA users and controls. However, DMPA users had decreased thickness of their superficial layer, but thicker upper IM[a] compared to controls (in follicular phase)	No-HC
Baseline samples taken at days 20–25 of menstrual cycle (luteal phase) and post-contraception initiation samples taken 6 weeks (±1 week) later (for DMPA and COC)	No change in thickness of the vaginal epithelium, number of cell layers or E-cadherin density with DMPA or OCP initiation.	Pre-contraceptive initiation
Baseline samples at days 18–26 of menstrual cycle and 6 weeks (±1 week) after first DMPA injection	↓ genes from the epidermal differentiation complex (EDC) (RPTN, LCE3D, LOR, SPRR2C), development of stratum corneum of the epidermis (TGM3, ALOX12B), cell junctional proteins (DSG1, DSC2, CDSN), SERPINB7, SPINK6, keratinocyte differentiation markers (KRT10, KRT1, DMKN, and SBSN) ↑ of other keratinocyte differentiation markers (KRT18 and KRT19) and CAPN14	Pre-contraceptive initiation
Not specified	Underabundant proteins were involved in maintenance and repair of epithelial barrier (TFF3, GRN, F11R, KLK7, APOD, TMPRSS11E), phagocytosis (CAPG, CALR, CDC42), and protease inhibition (KNG1, SPINT1, TIMP2, SERPINF2). Biological pathways involved with cell death, and injury pathways were overrepresented while those involved with fibroblast proliferation and connective tissue adhesion were underrepresented	No-HC
30–45 days following DMPA injection	↓ DSG-1 expression ↑ permissibility of ectocervical tissue	Pre-contraception initiation
Min. 6 months of DMPA/LNG-IUS use before sample collected and during the mid-secretory phase of menses for the non-hormonal contraceptive users	↑ gene expression involved with necrosis in the cervical TZ ↓ gene expression involved with the proliferation of cells and trending ↓ in the adhesion of blood cells in the cervical TZ	No-HC
Women used DMPA for 12 months, samples taken quarterly	No significant change in epithelial cell layers after 12 months of DMPA use Trending decreases in glycogen-positive cells and thickness, but significance not reached	Pre-contraceptive initiation

(Continues)

TABLE 4 (Continued)

Ref	Study	Cohort location and size	Cohort type	Study design	Sample type	Factors measured
53	Bahamondes et al 2014	São Paulo, Brazil (*N* = 46) *N* = 23 "long-term" DMPA users and *N* = 23 TCu380A IUD	Recruited healthy women with no evident vaginal conditions	Cross- sectional	Vaginal biopsy	Vaginal epithelial thickness (um)
55	Chandra et al 2013	Virginia, USA *N* = 15 used (from original study Mauck et al 1999)	Recruited women with no STIs, BV	Longitudinal	Vaginal biopsy	E-cadherin, KO-1, and Ki67+ cells
72	Simbar et al 2007	Tehran, Iran *N* = 68: *N* = 30 DMPA and *N* = 38 Cyclofem	Women seeking long-term contraception options	Longitudinal	Endometrial biopsy	Histology and Immunohisto chemistry for visualization of endometrial epithelium
56	Ildgruben et al 2003	Umea, Sweden *N* = 60: *N* = 15 in each HC group (COC, DMPA, and levonorgestrel subdermal implant) and *N* = 15 no-HC controls	Recruited healthy women using same contraceptive method for >1 year (control group no-HC for >8 weeks)	Cross-sectional	Vaginal biopsy	Vaginal epithelial thickness (um)
10	Miller et al 2000	Washington, USA *N* = 38 (all women using DMPA compared to pre-contraceptive initiation)	Recruited women who wanted to use DMPA, no evident vaginal conditions	Longitudinal	Vaginal biopsy (*n* = 10)	Superficial layers, cell layers, thickness, glycogen-positive cells
58	Bahamondes et al 2000	São Paulo, Brazil (*N* = 40) *N* = 20 DMPA and *N* = 20 no-HC controls	Recruited healthy women with no evident vaginal conditions	Cross-sectional	Vaginal biopsy	Vaginal epithelial thickness (mm)
59	Mauck et al 1999	Virginia, USA *N* = 16 women initiating DMPA use	Recruited women with no STIs, BV	Longitudinal	Vaginal biopsy	Epithelial thickness (height and cell layers)

Abbreviations: alpha SMA, alpha smooth muscle actin; PCNA, proliferating cell nuclear antigen; TZ, transformation zone; VSMC, vascular smooth muscle cell.

[a]Lower and upper IM refers to the lower and upper intermediate layer of the ectocervical epithelium.

Sample collection in terms of DMPA injection	Observations with DMPA use	Comparison group
DMPA: 90 ± 7 days following prior injection Controls: days 8–11 of menses	No significant difference between the vaginal epithelial thickness of women using DMPA and their matched IUD using controls	IUD use
Controls: days 22–26 of menstrual cycle (luteal phase) and days 8–12 of menstrual cycle (follicular phase) DMPA: 12 weeks following previous DMPA injection	↑ Ki67+ epithelial cells (cell proliferation marker) in DMPA users compared to both follicular and luteal phase controls No significant change in epithelial thickness, number of cell layers, E-cadherin and ZO-1 (tight-junction and adherens proteins)	Pre-contraceptive initiation
Biopsies were taken pre-contraception initiation and between 3–6 following first injection	↓ endometrial vascular density with DMPA use	Pre-contraception initiation
Sampling during follicular (days 8–13) and luteal phases (days 20–25) of the menstrual cycle in controls. DMPA and LNG users were sampled with matched intervals.	↑ Vaginal epithelial thickness of HC users (OC, LNG implant, and DMPA) compared to controls (greater increase within OC and DMPA users)	No-HC
Baseline samples collected 19–24 days following last menstrual cycle. Post-contraceptive samples collected 3 and 6 months post-initiation.	↓ % of superficial cells, cell layers, and thickness at 6 months post-DMPA injection compared to baseline ↓ Glycogen-positive epithelial thickness 6 months post-DMPA injection	Pre-contraceptive initiation
DMPA: 90 ± 7 days following prior injection Controls: days 20–25 of menses (luteal phase)	No significant difference between the vaginal epithelium thickness of women using DMPA and their no-HC controls.	No-HC
1 and 3 months (±1 week) after first DMPA injection	No significant change in thickness of the vaginal epithelium comparing post-DMPA injection to luteal phase of the menstrual cycle ↓ Epithelial vaginal wall thickness comparing follicular phase to post-DMPA injection (trend) Recovery of vaginal epithelial thickness at month 3 with DMPA use (trend)	Pre-contraceptive initiation

compartment continues to be an important area of research for reproductive health.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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