Author/s:
Hickey, M;Marino, JL;Tachedjian, G

Title:
Mechanisms of HIV Transmission in Depo-Provera Users: The Likely Role of Hypoestrogenism

Date:
2016-01-01

Citation:
Hickey, M., Marino, J. L. & Tachedjian, G. (2016). Mechanisms of HIV Transmission in Depo-Provera Users: The Likely Role of Hypoestrogenism. Journal of Acquired Immune Deficiency Syndromes, 71 (1), pp.1-7. https://doi.org/10.1097/QAI.0000000000000805.

Persistent Link:
https://hdl.handle.net/11343/219819

Title: Mechanisms of HIV transmission in Depo-Provera users: The likely role of hypoestrogenism

Authors: Martha HICKEY[1], BA(Hons), MSc, MBChB, FRCOG, FRANZCOG, MD; Jennifer L. MARINO[1], MPH, PhD; Gilda TACHEDJIAN[2,3,4,5], BSc(Hons), PhD

1. Department of Obstetrics and Gynaecology, The University of Melbourne, Parkville, Victoria, Australia and the Royal Women's Hospital, Victoria, Australia

2. Centre for Biomedical Research, Burnet Institute, Melbourne, Victoria, 3004, Australia

3. Department of Microbiology, Monash University, Clayton, Victoria, 3168, Australia

4. Department of Infectious Diseases, Monash University, Melbourne, Victoria, 3004, Australia

5. Department of Microbiology and Immunology, The University of Melbourne at the Peter Doherty Institute for Infection and Immunity, Parkville, Victoria, 3010, Australia

To whom correspondence should be addressed at: hickeym@unimelb.edu.au.

Postal address: Department of Obstetrics & Gynaecology, The University of Melbourne, Royal Women's Hospital, Level 7, 20 Flemington Rd, Parkville, Victoria, 3052, Australia

Phone: +61-3-8345 3700 Fax: +61-3-8345 3702

Words: 3096

Funding: MH is supported by Australian National Health and Medical Research Council Practitioner Fellowship 1058935. GT is supported by the Australian National Health and Medical Research Council Senior Research Fellowship 543105. GT gratefully acknowledges support of the Victorian Operational Infrastructure Support Program received by the Burnet Institute.

Conflict of Interest: The authors have no relevant conflicts of interest to disclose.

Running title: Mechanisms of HIV transmission in Depo-Provera users

Key words: HIV transmission, hypoestrogenism, Depo-Provera, vaginal estrogen

Abstract

Almost half of new HIV infections worldwide occur in women, and vaginal intercourse is the most common mode of transmission. Accumulating evidence suggests that depot medroxyprogesterone acetate (DMPA, Depo-Provera) may increase HIV transmission, but little is known about the underlying mechanisms. We propose that hypoestrogenism in DMPA may contribute to increased HIV transmission. We present supportive evidence and propose potential interventions to prevent or treat vaginal hypoestrogenism using vaginal estrogens.

Background

HIV infects around 2.3 million people annually, almost half of whom are women [1]. Worldwide, vaginal intercourse is the most common mode of transmission, accounting for more than 80% of new female cases [2]. In sub-Saharan Africa, women are infected 5 to 7 years younger than men [2].

Promotion of safe and effective contraception is central to the well-being and autonomy of women, and saves millions of lives worldwide by reducing maternal morbidity and mortality, and by preventing unplanned pregnancy and consequent infant mortality [3]. Effective contraception is particularly critical in areas of high HIV prevalence in order to prevent vertical transmission, because Prevention of Mother-to-Child Transmission (PMTCT) interventions have low coverage in resource-poor countries [4].

Injectable progestins are among the most common hormonal contraceptive methods, used by around 47 million women worldwide [5] of whom around 9 million are in Southern and Eastern Africa. Available products are depot medroxyprogesterone acetate (DMPA, Depo-Provera) and norethisterone enanthate (NET-EN). DMPA is much more widely used and has the advantage of 12-weekly administration (versus 4-weekly for NET-EN). Depo-Provera is popular amongst women, health care providers, rural health workers and international agencies due to low cost, safety, effectiveness, prolonged duration of action, reversibility, ease of use, privacy, and convenience.

In primate models, DMPA substantially enhances vaginal simian immunodeficiency virus (SIV) acquisition [6, 7]. Although human clinical studies have been inconsistent and there have been no adequately powered randomized controlled trials [3], there is

burgeoning evidence that DMPA may increase both acquisition and transmission of HIV [3, 8-10]. Despite limited data, existing evidence suggests that neither combined oral contraceptives (COC) nor NET-EN increase HIV risk [3, 10, 11]. Pooling ten studies, Ralph *et al.* found that DMPA increased risk of HIV acquisition (hazard ratio (HR) 1.40, 95% confidence interval (CI) 1.16-1.69), but COC did not (HR 1.00, 95% CI 0.86-1.16) [10]. In adjusted analysis of individual participant data from 18 studies, Morrison *et al.* also found that DMPA increased risk of HIV acquisition (HR 1.50, 95 % CI 1.24-1.83) but that there was no statistically significant association between HIV acquisition and COC (HR 1.03, 95% CI 0.88-1.20) or NET-EN (HR 1.24, 95% CI 0.84-1.82) [11].

The possibility that a widely used contraceptive promoted in populations with high HIV prevalence may increase transmission is of urgent public health and clinical concern [3]. This could potentially be responsible for 27,000-130,000 additional infections *per annum* globally, nearly all in resource-poor areas [12]. Limited contraceptive alternatives in these regions mean that avoidance of DMPA could increase unplanned pregnancy and maternal mortality. These concerns have prompted a review of guidelines on medical eligibility for contraceptive use by WHO [13, 14] and CDC [15] which concluded that there is continuing uncertainty about the relationship between DMPA and HIV acquisition, and a need for adequately powered randomized controlled trials to answer this question [16]. The ECHO Consortium (Evidence for Contraceptive Options and HIV Outcomes) is planning a three-armed comparison trial of around 9000 women randomized to DMPA, Jadelle (levonorgestrel contraceptive implant) and copper IUD in areas of high HIV prevalence [16]. Whilst there has been extensive debate about the feasibility and ethics of such a trial [17, 18], there is extensive support from local specialists and consumer advocates for definitive information to inform clinical practice, contraceptive access, and policy for women in high risk areas.

Hypoestrogenism and DMPA

The principal contraceptive action of DMPA is ovulation suppression. With continued use, circulating estradiol concentrations commonly fall into the postmenopausal range [19, 20]. Of key significance is that this uncompensated hypoestrogenism appears to be unique to DMPA and does not occur with other hormonal contraceptives, either because they contain estrogens (e.g. combined hormonal contraceptives, contraceptive vaginal ring), do not completely suppress endogenous estradiol (e.g. progestin-only implants), or do not consistently suppress ovulation (e.g. levonorgestrel-containing IUDs) [21]. NET-EN has a similar mechanism of action to DMPA, but limited data from NET-EN users suggests that estradiol levels remain in the normal premenopausal range [22, 23] (Figure 1). Systemic hypoestrogenism in DMPA users is related to body mass index (BMI), and estradiol concentrations show greater variability in obese women than normal weight women [24]. If hypoestrogenism contributes to HIV transmission in DMPA users, this may be of greater significance to women in resource-poor countries where BMI is generally lower.

Lactational amenorrhea (LAM) is a natural family planning method used by up to 20% of women in areas of high HIV prevalence [25]. During LAM, breast feeding suppresses ovulation via high circulating prolactin. Circulating estradiol concentrations are characteristically reduced during LAM, but women are not usually profoundly hypoestrogenic [26] with mean circulating estradiol concentrations of around 120 pmol/l in one prospective study [27]. Little is known about the relationship between LAM and HIV transmission.

Mechanisms by which DMPA may promote HIV infection in the vagina

Mechanisms of increased HIV acquisition with DMPA and other hormonal contraceptives are currently the subject of intense investigation to provide biological plausibility to observational data [3, 10, 11]. Elucidating these mechanisms would also increase understanding of the impact of sex hormones on HIV sexual transmission, and may inform the selection of safer alternative hormonal contraceptives and the development of targeted multipurpose prevention strategies to prevent pregnancy and HIV transmission.

The prevailing view has been that HIV infection is established via entry through the relatively superficial single layer of columnar ectocervical epithelium and the cervical transformation zone separating the ectocervix and endocervix [28]. This is the most immunologically active site in the reproductive tract with a relative abundance of lymphocytes and antigen presenting cells [29]. However, recent human and experimental data suggest that sexual transmission of HIV in women can occur throughout the reproductive tract including the squamous ectocervical and vaginal mucosae [28, 30, 31]. Potential mechanisms by which DMPA may increase the risk of HIV transmission via vaginal intercourse include disrupting physical barriers, induction of mucosal inflammation, decrease in protective innate and adaptive soluble and cellular immune responses, activation and recruitment of HIV target cells, alterations in vaginal microbiota, and promotion of HIV replication and transcytosis through female reproductive tract epithelial cells [32-35]. DMPA may also indirectly increase risk by changing sexual behavior or condom use, or by promoting genital herpes or other sexually transmitted infection [36]. The medroxyprogesterone (MPA) in DMPA binds with higher affinity to glucocorticoid receptor than do the norethindrone in NET-EN or

levonorgestrel in COC, [37] and has immunosuppressive effects on T cells and plasmacytoid dendritic cells not seen with other exogenous progestins [34, 37, 38].

Physical effects of hypoestrogenism in the vagina

The vagina is highly estrogen-sensitive and will be affected by a fall in circulating estradiol concentrations, although vaginal and circulating estrogen concentrations may differ. Numerous studies in postmenopausal women, in whom systemic (ovarian) estradiol production is very low, report that genitourinary symptoms of menopause include vaginal dryness, discomfort, pallor, and loss of rugae, sometimes accompanied by yellowish vaginal discharge. Objective assessments of hypoestrogenism include reduced acidity (increased pH), reduced superficial cellular maturity (Vaginal maturation index, VMI) and changes to microbiota, including reduction in lactobacilli [39]. However, little is known about how menopause affects vaginal epithelial thickness. A recent small study comparing vaginal wall thickness in biopsies from pre- and postmenopausal women with uterovaginal prolapse reported significantly **greater** vaginal wall thickness in postmenopausal women compared to premenopausal women, apparently due to a thicker muscular layer [40]. The authors also report substantial variation in thickness at different vaginal locations, suggesting that single biopsies may not be representative.

Whilst hypoestrogenism in DMPA users is well established, few studies have investigated whether this might influence HIV transmission. Physiological hypoestrogenism at menopause commonly results in vaginal hypoestrogenism [41], but little is known about how systemic hypoestrogenism in DMPA users impacts the female reproductive tract. DMPA has extensive effects in the vagina, but it is not known whether these are modulated via changes in systemic estrogen concentrations.

Further, the prevalence or nature of vaginal symptoms associated with hypoestrogenism have not routinely been collected in DMPA studies, although one recent study reports that vaginal dryness affects at least 10% [42] and that a characteristic “postmenopausal” vaginal appearance may be seen [43]. Vasomotor symptoms characteristic of menopause have not been reported in DMPA users, but this may be because DMPA is a moderately effective treatment for vasomotor symptoms [44].

Several small studies have measured vaginal epithelial thickness in DMPA users, most reporting little or no change [19, 20, 45, 46]. This contrasts with findings in non-human primates showing that DMPA promotes marked thinning of vaginal epithelium. Administration of DMPA is an established method of increasing SIV/SHIV infection in primate models for experimental studies [6, 7]. In addition to anatomical differences between primates and women, studies in primates may not have accounted for the modifying effect of BMI on hypoestrogenism associated with DMPA. Pigtail macaques, which have BMI similar to adult women, demonstrated only modest thinning of vaginal epithelium [47] on treatment with physiological DMPA concentrations.

Epithelial thinning is not the only potential mechanism by which DMPA could impair integrity of the female genital tract. Decreased vaginal epithelial integrity following DMPA use could allow HIV to migrate deeper into stratified vaginal epithelium and increase the probability of encountering target cells including dendritic cells (DCs) and CD4+ T cells [48, 49]. However, data regarding the impact of DMPA on the density of intercellular junctional proteins in vaginal epithelium is inconsistent [49]. Whether changes in vaginal epithelial integrity are observed following longer DMPA treatment requires further investigation. Although older age has been noted as a risk factor in vaginal transmission of HIV [50, 51], surprisingly, only one publication (a letter) has

addressed vaginal transmission risk in postmenopausal women [52]. This study reports three cases of women infected with HIV following fewer than 6 episodes of vaginal intercourse, and suggests that postmenopausal women are at higher risk than premenopausal women. Since the majority of postmenopausal women in developed countries are sexually active [39], the impact of hypoestrogenism at menopause on HIV transmission requires urgent evaluation in larger studies.

Immune modulatory effects of DMPA in the vagina

DMPA impairs vaginal immune defenses by a local reduction in the antimicrobial peptides human β-defensin-2 and -3 [46, 53], which have anti-HIV-1 activity [54]. It also increases vaginal levels of RANTES, a chemokine which competes with HIV for binding to the CCR5 co-receptor but also could recruit HIV target cells such as CD4+ T cells, DCs and macrophages to the mucosa [35, 53]. Higher RANTES levels in cervical samples from African women were predictive of HIV seroconversion [53]. DMPA suppresses the expression of key regulators of cellular and humoral immunity [35, 55]. This includes interferon alpha from plasmacytoid DCs, interferon gamma from activated T cells [55], and inflammatory cytokines and chemokines (e.g. MIP-1α) [34]. Interferon epsilon, a type I interferon constitutively expressed by female reproductive tract epithelial cells in mice and human cell lines, was shown to be protective against herpes simplex virus type 2 and *Chlamydia muridarum* [56]. Interferon epsilon is hormonally regulated although whether DMPA suppresses interferon epsilon in women and if it has inhibitory activity against HIV remain to be determined.

DMPA promotes activation and recruitment of immune cells. In contrast to suppressing soluble immune mediators, DMPA significantly alters vaginal immune cell populations by increasing numbers of T cells, macrophages, and HLA-DR- and CCR5-positive

cells which may increase susceptibility to HIV infection [49]. Activated CD4+ T-cells expressing CCR5 are elevated in the cervix of healthy postmenopausal women compared to premenopausal women [57]. However, recent evidence with long-term (≥ 6 to 24 months) DMPA treatment does not support this potential mechanism for DMPA enhancement of HIV transmission [55]. Instead a decrease in CD3+ T lymphocyte numbers was observed which might compromise adaptive immune defenses against HIV infection [55]. Whilst there is mounting evidence that immune changes due to DMPA could promote HIV infection, the effects of progesterone and estrogen on immune function in the female reproductive tract are complex. Both may influence immune function implicated in HIV transmission with distinct effects in the upper and lower tract, and the immune modulatory effects of estrogen are concentration dependent [33, 58-60]. Further *in vitro* studies using primary cells and tissues, *in vivo* studies in relevant animal models, and longitudinal studies in women will be required to explain any influence of DMPA on immune function to increase HIV susceptibility.

Effects of DMPA on the vaginal microbiota

Changes in the vaginal microbiota with DMPA are similar to those in postmenopausal women. Specifically, DMPA reduces vaginal colonization with H_2O_2-producing lactobacilli [19, 55]. A recent study reported colonization with such lactobacilli decreased by 49% (p=0.03) after 12 months on DMPA (n=32) [55]. Some *Lactobacillus* species (e.g. *L. crispatus*) are anti-inflammatory in vaginal epithelium and dampen pro-inflammatory cytokine and chemokine responses elicited by Toll-like receptor agonists which mimic viral and bacterial components [61-63] that could promote HIV infection by recruitment and activation of target cells. Vaginal lactobacilli contribute to local defenses against HIV infection [64, 65]. Apart from their immune modulatory effects,

lactobacilli produce lactic acid that acidifies the vagina [66] and potently inactivates HIV [67] and bacteria associated with bacterial vaginosis (BV) [68], which promote HIV acquisition [64]. BV is a common imbalance of vaginal flora marked by overgrowth of obligate anaerobic bacteria and depletion of lactobacilli [69, 70]. In contrast to studies [55, 60] showing that DMPA decreases vaginal lactobacilli, a recent systematic review reported that COC and DMPA reduce BV by 10-20 and 18-30%, respectively [71]. These findings were supported by a second systematic review and meta-analysis [72]. Estrogen in COC could increase glycogen and its breakdown products, an energy source for lactobacilli [73], and estrogen may also increase overgrowth of vaginal Candida species [71, 74]. While the role of progesterone in the decrease observed in the meta-analyses [71, 72] is not clear, it has been proposed that amenorrhea caused by progestin-only contraception could decrease exposure of BV-associated bacteria to iron, an essential energy source for these bacteria [72, 75]. Molecular studies using culture-independent methods based on 16S ribosomal RNA gene sequences, rather than Nugent scores, to characterize distinct *Lactobacillus* species with different vaginal activity [76] may provide better insight into the significance of vaginal microbiota changes mediated by DMPA.

Direct effects of DMPA on HIV replication

DMPA increases HIV replication in CD4+ T cells [34]. Enhancement of *in vitro* CC5-tropic HIV infection and replication by MPA [34] suggests that this mechanism could play a role in enhancing HIV susceptibility at the vaginal mucosa. In contrast, progesterone and estradiol have little and no effect, respectively, on HIV replication [34]. MPA could enhance HIV replication by two possible mechanisms. It could upregulate HIV transcription through binding of a MPA-glucocorticoid receptor complex to glucocorticoid response elements in the HIV long terminal repeat [77]. Alternatively, MPA

could block the production of antiviral cytokines that downmodulate the CCR5 chemokine receptor required for HIV entry [34]. Physiological levels of MPA were recently shown to increase HIV transcytosis by primary reproductive tract epithelial cells, resulting in enhanced infection of CD4+ T cells *in vitro* [35]. While progesterone also increased HIV transcytosis, the effect of MPA was greater and estradiol had no effect [35].

Treatment of vaginal hypoestrogenism with topical estrogens

In primate models, administration of estrogen, systemically or vaginally, protects against DMPA-induced SIV infection [58, 78, 79], which may be attributable to increased thickness of vaginal epithelium [79] or to protection of primary target immune cells [80, 81]. Vaginal estrogens are widely used by postmenopausal women and are an established, effective treatment for vaginal dryness and atrophic vaginitis secondary to hypoestrogenism [82, 83]. Available preparations contain estriol, estradiol or conjugated estrogen (Premarin) as creams, pessaries or a vaginal ring, and these are similarly effective in treating vaginal symptoms at menopause. Treatment regimens vary, but intermittent use (2-3 times weekly) reduces local inflammation, corrects vaginal pH, restores vaginal cytology to premenopausal levels and resolves vaginal dryness with minimal systemic absorption [84, 85]. In experimental models of menopause, topical estrogen increases vaginal epithelial thickness [86] and promotes extracellular matrix remodeling to restore structural integrity [87]. Small studies in postmenopausal women show that vaginal estrogen increases synthesis of mature collagen, decreased degradative enzyme activity, and increases thickness of the vaginal wall [88]. Systemic absorption of vaginal estrogen at therapeutic doses to treat vaginal atrophy is minimal [85]. Critically, primate data demonstrate that DMPA increases SIV transmission but that this can be prevented by vaginal estrogen treatment (as estriol) without detectable

systemic effects [79]. Since DMPA acts principally through the hypothalamic-pituitary axis to inhibit ovulation, the addition of exogenous estrogens does not impair the contraceptive efficacy of DMPA [89].

A trial of local (vaginal) replacement estrogens in DMPA users would elucidate the contribution of hypoestrogenism to HIV acquisition in this population. Improving our understanding of this potential contribution is important because it provides insight into mechanisms of vaginal HIV transmission and into HIV risk for sexually active postmenopausal women. Further, it would provide key information about whether other hormonal contraceptives which do not cause hypoestrogenism are likely to affect HIV transmission, which would inform practice and policy. If the trial proved successful, it could increase the safety of DMPA without compromising efficacy, thereby improving safe contraceptive options for women.

If HIV transmission in DMPA users is regulated by hypoestrogenism, then vaginal estrogens could be a simple method to reverse these vaginal changes and potentially protect against HIV. Vaginal estrogens are cheap, and stable at room temperature. Those containing estradiol (e.g. Vagifem®) cost around USD$16 per month and estriol cream (e.g. Ovestin®) around $10. The estradiol-releasing vaginal ring (Estring®) is more costly but can be left in situ. Currently, the lifetime cost in the US of treating one person infected with HIV is estimated at USD$379,668 [90]. Although generic antiretroviral drug costs are much lower in sub-Saharan Africa due to special terms in international trade law, HIV treatment is still costly at $747 per patient per year [91]. If a sexually active woman uses DMPA for contraception for 30 years (from ages 15 to 45), thrice weekly vaginal estrogen would cost an additional $4,000, the cost of five years' ART. Specifically manufactured products and similar trade arrangements could further reduce this cost.

Conclusion

Providing affordable, effective and accessible contraception to women in areas of high HIV prevalence is an international health priority. Planned randomized comparative trials of DMPA vs. other contraceptive options should include the collection of mechanistic data to further understand how DMPA may alter HIV risk. If hypoestrogenism with DMPA underlies this risk, vaginal estrogens could potentially be protective. This might contribute to maintaining contraceptive options for disadvantaged women at high risk of HIV with limited contraceptive options.

References

1. UNAIDS. *Global report: UNAIDS report on the global AIDS epidemic 2013* Geneva, Switzerland2013.
2. Abdool Karim Q, Sibeko S, Baxter C. Preventing HIV infection in women: a global health imperative. *Clinical Infectious Diseases.* 2010;50(S3):S122-129.
3. Polis CB, Curtis KM. Use of hormonal contraceptives and HIV acquisition in women: a systematic review of the epidemiological evidence. *Lancet Infect Dis.* 2013;13(9):797-808.
4. Tudor Car L, van-Velthoven MHMMT, Brusamento S, et al. Integrating prevention of mother-to-child HIV transmission (PMTCT) programmes with other health services for preventing HIV infection and improving HIV outcomes in developing countries. *Cochrane Database of Systematic Reviews.* 2011(6). http://onlinelibrary.wiley.com/doi/10.1002/14651858.CD008741.pub2/abstract.
5. United Nations Department of Economic and Social Affairs PD. National, regional and global estimates and projections of the number of women aged 15 to 49 who are married or in a union, 1970-2030. Vol 2013/2. New York, USA2013.
6. Marx PA, Spira AI, Gettie A, et al. Progesterone implants enhance SIV vaginal transmission and early virus load. *Nat Med.* Oct 1996;2(10):1084-1089.
7. Vishwanathan SA, Guenthner PC, Lin CY, et al. High susceptibility to repeated, low-dose, vaginal SHIV exposure late in the luteal phase of the menstrual cycle of pigtail macaques. *J Acquir Immune Defic Syndr.* Aug 1 2011;57(4):261-264.

8. Heffron R, Donnell D, Rees H, et al. Use of hormonal contraceptives and risk of HIV-1 transmission: a prospective cohort study. *Lancet Infect Dis.* 2012;12(1):19-26.

9. Crook AM, Ford D, Gafos M, et al. Injectable and oral contraceptives and risk of HIV acquisition in women: an analysis of data from the MDP301 trial. *Hum Reprod.* 2014;May 16. pii: deu113. [Epub ahead of print].

10. Ralph LJ, McCoy SI, Shiu K, Padian NS. Hormonal contraceptive use and women's risk of HIV acquisition: a meta-analysis of observational studies. *The Lancet Infectious Diseases.* 2015;15(2):181-189.

11. Morrison CS, Chen PL, Kwok C, et al. Hormonal Contraception and the Risk of HIV Acquisition: An Individual Participant Data Meta-analysis. *PLoS Med.* Jan 2015;12(1):e1001778.

12. Butler AR, Smith JA, Polis CB, Gregson S, Stanton D, Hallett TB. Modelling the global competing risks of a potential interaction between injectable hormonal contraception and HIV risk. *AIDS.* 2013;27(1):105-113.

13. WHO Department of Reproductive Health and Research. Hormonal contraception and HIV Technical Statement. Vol WHO/RHR/12.082012.

14. WHO. Hormonal contraceptive methods for women at high risk of HIV and living with HIV, 2014 Guidance Statement. Vol WHO/RHR/14.24. Geneva, Switzerland: WHO; 2014.

15. CDC. Update to CDC's *U.S. Medical Eligibility Criteria for Contraceptive Use, 2010*: Revised Recommendations for the Use of Hormonal Contraception among Women at High Risk for HIV Infection or Infected with HIV. *Morbidity and Mortality Weekly Report.* 2012;61(24):449-452.

16. Rees H, for the ECHO Consortium. DMPA and HIV: why we need a trial. *Contraception.* 2014;90:354–356.

17. Ralph LJ, McCoy, S. I., Hallett, T., Padian, N. Next steps for research on hormonal contraception and HIV. *Lancet.* 2013;382(9903):1467 - 1469,.

18. Jones HE. Time to focus on improving the contraceptive method mix in high HIV prevalence settings and let go of unanswerable questions. *Contraception.* Oct 2014;90(4):357-359.

19. Miller L, Patton DL, Meier A, Thwin SS, Hooton TM, Eschenbach DA. Depomedroxyprogesterone-induced hypoestrogenism and changes in vaginal flora and epithelium. *Obstet Gynecol.* 2000;96(3):431-439.

20. Bahamondes MV, Castro S, Marchi NM, et al. Human vaginal histology in long-term users of the injectable contraceptive depot-medroxyprogesterone acetate. *Contraception.* 2014 Feb 7. pii: S0010-7824(14)00034-1. doi: 10.1016/j.contraception.2014.01.024. [Epub ahead of print].

21. ESHRE Capri Workshop Group. Ovarian and endometrial function during hormonal contraception. *Hum Reprod.* 2001;16(7):527-535.

22. Meng YX, Virutamasen P. Effect of norethisterone enanthate on endometrial bleeding and changes in coagulation function, ovarian hormones and endometrial histology in Thai women. *Contraception.* 1991;44(1):73-82.

23. Lawrie TA, Hofmeyr GJ, De Jager M, Berk M, Paiker J, Viljoen E. A double-blind randomised placebo controlled trial of postnatal norethisterone enanthate: the effect on postnatal depression and serum hormones. *Br J Obstet Gynaecol.* Oct 1998;105(10):1082-1090.

24. Segall-Gutierrez P, Taylor D, Liu X, Stanzcyk F, Azen S, Mishell DR, Jr. Follicular development and ovulation in extremely obese women receiving

depo-medroxyprogesterone acetate subcutaneously. *Contraception.* Jun 2010;81(6):487-495.

25. Wanyenze RK, Tumwesigye NM, Kindyomunda R, et al. Uptake of family planning methods and unplanned pregnancies among HIV-infected individuals: a cross-sectional survey among clients at HIV clinics in Uganda. *J Int Aids Soc.* Jun 30 2011;14.

26. Velasquez EV, Trigo RV, Creus S, Campo S, Croxatto HB. Pituitary-ovarian axis during lactational amenorrhoea. I. Longitudinal assessment of follicular growth, gonadotrophins, sex steroids and inhibin levels before and after recovery of menstrual cyclicity. *Hum Reprod.* Apr 2006;21(4):909-915.

27. Campino C, Torres C, Rioseco A, et al. Plasma prolactin/oestradiol ratio at 38 weeks gestation predicts the duration of lactational amenorrhoea. *Hum Reprod.* Dec 2001;16(12):2540-2545.

28. Hladik F, Hope TJ. HIV infection of the genital mucosa in women. *Curr HIV/AIDS Rep.* 2009;6(1).

29. Pudney J, Quayle AJ, Anderson DJ. Immunological microenvironments in the human vagina and cervix: mediators of cellular immunity are concentrated in the cervical transformation zone. *Biol Reprod.* Dec 2005;73(6):1253-1263.

30. Stieh DJ, Maric D, Kelley ZL, et al. Vaginal Challenge with an SIV-Based Dual Reporter System Reveals That Infection Can Occur throughout the Upper and Lower Female Reproductive Tract. *PLoS Pathog.* Oct 2014;10(10):e1004440.

31. Smedley J, Turkbey B, Bernardo ML, et al. Tracking the luminal exposure and lymphatic drainage pathways of intravaginal and intrarectal inocula used in nonhuman primate models of HIV transmission. *PLoS One.* 2014;9(3):e92830.

32. Murphy K, S.C I, Herold BC. Research Gaps in Defining the Biological Link between HIV Risk and Hormonal Contraception. *Am J Reprod Immunol.* 2014; Feb 19. doi: 10.1111/aji.12209.

33. Hel Z, Stringer E, Mestecky J. Sex Steroid Hormones, Hormonal Contraception, and the Immunobiology of Human Immunodeficiency Virus-1 Infection. *Endocr Rev.* Feb 2010;31(1):79-97.

34. Huijbregts RP, Helton ES, Michel KG, et al. Hormonal contraception and HIV-1 infection: medroxyprogesterone acetate suppresses innate and adaptive immune mechanisms. *Endocrinology.* Mar 2013;154(3):1282-1295.

35. Ferreira VH, Dizzell S, Nazli A, et al. Medroxyprogesterone Acetate Regulates HIV-1 Uptake and Transcytosis but Not Replication in Primary Genital Epithelial Cells, Resulting in Enhanced T-Cell Infection. *J Infect Dis.* Dec 23 2014.

36. Achilles SL, Hillier SL. The complexity of contraceptives: understanding their impact on genital immune cells and vaginal microbiota. *AIDS.* Oct 2013;27 Suppl 1:S5-15.

37. Hapgood JP, Ray RM, Govender Y, Avenant C, Tomasicchio M. Differential glucocorticoid receptor-mediated effects on immunomodulatory gene expression by progestin contraceptives: implications for HIV-1 pathogenesis. *Am J Reprod Immunol.* Jun 2014;71(6):505-512.

38. Huijbregts RP, Michel KG, Hel Z. Effect of progestins on immunity: medroxyprogesterone but not norethisterone or levonorgestrel suppresses the function of T cells and pDCs. *Contraception.* Aug 2014;90(2):123-129.

39. Portman DJ, Gass ML. Genitourinary syndrome of menopause: new terminology for vulvovaginal atrophy from the International Society for the

Study of Women's Sexual Health and the North American Menopause Society. *Maturitas.* Nov 2014;79(3):349-354.

40. Lara LAD, da Silva AR, Rosa-e-Silva JC, et al. Menopause Leading to Increased Vaginal Wall Thickness in Women with Genital Prolapse: Impact on Sexual Response. *Journal of Sexual Medicine.* Nov 2009;6(11):3097-3110.

41. Mac Bride MB, Rhodes DJ, Shuster LT. Vulvovaginal Atrophy. *Mayo Clin Proc.* Jan 2010;85(1):87-94.

42. Veisi F, Zangeneh M. Comparison of Two Different Injectable Contraceptive Methods: Depo-medroxy Progesterone Acetate (DMPA) and Cyclofem. *J Family Reprod Health.* Sep 2013;7(3):109-113.

43. Walker C, Badawy SZ. Vaginal Atrophy following Long-Term Depot Medroxyprogesterone Acetate Use: A Case Report. *Case Rep Obstet Gynecol.* 2013;835316.(Epub 2013 Mar 6.).

44. Barton D, Loprinzi C, Quella S, Sloan J, Pruthi S, Novotny P. Depomedroxyprogesterone acetate for hot flashes. *J Pain Symptom Manage.* Dec 2002;24(6):603-607.

45. Bahamondes L, Trevisan M, Andrade L, et al. The effect upon the human vaginal histology of the long-term use of the injectable contraceptive Depo-Provera. *Contraception.* 2000;62(1):23-27.

46. Michel KG, Huijibregts RPH, Richter HE, Hel Z. Effect of depot medroxyprogesterone acetate on human β-defensin production and structural integrity of the human vaginal epithelium. *Lancet.* 3 Nov 2013 2013;382 Supp 2:S1-S25.

47. Radzio J, Hanley K, Mitchell J, et al. Physiologic doses of depot-medroxyprogesterone acetate do not increase acute plasma simian HIV

viremia or mucosal virus shedding in pigtail macaques. *AIDS.* 2014;28(10):1431-1439.

48. Carias AM, McCoombe S, McRaven M, et al. Defining the interaction of HIV-1 with the mucosal barriers of the female reproductive tract. *J Virol.* 2013;87(21):11388-11400.

49. Chandra N, Thurman AR, Anderson S, et al. Depot medroxyprogesterone acetate increases immune cell numbers and activation markers in human vaginal mucosal tissues. *AIDS Res Hum Retroviruses.* 2013;29(3):592-601.

50. Aaby P, Ariyoshi K, Buckner M, et al. Age of wife as a major determinant of male-to-female transmission of HIV-2 infection: A community study from rural West Africa. *AIDS.* Nov 1996;10(13):1585-1590.

51. Devincenzi I, Ancellepark RA, Brunet JB, et al. Comparison of Female to Male and Male to Female Transmission of HIV in 563 Stable Couples. *Brit Med J.* Mar 28 1992;304(6830):809-813.

52. Dwyer JM, Penny R, Gatenby PA, Learmont J. Susceptibility of postmenopausal women to infection with HIV during vaginal intercourse. *Med J Aust.* 1990;153(5):299.

53. Morrison C, Fichorova RN, Mauck C, et al. Cervical Inflammation and Immunity Associated With Hormonal Contraception, Pregnancy, and HIV-1 Seroconversion. *J Acquir Immune Defic Syndr.* 2014;66(2):109-117.

54. Wira CR, Patel MV, Ghosh M, Mukura L, Fahey JV. Innate immunity in the human female reproductive tract: endocrine regulation of endogenous antimicrobial protection against HIV and other sexually transmitted infections. *Am J Reprod Immunol.* Mar 2011;65(3):196-211.

55. Mitchell CM, McLemore L, Westerberg K, et al. Long-term Effect of Depot Medroxyprogesterone Acetate on Vaginal Microbiota, Epithelial Thickness and HIV Target Cells. *J Infect Dis.* 2014;210(4):651-655.

56. Fung KY, Mangan NE, Cumming H, et al. Interferon-epsilon protects the female reproductive tract from viral and bacterial infection. *Science.* Mar 1 2013;339(6123):1088-1092.

57. Meditz AL, Moreau KL, MaWhinney S, et al. CCR5 expression is elevated on endocervical $CD4^+$ T cells in healthy postmenopausal women. *J Acquir Immune Defic Syndr.* 2012;59(3):221-228.

58. Goode D, Aravantinou M, Jarl S, et al. Sex Hormones Selectively Impact the Endocervical Mucosal Microenvironment: Implications for HIV Transmission. *PLoS One.* May 15 2014;9(5).

59. Straub RH. The complex role of estrogens in inflammation. *Endocr Rev.* Aug 2007;28(5):521-574.

60. Wira CR, Fahey JV, Rodriguez-Garcia M, Shen Z, Patel MV. Regulation of mucosal immunity in the female reproductive tract: the role of sex hormones in immune protection against sexually transmitted pathogens. *Am J Reprod Immunol.* Aug 2014;72(2):236-258.

61. Fichorova RN, Yamamoto HS, Delaney ML, Onderdonk AB, Doncel GF. Novel vaginal microflora colonization model providing new insight into microbicide mechanism of action. *MBio.* 2011;2(6):e00168-00111.

62. Doerflinger SY, Throop AL, Herbst-Kralovetz MM. Bacteria in the vaginal microbiome alter the innate immune response and barrier properties of the human vaginal epithelia in a species-specific manner. *J Infect Dis.* 2014;209(12):1989-1999.

63. Rose WA, McGowin CL, Spagnuolo RA, Eaves-Pyles TD, Popov VL, Pyles RB. Commensal Bacteria Modulate Innate Immune Responses of Vaginal Epithelial Cell Multilayer Cultures. *PLoS One.* Mar 7 2012;7(3).

64. Atashili J, Poole C, Ndumbe PM, Adimora AA, Smith JS. Bacterial vaginosis and HIV acquisition: a meta-analysis of published studies. *AIDS.* Jul 31 2008;22(12):1493-1501.

65. Martin HL, Richardson BA, Nyange PM, et al. Vaginal lactobacilli, microbial flora, and risk of human immunodeficiency virus type 1 and sexually transmitted disease acquisition. *J Infect Dis.* Dec 1999;180(6):1863-1868.

66. O'Hanlon DE, Moench TR, Cone RA. Vaginal pH and microbicidal lactic acid when lactobacilli dominate the microbiota. *PLoS One.* 2013;8(11):e80074.

67. Aldunate M, Tyssen D, Johnson A, et al. Vaginal concentrations of lactic acid potently inactivate HIV. *J Antimicrob Chemother.* 2013;68(9):2015-2025.

68. O'Hanlon DE, Moench TR, Cone RA. In vaginal fluid, bacteria associated with bacterial vaginosis can be suppressed with lactic acid but not hydrogen peroxide. *BMC Infect Dis.* 2011;11:200.

69. Kenyon CR, Osbak K. Recent progress in understanding the epidemiology of bacterial vaginosis. *Curr Opin Obstet Gynecol.* Dec 2014;26(6):448-454.

70. Huang B, Fettweis JM, Brooks JP, Jefferson KK, Buck GA. The changing landscape of the vaginal microbiome. *Clin Lab Med.* Dec 2014;34(4):747-761.

71. van de Wijgert JH, Verwijs MC, Turner AN, Morrison CS. Hormonal contraception decreases bacterial vaginosis but oral contraception may increase candidiasis: implications for HIV transmission. *AIDS.* Aug 24 2013;27(13):2141-2153.

72. Vodstrcil LA, Hocking JS, Law M, et al. Hormonal contraception is associated with a reduced risk of bacterial vaginosis: a systematic review and meta-analysis. *PLoS One.* 2013;8(9):e73055.

73. Spear GT, French AL, Gilbert D, et al. Human alpha-amylase present in lower-genital-tract mucosal fluid processes glycogen to support vaginal colonization by Lactobacillus. *J Infect Dis.* Oct 1 2014;210(7):1019-1028.

74. Cruickshank R. The biology of the vagina in the human subject. II: The bacterial flora and secretion of the vagina in relation to glycogen in the vaginal epithelium. *J Obstet Gynecol Br Emp.* 1934;41:208-226.

75. Jarosik GP, Land CB, Duhon P, Chandler R, Jr., Mercer T. Acquisition of iron by Gardnerella vaginalis. *Infect Immun.* Oct 1998;66(10):5041-5047.

76. Ma B, Forney LJ, Ravel J. Vaginal microbiome: rethinking health and disease. *Annu Rev Microbiol.* 2012;66:371-389.

77. Ghosh D. Glucocorticoid receptor-binding site in the human immunodeficiency virus long terminal repeat. *J Virol.* Jan 1992;66(1):586-590.

78. Smith SM, Baskin GB, Marx PA. Estrogen protects against vaginal transmission of simian immunodeficiency virus. *Journal of Infectious Diseases.* Sep 2000;182(3):708-715.

79. Smith SM, Mefford M, Sodora D, et al. Topical estrogen protects against SIV vaginal transmission without evidence of systemic effect. *AIDS.* 2004;18(12):1637-1643.

80. Tasker C, Ding J, Schmolke M, Rivera-Medina A, Garcia-Sastre A, Chang TL. 17 beta-Estradiol Protects Primary Macrophages Against HIV Infection Through Induction of Interferon-Alpha. *Viral Immunol.* May 2014;27(4):140-150.

81. Rodriguez-Garcia M, Biswas N, Patel MV, et al. Estradiol reduces susceptibility of CD4+ T cells and macrophages to HIV-infection. *PLoS One.* 2013;8(4):e62069.

82. Suckling J, Lethaby A, Kennedy R. Local oestrogen for vaginal atrophy in postmenopausal women. *Cochrane Database Syst Rev.* 2006(4):CD001500. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=17054136.

83. Sturdee DW, Panay, N. Recommendations for the management of postmenopausal vaginal atrophy. *Climacteric.* 2010;13(6):509-522.

84. Al-Baghdadi O, Ewies AAA. Topical estrogen therapy in the management of postmenopausal vaginal atrophy: an up-to-date overview. *Climacteric.* 2009;12(2):91-105.

85. Santen RJ. Vaginal administration of estradiol: effects of dose, preparation and timing on plasma estradiol levels. *Climacteric.* Oct 18 2014:1-14.

86. Pessina MA, Hoyt Jr. RF, Goldstein I, Traish AM. Differential effects of estradiol, progesterone, and testosterone on vaginal structural integrity. *Endocrinology.* 2006;14(1):61-69.

87. Slayden OD, Hettrich K, Carroll RS, Otto LN, Clark AL, Brenner RM. Estrogen enhances cystatin C expression in the macaque vagina. *J Clin Endocrinol Metab.* 2004;89(2):883-891.

88. Rahn DD, Good MM, Roshanravan SM, et al. Effects of preoperative local estrogen in postmenopausal women with prolapse: a randomized trial. *J Clin Endocrinol Metab.* Oct 2014;99(10):3728-3736.

89. Abdel-Aleem H, d'Arcangues C, Vogelsong KM, Gulmezoglu AM. Treatment of vaginal bleeding irregularities induced by progestin only contraceptives. *Cochrane Database Syst Rev.* 2007(4):CD003449.

90. CDC. HIV Cost-effectiveness. 2013; www.cdc.gov/hiv/prevention/ongoing/costeffectiveness/. Accessed April 29, 2015.

91. Office of U.S. Global AIDS Coordinator. 2014 Report on Costs of Treatment in the President's Emergency Plan for AIDS Relief (PEPFAR). 2015; http://www.pepfar.gov/documents/organization/223163.pdf. Accessed April 29, 2015.

92. Sturgeon SR, Potischman N, Malone KE, et al. Serum levels of sex hormones and breast cancer risk in premenopausal women: a case-control study (USA). *Cancer Causes Control.* Feb 2004;15(1):45-53.

93. Hidalgo MM, Hidalgo-Regina C, Bahamondes MV, Monteiro I, Petta CA, Bahamondes L. Serum levonorgestrel levels and endometrial thickness during extended use of the levonorgestrel-releasing intrauterine system. *Contraception.* Jul 2009;80(1):84-89.

94. Makarainen L, van Beek A, Tuomivaara L, Asplund B, Bennink HC. Ovarian function during the use of a single contraceptive implant: Implanon compared with Norplant. *Fertil Steril.* Apr 1998;69(4):714-721.

95. Hidalgo MM, Lisondo C, Juliato CT, Espejo-Arce X, Monteiro I, Bahamondes L. Ovarian cysts in users of Implanon and Jadelle subdermal contraceptive implants. *Contraception.* May 2006;73(5):532-536.

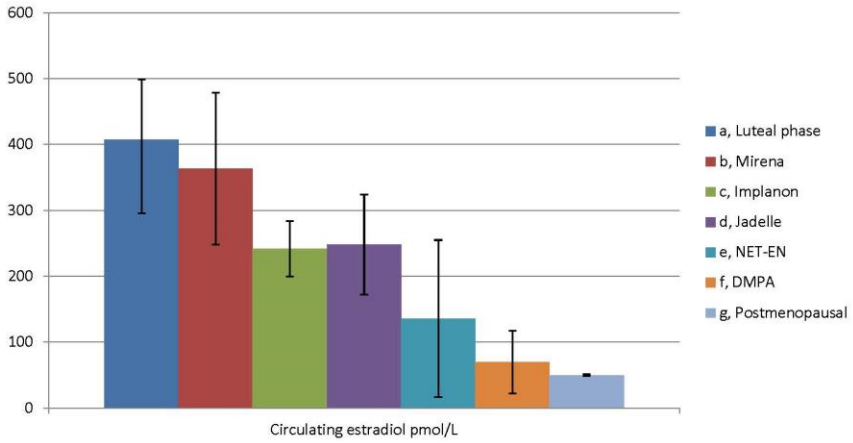
96. Clark MK, Sowers M, Levy BT, Tenhundfeld P. Magnitude and variability of sequential estradiol and progesterone concentrations in women using depot

medroxyprogesterone acetate for contraception. *Fertil Steril.* May 2001;75(5):871-877.

97. Key TJ, Appleby PN, Reeves GK, et al. Circulating sex hormones and breast cancer risk factors in postmenopausal women: reanalysis of 13 studies. *Br J Cancer.* Aug 23 2011;105(5):709-722.

Figure Legend

Figure 1. Mean circulating estradiol in (a) normal premenopausal contracepting women (N=78) [92]; (b-f) premenopausal women using progestin-only contraceptives (b: Mirena, N=86 [93]; c: Implanon, N=11 [94]; d: Jadelle, N=88 [95]; e: NET-EN, N=73 [23]; f: DMPA, N=31) [96]; and normal postmenopausal women (g, N=1446) [97]. Dashed bars indicate 95% confidence intervals and solid bars standard deviation.


600
500
400
300
200
100
0
Circulating estradiol pmol/L
a, Luteal phase
b, Mirena
c, Implanon
d, Jadelle
e, NET-EN
f, DMPA
g, Postmenopausal