A review on HIV transmission in the female reproductive tract and preformulation considerations for intravaginal drug delivery of prevention technologies

.

|  |
| --- |
| **ABSTRACT:**The female reproductive tract consists of several anatomical and physiological features that present high risk for HIV and sexually transmitted infections in women.Clinical and epidemiological research has shown significant variation in risk of transmission and co-infections for different age groups and pregnancy status.Around 63% of all newly acquired HIV infections are in women aged 15-24 years and access to prevention product options that are safe, user friendly and comfortable remains low. Design and development of intravaginal drug delivery products provides better option for discreet and user-friendly methods for HIV prevention in women. Products that have good bioadhesive properties, optimal mucosal penetration, controlled release, high efficacy in inflamed vaginal conditions can improve HIV prevention in this target group. Key preformulation considerations must be taken to achieve optimal delivery of safe microbicides via the vaginal route and to further development of products in this category. |

*Keywords: microbicides, HIV transmission,* *female reproductive tract, cervicovaginal mucus*

1. INTRODUCTION

Human immune deficiency virus (HIV) acquired through heterosexual transmission remains a challenge to female reproductive health for lower middle-income countries remains particularly in the age ranges of 15-24 years (1). In sub-Saharan Africa, adolescent, and young women have at least three times higher infection rates than the male counterparts. On average, 4,000 adolescent girls and young women acquire HIV every week, only 42% of districts with an inflated HIV incidence in Sub-Saharan Africa are currently covered with dedicated prevention programs for adolescent girls and young women (1,2).

Clinical research has proven the correlation between increased risk of co-infection of HIV and STIs, HIV transmission due to vaginal lesions and viral shedding, HIV and cervical cancer, fertility and HIV mother to child transmission during pregnancy (3). The objective to end the pandemic remains clear and with it comes opportunity for innovation and technology, not only in treatment, but in multipurpose prevention platforms that can reduce new HIV infections and address reproductive healthcare needs for women.

The introduction of anti-retroviral drugs for prevention and treatment has reduced infection rates and improved survival of HIV/AIDS patients, however drug resistance, low adherence due to adverse effects, poor biopharmaceutical properties of traditional formulations, social and cultural context that women exist in have been a challenge in curtailing the pandemic. Long acting injectables for prevention have been developed with inhibitory high product cost (>$40,000.00 USD/year) requiring donor aid subsidy, systemic side effects and use of an invasive route of administration. HIV vaccines and broad neutralizing antibodies are also in the pipeline.

In this paper we review the role of the female reproductive tract in risk and protection for HIV infections, different antiretroviral drug classes and their potential as novel dosage forms for prevention of HIV heterosexual transmission, co-infections, and the critical considerations in preclinical work required in translating benchtop products to clinical use. Numerous studies have shown women to opt for prevention products designed and suited for personal use without consent of a sexual partner (4–6). This has led to much interest in the development of microbicides for vaginal delivery.

The vaginal route has several distinct advantages for localised or systemic drug delivery. With a large surface area, vasculature, a lymphatic drainage system and the ability to bypass the first pass effect makes it a valuable route for drug delivery (5,7,8). When products are formulated for this route, design considerations have to be made, these include the drug release profile (immediate/controlled), compatibility with vaginal microbiota, environment pH, small volume, anatomical space, safety and tolerability of drug formulation as well as excipients of choice used in product formulation (8,9).

The pattern from successful programs that reduced infection rates caused by sexual transmission are proof that targeted interventions drive the incidence rates down (6). Reduction in discrepancy of newly acquired infections between genders of the 15 - 24-year-old age group, is possible by developing appropriate products (6). For women in Sub-Saharan Africa, what remains of great importance in pre- and post-exposure prophylaxis is the opportunity to reduce the rate of newly acquired HIV infection and the availability to select a product of their choice.

**2. HIV infections and the female reproductive tract**

This section will review the different factors that increase the risk of transmission of HIV and co-infections in the female reproductive tract and implications to topical microbicide product design. The main driver for HIV transmission is mucosal exposure which accounts for over 80% of all infections with 40% of the infections occurring in the female reproductive system (3,10,11). Women are still a high-risk group for new HIV infections in rates higher than male counterparts and this presents an opportunity for the development of tailor-made prevention products. The specific steps and mechanisms that result in successful HIV infection following exposure in the female genital tract are still a grey area requiring research. Several models have been used to further understand HIV route of transmission and studies in non-human primates have proved that infection can occur in the upper or lower female genital tract (3,12).

Several physiological and anatomical features exist that regulate reproductive life cycle, immunobiology and hormonal regulation, growth and maturity of the cervix and epithelial cells at different ages all put women at high risk for HIV transmission and will be briefly discussed in this section.

 

**Squamous epithelium Columnar epithelium**

**Squamocolumnar junction**

**Fig 1: The female reproductive tract** (Atlas of visual inspection of the cervix with acetic acid for screening, triage, and assessment for treatment, June 2025(13)

Epithelial cells in the reproductive tract are the primary route for HIV and other infections with differences in structures of the tissue at different parts of the reproductive tract (3,14). The lower tract consists of stratified squamous cervical and vaginal epithelium 25-cell layers or thicker. The epithelia for the upper reproductive tract are made up of endocervix, uterus, fallopian tubes and endometrium is columnar, consisting of a single layer of cells that secrete mucus and are a natural defence against pathogens and potential infections (3). The columnar epithelium tissue is characterized by tight junctions between cells that makes it impermeable to entry of any large molecules and particulate matter, including pathogens (3,14,15). The thick stratified epithelium of the lower reproductive tract, although not impermeable, is robust and provides a substantive physical barrier compared to the delicate single layer of columnar epithelium of the upper reproductive tract (3,16).

The section where the squamous and columnar epithelium meet is described as the transformational zone. In addition to the physical barrier innate immune response to infections apart will also include mucus and antimicrobial peptides that are a form a chemical and biological barrier, and the Lactobacillus-rich vaginal milieu creates a biological barrier (3,14). The mucus which is mainly secreted from the endocervical epithelium has thick gel like consistency and contains electrolytes, mucin associated fatty acids and cellular debris from dead cells or material cleared by the neutrophils (14,15).

The lower female reproductive tract has a permeable thick stratified epithelium which forms a substantive physical barrier compared to the delicate single layer of columnar epithelium of the upper reproductive tract (3). Through the continuous sloughing of the superficial layers of the stratified epithelium of the vagina and ectocervix this process prevents many pathogens from colonizing and establishing infections, thus providing a better mechanical protection against HIV invasion than the single layer columnar epithelium that lines the upper reproductive tract (11,12,14). However, the greater surface area of the vaginal wall and ectocervix arguably allows greater access for HIV entry, particularly when breaches occur in the epithelium, such as during sexual intercourse.

The changes in morphology, physiological function and immunological responses in the female reproductive tract in adolescent (10-19 years), reproductive (15-49 years) and menopause ages (45-55 years) affect the risk of HIV acquisition and have an implication on product design.

**2.1 Adolescent age group:**

The risk of HIV transmission for women in this age group is 4-7 times higher for Sub-Saharan Africa compared to male counterparts. Cervical ectropion known as ectopy or eversion commonly occurs in adolescents were erythrocytes in the endocervix appear on the outer surface of the ectocervix (10). Several studies have shown that it is not independently associated with the acquisition of HIV infections, though prevalence of HIV is higher in adolescent girls with cervical ectopy (10,14). The condition is associated with different sexually transmitted infections including human papilloma virus and gonorrhoea. The STIs can possibly alter cervical tissue function. The prevalence of ectopy is higher in female adolescent populations that haven’t engaged in sexual intercourse and places them at a greater risk of infection from HIV and STIs (10,17).

The mature cervix for women consists of stratified squamous multiple cell layers that provide a physical barrier for protection against pathogens. The single-layer of columnar epithelium in adolescents is easier to breach during sexual intercourse, which can allow the entry pathogens.

The presence of elevated levels of inflammatory cytokines in adolescent girls compared to adult females is also linked to a higher risk of HIV transmission. The chemokines IL-6 and TNF-α contribute to genital inflammation creating conditions favourable for HIV acquisition and replication. MIP-1α and MIP-1β promote recruitment of CCR5 expressing cells to the genital tract including T-cells and macrophages which are targets for HIV virus (10,15). Previous studies have shown that higher pro-inflammatory biomarker TNF-α, as well as a distinct inflammation-associated immune clustering in sexually inactive adolescent girls, can potentially increase risk for infections including HIV upon sexual debut (10–12).

Literature on the level of antimicrobial peptides in this age group is limited, though they are important for immunomodulation, maintenance of tissue homeostasis, protection against pathogens among other roles they play in protecting the female reproductive tract (15). What is clear is that vaginal secretions vary with age increasing during reproductive age and lower before puberty and post menopause due to hormonal (estrogen) changes, pregnancy and local pathological states that may affect the level of antimicrobial peptides(15,17).

**2.2 Reproductive age and the menstrual cycle**

The changes in estradiol and progesterone hormonal levels during menstrual cycles affect immune function and regulation in the female reproductive tract. Several studies suggest highest risk of HIV transmission in the luteal phase of the cycle especially after ovulation while some research has also shown high transmission risk in the first half of the cycle due to accumulation of immune cells susceptible to HIV (10,17,18). Sex hormones play a role in increasing migration and response of immune cells and the expression of adhesion molecules and chemotactic factors. Epithelial cells, in addition to providing barrier protection, transport immunoglobulins (IgA and IgG) into FRT secretions, and produce antimicrobials that are both bactericidal and viricidal (12,18).

Research done in this age group has proven the role of several AMPs, Macrophage Inflammatory Protein including (MIP)-3α, RANTES (regulated upon activation, normally T-cell expressed and secreted), human beta defensin (HBD)-2, elafin, and several other immune mediators to be protective against HIV infections (17).

**2.3 Pregnancy**

The research done on large populations on pregnant and non-pregnant women and risk for HIV acquisition showed no substantial variations (17). Physiological changes occur during pregnancy to the physical, chemical and biological barriers in the FRT that are potential risk factors. AMPs play an important role in implantation, preventing pathogen infections and inflammation during pregnancy. The cervical epithelial cells play an important role in ensuring a physical and immunological barrier to infection is provided for the upper reproductive tract(10,17) . The cells will constantly undergo changes during each trimester of pregnancy.

Cooley, Anne et al. 2023 studied a series of images over a period of time to track changes in the structure of the cervical epithelia during pregnancy. From estrus cycle to 19 days in the first trimester significant changes in the stratified epithelium occur in the proliferation, differentiation, and apoptosis biological processes and an increase in the secretory epithelia (19).

During pregnancy, cell morphology and proliferation are similar between endo and ectocervix while cell death occurs primarily in the endocervix. If the physical barrier of the epithelial layer is damaged during pregnancy or vaginal mucus permeability increases risk for infection increases (10,19).

In vitro studies using TZM-bl assays have shown HIV inhibitory effect of cervicovaginal mucus secretions for pregnant and non-pregnant females, and anti-HIV activity did change between trimesters in pregnant women with the same assay (20).

**2.4 Physiological role of lactobacilli in the vaginal microenvironment**

Lactobacilli have different function it plays as microbiota in the vaginal microenvironment from prevention of local infections to reproductive roles in conception and pregnancy (21).The microbiota is commonly dominated by Lactobacilli species at low pH (<4.5) which exhibit probiotic effect and are part of the natural defence against reproductive pathogens like Chlamydia trachomatis, herpes simplex virus (HSV-2) and HIV-1 (21,22). Variable amounts of lactic acid, biosurfactants, bacteriocin-like chemicals, and hydrogen peroxide are produced by various strains of the lactobacilli species that help prevent proliferation of pathogens and maintain an acidic vaginal microenvironment for a normal microbiota (22).

The balance of vaginal flora can be affected by multiple factors including age, hormonal changes, smoking, use of oestrogen-based contraceptives, diabetic conditions and menstrual flow in women. The most commonly occurring vaginal infection due to change in vaginal flora composition in women of reproductive age is Bacterial vaginosis (BV) with an incidence rate of 15% to 50% (21,22). Half of BV patients show no symptom; clinical symptoms of BV include a burning feeling during urination, itching around the outside of the vagina, and an increased vaginal pH (>4.5), abnormal color of vaginal discharge, and an unpleasant fishy odor (21). The infection reduces the effectiveness of the physical barrier and innate immunity leading to an increased risk of acquisition of HIV and other STIs through sexual intercourse.

**3. Intravaginal drug delivery for HIV Prevention**

Localised vaginal delivery of topical microbicides with PrEP agents offers a great opportunity for non-systemic products that can be absorbed and distributed into vaginal epithelium. Though physical, biological and chemical barrier of the female reproductive tract are important for protection from pathogens, they pose a significant challenge to intravaginal delivery and efficacy of anti-HIV molecules and other modalities used on multipurpose platforms to prevent pregnancy, STIs and other infections (23,24). Furthermore, for antiretroviral drugs the window phase is highly depended on the class of molecules, and physicochemical properties of the drug affect penetration of the cervicovaginal mucus, and drug delivery at site of interest as this affects efficacy and HIV acquisition (25).



**Fig. 2: HIV infection timeline and protection of different ARV drug classes** adapted from Miller and Shattock, (2003)(25)

The vaginal wall transudate, vulva secretions, exfoliated epithelial cells, and secretions from bacterial flora collectively form cervicovaginal fluid together with cervical mucus form the cervicovaginal mucus (CVM) (26). The composition and physical of the CVM affect drug penetration, permeability, distribution and retention within the vaginal epithelium which also serves as physical barrier with innate immunity mechanism (23,26).

Data from preclinical and clinical work done in developing anti-HIV drug agents has shown several potential sites that can be targeted by small or large molecules in the inhibition of the retrovirus replication (25). Antiretrovirals can block one or several steps in the viral lifecycle. Understanding mechanism of transmission, target tissue and cells for virus, viral resistance and time to successful infection is important for the development of appropriate microbicides (27).

Drug resistance may occur but is currently low for those diagnosed taking oral prevention pills tenofovir and lamivudine containing preparations. Long acting cabotegravir injection has a few emerging cases of cross drug resistance among patients on HIV prophylaxis with integrase inhibitors in the same class (28). Dapivirine vaginal ring a non-nucleoside reverse transcriptase inhibitor showed low risk of transmission for drug resistant HIV for breakthrough infections occurring among PrEP users in a phase 3 study (29).

 

**Fig. 3: HIV life cycle and potential targets for inhibition in drug development** (30)

Vaginal drug delivery offers an opportunity of controlled drug delivery that is discreet, economical, user friendly, good quality with the option for multipurpose prevention products that can not only prevent HIV infection but STIs, cervical cancer and/or pregnancy. Compared to traditional oral drug administration the potential to improve adherence, overcome social barriers to using PrEP, reduce systemic side effects while improving efficacy and more importantly giving end user better options for their choice of prevention product to use (5). Several product types have been developed from preclinical stage to clinal use including intravaginal rings, tablets, fast dissolving inserts, hydrogels and polymeric films.

To enhance drug delivery with various dosage forms, APIs are incorporated or fabricated as nanoparticles. Based on previous research work done the ideal target product profile for NPs that can improve drug retention, reduce clearance through opsonisation and increase bio-adhesion in the vaginal lumen is shown in the table 1 and 2 (31,32).

Multiple considerations should be made in the product design of a microbicide as summarised in table 1.

**Table 1: A summary of considerations to be made in formulation of microbicides for HIV prevention**

|  |
| --- |
| Product formulation, physicochemical characterization of a topical microbicide in vitro, in vivo and ex vivo testing (7,8,32,33)  |
| Component | **Equipment required and/ rationale** |
| Preformulation characterization | To understand and define the API, selection criteria for vaginal drug delivery and HIV prevention. Analytical method development (HPLC and LCMS), excipient and API compatibility. Thermal analytical methods using DSC for crystallinity and thermal behaviour. |
| Formulation development | Dosage form selection, Design of Experiment and process optimization, target product profile |
| In vitro drug release studies | Different equipment setup depending on dosage form type IV dissolution usually employed and biorelevant media as per USP guide. To determine and optimize drug release as target product profile |
| Physicochemical and mechanical properties | Rheometer (Viscosity determination), texture analysis, mucoadhesive properties  |
| In vitro, in vivo and ex vivo efficacy and toxicity testing (free drug vs drug loaded carrier vs blank carrier vs control drug vs media) | - To determine in vivo toxicity of products administered intravaginally to non-human primates (NHP) and 24 hours histopathological exams are carried using high power microscopes with a digital camera- drug retention and distribution in vaginal lumen using ex vivo NHP model - drug-cell association determination using fluorescent activated cell sorting (FACS) was used to assess particle-cell associations and cell-specific particle distribution on ex vivo vaginal animal tissue - Retention profile of microbicide in reproductive tissue (ex vivo animal model) |
| Lactobacillus compatibility | Microbicide safety test with nonoxynol-9 control |
| Product efficacy studies | against HIV-1BaL in the TMZ-bl antiviral in vitro assay |
| Ex vivo permeability (ADC vs free drug vs antibody) | Comparative product permeability study using the Ex-Vivo Ectocervical Tissue Model in an In-Line Set-Up. Reduce use of animal models. |
| in vivo pharmacokinetics in rodent model | Confirm safety and pharmacokinetic profile of product. Alternative to NHP when an existing molecule on the market is repurposed from treatment to prevention. |
| Accelerated Stability testing | ICH compliant stability chambers |

**Table 2: Target product profile for nanoparticles used in vaginal delivery** (31,32)

|  |  |  |
| --- | --- | --- |
| Parameter  | Value  | Importance |
| Particle size and morphology | (100 – 200 nm); spherical to near spherical shape | Improved penetration and retention in the CVM. |
| Surface charge | Close to neutral, slightly negative or positive charge | Improved interaction with vaginal mucosa and retention of drug. |
| pdI |  pdI (<0.15) | Narrow particle size range to assure uniform drug distribution per dosage unit |
| Encapsulation efficiency | >60% | Higher payload and smaller dosage units |
| Polymer properties | Biocompatabile, degradable and bioadhesive  | Retention of drug, penetration and slow release. Safety and clearance from the reproductive system |

4. Conclusion

The need to develop prevention products for the female population can never be overstated. Gaps still exist in knowledge of the biological, chemical and physical roles that the female reproductive tract plays for fertility, protection from pathogens and cell regulation at different ages and hormonal cycles throughout the life of an individual. HIV infections and transmission rates remain higher in the adolescent to young adult age groups where risk factors tend still remain unclear from a clinical perspective. Cervical ectopy, early sexual debut, elevated levels of inflammatory cytokines and changes in cervical epithelia all play role in increased risk of HIV acquisition.

Potential targets for drugs in the HIV life cycle exist, but the infection timeline from crossing the vaginal lumen to infection of regional lymph nodes and systemic circulation of the virus gives a prevention window from hours to a few days in which they may have greatest efficacy. These considerations are important in product design and selection of the active pharmaceutical ingredient. The ability to penetrate cervicovaginal mucus and reduce rate of clearance is also important in developing a microbicide. A rational approach to formulation development process, understanding the pharmacokinetics antiviral activity, mucoadhesion, safety, toxicity and patient acceptability all have an impact on product uptake and therapeutic efficacy.

**Study HIGHLIGHTS:**

* Natural barriers (biological, chemical and physical) to sexually transmitted infections
* The different factors that increase the risk of transmission of HIV and co-infections in the female reproductive tract
* Opportunities and challenges for intravaginal drug delivery in HIV prevention technologies
* Preformulation and formulation methods for vaginal dosage forms designed for HIV prevention

**Disclaimer (Artificial intelligence)**

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

Option 2:

Author(s) hereby declare that generative AI technologies such as Large Language Models, etc. have been used during the writing or editing of manuscripts. This explanation will include the name, version, model, and source of the generative AI technology and as well as all input prompts provided to the generative AI technology

Details of the AI usage are given below:

1.

2.

3.

References

1. UNAIDS. UNAIDS 2023 epidemiological estimates, UNAIDS [Internet]. UNAIDS; 2023 [cited 2024 Apr 16]. Available from: https://thepath.unaids.org/wp-content/themes/unaids2023/assets/files/2023\_report.pdf

2. van Schalkwyk C, Mahy M, Johnson LF, Imai-Eaton JW. Updated Data and Methods for the 2023 UNAIDS HIV Estimates. J Acquir Immune Defic Syndr 1999. 2024 Jan 1;95(1S):e1–4.

3. Kaushic C. HIV-1 Infection in the Female Reproductive Tract: Role of Interactions between HIV-1 and Genital Epithelial Cells. Am J Reprod Immunol. 2011 Mar 1;65(3):253–60.

4. Cutler B, Justman J. Vaginal microbicides and the prevention of HIV transmission. Lancet Infect Dis. 2008 Nov;8(11):685–97.

5. Baeten JM, Hendrix CW, Hillier SL. Topical Microbicides in HIV Prevention: State of the Promise. Annu Rev Med. 2020 Jan 27;71:361–77.

6. Irungu E, Khoza N, Velloza J. Multi-level Interventions to Promote Oral Pre-exposure Prophylaxis Use Among Adolescent Girls and Young Women: a Review of Recent Research. Curr HIV/AIDS Rep. 2021 Dec;18(6):490–9.

7. Gosecka M, Gosecki M. Antimicrobial Polymer-Based Hydrogels for the Intravaginal Therapies-Engineering Considerations. Pharmaceutics. 2021 Sep 2;13(9).

8. Kramzer LF, Hamorsky KT, Graebing PW, Wang L, Fuqua JL, Matoba N, et al. Preformulation Characterization of Griffithsin, a Biopharmaceutical Candidate for HIV Prevention. AAPS PharmSciTech. 2021 Feb 24;22(3):83.

9. Leyva-Gómez G, Piñón-Segundo E, Mendoza-Muñoz N, Zambrano-Zaragoza ML, Mendoza-Elvira S, Quintanar-Guerrero D. Approaches in Polymeric Nanoparticles for Vaginal Drug Delivery: A Review of the State of the Art. Int J Mol Sci. 2018 May 23;19(6).

10. Rodriguez-Garcia M, Connors K, Ghosh M. HIV Pathogenesis in the Human Female Reproductive Tract. Curr HIV/AIDS Rep. 2021 Apr 1;18(2):139–56.

11. Monin L, Whettlock EM, Male V. Immune responses in the human female reproductive tract. Immunology. 2020 Jun 1;160(2):106–15.

12. Byrareddy SN. Immune landscape of female reproductive tract and HIV susceptibility. eBioMedicine [Internet]. 2021 Aug 1 [cited 2025 Jul 17];70. Available from: https://doi.org/10.1016/j.ebiom.2021.103497

13. Atlas of visual inspection of the cervix with acetic acid for screening, triage, and assessment for treatment [Internet]. [cited 2025 Jul 24]. Available from: https://screening.iarc.fr/atlasviadetail.php?Index=14&e=

14. Shen R, Richter HE, Smith PD. Interactions between HIV-1 and Mucosal Cells in the Female Reproductive Tract. Am J Reprod Immunol. 2014 Jun 1;71(6):608–17.

15. Yarbrough VL, Winkle S, Herbst-Kralovetz MM. Antimicrobial peptides in the female reproductive tract: a critical component of the mucosal immune barrier with physiological and clinical implications. Hum Reprod Update. 2015 May 1;21(3):353–77.

16. Calenda G, Villegas G, Reis A, Millen L, Barnable P, Mamkina L, et al. Mucosal Susceptibility to Human Immunodeficiency Virus Infection in the Proliferative and Secretory Phases of the Menstrual Cycle. AIDS Res Hum Retroviruses. 2019 Mar 1;35(3):335–47.

17. Hughes BL, Dutt R, Raker C, Barthelemy M, Rossoll RM, Ramratnam B, et al. The impact of pregnancy on anti-HIV activity of cervicovaginal secretions. Am J Obstet Gynecol. 2016 Dec 1;215(6):748.e1-748.e12.

18. 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. 2014 Aug 1;72(2):236–58.

19. Cooley A, Madhukaran S, Stroebele E, Caraballo MC, Wang L, Hon GC, et al. Dynamic states of cervical epithelia during pregnancy and epithelial barrier disruption. bioRxiv. 2022 Jan 1;2022.07.26.501609.

20. Anderson BL, Ghosh M, Raker C, Fahey J, Song Y, Rouse DJ, et al. In vitro anti-HIV-1 activity in cervicovaginal secretions from pregnant and nonpregnant women. Am J Obstet Gynecol. 2012 Jul;207(1):65.e1-10.

21. Amabebe E, Anumba DOC. The Vaginal Microenvironment: The Physiologic Role of Lactobacilli. Front Med [Internet]. 2018;Volume 5-2018. Available from: https://www.frontiersin.org/journals/medicine/articles/10.3389/fmed.2018.00181

22. Pendharkar S, Skafte-Holm A, Simsek G, Haahr T. Lactobacilli and Their Probiotic Effects in the Vagina of Reproductive Age Women. Microorganisms. 2023;11(3).

23. Patil P, Bhopale P, Saudagar RB. Intravaginal Drug Delivery System: Compherensive Approach to Vaginal Formulations. J Drug Deliv Ther. 2019 Sep 15;9(5):171–4.

24. Rafiei F, Tabesh H, Farzad S, Farzaneh F, Rezaei M, Hosseinzade F, et al. Development of Hormonal Intravaginal Rings: Technology and Challenges. Geburtshilfe Frauenheilkd. 2021 Jul;81(7):789–806.

25. Miller CJ, Shattock RJ. Target cells in vaginal HIV transmission. Microbes Infect. 2003 Jan;5(1):59–67.

26. Sanchez Armengol E, Veider F, Millotti G, Kali G, Bernkop-Schnürch A, Laffleur F. Exploring the potential of vaginal drug delivery: innovations, efficacy, and therapeutic prospects. J Pharm Pharmacol. 2025 Jun 26;rgaf045.

27. Garg A, Nuttall J, Romano J. The Future of HIV Microbicides: Challenges and Opportunities. Antivir Chem Chemother. 2009 Feb 1;19:143–50.

28. Parikh UM, Koss CA, Mellors JW. Long-Acting Injectable Cabotegravir for HIV Prevention: What Do We Know and Need to Know about the Risks and Consequences of Cabotegravir Resistance? Curr HIV/AIDS Rep. 2022 Oct;19(5):384–93.

29. Nel A, van Niekerk N, Van Baelen B, Malherbe M, Mans W, Carter A, et al. Safety, adherence, and HIV-1 seroconversion among women using the dapivirine vaginal ring (DREAM): an open-label, extension study. Lancet HIV. 2021 Feb 1;8(2):e77–86.

30. NIAD. HIV Replication Cycle [Internet]. NIAD; 2018 [cited 2024 Apr 16]. Available from: https://www.niaid.nih.gov/diseases-conditions/hiv-replication-cycle

31. Iqbal Z, Dilnawaz F. Nanocarriers For Vaginal Drug Delivery. Recent Pat Drug Deliv Formul. 2019;13(1):3–15.

32. Tong X, Patel SK, Li J, Patton D, Xu E, Anderson PL, et al. Development and Evaluation of Nanoparticles-in-Film Technology to Achieve Extended In Vivo Exposure of MK-2048 for HIV Prevention. Polymers. 2022 Mar 16;14(6).

33. Kumar A, Valamla B, Thakor P, Chary PS, Rajana N, Mehra NK. Development and evaluation of nanocrystals loaded hydrogel for topical application. J Drug Deliv Sci Technol. 2022 Aug 1;74:103503.