CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

http://doi.org/10.5281/zenodo.1489183

Available online at: http://www.iajps.com Research Article

INFLUENCE OF ANTIRETROVIRAL THERAPY DURATION HIV RNA SHEDDING IN SEMEN

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Abstract:

The semen of an HIV-infected man is the most widely recognized method of HIV transmission. Highly Active Antiretroviral Therapy (HAART) frequently results in an imperceptible blood HIV RNA viral load (VL), and it has been proposed that there might be no danger of sexual transmission in this specific situation. Be that as it may, the effect of viable HAART on HIV levels in semen requires additionally consider. We played out an imminent, longitude semen of an HIV-contaminated man is the most widely recognized method of HIV transmission. Highly Active Antiretroviral Therapy (HAART) regularly results in an imperceptible blood HIV RNA viral load (VL), and it has been proposed that there might be no danger of sexual transmission in this unique situation. Be that as it may, the effect of viable HAART on HIV levels in semen requires additionally think about. We played out a forthcoming, longitudinal investigation of semen and blood HIV RNA levels after HAART commencement and a cross-sectional examination in men on long-haul viable HAART. Compelling HAART was characterized as the accomplishment of an imperceptible blood VL. udinal investigation of semen and blood HIV RNA levels after HAART commencement, and a cross-sectional examination in men on long-haul powerful HAART. Viable HAART was characterized as the accomplishment of an imperceptible blood VL. These findings demonstrate that neither systemic nor mucosal HIV-specific CD8+ responses, when assayed with IFN production as an endpoint, were associated with reduced HIV RNA levels in blood or semen. Semen HIV RNA levels did correlate with local inflammatory cytokines and CMV reactivation. Furthermore, despite effective HAART a significant proportion of HIV-infected men continued to shed HIV RNA in semen.

Keywords: *Effective antiretroviral therapy, HIV sexual transmission, genital HIV levels*

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Please cite this article in press Maryam Tariq o et al., ***Influence Of Antiretroviral Therapy Duration HIV RNA Shedding in Semen.,*** *Indo Am. J. P. Sci, 2018; 05(11).*

INTRODUCTION

There were an estimated 2.5 million new HIV infections in 2007, and most resulted from sexual contact with the semen of an infected man[37]. The level of HIV RNA in blood is a critical determinant of the probability of sexual HIV transmission, likely because this tends to correlate with virus levels in the genital tract. Highly Active Antiretroviral Therapy (HAART) will often suppress both blood[244] and genital[135] HIV RNA levels to below the limit of detection, and therefore may reduce HIV transmission at a population level. Based on these observations, it has recently been stated that individuals receiving effective HAART (i.e.: those with an undetectable blood HIV RNA viral load (VL) on therapy) are sexually non-infectious [1-5].

The latter statement has caused controversy in the HIV prevention field[6]. A model-based analysis demonstrated the potential for increased HIV incidence in the absence of condom use, particularly among men who have sex with men, despite substantial and consistent reductions in the genital VL on HAART. In addition, the impact of therapy on genital tract virus levels appears to be quite heterogeneous, since semen HIV shedding may be present despite HAART. While the mechanisms for this phenomenon are poorly defined, discordant HAART effects in blood and semen could include compartmentalization of drug-resistant viral strains in the genital tract, incomplete semen penetration of antiretroviral drugs, or genital mucosal inflammation resulting from localized co-infections or other causes. Based on these concerns, the Joint United Nations Programme on HIV/AIDS (UNAIDS) has stated that "more research is needed to determine the association between the viral load in blood and the viral load in semen[6-11]". Our results strongly suggest that, while HAART is likely to reduce HIV sexual transmission at a population level, individual counselling must continue to emphasize the importance of safe sex.

METHODS

HIV Acquisition and the Genital Mucosa

The majority of HIV is transmitted sexually as HIV infected individuals tend to shed cell free (HIV RNA) and cell-associated viral particles in their genital secretions. Even though not all the potential sites of entry or their relative contributions to HIV transmission or acquisition are known, understanding the immune-pathogenesis of HIV transmission is pivotal in the development of more targeted and effective prevention interventions. Below is an overview of some of the known mechanisms utilized by HIV to enter and establish infection across the genital and rectal mucosa[12-20].

The Female Genital Tract

Between 30% and 40% of all new HIV infections are acquired across the female genital mucosa[1]. Therefore, a great deal of work has been done to better understand how the anatomy of the female genital tract relates to HIV acquisition at this site. As in vivo human studies are difficult to perform, several alternative models; including the use of non-human primate models, *ex vivo* and *in vitro* organ culture systems using cervicovaginal tissues obtained from human surgical samples have been used.

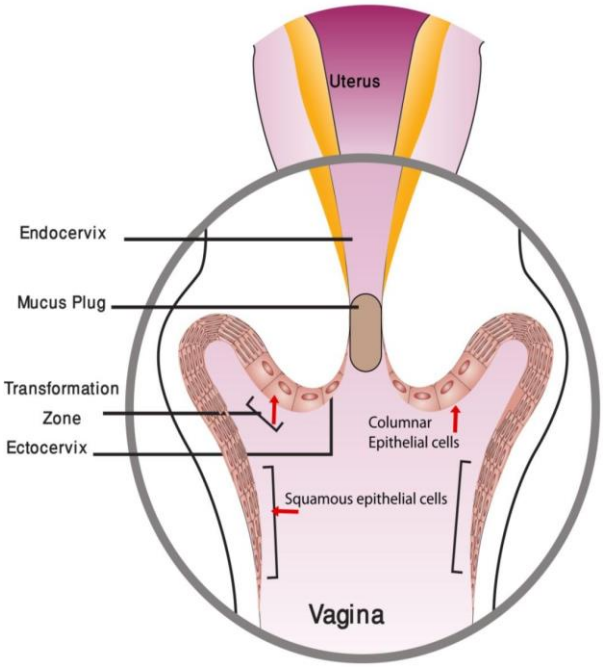
The lower female genital tract consists of the vagina, ectocervix and the endocervix, all of which have very different anatomic and physiological properties. Although the relative risk(s) of acquiring HIV at the various anatomical sites in the FGT remains unclear, it is generally believed that the majority of HIV is acquired through the endocervix. Unlike the endocervix, which is lined with a thin mono-layer of columnar epithelial cells, the vagina and the ectocervix have multilayered squamous epithelial cells (between 20 and 45 cells thick) lining the outside, which offers much better protection from pathogens including HIV. However, the vagina and ectocervix are prone to trauma, particularly micro-abrasions during sexual intercourse, which may compromise the patency of the epithelial lining and provide a portal of entry for HIV [21-28].

Despite the thick lining of squamous epithelial cells surrounding the vaginal mucosa, vaginal acquisition of HIV has been demonstrated in vitro in the absence of sexual trauma and/or microabrassions. One possible mechanism for viral entry is hypothesized to be through specialized antigen presenting cells located in the vaginal mucosa and the skin called Langerhan cells (LC‟s). LC‟s are capable of directly binding HIV, and are found in abundance in the vaginal epithelial layers. LC‟s, like other dendritic cells, are immune sentinels that monitor the periphery often extending their processes across epithelial tight junctions into the vaginal lumen[29-35]. Upon antigen recognition the vaginal LC‟s home to the draining iliac lymph node and activate CD4+ and CD8+ T cells. Several in vitro studies using human tissue and in vivo studies carried out in the SIV model of infection at least two pathways of HIV-DC interaction have been

suggested. HIV can either infect LC through a CD4-CCR5 mediated pathway or be internalized by LC through DC-SIGN or other C-type lectins, which facilitate binding and internalization of HIV[130-132]. Upon activation LC's migrate to draining lymph nodes, which are rich in HIV target cells thereby resulting in systemic dissemination of HIV. Although the role of LC in mediating HIV entry is not clear, it represents a potential mechanism of entry in the absence of vaginal trauma [36-44].

The endocervix is located proximal to the ectocervix in the female genital tract and is covered by a thin monolayer of simple columnar epithelial cells separating the lamina propria and the sub-mucosal layer from the cervical lumen. Although, it is unclear if the endocervix comes into direct contact with HIV following sexual intercourse, the endocervix presents an ideal site for HIV infection to occur as the cervical sub-mucosa is rich in CD4+ cells, particularly the transformation zone. The transformation or transitional zone is a region of the cervix where the epithelial lining transitions from a thin layer of simple columnar cells (cervix) to the thicker stratified squamous epithelial cell lining (vagina)[135]. The increased risk of acquiring HIV (and other sexually transmitted infections) observed in adolescent women may be partly because premenarchal women have an enlarged transformation zone that extends into the vagina, which provides a larger surface area for contact with HIV during sexual intercourse[138]. The single mono-layer of columnar cells of the transformation zone makes it particularly susceptible to HIV as it affords little protection to the target cells (CD4+ T cells and macrophages) in the sub-mucosa[45-55].

Although the epithelium in the cervix is not as thick as that of the vagina, endocervical epithelial cells are covered by a layer of mucus, which may serve to immobilize viral particles and prevent contact/entry with epithelial cells. Alternatively, mucous trapped virions may increase the exposure time of HIV to the cervical mucosa and therefore enhance HIV transmission at this site [56-70].



Potential sites of HIV entry in the female genital tract.

Several potential sites of HIV entry in the female genital tract have been described and the above figure highlights the vaginal epithelium, the transformation zone and the cervix, all of which represent potential sites of HIV entry (designated by red arrows). The schematic illustrates the differences in the epithelial architecture at each anatomical site[71-79].

The Male Genital Tract (MGT)

Although the surface area of the MGT exposed to virus is much smaller than the FGT, almost 70% of HIV infections in adult men are acquired through the penis, specifically the penile foreskin. One of the earliest practices to protect men against sexually transmitted infections (e.g. syphilis) was the removal of the penile foreskin. Circumcision has been shown to reduce rates of some but not all STI's[147], with the most dramatic reduction observed in HIV acquisition through the penis[148]. The earliest suggestions that circumcision reduced the risk of acquiring HIV came from ecological studies in Africa where countries and regions, with similar sexual practices had very different rates of HIV prevalence. The most striking evidence was from Kenya, where HIV seroprevalence rates were four-times higher in western Kenya (Kisumu), where <20% of men are circumcised, compared to the rest of Kenya where >80% of men are circumcised[15, 45].

Observations suggesting the potential link between circumcision and risk of HIV acquisition provided the impetus to conduct randomized clinical trials to investigate the relationship between circumcision and HIV. Large trials in both the Rakai district of Uganda and the Kisumu district in Western Kenya have recently demonstrated that circumcision reduced HIV acquisition rates by 50 – 60%.

The increased risk of acquiring HIV through the foreskin is because the inner foreskin is lined with a thin layer of poorly keratinized epithelial cells, while the outer foreskin is covered with a thick layer of highly keratinized epithelial cells providing an almost impenetrable barrier to HIV. Detailed histological studies of adult foreskins have suggested that although the inner and outer foreskin contain similar concentrations and numbers of CD4+ T cells co-expressing CCR5 and CXCR4, the inner foreskin contains more superficial Langerhan DC‟s[144, 152]. The thin layer of keratin over the epithelial monolayer and the superficial Langerhan DC‟s make the inner foreskin particularly susceptible to HIV[152], as these cells are prone to infection and contain surface receptors that support viral attachment[130]. Similar to the FGT, after activation LC‟s home to local draining lymph nodes, which are rich in potential target cells for HIV including macrophages and CD4+ T cells. It is important to note that although the relative risk of acquiring HIV is much lower in circumcised men compared to their uncircumcised counterparts, it is still possible for circumcised men to acquire HIV through the penis, primarily through the urethera and penile salcus, and so safe-sex practices should always be followed during sexual encounters.

Source of HIV RNA shed in Genital secretions

Sexual transmission of HIV occurs because HIV infected men and women shed HIV in their genital secretions[65, 66]. The source(s) of HIV RNA that is shed in genital secretions of men and women are not entirely clear. Current evidence suggests that the majority of HIV shed in the female genital secretions originates primarily from the cervix and the upper genital tract, although HIV RNA has been measured in the genital secretions of women that have undergone a hysterectomy. So, even though the majority of HIV RNA shed in female genital secretions may originate from the cervix, other sites must contribute to HIV shedding in the genital secretions of HIV-infected women.

Similarly, the source of HIV in the MGT has been difficult to pin-point, as the MGT is not accessible to direct sampling. However, studies carried out in HIV infected men suggest that the majority of cell free HIV RNA shed in semen plasma originates from the prostate and the bulbo-uretheral gland. These sites are distal to the seminiferous tubules as vasectomised men shed similar HIV RNA levels in seminal plasma as non-vasectomized men. Recent studies in acute and chronic SIV-infected non-human primates further confirmed earlier findings carried out in HIV infected men, demonstrating that CD4+ T cells in the prostate and the bulbo-uretheral gland of non- human primates contained 100-fold more SIV RNA than CD4+ T cells isolated from other organs of the MGT including the testes and the epididymus.

HIV shed in genital secretions contains both cell free (as HIV RNA) and cell- associated virus (HIV proviral DNA)[174]. Furthermore, even though the relative contribution of HIV RNA or cell associated HIV DNA to HIV transmission remains unclear, both cell-free[175] and cell-associated virus have been shown to be infectious. Despite the strong correlation between HIV RNA levels in blood and genital secretions, several studies have documented compartmentalized sequences of HIV in genital secretions, implying a local reservoir of replication. Typically, HIV RNA or HIV DNA is detected in the genital tracts of 10-20 % of men and women on Highly Active Antiretroviral Therapy (HAART) despite undetectable viremia, implying that the HIV virus is able to replicate within local organs of the male and female genital tracts.

Statistical analysis

SPSS 12 for windows XP was utilized. Examinations among blood and semen were performed utilizing a non-parametric Wilcoxon Signed Ranks matched t test. Dichotomous factors were thought about between gatherings by Chi squared tests. Constant factors were looked at between gatherings utilizing the Mann Whitney non-parametric test.

RESULTS:

Participant demographics

Twenty-five therapy-naïve participants were enrolled in the prospective study of HAART initiation (Table 1), with a median absolute CD4+ T cell count of 213 cells/mm^3 (range; 60-590/mm^3), and a blood HIV RNA VL of 50,000 copies/mL (range; 120 – >500,000 copies/mL). The median semen HIV VL prior to therapy initiation was 3,834 RNA copies/mL (range; <300 – 86,856 copies/mL), and high-level semen shedding (>5,000 RNA

copies/mL; see above) was present in 13/25 participants (52%). All participants started HAART within 48 hours of enrolment and provided paired blood and semen samples at each of the eight scheduled time points, for a total of 200 study visits. No *C. trachomatis* infection, *N. gonorrhoeae* infection, syphilis or clinical STI/urethritis were detected at any time point. The HSV-2 seroprevalence was 9/25 (36%), and all participants were CMV seropositive. No major resistance mutations to any of the three main antiretroviral drug classes were detected in blood genotypes performed prior to therapy.

Impact of effective HAART on HIV RNA levels in the blood and semen.

All participants had achieved an undetectable blood viral load (<50 RNA copies/ml) by week 16, thereby meeting the definition of effective HAART, and all but two participants had suppressed semen VL to undetectable levels (<300 RNA copies/ml) by the same time point (Figure 1a). One of these participants never suppressed semen virus despite consistently effective HAART (Figure 1b). In the other, blood VL was undetectable by week 4, but rebound was seen at week 16 despite initial suppression, and the participant admitted to suboptimal medication compliance; after appropriate counseling, complete suppression was achieved in both the blood and semen compartments.

Although semen HIV RNA levels initially became undetectable in 24/25 (96%) participants, there was substantial inter-individual heterogeneity in the consistency of semen VL suppression. Specifically, semen HIV shedding despite an undetectable blood viral load (isolated semen HIV shedding) was detected during at least one visit for 12/25 (48%) participants (Table 2). This occurred after the initial achievement of an undetectable semen VL in 9/12 (75%) participants. Overall, isolated semen HIV RNA shedding was detected during 19/116 (16.4%) study visits on effective HAART, and was present at a high level during 5/19 of these visits (26%; Figure 5.1b-d for representative examples). High-level isolated semen HIV RNA shedding was observed at any time in 4/25 (16%) participants, with peak semen HIV RNA levels ranging from 6,672–16,026 RNA copies/mL. While the proportion of participants at any given time point with an undetectable blood HIV VL reached 100% by six months, the proportion with undetectable semen virus appeared to plateau at just over 80% (Figure 2).

Associations of isolated semen HIV RNA shedding.

No association was seen between isolated semen HIV RNA shedding and specific antiretroviral agents or classes (Table 1). Just over half the participants were taking a boosted protease inhibitor (PI; 13/25; 52%), most commonly lopinavir or atazanavir, and the remainder were taking a non-nucleoside reverse transcriptase inhibitor (NNRTI; efavirenz in 10/12 cases). All participants were also taking a dual nucleoside reverse transcriptase inhibitor backbone, most commonly tenofovir/FTC or abacavir/3TC. Seven (7/12) participants with isolated HIV shedding were taking an NNRTI vs. 5/13 of those without (p=0.32); of those participants with high-level isolated semen shedding, 1/4 was taking an NNRTI (p=0.31).

Clinical parameters routinely tested prior to HAART initiation were not useful in predicting subsequent isolated semen shedding (Table 3). Specifically, the pre-therapy blood VL did not differ between groups (medians; 4.65 and 4.50 log10 RNA copies/ml in men with vs. without isolated shedders, respectively; p=0.86), nor did the baseline CD4+ T cell count (medians; 195 vs. 230/mm^3; p=0.97). The seroprevalence of HSV-2 in ever and never isolated shedders was similar (5/13 vs. 4/12; p=0.57), and all participants were CMV infected. However, although not standard clinical test, the baseline semen HIV RNA load was ten-fold higher in those participants with subsequent isolated semen shedding (median 4.42 vs. 3.41 log10 RNA copies/ml; p=0.03).

Viral characteristics of isolated semen viral isolates.

Sequence based screening of HIV RNA in blood for antiretroviral resistance mutations was performed at baseline for all participants. In addition, semen HIV isolates were tested at baseline and on therapy for three out of four participants with high-level isolated semen shedding (>5,000 HIV RNA copies/ml). No major or minor drug resistance mutations to any of the three major drug classes (nucleoside reverse transcriptase inhibitors, protease inhibitors or non-nucleoside reverse transcriptase inhibitors) were detected in the blood or semen HIV isolates from these individuals, either before or during HAART therapy. Limitations in PCR amplification did not permit analysis of semen HIV isolates with lower viral loads and in 1/4 participant with high- level isolated semen shedding.

Infection assays using enriched CD4+ T cells were

performed to determine whether semen HIV RNA detected on suppressive HAART was infectious in vitro. Infectivity assays were set up for three participants using semen plasma from time points with high- level (>5,000 RNA copies/mL) isolated semen shedding. Despite the substantial toxicity of seminal plasma on target cells in this in vitro system, high-level p24 production was apparent in 1/3 (33%) participants, demonstrating the infectious potential of isolated semen viruses. Although *in vitro* infection was only demonstrated in 1/3 participants from HIV RNA from isolated semen shedding episode, infection assays were particularly difficult to perform. Semen plasma reduced cell CD4+ T cell activation and viability and we observed that even baseline (prior to HAART) semen samples were unable to cause productive infections in vitro.

Isolated semen shedding after very long-term suppression on HAART.

Participants in this prospective study were only followed for six months after HAART initiation. However, it has been suggested that men may be sexually non- infectious after more than six months of complete suppression of the blood VL. To address this issue specifically, we examined semen HIV RNA levels in a group of men who had been on uninterrupted HAART with an undetectable blood HIV viral load for at least 4 years, and who have been shown to have unusually complete normalization of HIV-associated immune defects in both the blood and gastrointestinal mucosa[409]. Semen samples were collected at a single time point from 13 such individuals without STIs or evidence of urethral inflammation. Despite a median duration of complete blood VL suppression of 82 months (range; 48-216 months; Table 3), isolated semen HIV RNA shedding was detected in 4/13 participants (31%) at a median level of 564 RNA copies/ml (range, 336-828 HIV RNA copies/ml). Although isolated shedding was common, high level isolated virus was not detected in any participant on long-term completely suppressive HAART.

While, isolated semen shedding was seen in association with both NNRTI-based and boosted PI-based HAART regimens (Table 3), these was an association with HSV- 2 seropositivity: 4/8 HSV-2 infected participants demonstrated isolated semen shedding, vs. 0/5 HSV-2 uninfected (p=0.03). No participant had clinical evidence of HSV-2 reactivation, although microbiological screening for HSV-2 reactivation was not performed.

DISCUSSION:

Blood HIV levels predict the likelihood of HIV sexual transmission[181] and tend to correlate with virus levels in the genital tract. However, while the suppression of HIV blood VL on HAART is likely to correlate with a substantially reduced probability of sexual transmission, data are lacking to support the recent statement that such individuals are sexually non-infectious. Our prospective study confirms earlier reports that effective HAART substantially reduces HIV semen RNA levels. Just over half of our study participants never had detectable semen HIV RNA after suppression of blood viremia to undetectable levels, and this reduction in semen virus levels would be expected to have a substantial impact on HIV sexual transmission at a population level. However, isolated HIV RNA shedding was detected in the semen of almost half of participants, despite suppression in blood, and often at high enough levels to pose a potential significant transmission risk. Although semen plasma had toxic effects in our *in vitro* PBMC infectivity assay system, precluding detailed analysis of relative semen infectivity, we did demonstrate that HIV RNA detected during one episode of isolated shedding was associated with *in vitro* infectiousness.

Standard pre-therapy clinical and laboratory investigations were not useful in predicting which participants would develop isolated semen shedding after HAART initiation: blood VL and CD4 count were well matched between groups, and neither CMV nor HSV-2 infection status were predictive. The only robust predictor of the phenomenon was the pre-therapy HIV RNA semen viral load, which was ten-fold higher in participants with subsequent isolated semen shedding. However, semen HIV RNA VL testing is only available as a research tool, and not as a tool for clinicians or their patients. Furthermore, it should be noted that semen shedding despite effective HAART was observed in one individual with a pre-therapy semen VL of just 384 copies/ml. Although not associated with isolated shedding in the prospective study, HSV-2 infection was a significant risk factor for low-level semen virus in the small, cross-sectional study of participants with very long-term suppression of blood virus. Globally, the majority of HIV-infected people are co-infected by HSV-2, and HIV/HSV-2 co-infection substantially enhances HIV sexual transmission [200]. Therefore, despite the relatively low level of semen virus detected, the potential for HSV-2 to drive ongoing HIV sexual transmission despite effective HAART may merit further study.

Isolated semen shedding was not associated with specific antiretroviral agents. Tenofovir, which has excellent semen penetration[411], was included in the regimen of most participants with semen HIV detected despite effective HAART. In all cases this was combined with 3TC or FTC, as well as with at least one other recommended first line agent, most commonly efavirenz or a boosted protease inhibitor (lopinavir or atazanavir). Although NNRTIs and boosted PIs had low semen levels, as has been previously described, concentrations of 3TC in semen were on average 100 fold higher in semen than blood. Furthermore, the phenomenon of isolated semen HIV RNA shedding while on effective HAART was not associated with inter-individual differences in antiretroviral semen penetration, and so there was no suggestion that switching to an alternate antiretroviral agent(s) would prevent isolated semen shedding.

Semen HIV shedding while taking effective HAART was not generally related to a persistent lack of antiretroviral activity in this compartment. In all but one participant with isolated shedding, there had been initial reduction of semen HIV RNA to undetectable levels. It is also unlikely that medication non-compliance played a significant role, since (in addition to self-reported compliance) the blood VL remained persistently undetectable after initial suppression in all but one participant, perhaps suggesting reactivation of latent virus rather than active replication, similar to the phenomenon of blood plasma viral blips. In many cases isolated HIV shedding was detected after initial complete suppression of semen HIV RNA, suggesting that isolated semen HIV shedding may be related to reactivation of latent viruses (CMV) or increase in levels of inflammation (IL8, IL6), which have been demonstrated to be elevated in HIV infected individuals and enhance HIV replication in vitro.

No HIV antiretroviral resistance was detected during isolated semen shedding. However, the time to development of resistance on a non-suppressive regimen is quite variable, and participants were only followed up to 24 weeks in our prospective study protocol. In HIV infected women, compartmentalized viruses may be present within the blood and genital tract, with subsequent recombination[48]. Therefore, whether isolated shedding of drug resistant isolates might develop over time, and whether this might lead to a failure of blood VL suppression, are important questions that will require further study.

This longitudinal, prospective study clearly demonstrates the potential for HIV transmission through semen despite attaining an undetectable blood HIV viral load on HAART. While effective antiretroviral therapy is likely to substantially reduce HIV transmission at a population level, we believe that health care providers must continue to provide HIV-infected individuals with a strong and consistent message regarding the need for ongoing safe sex in this context.

Table 1. Characteristics of study participants initiating HAART.

Subject	Baseline CD4+ T cell count (/mm^3)	Baseline blood HIV VL (RNA copies/mL)	Initial HAART regimen
001	300	123,851	ABC/3TC/RTV/ATZ
002*	310	190,532	ABC/3TC/EFV
003	590	8,402	ABC/3TC/AZT/RTV/LPV
004*	140	>500,000	TDF/3TC/EFV
005*	200	50,000	ABC/3TC/TFV/RTV/ATZ
006*	127	20,657	TDF/3TC/RTV/LPV
007*	397	39,564	ABC/3TC/AZT/RTV/LPV
008*	320	53,585	TDF/3TC/RTV/LPV
009*	360	120	ABC/3TC/NVP
010	681	150,716	TDF/FTC/RTV/LPV
011	070	15,242	ABC/3TC/EFV
012*	340	24,085	TDF/FTC/EFV
013	240	150,827	ABC/3TC/RTV/LPV
014	060	4,604	ABC/3TC/RTV/ATZ
015*	170	406,990	TDF/FTC/EFV
016	220	319,304	TDF/3TC/EFV
017	105	67,893	ABC/3TC/RTV/ATZ
018	280	53,531	TDF/FTC/EFV
019	440	78,663	TDF/FTC/EFV
020	200	287,382	TDF/FTC/RTV/SQV
021*	180	195,663	TDF/FTC/EFV
022	310	2,766	TDF/FTC/EFV
023	060	55873	TDF/FTC/RTV/SQV
024*	140	4,882	ABC/3TC/RTV/ATZ
025*	190	31,507	TDF/FTC/NVP

*Participant demonstrated subsequent isolated HIV semen shedding (Table 1b for details).
**Antiretroviral abbreviations: ABC, abacavir; 3TC, lamivudine; FTC, emtricitabine; RTV, ritonavir; ATZ, atazanavir; EFV, efavirenz; AZT, zidovudine; LPV, lopinavir; TDF, tenofovir; NVP, nevirapine; SQV, saquinavir.

Table 2: Longitudinal HIV RNA levels in blood and semen plasma of participants with isolated semen viral shedding†

		Time on HAART (weeks)							
ID#	**Sample**	**baseline**	**2**	**4**	**8**	**12**	**16**	**20**	**24**
002	Blood	190,532	488	241	163	<50	**<50**	<50	<50
	Semen	14,082	<300	<300	<300	<300	**870**	<300	<300
004	Blood	>500,000	5,135	3,543	523	<50	<50	**<50**	**<50**
	Semen	57,395	2,496	<300	1,860	<300	<300	**8,418**	**16,026**
005	Blood	20,657	970	**<50**	**<50**	**<50**	18,056	201	<50
	Semen	82,836	447,660	**4,692**	**6,672**	**2,286**	7,026	<300	<300
006	Blood	50,000	1785	416	104	<50	**<50**	<50	<50
	Semen	13,230	20,685	2,718	<300	<300	**6,988**	<300	<300
007	Blood	39,564	333	119	61	**<50**	<50	<50	<50
	Semen	384	<300	<300	<300	**678**	<300	<300	<300
008	Blood	53,585	715	**<50**	<50	<50	<50	<50	<50
	Semen	52,494	19,296	**900**	<300	<300	<300	<300	<300
009	Blood	120	68	**<50**	<50	<50	<50	<50	<50
	Semen	1,152	<300	**714**	<300	<300	<300	<300	<300
012	Blood	24,085	163	**<50**	<50	<50	<50	<50	<50
	Semen	31,242	1,488	**1,518**	<300	<300	<300	<300	<300
015	Blood	406,990	1,722	556	265	503	**<50**	<50	<50
	Semen	86,856	4,206	<300	2,688	<300	**408**	<300	<300
021	Blood	54,495	274	52	51	**<50**	**<50**	**<50**	**<50**
	Semen	22,386	564	576	1,512	**1,098**	**1,092**	**3,546**	**2,124**
024	Blood	4,882	280	108	74	**<50**	<50	**<50**	50
	Semen	32,352	<300	<300	<300	**7,740**	<300	**468**	<300
025	Blood	31,507	383	357	85	<50	**<50**	<50	<50
	Semen	846	1,164	<300	1770	<300	**360**	<300	<300

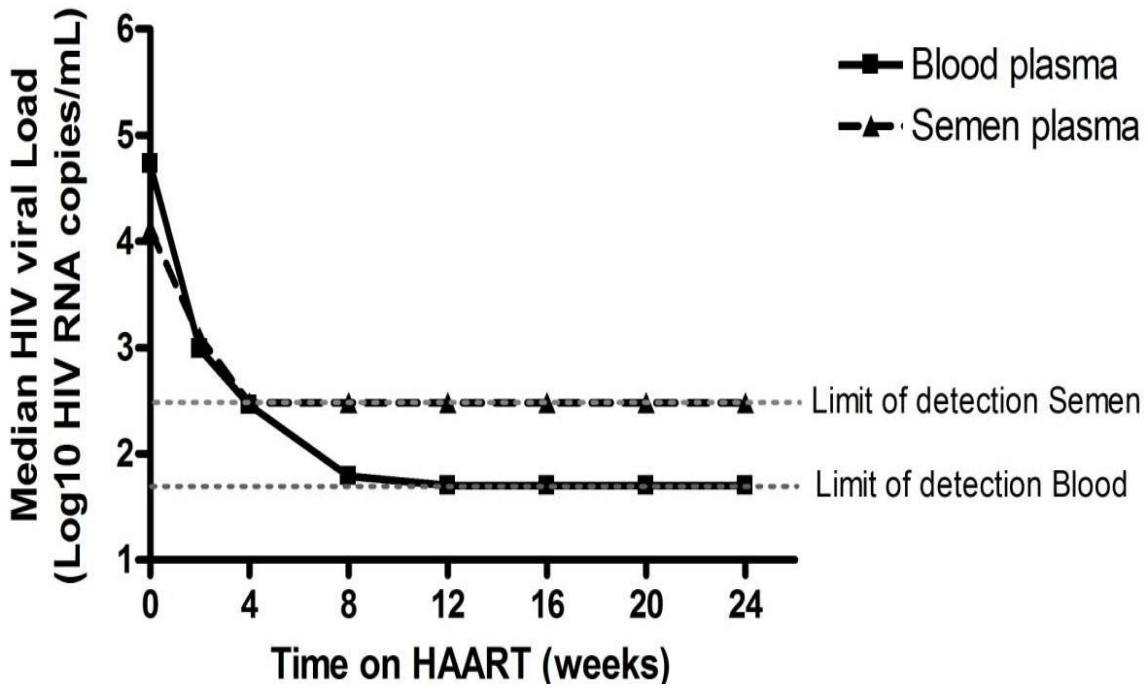
† Time points with isolated semen viral shedding are indicated in bold.
* Upper limit of detection in blood was 500,000 copies/mL
** Lower limit of detection in blood was 50 HIV RNA copies/mL.
*** Limit of detection in semen was 300 HIV RNA copies/mL.

Table 3: Characteristics of study participants on long-term effective therapy.

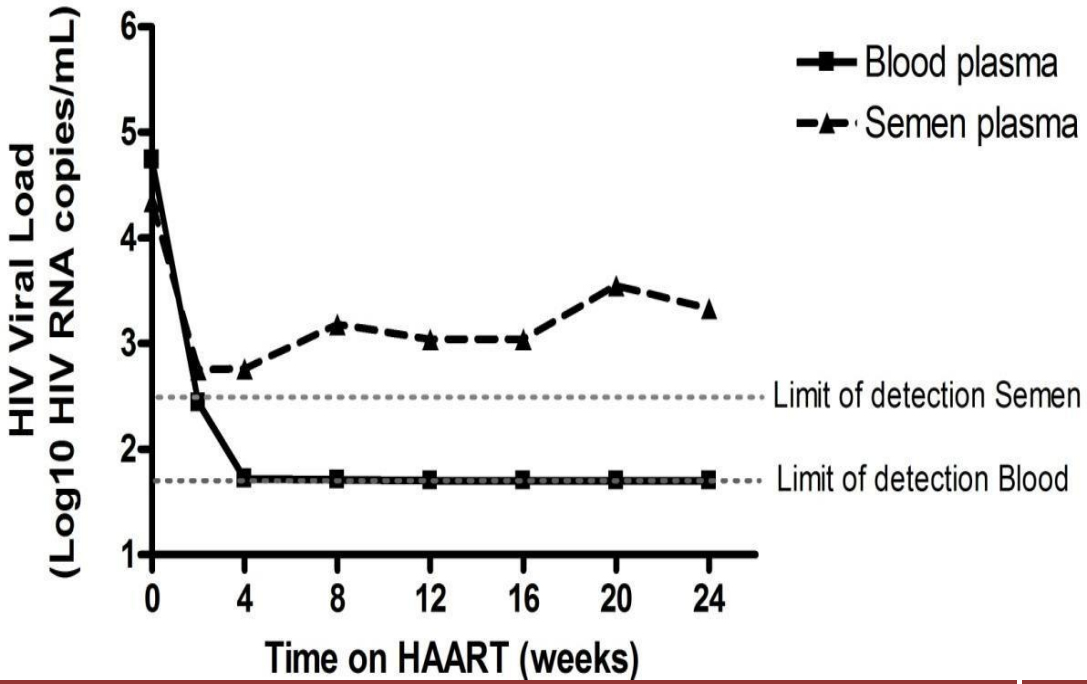
ID	CD4+ count*	Duration HAART (months)	Duration blood VL undetectable (months)	HAART regimen	HSV-2 serostatus	Semen HIV RNA VL (copies/mL)
1	540	132	120	ABC/EFV/3TC	Positive	378
2	440	86	75	ABC/3TC/RTV/SQV	Positive	<300
3	970	192	103	ABC/3TC/SQV/LPV	Negative	<300
4	730	168	102	ABC/3TC/SQV/RTV	Positive	<300
5	560	180	112	TDF/3TC/EFV	Negative	<300
6	970	120	115	ABC/3TC/RTV/SQV	Positive	750
7	710	116	54	TDF/3TC/LPV/RTV	Negative	<300
8	580	198	57	3TC/EFV/LPV/RTV	Positive	<300
9	610	216	82	ABC/3TC/ZDV/TDF/EFV	Positive	<300
10	580	102	70	EFV/AZT/3TC	Positive	828
11	590	85	69	ABC/3TC/ATV/RTV	Negative	<300
12	780	51	49	ABC/3TC/LPV/RTV	Negative	<300
13	490	114	48	AZT/3TC/RTV/SQV	Positive	336

*Absolute blood CD4+ T cell count

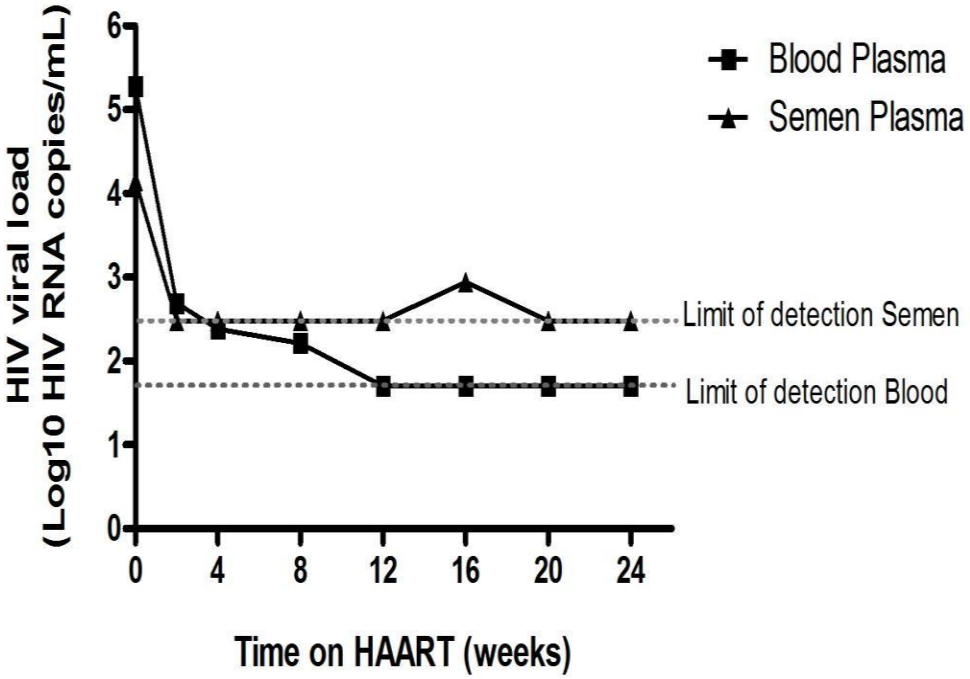
A.


Median HIV viral Load
(Log10 HIV RNA copies/mL)
6
5
4
3
2
1
0
4
8
12
16
20
24
Time on HAART (weeks)
Blood plasma
Semen plasma
Limit of detection Semen
Limit of detection Blood

B.


HIV Viral Load
(Log10 HIV RNA copies/mL)
6
5
4
3
2
1
0
4
8
12
16
20
24
Time on HAART (weeks)
Blood plasma
Semen plasma
Limit of detection Semen
Limit of detection Blood

C.



D.

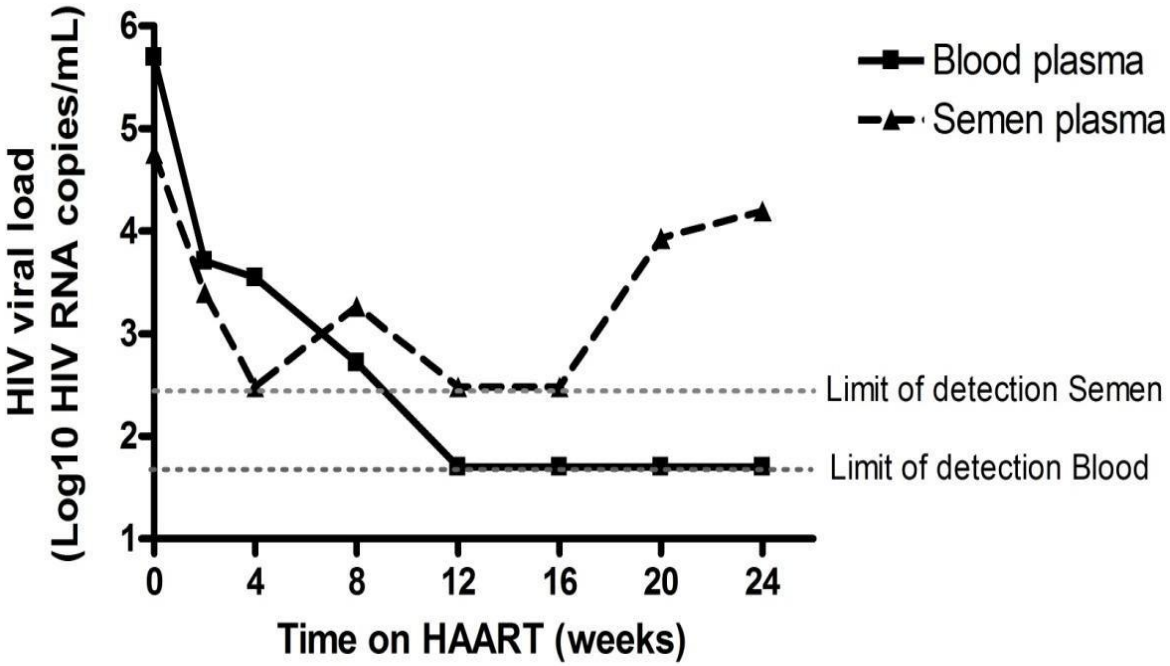


Figure 1 – HIV levels in blood and semen after HAART initiation.

Median levels of HIV RNA in the blood and semen of all 25 participants (Figure 1a), demonstrating that complete viral suppression below the level of detection in each compartment was the rule. However, this masked significant inter-individual heterogeneity. One participant had sustained semen shedding despite effective HAART (Figure 1b). Other individuals developed isolated semen shedding of HIV RNA despite initial semen suppression, both at low levels (<5,000 RNA copies/ml; Figure 1c) and high levels (□5,000 HIV RNA copies/ml; Figure 1d).

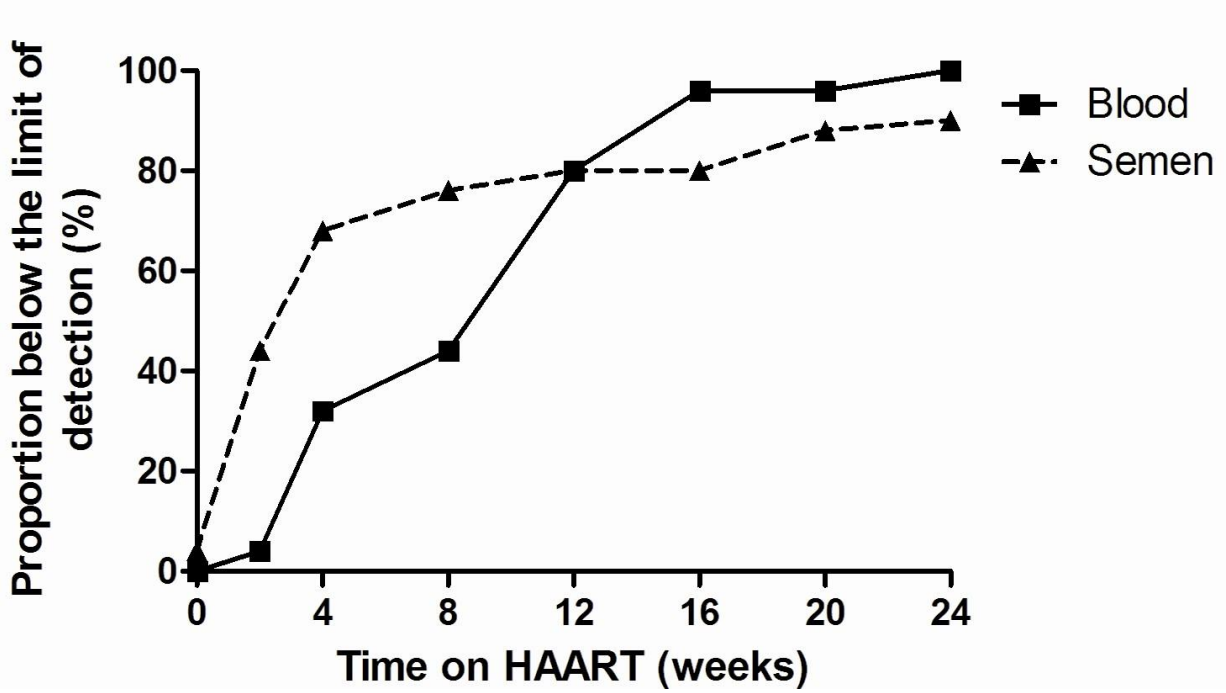
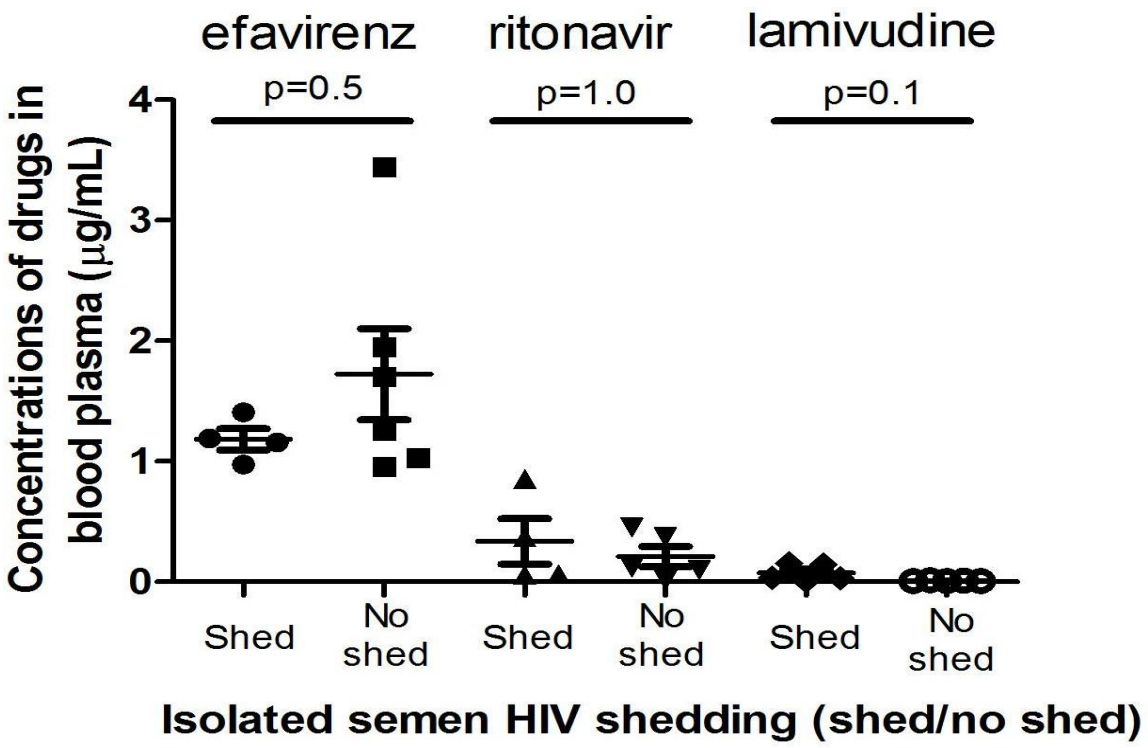
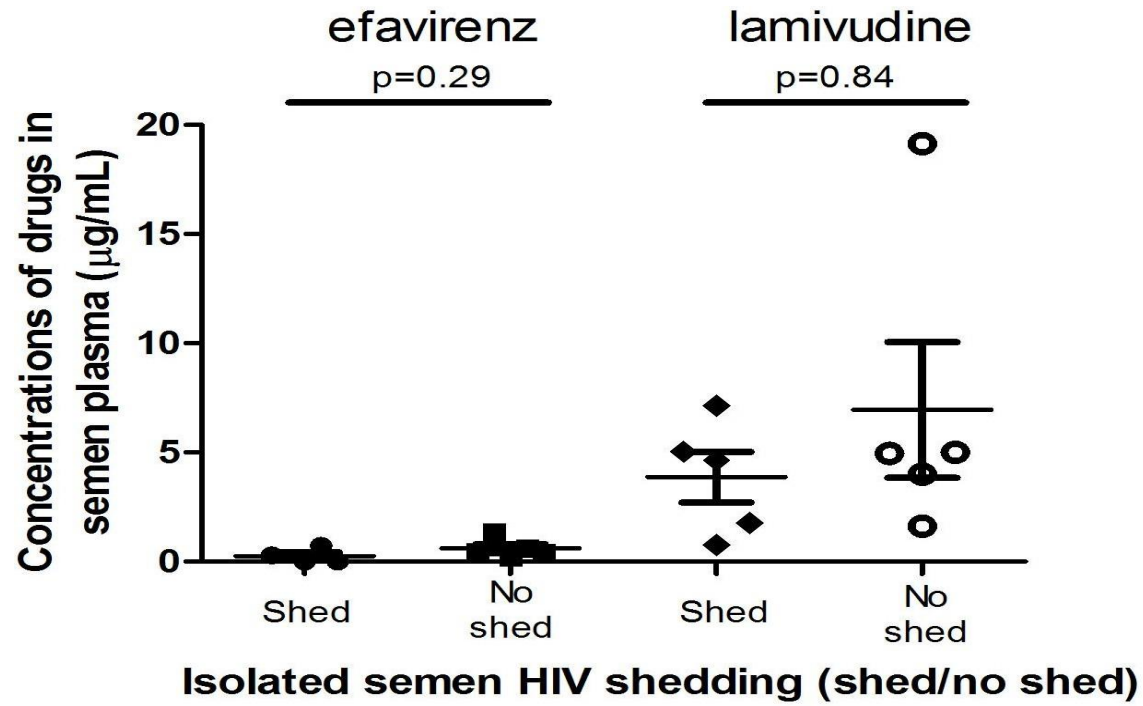


Figure 2 –Participants with undetectable HIV RNA in blood and semen plasma, by study time point. The proportion of study participants with HIV RNA levels below the limit of detection in blood (< 50 copies/mL) and semen (< 300 copies/mL) up to 24 weeks after HAART initiation.

A.


efavirenz
ritonavir
lamivudine
p=0.5
p=1.0
p=0.1
Concentrations of drugs in blood plasma (μg/mL)
4
3
2
1
0
Shed
No shed
Shed
No shed
Shed
No shed
Isolated semen HIV shedding (shed/no shed)

B.


efavirenz
lamivudine
p=0.29
p=0.84
Concentrations of drugs in semen plasma (μg/mL)
20
15
10
5
0
Shed
No shed
Shed
No shed
Isolated semen HIV shedding (shed/no shed)

C.

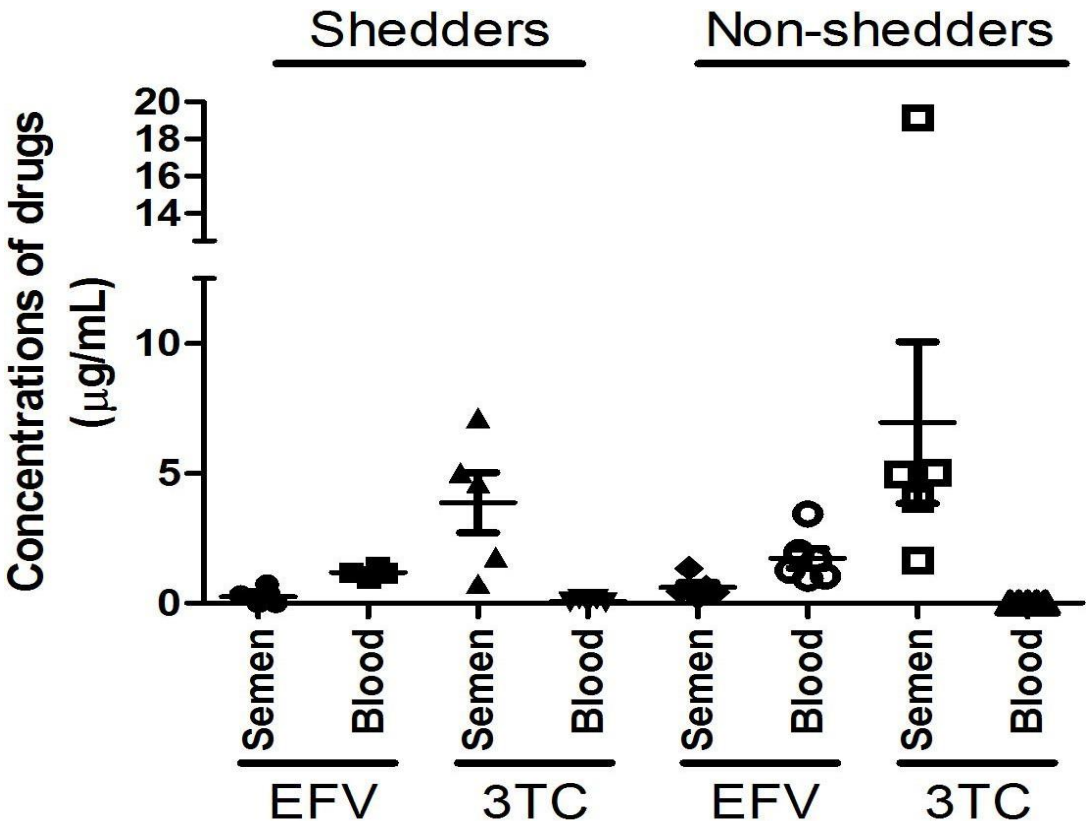


Figure 3 – Comparing concentrations of antiretroviral agents in blood and semen plasma of HIV-infected patients five months after HAART initiation. Similar levels of antiretroviral drugs in blood (A) and the semen (B) of HIV infected individuals on completely suppressive HAART. Drug levels were quite varied in blood and semen plasma, as some drugs (EFV) were found in higher concentration in blood while others (3TC) were found at much higher levels in semen plasma (C). Drug concentrations in blood or semen were not associated with isolated semen HIV shedding.

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