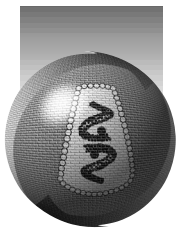

LEADING ARTICLE

Antiretroviral therapy to reduce the sexual transmission of HIV

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ABSTRACT

Sexual transmission of HIV is the principal mode of spread of HIV throughout the world. Whether the use of combination antiretroviral therapy will affect the sexual transmission of HIV remains to be seen. For an effect to occur, the viral load reductions seen within the blood need to be mirrored at a mucosal level, within the rectal tissues, the female genital tract and the semen of HIV-1-infected men. In part, this will be determined by the local concentrations of drugs within the genital tract. In this overview, we summarise the current knowledge on antiretroviral drug penetration into the male and female genital tracts. We also review studies that have investigated the effect of antiretroviral therapy on genital-tract shedding. The clinical implications of these studies are discussed. We conclude that the risk of HIV transmission exists as a spectrum, with many factors, both behavioural and biological, at interplay. It is conceivable that the use of antiretroviral therapy could reduce the spread of HIV from certain individuals. However, this idea is based on biological plausibility and surrogate marker data, rather than prospective population studies. In the worst-case scenario, antiretroviral therapy may simply increase the transmission of drug-resistant virus.

INTRODUCTION

Combination antiretroviral therapy has brought about individual health benefits in terms of reducing morbidity and mortality [1]. However, whether this treatment will affect the sexual transmission of HIV remains to be seen. For this to be the case, the viral load reductions seen within the blood plasma need to be mirrored at a mucosal level, within the rectal tissues, the female genital tract and the semen of HIV-1 infected men. In part, this will be determined by the local concentrations of drugs within the genital tract. Here, we summarise current knowledge of antiretroviral drug penetration into the male and female genital tracts. We also review studies that have investigated the effect of antiretroviral therapy on genital tract shedding. Finally, we discuss the potential clinical implications of these studies.

BACKGROUND

The efficacy of a pathogen in maintaining an epidemic depends on its basic reproductive rate (R_0). R_0 designates the number of individuals that are, on average, infected by one single person. As long as R_0 exceeds 1, the infection will continue to spread [2]. For a sexually transmitted pathogen, such as HIV, R_0 is a function of the infection risk per partnership (β), the number of sexual partners per unit of time (c) and the duration of the infectiousness (D). Thus:

$$R_0 = \beta \times c \times D$$

Furthermore, the infection risk per partnership depends on the infectiousness of the infected individual (reviewed in detail in [3] and [4]), the mode of the sexual contact and the susceptibility of the uninfected partner (reviewed in [5]). Reducing the latter is the main focus of preventive vaccine and vaginal microbicide programmes, whereas promotion of condom use and safer sex campaigns are the major behavioural strategies aimed at reducing the risks of HIV transmission (Table 1). In addition, the treatment of sexually transmitted infections can affect both the potential infectiousness of an individual [6–10] and the person's susceptibility to infection [11,12].

However, what we are considering in this review is whether treatment of an individual with antiretroviral therapy will render that person less infectious and, hence, could this treatment affect the spread of the epidemic? Classically, the parameter D, the duration of infectiousness, has been considered to be the length of time a person remains infected with HIV (i.e. in the absence of a cure, the

Table 1: Factors and interventions that may increase or reduce the sexual transmission of HIV.

Factors likely to increase sexual transmission of HIV	Factors likely to decrease sexual transmission of HIV
• Deferral of therapy	• Using antiretrovirals that effectively reduce the genital tract viral load
• Sex during primary infection and late disease	• Treatment during primary infection
• High seminal, cervical and rectal viral load	• Identification and treatment of genital supershedders of HIV
• Structured treatment interrruptions	• Continual maximally suppressive therapy
• Genital inflammatory conditions and ulceration	• Treatment of sexually transmitted diseases
• Increase in risk-taking behaviours	• Reduction in risk-taking behaviours
• Increase in numbers of sexual partners	• Decrease in number of sexual partners
• Not using condoms	• Consistent condom use

life of the individual). However, it is also hypothetically feasible that antiretroviral therapy may also modulate the effective duration of infectiousness at an individual level.

Factors influencing infectiousness

Epidemiological studies have identified the factors that influence infectiousness: advanced stages of HIV disease, a low CD4 count [13–18] and the presence of sexually transmitted infections [19–21] are all associated with an increased risk of transmission. However, the most powerful predictor of transmission so far identified is the blood viral load in the infected partner [22–24]. In addition, theoretical, biological and epidemiological evidence all indicate that primary HIV infection is a major risk for the sexual transmission of HIV (Table 1) [25–27].

Several studies have also shown convincingly that these risk factors for sexual transmission are associated with increased shedding of HIV in the genital tract [7,28–35]. This is unlikely to be purely coincidental. Rather, the factors that increase genital shedding, in essence, render individuals more infectious to their sexual partners.

Will the corollary be true? Will drugs that are effective in reducing the plasma viral load, increasing the CD4 count and reducing symptoms associated with HIV reduce the sexual transmission risk? This will, in part, depend on whether reductions in the plasma viral load are mirrored in the genital tract. If this is the case, mathematical models have predicted that the probability of sexual transmission can be directly related to the seminal viral load in the index case [36]. However, this benefit may be offset by any increase in the frequency of unsafe sexual behaviours in infected individuals.

A number of studies have now investigated the effect of antiretroviral therapy in reducing genital tract secretions and the findings are summarised below. However, many issues remain unanswered. Do all antiretrovirals penetrate the genital tract to the same extent? Is this reflected in the genital tract viral load suppression or in the efficiency of viral clearance? Does residual viral replication occur in the genital tract in the face of a suppressed plasma viral load? Ultimately, will this lead to the differential development and sexual transmission of resistant HIV?

The potential importance of therapeutic drug concentrations in the genital tract

Several groups have investigated the penetration of antiretrovirals into the male genital tract and it is now clear that this penetration is highly drug-specific [37–56]. In fact, concentrations can vary from drugs that are virtually undetectable to drugs that appear to concentrate in genital secretions. The potential clinical implications of these differences are summarised in Table 2 and discussed below.

Drugs that achieve high concentrations in genital secretions may be good candidates for pre- or postexposure sexual prophylaxis because these are the sites that are involved with the first contact of the virus. Drugs that achieve high concentrations in the female genital tract may be particularly effective at preventing mother-to-child transmission, provided most transmission occurs during passage through the birth canal [57]. In addition, if the genital tract truly represents a separate compartment, as many studies now suggest [58–65], then drugs that achieve therapeutic concentrations within this compartment may well be important for controlling local viral replication. This will certainly have implications for the prevention of sexual transmission of the virus and also for the local development of drug-resistant HIV. It has been postulated by Kepler and Perelson [66] that the differential penetration of drugs into body compartments such as the genital tract is likely to promote the generation of resistant HIV. Whether this is due to the concept of 'compartmental monotherapy' or simply ongoing replication in the face of suboptimal drug concentrations is currently unknown.

Table 2: The potential clinical implications of therapeutic versus subtherapeutic drug concentrations in the genital tract.

Rapid and therapeutic drug distribution to rectal, vaginal and cervical tissues may be crucial for agents that may be used for pre- or postsexual prophylaxis regimens.
Therapeutic drug concentrations in the female genital tract may be important for preventing mother-to-child HIV transmission.
Therapeutic drug concentrations in the genital tract may be important for controlling local viral replication within the genital tract, and hence for the potential to transmit virus sexually.
Subtherapeutic drug concentrations may encourage the local development of drug resistance, which may either seed back into the systemic circulation or be sexually transmitted.

Drug penetration into the male genital tract

The studies that have investigated the penetration of antiretroviral drugs into the male genital tract are summarised in Tables 3, 4 and 5. In general, nucleoside reverse transcriptase inhibitors (NRTIs) achieve concentrations in the semen of HIV-1-infected men that are of a similar magnitude or greater to those achieved in matched blood plasma samples. In particular, zidovudine (ZDV) and lamivudine (3TC) appear to concentrate in seminal plasma to levels at least double those in plasma and often much higher [37,43,52,53,67]. It has been proposed that this apparent concentration of certain NRTIs may be due to as yet undefined active drug transporters. Notably, it is the intracellular triphosphorylated moiety that has antiviral activity. To date, the only study to examine intracellular levels of ZDV and 3TC triphosphate has shown that 3TC triphosphate concentrations in seminal mononuclear cells were equivalent to those found in peripheral blood monocytes. In contrast, seminal ZDV triphosphate concentrations were only a fraction of those in blood [68]. The significance of these findings requires further investigation.

Data on all three of the currently licensed non-nucleoside reverse transcriptase inhibitors (NNRTIs) have been published or presented at international conferences [43,44, 54,69]. Nevirapine (NVP) has been shown to reach the highest concentrations in semen, reported as 60–100% of plasma levels irrespective of time after the dose [43]. These

Table 3: Median nucleoside reverse transcriptase inhibitor concentrations in the genital tract of male and female HIV-1-positive subjects.

	Males				Females			
	Reference	Semen, ng/ml (range)	Time after dose (h)	SP/BP (range)	Reference	CV fluid, ng/ml (range)	Time after dose (h)	CV/BP (range)
Abacavir	[104]	370 (270–3360), *n* = 5	4–8	5.6 (3.3–19.7)				
	[49]	1223 (925–2051*), *n* = 4	2	NS				
Lamivudine	[43]	1224 (391–1606), *n* = 3	8–12	8.7 (4–16.3)	UD	6770 (730–16730), *n* = 5	5–6	4.10 (1.26–8.62)
	[52]	2701 (1460–4320), *n* = 82	0–12	12.4 (6.8–18.8*)	[73]	69, *n* = 1	NS	NS
	[55]	NS	0–12	AUC 6.6 (4–8.1)				
Zidovudine	[52]	292 (194–438), *n* = 79	~6	15.7 (9.4–27*)	UD	730 (50–1230), *n* = 4	5–6	1.85 (0.03–5.40)
	[37]	NS	0–12	AUC 2.2 (1.6–2.8)	[73]	29.3±21.1, *n* = 5	NS	NS
	[55]	NS	0–12	AUC 3.31				
Stavudine	[43]	<25 (<25–388), *n* = 5	8–12	3.5 (1–6)				
Didanosine	[50]	430 (<50–1240), n = 5	4–12					

Where possible, the absolute drug concentrations given are those obtained at the end of the dosing interval (i.e. the approximate trough concentration); the median semen or cervicovaginal fluid/blood plasma ratios given may have been collected over a wide range of time intervals and may not represent the trough ratio. *Interquartile range. The didanosine dose was 400 mg once a day. AUC, area under the curve ratio; BP, blood plasma; CV, cervicovaginal; *n*, number of samples; NS, not stated; SP, seminal plasma; UD, unpublished data (M Boffito *et al.*, 2003).

Table 4: Median non-nucleoside reverse transcriptase inhibitor concentrations in the genital tract of male and female HIV-1-positive subjects.

	Males					Females			
	Reference	Semen, ng/ml (range)	Time after dose (h)	SP/BP (range)	EC_{50} (MEC)	Reference	CV fluid, ng/ml (range)	Time after dose (h)	CV/BP (range)
Nevirapine	[43]	3900, *n* = 6 (1300–9100)	8–12	0.61 (0.59–0.78)	39.9 (3400)	UD	1515, *n* = 2 (900–2130)	5–6	0.27 (0.25–0.30)
						[75]	Not stated	3–4	1.3
							Not stated	12	0.8
Efavirenz	[44]	238, *n* = 13 (49–1256)	24	0.09 (0.03–0.43)	4.4 (1100)				
	[54]	*n* = 147	0–24	AUC 0.034 (0.02–0.05*)					
Delavirdine	[69]	1025, *n* = 4 (819–1210)	6–9	0.16 (0.12–0.21)		[75]	Not stated	3–4	0.05
						[73]	Not stated	12	0.5

Where possible, the absolute drug concentrations given are those obtained at the end of the dosing interval (i.e. the approximate trough concentration); the median semen or cervicovaginal fluid/blood plasma ratios given may have been collected over a wide range of time intervals and may not represent the trough ratio. *Interquartile range. AUC, area under the curve ratio; BP, blood plasma; CV, cervicovaginal; EC_{50}, protein-corrected 50% effective drug concentration previously proposed for blood plasma and based on *in vitro* determinations of 50% inhibitory concentrations (IC_{50}) with a correction factor for plasma protein binding from [105]; MEC, minimum effective plasma concentration; *n*, number of samples; SP, seminal plasma; UD, unpublished data (M Boffito *et al.*, 2003).

concentrations are approximately 100-fold the calculated protein-corrected effective concentration that inhibits viral replication by 50% (EC_{50}) for NVP of 39.9 ng/ml [70], and are equivalent to the suggested blood plasma minimum effective concentration (MEC) of 3400 ng/ml [71]. Similarly, efavirenz (EFV) seminal plasma concentrations can be said to exceed a proposed calculated plasma protein-corrected EC_{50} of 4.4 ng/ml [70] by 50-fold. In contrast, if the blood plasma proposed MEC of 1100 ng/ml is used [72], then the seminal plasma EFV concentrations are only 20% of this value [44]. This huge range in potential therapeutic levels within the genital tract emphasises the complexities in trying to establish the actual drug concentrations required for antiviral activity within the genital tract. Furthermore, no studies have been able to determine the effect of seminal protein binding on drug activity, due to the technical difficulties involved with these experiments.

Among the protease inhibitors (PIs), irrespective of the target concentrations used, results from four studies have shown that indinavir (IDV) achieves therapeutic trough seminal plasma concentrations in excess of blood plasma values [40,42,45,47]. These are even higher if IDV is co-administered with ritonavir (RTV), possibly due to inhibition of P-glycoprotein or other drug transporters at the blood–genital tract interface [48]. One postulated reason for the effective penetration of IDV is its relatively low protein binding (60%). This hypothesis is supported by the fact that amprenavir (APV), which is approximately 90% protein-bound, reaches semen concentrations that are around 20% of blood plasma levels and these are close to the protein-corrected EC_{95} and MEC for APV [53].

The remaining PIs generally reach the semen at <7% of blood concentrations. However, absolute concentrations, which are the more important measure, are achieved in the order lopinavir (LPV) >> RTV > saquinavir (SQV) =

Table 5: Median protease inhibitor concentrations in the genital tract of male and female HIV-1-positive subjects.

	Males					Females			
	Reference	Semen, ng/ml (range)	Time after dose (h)	SP/BP (range)	EC_{95} (MEC)	Reference	CV fluid, ng/ml (range)	Time after dose (h)	CV/BP (range)
Indinavir	[45]	558, *n* = 7 (272–3178)	6–8	1.44 (0.67–5.79)	42 (100)	[73]	0.31±0.09*, *n* = 7	NS	NS
						[75]	NS	3–4	0.7
	[40]	714±170**, *n* = 12	NS	1.9			NS	12	1.45
Indinavir/r	[48]	1634, *n* = 9 (1109–2431*)	8–12	NS		[74]	1800, *n* = 14 (280–5410)	3–4	3.8 (0.99–10)
	[47]	448, *n* = 39 (240–1015)	8	NS			2040, *n* = 15 (480–28,100)	12	1.32 (0.08–5.62)
Ritonavir	[45]	295, *n* = 9 (<25–870)	9–12	<0.04 (<0.02–0.11)	1514 (2100)	[75]	NS	3–4	0.02
							NS	12	0.03
Saquinavir/r	[45]	<20, *n* = 7	9–12	<0.04 (<0.02–<0.06)	278 (100)				
Lopinavir/r	[56]	230, *n* = 14 (46–3900)	1–12	0.034 (0.02–0.07*)	64.2† (1000)	[75]	NS	3–4	0.05
							NS	12	0.05
	[40]	1743±1066**, *n* = 7	NS	0.07		[74]	480, *n* = 4 (360–720)	3–4	0.07 (0.026–0.091)
							480, *n* = 7 (300–600)	12	0.062 (0.013–0.42)
Amprenavir	[53]	319, *n* = 43 (73–929)	0–12	0.2 (0.1–0.39)	102 (400)	[75]	NS	3–4	0.5
Nelfinavir	[40]	156±48**, *n* = 7	NS	0.07	567 (800)				

Where possible, the absolute drug concentrations given are those obtained at the end of the dosing interval (i.e. the approximate trough concentration); the median semen or cervicovaginal fluid/blood plasma ratios given may have been collected over a wide range of time intervals and may not represent the trough ratio. *Interquartile range; **mean; †EC_{50} value. BP, blood plasma; CV, cervicovaginal; EC_{95}, protein-corrected 95% effective drug concentration previously proposed for blood plasma and based on *in vitro* determinations of 95% inhibitory concentrations (IC_{95}) with a correction factor for plasma protein binding from [105]; MEC, minimum effective plasma concentration; *n*, number of samples; NS, not stated; r, low-dose ritonavir; SP, seminal plasma.

nelfinavir (NFV) [40,45]. Although LPV seminal/blood plasma concentration ratios are low, the absolute values may be high enough to exert antiviral pressure. Whether this results in adequate suppression of genital tract viral load or selects for viral resistance remains to be seen.

Drug concentrations in the female genital tract

While it is clear that antiretroviral drug concentrations can be directly measured in seminal plasma, very few studies have evaluated measurements in the female genital tract [73–75].

Kashuba *et al.* [75] investigated different class antiretroviral drug concentrations in blood plasma and cervicovaginal secretions (CVS) in nine HIV-infected women. The overall ranking of antiretroviral drug penetration into the female genital tract, among the drugs studied, was IDV> NVP> APV > delavirdine (DLV) > LPV = RTV. This is similar to the pattern observed in men and confirmed the negative impact of protein binding on compartmental penetration.

More recently, the diffusion of IDV and LPV in genital secretions of 19 HIV-infected women has been further investigated. A total of 70 blood plasma/CVS sample-pairs were analysed. Median CVS:blood plasma ratios were low for LPV (0.06 trough and 0.07 peak concentration) and high for IDV (1.32 and 3.8, respectively) [74]. Interestingly, IDV concentrations in the 14 CVS samples with detectable IDV at the end of the dosing interval were above the MEC able to suppress HIV wild type in plasma (100 ng/ml) [76]. This suggests that IDV reaches therapeutic concentrations in the female genital tract, as it does in the male genital tract.

At the University of Turin, Italy, a study to assess CVS and blood plasma antiretroviral drug concentrations in HIV-infected women is currently under way. Preliminary results in five women (Tables 3 and 4) show a moderate diffusion of NVP, and diffusion of the NRTIs ZDV and 3TC similar to that observed in seminal plasma. All samples (both blood and CVS) were taken 4–6 hours after drug intake. Blood plasma and CVS viral loads were measured at the same time and found to be undetectable in all except one blood plasma sample (unpublished data, M Boffito *et al.*, 2003).

The effect of antiretroviral therapy on genital tract shedding

Tables 6 and 7 summarise the studies that have investigated the effect of antiretroviral drugs on genital tract shedding in males and females, respectively. Many more groups have studied the effects of antiretrovirals on HIV shedding in the male than in the female genital tract. In general, early studies using monotherapy regimens revealed only a modest effect on genital tract shedding [28,77]. However, as combination therapy has become the norm (in developed countries), the effect on seminal shedding has also

appeared to improve. In the best-case scenario in one of the largest studies looking at the effect of combination antiretroviral therapy on seminal HIV shedding, seminal plasma HIV RNA was detectable in only two of 114 men (<2%). However, cell-associated DNA could still be detected in 16% of men [78]. The duration of effective therapy also appears important. Leruez-Ville *et al.* [79] observed a reduction in both HIV-1 RNA and DNA over time, so that by 18 months of effective antiretroviral therapy, all patients in that study had <400 HIV-1 RNA copies/ml in seminal plasma and none had detectable HIV DNA.

However, these studies appear to represent the best cases. Of more concern are patients in whom the seminal plasma viral load remains detectable in the face of a suppressed or low blood plasma viral load. This has been found most frequently in studies using suboptimal antiretroviral regimens [10,40, 80–84]. Furthermore, replication-competent virus can be recovered from seminal cells in patients with an undetectable seminal plasma viral load [85]. Thus, while men on antiretroviral therapy will probably be less infectious than untreated men, they must still be considered able to transmit HIV.

The effect of antiretroviral therapy in the female genital tract

Studies on the effect of antiretroviral therapy in the female genital tract are far less complete than those for the male (Table 7). Also, they are of much greater concern regarding drug efficacy and viral clearance. In general, the use of antiretroviral therapy appears to reduce genital shedding in the female genital tract. However, the published data so far are less impressive with regard to viral clearance. In the largest study to date [86], of 311 women, 33% (27/83) with plasma viral loads <400 HIV-1 RNA copies/ml still had detectable viral loads in cervicovaginal lavage samples. This was despite the fact that 52% of these women were on antiretroviral regimens containing a PI. Unlike previous work in the male genital tract, these authors analysed HIV-1 RNA in an extract of the whole genital secretion, including cellular components. Thus, the detection of HIV-1 RNA originated from intracellular (messenger RNA) HIV as well as from cell-free HIV-1 RNA. Therefore, residual HIV-1 RNA in this work may also signify detection of HIV-infected lymphocytes in cervicovaginal lavage [86]. Similarly, Si-Mohamed *et al.* [73] found that although combination antiretroviral therapy on the whole was more effective at reducing HIV-1 RNA in cervicovaginal lavage than mono- or dual therapy, proviral HIV-1 DNA was detectable in 50% of the cervicovaginal lavage samples. Moreover, of 14 cervicovaginal samples analysed for HIV resistance, 12 contained resistance-associated mutations [73]. Therefore, to date, on the basis of published studies, the effect of antiretroviral therapy in reducing the potential infectiousness of women is not as robust as in the male.

Does the composition of the regimen matter?

So far, differences found in the penetration of PIs into the genital compartment have not been convincingly associated with either rates of HIV-1 RNA detection in the genital tract or the development of resistance, as has been postulated [66]. However, PIs are usually given in combination with NRTIs which, in general, penetrate well in to the genital compartment, as described above [46]. However, one published study has presented data suggesting that drug penetration may be important in controlling compartmental replication. Laffeuillade *et al.* [40] studied 42 HIV-positive men on antiretroviral regimens for at least 6 months. These contained two NRTIs plus NFV or LPV/RTV or IDV. Six patients in this study had a detectable seminal plasma viral load despite therapy. Importantly, three of these had a detectable seminal plasma viral load despite a blood plasma viral load of <50 HIV-1 RNA copies/ml, two on NFV and one on LPV. In particular, all three patients had NRTI-associated mutations in the seminal plasma. The authors postulate that the poor penetration of NFV and LPV into semen may have resulted in a therapeutic state of only two drugs (NRTIs) rather than three or four antiretrovirals in this compartment, leading to viral escape and the development of resistance [40].

Differences in genital compartment penetration might need to be reconsidered if regimens using PIs only are used. In a small 48-week pilot study, treatment with a single PI alone did result in similar success rates in semen as treatment with a standard PI/NRTI combination [87]. However, treatment in the PI-only arm was with APV, which does reach therapeutic drug levels in the male genital tract, unlike other PIs such as RTV, SQV and NFV.

Furthermore, a recently presented pilot study of 12 patients treated with RTV-boosted IDV alone showed persistent viral suppression for up to 48 weeks [88]. It remains to be shown whether PI-only regimens that do not enter the genital compartment as efficiently as IDV or APV will allow persistent genital HIV shedding, or even lead to the local development of drug-resistant viruses.

Will antiretroviral therapy reduce sexual transmission?

To date, only one published study has formally addressed this question, and that was in the monotherapy era. Musicco *et al.* [18] followed 436 monogamous HIV-negative females who were in sexual relationships with HIV-positive men. Over the duration of the study, 27 women seroconverted. This was more likely with inconsistent condom use, advanced stage of disease in their partner and without use of ZDV. When the analysis took disease stage into account, the rate of transmission from ZDV-treated men was lower than from untreated men (relative risk 0.5, 95% confidence interval 0.1–0.9) [18].

The more recent findings of effective suppression of HIV-1 RNA and DNA levels in the male genital tract certainly give rise to the hope that antiretroviral therapy may at least reduce the infectiousness of some males infected with HIV. However, given the current lack of hard evidence, in addition to the cost, toxicities and complexity of current antiretroviral therapy, it cannot be considered worthwhile as a preventive public health method at this time. Nevertheless, the effect of treatment on the sexual transmissibility of HIV

Table 6: Studies looking at the effect of antiretroviral therapy on seminal HIV shedding.

Ref., study type (*n*)	Source, semen HIV detection	Detection HIV in SP/NSC	Detection HIV in BP (related SP)	ART, effects on SP HIV	Results and key findings/conclusions of study
Krieger *et al.* 1991 [77] CS (34), LS (6)	NSC culture	28% +ve (CS) 50% at least 1 +ve (LS)	88% +ve (PBMCs)	ZDV 22, 23% +ve not on ZDV, 41% +ve on ZDV	Seminal shedding not associated with CD4 or CD8 counts, disease stage or ZDV therapy.
Hamed *et al.* 1993 [106] CS (36), LS (6)	DNA PCR on NSC RT PCR on SP	DNA +ve 67% RNA +ve 44%	6/6 DNA +ve in PBMCs in LS	None (9 pts), 1 (23), 2 (4 pts); treated DNA +ve 78%, RNA 48%	Seminal shedding not associated with disease stage, CD4 count or ART. In LS a decrease in SP VL was noted in only 2/6 men.
Kroodsma *et al.* 1994 [92] CS (16)	RT PCR on SP DNA PCR on NSC	44% SP RNA +ve RNA and DNA sequenced	68% BP RNA +ve	ZDV/ddI (3), ZDV (7) ddI (6); drug-resistant variants in SP	25% of drug-resistant genotypes discordant in SP and BP for ZDV- and ddI-associated mutations. In LS, mutations found first in SP/BP and before detection in NSCs/PBMCs.
Krieger *et al.* 1995 [31] LS (56)	NSC and SP culture	67% from NSC 14% from SP	Not stated	No drug (9), ZDV or ddI (30)	ZDV or ddI had little effect on seminal shedding. CMV detected in 30% of semen samples.
Vernazza *et al.* 1997 [107] CS (44)	NSC MC NASBA on SP	37% culture +ve 68% SP RNA +ve Median SPVL 3.91 $\log_{10}$	Median BPVL 4.96	1NRTI (2), 2 NRTI (5) 2NRTI+PI or NNRTI (22); +TX 12% culture +ve, 27% SP RNA +ve	Treatment-induced changes in BPVL generally associated with similar SP changes. All pts with undetectable BPVL also had undetectable SPVL.
Gupta *et al.* 1997 [33] CS (34), LS (14)	NASBA on SP + whole semen	66% RNA +ve Median SP 5238 c/ml, semen 11,000 c/ml	32/34 RNA +ve Median BPVL 33,000 c/ml	IDV±EFV in LS. All pts BLD in SP post-TX	SPVL 10–1000-fold higher than previous estimates; whole semen VL > SPVL. RT PCR suffered inhibition by seminal plasma. NASBA RNA↑ all samples. BPVL/SPVL highly correlated (P <0.001). Seminal shedding not associated CD4.
Gilliam *et al.* 1997 [108], LS (22), ART (11)	NSC MC NASBP on SP	50% culture +ve before TX	100%	No drug (6), others 1/2 NRTIs ± NNRTIs 78% pts BLD post-TX	Only 0.1% samples culture +ve post-TX. SPVL↓ ~1 $\log_{10}$. ART effectively reduced seminal shedding.
Zang *et al.* 1998 [85] CS (7)	RT PCR on SP DNA PCR on NSC+ PBMCs; NSC culture	SP RNA BLD 7/7; DNA detectable 4/7 NSCs; 2/7 NSCs culture +ve	BP RNA BLD 7/7, DNA detectable 3/7; 3/7 PBMCs culture +ve	2 NRTI + PI 5–41 mths SP RNA BLD; no resistance mutations detected	HIV DNA still in 3/7 NSCs and replication-competent virus cultured in 2 despite long-term HAART. WT sequences recovered on sequencing seminal cell-associated virus.
Taylor *et al.* 1998 [42] LS (7)	NASBA on SP HPLC on SP for SQV/RTV	4/7 detectable SP RNA pre-TX; SPVL median 4600 c/ml	7/7 detectable BP VL pre-TX; median BPVL 18,000 c/ml	RTV/SQV+d4T; 6/7 pts BLD at 24 wks	Poor penetration RTV/SQV in semen (<5% of BP levels). Despite this, SPVL suppressed BLD in all patients.
Dornadula *et al.* 1999 [109] CS (20)	Ultrasensitive RNA assay <5 c/ml	8/20 pts SP RNA >5 but <50 c/ml despite HAART	20/20 pts BP still detectable (mean 17 c/ml) on HAART	3 drugs, all BPVL <50 c/ml	With HAART, low-level BP and SP RNA still detectable at >5 to <50 c/ml despite BP RNA levels <50 c/ml.
Luizzi *et al.* 1999 [110] LS (17)	SP RNA↓ at 1, 3, 6 (4) mths after start TX	ZDV 0.7, 0.5, 0.2; ZDV/ddI 1.1, 1.4, 1.3; 2 NRTIS + PI 1.2, 2.2, 3 (mth 4)	ZDV 0.6, 0.4, 0.2; ZDV/ddI 1.4, 1.3, 1.1; 2 NRTIS + PI 1.8, 1.7, 2.4 (mth 4)	1, 2 or 3 drugs (values in previous 2 columns are $\log_{10}$)	HIV-1 RNA levels in BP/SP related to duration/type/no. drugs used. With PI + 2 NRTIs, strong ↓ in cell-free HIV-1 RNA in semen.
Mayer *et al.* 1999 [83] LS (22)	SP RNA, cell RNA and cell DNA PCR baseline + 6 mths	SP RNA (400,000 c/ml), SP DNA +ve 59% pre-TX; cell RNA 10,000 c/ml, cell DNA 70,000	Not stated	SP RNA/DNA +ve in 31% (RNA 27,000 c/ml); cell RNA 80,000 c/ml; cell DNA 3000 c/ml	When IDV added to regimens with 2 NRTIs only, DNA/RNA persisted in semen in 31%, but no +ve HIV cultures isolated from semen. PI resistance mutations identified in seminal leukocyte DNA from 2 pts which differed from mutations in PBMCs.
Tachet *et al.* 1999 [84] LS (22)	RT PCR on SP DNA PCR on NSC and SPZ	87% RNA +ve 57% NSCs +ve	98% median BPVL 4.2 $\log_{10}$	21% pts on TX; no significant difference between RX and no RX	1/3 of men had SP RNA similar/equal BP RNA. Cell-associated DNA in men with –ve SP RNA. SPVL/BPVL correlated (r_s = 0.56, P<0.0001). DNA in SPZ, contamination?
Winter *et al.* 1999 [10] CS (94)	NASBA on SP	58% pts no TX had median SPVL 4.7 $\log_{10}$; 10% pts on TX had median SPVL 2.9 $\log_{10}$	100% pts no TX had median BPVL 4.7 $\log_{10}$; pts on TX had BPVL 2.6 $\log_{10}$	13% 2 drugs, 38% no drug, 49% 3/4 drugs; if no TX, 11× more likely to shed HIV in semen	Seminal shedding associated with BPVL (AOR 19.3, CI 2.6–144 per 10-fold ↑ BPVL). Asymptomatic urethritis in 9%, significantly associated seminal shedding. 53 men on ART tested for urethritis; men urethritis +ve were >8× more likely to shed HIV in semen. BPVL/SPVL correlated (r_s= 0.6; Spearman's rank P<0.001).
Speck *et al.* 1999 [111] CS (49)	HIV RNA, HIV/CMV coculture	SP detection ↑ with ↑ polymorph nuclear count, +ve CMV coculture, CD4 <200	Not stated	SP detection ↓ with ≥2 drugs	Best multivariate model predicting +ve semen VL had 2 systemic host factors, CD4 <200 (OR 3.0; 95% CI 1.3, 6.9) and NRTI (1 drug OR 0.5; 95% CI 0.3, 1.0; ≥2 drugs OR 0.4; 95% CI 0.2, 0.9), and +ve CMV coculture (OR 1.7; 95% CI 1.0, 3.0).
Ball *et al.* 1999 [80] LS (12)	NASBA on SP and BP	7/12 pts RNA +ve pre-TX SPVL median 4.56 $\log_{10}$	9/12 pts RNA +ve pre-TX SPVL median 4.81 $\log_{10}$	2NRTIs, IDV added 5/12 BPVL +ve and 2/12 SPVL +ve	Stable ↓ BP/SP VL not achievable in significant proportion of NRTI-experienced men when IDV added to a failing regimen.
Barroso *et al.* 2000 [81] LS (93)	NASBA on SP and BP	69/73 (74%) SP RNA +ve pre-TX	889/93 (96%) BP RNA +ve pre-TX	6 mths post 2/3 drugs BP RNA +ve 38%, SP RNA 33%	ART reduces HIV shedding in semen. A substantial number of pts still have detectable RNA, may remain infectious.
Eron *et al.* 2000 [87] LS (30)	SP and BP RNA	Pre-TX 4/30 pts SPVL >6.10 $\log_{10}$	Not stated	19 APV alone, 11 APV/ZDV, 3TC; post-TX, 77% SPVL BLD	First to show that 1PI (APV) ↓ seminal shedding but 8 (27%) had +ve SP HIV RNA at last visit. Persistent HIV replication in genital tract may ↑ transmission of resistant virus.
Vernazza *et al.* 2000 [78] CS (114 TX, 55 controls)	HIV RNA and DNA	SP RNA in 2/114 on TX, 67% no TX; DNA +ve 16% TX, 38% no TX	BP RNA detectable in 0/114 on TX	114 men stable on HAART versus 55 controls	Optimal TX-induced suppression of BPVL leads to undetectable semen HIV (<2%); seminal shedding of cell-free/cell-associated HIV significantly ↓ versus asymptomatic no TX.

Table 6: Studies looking at the effect of antiretroviral therapy on seminal HIV shedding *(continued)*

Ref., study type (*n*)	Source, semen HIV detection	Detection HIV in SP/NSC	Detection HIV in BP (related SP)	ART, effects on SP HIV	Results and key findings/conclusions of study
Taylor *et al.* 2001 [44] LS (19)	NASBA on SP and BP; EFV in SP and BP	Median pre-TX in naive pts SPVL 3.48 $\log_{10}$, 4.51 $\log_{10}$ post TX	Pre-TX BPVL in naive pts 4.9 $\log_{10}$, 5.88 $\log_{10}$ with post TX	EFV regimens; by 24 wks, BPVL in 16/18 <50 c/ml, SPVL in 18/18 <400 c/ml	Prospective study of EFV effect on SP RNA. SP EFV only 10% of BP levels. SPVL suppressed by 24 wks of TX in all patients. Authors concluded that EFV-containing regimens have antiviral activity in male genital tract.
Gunthard *et al.* 2001 [112] LS (28)	RT PCR and NASBA on BP and SP	3/23 samples detected HIV RNA >50 but <400 c/ml	BPVL BLD all pts	IDV/3TC/ZDV >2 years; 20/23 samples BLD	Lymphoid compartment is important reservoir of residual HIV in pts with successful ART for >2 years, but HIV RNA undetectable in all CSF samples and 20/23 SP samples.
Boulet *et al.* 2001 [113] (40; 23 ART)	RT PCR on BP, SP and saliva	SP RNA +ve in 17/44 (39%) of samples	BP RNA +ve in 37/46 (80%) of samples	33/40 pts on ART >3 mths; 11 on 2 drugs, 18 on 3 drugs	BPVL/SPVL strong positive correlation (r_S=0.59, P<0.0001); BP/saliva weak correlation (r_S = 0.31, P<0.05); none for saliva/semen. Mean BPVL and SPVL significantly affected by no. of drugs (P<0.001 and P<0.05, respectively).
Taylor *et al.* 2001 [45] CS (23)	NASBA on SP and BP; IDV/RTV/SQV in SP and BP	7 IDV pts SPVL <800 c/ml; 12/16 RTV/SQV pts <400 c/ml	7 IDV pts BPVL <400 c/ml; 12/16 RTV/SQV pts <400 c/ml	IDV+2NRTIs (7) or regimen containing RTV/SQV (16)	SP IDV > therapeutic level; SQV/RTV high in BP but sub-therapeutic in SP. SPVL/BPVL detected in 4 pts, 3 on ART 4 wks only; 4th pt on TX 24 weeks, had SPVL 42,000 c/ml and BPVL 1100 c/ml.
Kalichman *et al.* 2001 [82] CS (44)	NASBA on semen (whole or SP not given); RT PCR BP	SPVL 1.7±2 if SPVL =BPVL (29 pts), 5.2± 0.7 if SP>BP (15 pts)	53% with no BPVL but SPVL detectable; 31% vice versa	Various ART (2/3 drugs)	BPVL/SPVL not associated. SPVL > BPVL in 34% pts. BPVL suppressed in 15/35 (=suboptimal ART?). 53% with undetectable BPVL had detectable SPVL.
Celum *et al.* 2001 [114] (89)	RT PCR on BP NASBA on SP	67% SPVL +ve if BP VL >50,000 c/ml, 61% +ve if BPVL 10,000–50,000, 40% +ve if <10,000	In general, ↑ BPVL associated with ↑ SPVL	88% of 54 on TX had undetectable SPVL; 12% detectable SPVL despite ART	SPVL more variable than BPVL. SPVL usually 1 $\log_{10}$ lower than BPVL. When BPVL <5000 c/ml, 32% with no TX, 13% on NRTIs and 7% on PIs still had detectable SPVL.
Pilcher *et al.* 2001 [102] LS (8)	Roche Amplicor for BP; NASBA for SP	Mean SPVL in sero-converters 3.98± 1.43 $\log_{10}$ versus 3.61±1 $\log_{10}$	Mean BPVL in sero-converters 5.49±0.8 $\log_{10}$ versus 4.28±0.8 $\log_{10}$	After diagnosis, pts started 4 drugs with NVP, ddI, d4T, HU	SPVL highly variable; in 3/8 pts SPVL > BPVL and needed >24 wks TX to be undetectable. SPVL found before symptoms (virus in genital tract early?). High seminal shedding of HIV associated with other STD co-infection.
Sadiq *et al.* 2002 [8] LS (24 cases, 16 controls)	RT PCR and NASBA on BP and SP in men with/ without urethritis	I8/24 men on TX +urethritis had SPVL <1000 c/ml; 6 had detectable SPVL	5/6 men with detectable SPVL had detectable BPVL	ART ≥2 drugs for ≥3 mths in men with/without urethritis	Effective ART limited urethritis effect on SPVL, but if poor control of BPVL, ↑ SPVL during urethritis. In 1 pt, SPVL ↓ 20-fold after TX for gonococcal urethritis.
Leruez-Ville *et al.* 2002 [79] LS (39)	RT PCR in SP and BP, RNA NSC DNA	92% RNA +ve and 66% DNA +ve at baseline (median DNA VL 1.1 $\log_{10}$)	100% baseline DNA +ve, 100% VL ↓ 2 $\log_{10}$ (mth 1), mth 18 still +ve	HAART 18 mths, SP RNA ↓1.8 $\log_{10}$ mth 1, all NSC DNA –ve by mth 18	By mth 6, SPVL detectable in only 11% pts; by mth 18 of HAART, all pts <400 c/ml, all NSC DNA –ve, but SPVL 292, 100, 10 c/ml in 3 pts. Do infected cells in male genital compartment come from intermittent blood lymphocyte passage?
Reddy *et al.* 2002 [54] LS (21)	Roche Amplicor on BP, NASBA on SP	No SPVL found in 8 (89%) EFV-naive, nor 10 others (83%)	BPVL <2.6 in 4/9 (44%) EFV-naive and 5/12 others	EFV in regimen (9 EFV-naive, 10 with previous EFV)	Prospective study of EFV effect on SP RNA. EFV > HIV-1 WT IC_{50} over whole dose interval. Authors conclude that EFV in TX regimen can suppress SPVL.
Nunnari *et al.* 2002 [115] CS (28)	Detection of 2LTR circles; viral coculture assay	Viral growth in 5/28 (18%) NSCs; 0/28 samples 2LTR +ve	Viral growth in 16/26 (57%) PBMCs; 18/28 2LTR +ve	Men on fully suppressive HAART; VL <50 c/ml	With suppressive HAART, new cellular infections occurred in PBMCs but not seminal cells *in vivo* (in which mainly latent HIV-1 found). Is this a compartment-specific mechanism of residual HIV-1 disease?
Laffeuillade *et al.* 2002 [40] CS (41)	Roche Amplicor on HIV RNA plus PI analyses	SPVL <50 c/ml in 28/34 pts; 6 had median 556 c/ml (3 with BPVL <50 c)	BPVL detectable in 4 men on IDV, 5 NFV, 4 LPV	2NRTIs + IDV (16) 2NRTIs + NFV (13) 2NRTIs + LPV/r (12)	SP/BP ratio 1.9 IDV, 0.07 NFV, 0.07 LPV. Detectable SP VL in 6 pts associated with detectable BPVL in 3 (NFV 1, LPV 2) and BPVL <50 c/ml in 3 (NFV 2, LPV 1). 3 semen samples of latter had NRTI mutations (=low PI in semen?).
Liuzzi *et al.* 2003 [116] LS (12)	Branched DNA assay BP and SP HIV RNA in pts with STI	In STI, all SPVL rebounded (1136–1,000,000 c/ml)	In STI, all BPVL rebounded (69–>500,000 c/ml)	Various 3-drug regimens. STI for median 4 mths. 7 pts restarted TX after 34–192 days	BPVL and SPVL rebound in 12/12 pts with STI of effective HAART. BPVL rebound > SPVL rebound, but highest VL in semen (2 pts). Both VLs ↓ with restart TX (after 1–2 mths, 4/7 pts BLD in semen).
Barroso *et al.* 2003 [117] LS (93)	NASBA in BP and SP for HIV RNA	Baseline: median SPVL 7800 c/ml (24/93 pts BLD)	Baseline: median BPVL 47,000 c/ml (4/93 pts BLD)	2/3 drugs; after 6 mths, 20 shed HIV in semen (VL 11,000 c)	After 6 mths TX, median BPVL in semen HIV shedders was 22,000 c/ml, BLD in non-shedders. Strong association of poor adherence with persistent HIV seminal shedding.
Laffeuillade *et al.* 2003 [97] CS (23)	RT PCR and NASBA for HIV RNA	SPVL found in 3 (median 704 c/ml, range 274–5100)	BPVL BLD in 13/23 detectable in 10 (median 357 c/ml)	2NRTIs + LPV/r (19) 2NRTIs + EFV + LPV/r (4 pts)	LPV poor level in SP (6% of BP). SPVL BLD in 87% pts on salvage regimen with LPV/r. Pts with ↑ LPV resistance may be BLD in BP but not SP due to insufficient SP IQ.

AOR, adjusted odds ratio; APV, amprenavir; ART, antiretroviral therapy; BLD, below the limit of detection; BP, blood plasma; CI, confidence interval; CMV, cytomegalovirus; CS, cross-sectional study; CSF, cerebrospinal fluid; ddI, didanosine; EFV, efavirenz; HAART, highly active antiretroviral therapy; HPLC, high-performance liquid chromatography; IC_{50}, 50% inhibitory concentration; IDV, indinavir; LPV, lopinavir; LS longitudinal study; LTR, long terminal repeat; MC, microculture; mth, month; NASBA, nucleic acid sequence-based amplification; NFV, nelfinavir; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; NSC, non-seminal cells; OR, odds ratio; PBMC, peripheral blood monocyte; PCR, polymerase chain reaction; PI, protease inhibitor; pts, patients; r, low-dose ritonavir; ref., reference; RT, reverse transcriptase; RTV, ritonavir; SP, seminal plasma; SPZ, spermatozoal fraction; SQV, saquinavir; STI, structured treatment interruption; 3TC, lamivudine; TX, treatment with antiretroviral therapy; +ve, positive; VL, viral load (measured as HIV-1 RNA copies/ml or as $\log_{10}$ HIV-1 RNA copies/ml ($\log_{10}$); wk, week, WT, wild-type HIV; ZDV, zidovudine.

Table 7: Studies looking at the effect of antiretroviral therapy on HIV shedding in the female genital tract.

Reference, study type (*n*)	Method used to collect CVS	No. of women enrolled	ARV regimens used	Summary of results and key findings of study
Loussert-Ajaka *et al.* 1997 [118]	CV lavage	43 (pregnant HIV-infected women)	ZDV monotherapy	HIV-1 RNA was detected in 10/43 CV samples and ranged from 280 to 36,080 copies/mL (median: 1320 copies/ml). ZDV therapy at the time of sampling was associated with a lower rate of HIV DNA detection in the CVS suggesting that ART can reduce HIV excretion in the FGT.
Hart *et al.* 1999 [119]	CV lavage and cytobrush	*n* = 52	31/52 women stable on ART regimens; 11 started or changed ART (2NRTIs and IDV)	Cell-free vaginal HIV-1 RNA was detected in 32 (46%) of the 52 lavage samples with a range of <1000 to 122,000 copies/lavage (median: 2600 copies/lavage). The initiation of ART significantly reduced the amount of HIV RNA, both in plasma and CVS.
Di Stefano *et al.* 1999 [94]	CV lavage	*n* = 2	The patients were multiexperienced to various ART regimens	Multidrug-resistant HIV-1 variants were detected in the CVS of two women treated with different ART regimens.
Cu-Uvin *et al.* 2000 [120]	CV lavage	*n* = 205	*n* = 74, not on treatment *n* = 65, on NRTIs *n* = 67, on HAART	51 (25%) had HIV-1 RNA detected in both plasma and GT secretions. The CV HIV-1 RNA load was positively correlated with the plasma viral load and negatively with the CD4 cell count. The use of ART was associated with low HIV replication in both plasma and CVS.
Si-Mohamed *et al.* 2000 [73]	CV lavage	*n* = 58 (samples obtained from 54 women have been analysed)	*n* = 12, treatment-naive *n* = 12, ZDV on monotherapy *n* = 12, 2 NRTI *n* = 18, HAART (various combination therapies)	In total, HIV RNA found in 21/54 (39%) of CV samples. CV HIV-1 RNA was detectable in 9/12 of treatment-naive patients (2.95±0.40 $\log_{10}$ c/ml), 8/12 on ZDV monotherapy (2.88±0.30 $\log_{10}$ c/ml), 2/12 on 2NRTIs (1.57±0.14 $\log_{10}$ c/ml) and 2/18 on HAART (1.66±0.20 $\log_{10}$ c/ml). Persistence of cell-associated DNA provirus in the FGT was detected in 50% of CVS samples, suggesting that treated women remain potentially infectious during heterosexual intercourse. 12/14 detectable CV samples contained resistance-associated mutations.
Kovacs *et al.* 2001 [86]	CV lavage and pap smear	*n* = 311	Various ART combination therapies	Plasma RNA concentration was the most important factor in predicting genital HIV-1 shedding, even among women receiving ART. Of concern 33% (27/83) women with BPVL <500 still had detectable HIV-1 shedding in the genital tract. 74% of these were on combination ART and 52% were receiving ART including a PI, suggesting that a separate reservoir of HIV-1 replication may exist in some women.
Debiaggi *et al.* 2001 [121]	CV lavage and CV swabs	*n* = 37 (total number of samples: 128 pairs blood and CV)	*n* = 26, 2 NRTIs and IDV *n* = 11, 2 NRTIs	HIV RNA was detectable in 40 (31%) CV samples. The prevalence of HIV-related nucleic acids in CVS was significantly associated with the presence of HIV RNA in plasma. However, the positive correlation observed in this study provides no assurance that HIV shedding does not occur in the FGT with undetectable VL in plasma. Therefore, the impact of systemic ART on HIV shedding needs further investigation.
Ellerbrock *et al.* 2001 [122]	CV lavage and cytobrush	*n* = 15	ZDV or d4T plus 3TC and IDV added to the regimen after 6 months of therapy	Differences in the development of drug resistance-associated mutations in cell-free HIV-1 in blood compared with that in genital tract secretions were observed. These suggest that local HIV-1 replication kinetics or drug availability in the FGT may be decreased, compared with that in plasma. These differences suggest that locally produced, cell-free HIV-1 in genital secretions was under different selective pressures than in blood.
Coombs *et al.* 2001 [123]	SNO-STRIP™ wicks, cytobrushes (endocervix) and CV lavage (ectocervix)	*n* = 55	Three quarters of the patients were on a PI-containing regimen	The general pattern of HIV-1 shedding showed the lowest levels of viral RNA in the follicular phase immediately following menses with a gradual increase throughout the luteal phase until the next menses. HIV-1 shedding was dependent on the genital subcompartment sampled and was associated with ART, >plasma viral RNA and the presence of genital tract infections. A greater natural short-term variation of HIV-1 RNA in the genital tract (13-fold) compared with blood (7-fold) was observed. The authors conclude that further studies are warranted to better understand the impact of systemic and topical ART to prevent both horizontal and vertical transmission.

ART, antiretroviral therapy; BP, blood plasma; CV, cervicovaginal; CVS, cervicovaginal secretions; FGT, female genital tract; HAART, highly active antiretroviral therapy; IDV, indinavir; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; VL, viral load, measured as HIV-1 RNA copies/ml or as $\log_{10}$ HIV-1 RNA copies/ml ($\log_{10}$); ZDV, zidovudine.

could, theoretically, have a relevant effect on the spread of the epidemic. The magnitude of the treatment effect is likely to vary considerably. Further, it may be inextricably linked to the coexistence of sexually transmitted infections [6–8, 10,12,89,90] and the change in sexual behaviours of treated individuals [91]. In the best case, highly motivated men, who are on maximally suppressive antiretroviral therapy and are free from genital infections, are probably less infectious to their sexual partners. Nevertheless, this is relative, and they will still pose a transmission risk.

Antiretroviral therapy in the transmission of resistance

The presence of drug-resistant HIV has been documented in both male and female genital secretions [40,59,62,64, 83,92–97]. The increase in the transmission of drug-resistant viruses is evidence of their transmissability [62,98–100]. The influence of differential penetration of antiretroviral therapy on the development of resistance, as described above, has not yet been proved conclusively, but remains plausible. Differences in resistance mutations have

been detected between the blood and genital tract [40,64, 92,101]. However, several groups have also found identical mutations in both compartments [59,62,73], suggesting that the degree of compartmentalisation may vary between individuals. Of greater concern for the transmission of resistance may be the concentration of resistant virus in the genital tract. Hence, the poorly adherent patient, on suboptimal therapy, with a sexually transmitted infection, who engages in unsafe sexual acts, probably represents the worst-case scenario in terms of the rapid spread of resistant HIV [95]. In such cases, antiretroviral therapy could paradoxically increase the spread of resistant HIV.

Given the adverse consequences for the transmission of resistance by poorly motivated individuals or those taking suboptimal regimens, the widespread use of antiretroviral therapy as a method of limiting the spread of HIV may be inappropriate.

Targeting antiretroviral therapy for people at high risk of transmission

The concept of preventing HIV transmission by reducing the infectiousness of the infected individual might be a valid option if treatment is targeted towards core groups, whose potential for transmitting HIV is high. Such targeted preventive efforts might be appropriate for patients with primary HIV infection and those with large numbers of sexual partners and a limited uptake of safer sex recommendations. Furthermore, a small proportion of individuals with HIV infection appear to have increased levels of genital HIV shedding [8,62,82,84,87,102]. This group, also known as 'super-shedders' [103], might be an alternative target for such a strategy, once genital infections are ruled out. However, for the intervention to be effective, adherence to medication is a prerequisite and this, again, requires motivation from the individuals involved.

Conclusions

The risk of HIV transmission exists as a spectrum. Many factors, both behavioural and biological, are interacting. It is feasible that the use of antiretroviral therapy could reduce the spread of HIV from certain people. However, this is based on biological plausibility and surrogate marker data, rather than prospective population studies.

The drug-specific nature of drug penetration into both the male and female genital tracts is intriguing. The use of wide-reaching drugs is intuitively attractive. However, no major clinical studies, to date, have conclusively demonstrated a benefit from a penetrating rather than a non-penetrating regimen, neither in terms of efficacy nor in genital tract suppression.

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References

1. Egger M, Hirschel B, Franciolo P *et al.* Impact of new antiretroviral combination therapies in HIV infected patients in Switzerland: prospective multicentre study. Swiss HIV Cohort Study. *BMJ*, 1997, **315**, 1194–1199.
2. Anderson RM and May RM. Epidemiological parameters of HIV transmission. *Nature*, 1988, **333**, 514–519.
3. Vernazza PL, Eron JJ, Fiscus SA and Cohen MS. Sexual transmission of HIV: infectiousness and prevention. *AIDS*, 1999, **13**, 155–166.
4. Baeten JM and Overbaugh J. Measuring the infectiousness of persons with HIV-1: opportunities for preventing sexual HIV-1 transmission. *Curr HIV Res*, 2003, **1**, 69–86.
5. Buchacz KA, Wilkinson DA, Krowka JF *et al.* Genetic and immunological host factors associated with susceptibility to HIV-1 infection. *AIDS*, 1998, **12** (suppl A), S87–S94.
6. Cohen MS, Hoffman IF, Royce RA *et al.* Reduction of concentration of HIV-1 in semen after treatment of urethritis: implications for prevention of sexual transmission of HIV-1. AIDSCAP Malawi Research Group. *Lancet*, 1997, **349**, 1868–1873.
7. Dyer JR, Eron JJ, Hoffman IF *et al.* Association of CD4 cell depletion and elevated blood and seminal plasma human immunodeficiency virus type 1 (HIV-1) RNA concentrations with genital ulcer disease in HIV-1-infected men in Malawi. *J Infect Dis*, 1998, 177, 224–227.
8. Sadiq ST, Taylor S, Kaye S *et al.* The effects of antiretroviral therapy on HIV-1 RNA loads in seminal plasma in HIV-positive patients with and without urethritis. *AIDS*, 2002, **16**, 219–225.
9. Wawer MJ, Sewankambo NK, Serwadda D *et al.* Control of sexually transmitted diseases for AIDS prevention in Uganda: A randomised community trial. *Lancet*, 1999, **335**, 525–535.
10. Winter AJ, Taylor S, Workman J *et al.* Asymptomatic urethritis and detection of HIV-1 RNA in seminal plasma. *Sex Transm Infect*, 1999, **75**, 261–263.
11. Fleming DT and Wasserheit JN. From epidemiological synergy to public health policy and practice: the contribution of other sexually transmitted diseases to sexual transmission of HIV infection. *Sex Transm Infect*, 1999, **75**, 3–17.
12. Wasserheit JN. Epidemiological synergy: interrelationships between human immunodeficiency virus infection and other sexually transmitted diseases. *Sex Transm Dis*, 1992, **19**, 61–77.
13. Nicolosi A, Musicco M, Saracco A and Lazzarin A. Risk factors for woman-to-man sexual transmission of the human immunodeficiency virus. Italian Study Group on HIV Heterosexual Transmission. *J Acquir Immune Defic Syndr*, 1994, **7**, 296–300.
14. Seidlin M, Vogler M, Lee E *et al.* Heterosexual transmission of HIV in a cohort of couples in New York City. *AIDS*, 1993, 7, 1247–1254.
15. de V I. A longitudinal study of human immunodeficiency virus transmission by heterosexual partners. European Study Group on Heterosexual Transmission of HIV. *N Engl J Med*, 1994, **331**, 341–346.
16. Nicolosi A, Correa Leite ML, Musicco M *et al.* The efficiency of male-to-female and female-to-male sexual transmission of the human immunodeficiency virus: a study of 730 stable couples. Italian Study Group on HIV Heterosexual Transmission. *Epidemiology*, 1994, **5**, 570–575.
17. O'Brien TR, Busch MP, Donegan E *et al.* Heterosexual transmission of human immunodeficiency virus type 1 from transfusion recipients to their sex partners. *J Acquir Immune Defic Syndr*, 1994, 7, 705–710.
18. Musicco M, Lazzarin A, Nicolosi A *et al.* Antiretroviral treatment of men infected with human immunodeficiency virus type 1 reduces the incidence of heterosexual transmission. Italian Study Group on HIV Heterosexual Transmission. *Arch Intern Med*, 1994, **154**, 1971–1976.
19. Deschamps MM, Pape JW, Hafner A and Johnson WD Jr. Heterosexual transmission of HIV in Haiti. *Ann Intern Med*, 1996, **125**, 324–330.
20. Grosskurth H, Mosha F, Todd J *et al.* Impact of improved treatment of sexually transmitted diseases on HIV infection in rural Tanzania: randomised controlled trial. *Lancet*, 1995, **346**, 530–536.

21. Lazzarin A, Saracco A, Musicco M and Nicolosi A. Man-to-woman sexual transmission of the human immunodeficiency virus: risk factors related to sexual behavior, man's infectiousness, and woman's susceptibility. Italian Study Group on HIV Heterosexual Transmission. *Arch Intern Med*, 1991, **151**, 2411–2416.
22. Quinn TC, Wawer MJ, Sewankambo N *et al.* Viral load and heterosexual transmission of human immunodeficiency virus type 1. Rakai Project Study Group. *N Engl J Med*, 2000, **342**, 921–929.
23. Lee TH, Sakahara N, Fiebig E *et al.* Correlation of HIV-1 RNA levels in plasma and heterosexual transmission of HIV-1 from infected transfusion recipients. *J Acquir Immune Defic Syndr Hum Retrovirol*, 1996, **12**, 427–428.
24. Ragni MV, Faruki H and Kingsley LA. Heterosexual HIV-1 transmission and viral load in hemophilic patients. *J Acquir Immune Defic Syndr Hum Retrovirol*, 1998, **17**, 42–45.
25. Pilcher CD, Eron JJ, Jr., Vemazza PL *et al.* Sexual transmission during the incubation period of primary HIV infection. *JAMA*, 2001, **286**, 1713–1714.
26. Yerly S, Vora S, Rizzardi P, Chave JP, Vernazza PL, Flepp M *et al.* Acute HIV infection: impact on the spread of HIV and transmission of drug resistance. *AIDS*, 2001, **15**, 2287–2292.
27. Koopman JS, Jacquez JA, Welch GW *et al.* The role of early HIV infection in the spread of HIV through populations. *J Acquir Immune Defic Syndr Hum Retrovirol*, 1997, **14**, 249–258.
28. Anderson DJ, O'Brien TR, Politch JA *et al.* Effects of disease stage and zidovudine therapy on the detection of human immunodeficiency virus type 1 in semen. *JAMA*, 1992, **267**, 2769–2774.
29. Vernazza PL, Eron JJ, Cohen MS *et al.* Detection and biologic characterization of infectious HIV-1 in semen of seropositive men. *AIDS*, 1994, **8**, 1325–1329.
30. Vernazza PL, Gilliam BL, Dyer J *et al.* Quantification of HIV in semen: correlation with antiviral treatment and immune status. *AIDS*, 1997, 11, 987–993.
31. Krieger JN, Coombs RW, Collier AC *et al.* Seminal shedding of human immunodeficiency virus type 1 and human cytomegalovirus: evidence for different immunologic controls. *J Infect Dis*, 1995, **171**, 1018–1022.
32. Liuzzi G, Chirianni A, Clementi M *et al.* Analysis of HIV-1 load in blood, semen and saliva: evidence for different viral compartments in a cross-sectional and longitudinal study. *AIDS*, 1996, **10**, F51–F56.
33. Gupta P, Mellors J, Kingsley L *et al.* High viral load in semen of human immunodeficiency virus type 1-infected men at all stages of disease and its reduction by therapy with protease and nonnucleoside reverse transcriptase inhibitors. *J Virol*, 1997, **71**, 6271–6275.
34. Xu C, Politch JA, Tucker L *et al.* Factors associated with increased levels of human immunodeficiency virus type 1 DNA in semen. *J Infect Dis*, 1997, **176**, 941–947.
35. Coombs RW, Reichelderfer PS and Landay AL. Recent observations on HIV type-1 infection in the genital tract of men and women. *AIDS*, 2003, **17**, 455–480.
36. Chakraborty H, Sen PK, Helms RW *et al.* Viral burden in genital secretions determines male-to-female sexual transmission of HIV-1: a probabilistic empiric model. *AIDS*, 2001, **15**, 621–627.
37. Anderson PL, Noormohamed SE, Henry K *et al.* Semen and serum pharmacokinetics of zidovudine and zidovudine-glucuronide in men with HIV-1 infection. *Pharmacotherapy*, 2000, **20**, 917–922.
38. Henry K, Chinnock BJ, Quinn RP *et al.* Concurrent zidovudine levels in semen and serum determined by radioimmunoassay in patients with AIDS or AIDS-related complex. *JAMA*, 1988, **259**, 3023–3026.
39. Kashuba AD, Dyer JR, Kramer LM *et al.* Antiretroviral-drug concentrations in semen: implications for sexual transmission of human immunodeficiency virus type 1. *Antimicrob Agents Chemother*, 1999, **43**, 1817–1826.
40. Lafeuillade A, Solas C, Halfon P *et al.* Differences in the detection of three HIV-1 protease inhibitors in non-blood compartments: Clinical correlations. *HIV Clin Trials*, 2002, **3**, 27–35.
41. Solas C, Lafeuillade A, Halfon P *et al.* Discrepancies between protease inhibitor concentrations and viral load in reservoirs and sanctuary sites in human immunodeficiency virus-infected patients. *Antimicrob Agents Chemother*, 2003, **47**, 238–243.
42. Taylor S, Back DJ, Workman J *et al.* Poor penetration of the male genital tract by HIV-1 protease inhibitors. *AIDS*, 1999, 13, 859–860.
43. Taylor S, van Heeswijk RP, Hoetelmans RM *et al.* Concentrations of nevirapine, lamivudine and stavudine in semen of HIV-1-infected men. *AIDS*, 2000, **14**, 1979–1984.
44. Taylor S, Reynolds H, Sabin CA *et al.* Penetration of efavirenz into the male genital tract: drug concentrations and antiviral activity in semen and blood of HIV-1-infected men. *AIDS*, 2001, **15**, 2051–2053.
45. Taylor S, Back DJ, Drake SM *et al.* Antiretroviral drug concentrations in semen of HIV-infected men: differential penetration of indinavir, ritonavir and saquinavir. *J Antimicrob Chemother*, 2001, **48**, 351–354.
46. Taylor S and Pereira AS. Antiretroviral drug concentrations in semen of HIV-1 infected men. *Sex Transm Infect*, 2001, 77, 4–11.
47. Van Praag RM, Repping S, de Vries JW *et al.* Pharmacokinetic profiles of nevirapine and indinavir in various fractions of seminal plasma. *Antimicrob Agents Chemother*, 2001, **45**, 2902–2907.
48. Van Praag RM, Weverling GJ, Portegies P *et al.* Enhanced penetration of indinavir in cerebrospinal fluid and semen after the addition of low-dose ritonavir. *AIDS*, 2000, **14**, 1187–1194.
49. Van Praag RM, van Heeswijk RP, Jurriaans S *et al.* Penetration of the nucleoside analogue abacavir into the genital tract of men infected with human immunodeficiency virus type 1. *Clin Infect Dis*, 2001, **33**, e91–e92.
50. Gatti G, Bonora G, Liuzzi G *et al.* ddI concentrations in seminal plasma. *Eighth European Conference on Clinical Aspects and Treatment of HIV-Infection,* Athens, 2001, Abstr. p157.
51. Liuzzi G, Chirianni A, Casazza R *et al.* Concentrations of stavudine in blood and semen from HIV-1-infected patients. *AIDS*, 2000, **14** (suppl 4), S96–S97 (Abstr. 279).
52. Pereira AS, Kashuba AD, Fiscus SA *et al.* Nucleoside analogues achieve high concentrations in seminal plasma: relationship between drug concentration and virus burden. *J Infect Dis*, 1999, **180**, 2039–2043.
53. Pereira AS, Smeaton LM, Gerber JG *et al.* The pharmacokinetics of amprenavir, zidovudine, and lamivudine in the genital tracts of men infected with human immunodeficiency virus type 1 (AIDS clinical trials group study 850). *J Infect Dis*, 2002, **186**, 198–204.
54. Reddy YS, Gotzkowsky SK, Eron JJ *et al.* Pharmacokinetic and pharmacodynamic investigation of efavirenz in the semen and blood of human immunodeficiency virus type 1-infected men. *J Infect Dis*, 2002, **186**, 1339–1343.
55. Reddy YS, Kashuba A, Gerber J and Miller V. Round table report: importance of antiretroviral drug concentrations in sanctuary sites and viral reservoirs. *AIDS Res Hum Retroviruses*, 2003, **19**, 167–176.
56. Sankatsing SU, Droste J, Burger D *et al.* Limited penetration of lopinavir into seminal plasma of HIV-1-infected men. *AIDS*, 2002, **16**, 1698–1700.
57. Newell ML, Dunn DT, Peckham CS *et al.* Vertical transmission of HIV-1: maternal immune status and obstetric factors. The European Collaborative Study. *AIDS*, 1996, **10**, 1675–1681.
58. Byrn RA, Zhang D, Eyre R *et al.* HIV-1 in semen: an isolated virus reservoir. *Lancet*, 1997, **350**, 1141.
59. Choudhury B, Pillay D, Taylor S and Cane PA. Analysis of HIV-1 variation in blood and semen during treatment and treatment interruption. *J Med Virol*, 2002, **68**, 467–472.
60. Delwart EL, Mullins JI, Gupta P *et al.* Human immunodeficiency virus type 1 populations in blood and semen. *J Virol*, 1998, **72**, 617–623.
61. Gupta P, Leroux C, Patterson BK *et al.* Human immunodeficiency virus type 1 shedding pattern in semen correlates with the compartmentalization of viral quasi species between blood and semen. *J Infect Dis*, 2000, **182**, 79–87.
62. Taylor S, Cane P, Hue S *et al.* Identification of a transmission chain of HIV type 1 containing drug resistance-associated mutations. *AIDS Res Hum Retroviruses*, 2003, **19**, 353–361.
63. Overbaugh J, Anderson RJ, Ndinya-Achola JO and Kreiss JK. Distinct but related human immunodeficiency virus type 1 variant populations in genital secretions and blood. *AIDS Res Hum Retroviruses*, 1996, **12**, 107–115.
64. Eyre RC, Zheng G and Kiessling AA. Multiple drug resistance mutations in human immunodeficiency virus in semen but not blood of a man on antiretroviral therapy. *Urology*, 2000, **55**, 591.
65. Ball JK, Curran R, Irving WL and Dearden AA. HIV-1 in semen: determination of proviral and viral titres compared to blood, and

quantification of semen leukocyte populations. *J Med Virol*, 1999, **59**, 356–363.
66. Kepler TB and Perelson AS. Drug concentration heterogeneity facilitates the evolution of drug resistance. *Proc Natl Acad Sci USA*, 1998, **95**, 11514–11519.
67. Pereira AS, Kenney KB, Cohen MS *et al*. Simultaneous determination of lamivudine and zidovudine concentrations in human seminal plasma using high-performance liquid chromatography and tandem mass spectrometry. *J Chromatogr B Biomed Sci Appl*, 2000, **742**, 173–183.
68. Reddy YS, Troiani L, Kim J *et al*. Differential phosphorylation of zidovudine and lamivudine between semen and blood mononuclear cells in HIV infected men. *10th Conference on Retroviruses and Opportunistic Infections,* Boston, 2003, Abstr. 530.
69. Taylor S, Reynolds H, Sabin C *et al*. Penetration of non-nucleoside reverse transcriptase inhibitors (NNRTIs) into the male genital tract: variable drug concentrations in semen and blood plasma of HIV-1 infected men. *Second International Workshop on Clinical Pharmacology of HIV Therapy,* Noordwijk, 2001, Abstr. 2.2.
70. Hsu A. Pharmacokinetic/pharmacodynamic modeling in HIV therapy. *Second International Workshop on Clinical Pharmacology of HIV Therapy,* Noordwijk, 2001, Abstr. 5.3.
71. Veldkamp AI, Weverling GJ, Lange JM *et al*. High exposure to nevirapine in plasma is associated with an improved virological response in HIV-1-infected individuals. *AIDS*, 2001, **15**, 1089–1095.
72. Marzolini C, Telenti A, Decosterd LA *et al*. Efavirenz plasma levels can predict treatment failure and central nervous system side effects in HIV-1-infected patients. *AIDS*, 2001, **15**, 71–75.
73. Si-Mohamed A, Kazatchkine MD, Heard I *et al*. Selection of drug-resistant variants in the female genital tract of human immunodeficiency virus type 1-infected women receiving antiretroviral therapy. *J Infect Dis*, 2000, **182**, 112–122.
74. Launay O, Tod M, Louchahi K *et al*. Differential diffusion of indinavir (IDV) and lopinavir (LPV) in genital secretions of HIV-infected women. *42nd International Conference on Antimicrobial Agents and Chemotherapy*, San Diego, 2002, Abstr. N: H-1712.
75. Kashuba AD, Minn SS, Corbett AH *et al*. Comparison of protease inhibitor and non-nucleoside reverse transcriptase inhibitor concentrations in the male and female genital tract. *Third International Workshop on Clinical Pharmacology of HIV Therapy*, Washington DC, 2002, Abstr. 5.3.
76. Back D, Gatti G, Fletcher C *et al*. Therapeutic drug monitoring in HIV infection: current status and future directions. *AIDS*, 2002, **16**, (suppl 1), S5–S37.
77. Krieger JN, Coombs RW, Collier AC *et al*. Recovery of human immunodeficiency virus type 1 from semen: minimal impact of stage of infection and current antiviral chemotherapy. *J Infect Dis*, 1991, **163**, 386–388.
78. Vernazza PL, Troiani L, Flepp MJ *et al*. Potent antiretroviral treatment of HIV-infection results in suppression of the seminal shedding of HIV. The Swiss HIV Cohort Study. *AIDS*, 2000, **14**, 117–121.
79. Leruez-Ville M, Dulioust E, Costabliola D *et al*. Decrease in HIV-1 seminal shedding in men receiving highly active antiretroviral therapy: an 18 month longitudinal study (ANRS EP012). *AIDS*, 2002, **16**, 486–488.
80. Ball JK, Rowe T, Curran R *et al*. Poor reduction of HIV-1 RNA titres in nucleoside reverse transcriptase inhibitor experienced patients treated with indinavir combination therapy. *Sex Transm Infect*, 1999, **75**, 337–339.
81. Barroso PF, Schechter M, Gupta P *et al*. Effect of antiretroviral therapy on HIV shedding in semen. *Ann Intern Med*, 2000, **133**, 280–284.
82. Kalichman SC, Cage M, Barnett T *et al*. Human immunodeficiency virus in semen and plasma: investigation of sexual transmission risk behavioral correlates. *AIDS Res Hum Retroviruses*, 2001, **17**, 1695–1703.
83. Mayer KH, Boswell S, Goldstein R *et al*. Persistence of human immunodeficiency virus in semen after adding indinavir to combination antiretroviral therapy. *Clin Infect Dis*, 1999, **28**, 1252–1259.
84. Tachet A, Dulioust E, Salmon D *et al*. Detection and quantification of HIV-1 in semen: identification of a subpopulation of men at high potential risk of viral sexual transmission. *AIDS*, 1999, **13**, 823–831.
85. Zhang H, Dornadula G, Beumont M *et al*. Human immunodeficiency virus type 1 in the semen of men receiving highly active antiretroviral therapy. *N Engl J Med*, 1998, **339**, 1803–1809.
86. Kovacs A, Wasserman SS, Burns D *et al*. Determinants of HIV-1 shedding in the genital tract of women. *Lancet*, 2001, 358, 1593–1601.
87. Eron JJ, Jr., Smeaton LM, Fiscus SA *et al*. The effects of protease inhibitor therapy on human immunodeficiency virus type 1 levels in semen (AIDS clinical trials group protocol 850). *J Infect Dis*, 2000, **181**, 1622–1628.
88. Hupfer M, Wagels T and Kahlert C. Ritonavir boosted indinavir treatment as a simplified maintenance 'mono'-therapy for HIV infection. *2nd IAS Conference on HIV Pathogenesis and Treatment*, Paris, 2003, Abstr. 589.
89. Grosskurth H, Mosha F, Todd J *et al*. Impact of improved treatment of sexually transmitted diseases on HIV infection in rural Tanzania: randomised controlled trial. *Lancet*, 1995, 346, 530–536.
90. Schacker T, Ryncarz A, Goddard J *et al*. Frequent recovery of HIV from genital herpes simplex lesions in HIV infected persons. *JAMA*, 1998, **280**, 61–66.
91. Desquilbet L, Deveau C, Goujard C *et al*. Increase in at-risk sexual behaviour among HIV-1-infected patients followed in the French PRIMO cohort. *AIDS*, 2002, **16**, 2329–2333.
92. Kroodsma KL, Kozal MJ, Hamed KA *et al*. Detection of drug resistance mutations in the human immunodeficiency virus type 1 (HIV-1) pol gene: differences in semen and blood HIV-1 RNA and proviral DNA. *J Infect Dis*, 1994, **170**, 1292–1295.
93. Eron JJ, Vernazza PL, Johnston DM *et al*. Resistance of HIV-1 to antiretroviral agents in blood and seminal plasma: implications for transmission. *AIDS*, 1998, **12**, F181–F189.
94. Di Stefano M, Fiore JR, Monno L *et al*. Detection of multiple drug-resistance-associated pol mutations in cervicovaginal secretions from women largely treated with antiretroviral agents [letter]. *AIDS*, 1999, **13**, 992–994.
95. Taylor S, Sadiq ST, Weller I *et al*. Drug-resistant HIV-1 in the semen of men receiving antiretroviral therapy with acute sexually transmitted infections. *Antiviral Ther,* 2003, **8**, 29–33.
96. Wainberg MA, Beaulieu R, Tsoukas C and Thomas R. Detection of zidovudine-resistant variants of HIV-1 in genital fluids. *AIDS*, 1993, **7**, 433–434.
97. Lafeuillade A, Solas C, Chadapaud S *et al*. HIV-1 RNA levels, resistance, and drug diffusion in semen versus blood in patients receiving a lopinavir-containing regimen. *J Acquir Immune Defic Syndr*, 2003, **32**, 462–464.
98. Little SJ, Daar ES, D'Aquila RT *et al*. Reduced antiretroviral drug susceptibility among patients with primary HIV infection. *JAMA*, 1999, **282**, 1142–1149.
99. UK Resistance Collaborative Group. Analysis of prevalence of HIV-1 drug resistance in primary infections in the United Kingdom. *BMJ*, 2001, **322**, 1087–1088.
100. Yerly S, Kaiser L, Race E *et al*. Transmission of antiretroviral-drug-resistant HIV-1 variants. *Lancet*, 1999, **354**, 729–733.
101. Eron JJ, Vernazza PL, Johnston DM *et al*. Resistance of HIV-1 to antiretroviral agents in blood and seminal plasma: implications for transmission. *AIDS*, 1998, **12**, F181–F189.
102. Pilcher CD, Shugars DC, Fiscus SA *et al*. HIV in body fluids during primary HIV infection: implications for pathogenesis, treatment and public health. *AIDS*, 2001, **15**, 837–845.
103. Taylor S, Sadiq ST, Sabin C *et al*. Seminal supershedding of HIV: implications for sexual transmission. *10th Conference on Retroviruses and Opportunistic Infections*, Boston, 2003, Abstr. 454.
104. Taylor S, Veldkamp A, Kapplehoff B *et al*. Abacavir and lamivudine reach high concentrations in the semen of HIV-1 infected men. *Sixth International Congress on Drug Therapy in HIV Infection*, Glasgow, 2002, Abstr. P 168.
105. Molla A, Vasavanonda S, Kumar G *et al*. Human serum attenuates the activity of protease inhibitors toward wild-type and mutant human immunodeficiency virus. *Virology*, 1998, **250**, 255–262.
106. Hamed KA, Winters MA, Holodniy M *et al*. Detection of human immunodeficiency virus type 1 in semen: effects of disease stage and nucleoside therapy. *J Infect Dis*, 1993, **167**, 798–802.
107. Vernazza PL, Gilliam BL, Flepp M *et al*. Effect of antiviral treatment on the shedding of HIV-1 in semen. *AIDS*, 1997, **11**, 1249–1254.
108. Gilliam BL, Dyer JR, Fiscus SA *et al*. Effects of reverse transcriptase inhibitor therapy on the HIV-1 viral burden in semen. *J Acquir Immune Defic Syndr Hum Retrovirol*, 1997, **15**, 54–60.

109. Dornadula G, Zhang H, VanUitert B *et al.* Residual HIV-1 RNA in blood plasma of patients taking suppressive highly active antiretroviral therapy. *JAMA*, 1999, **282**, 1627–1632.
110. Liuzzi G, Chirianni A, Bagnarelli P *et al.* A combination of nucleoside analogues and a protease inhibitor reduces HIV-1 RNA levels in semen: implications for sexual transmission of HIV infection. *Antiviral Ther*, 1999, **4**, 95–99.
111. Speck CE, Coombs RW, Koutsky LA *et al.* Risk factors for HIV-1 shedding in semen. *Am J Epidemiol*, 1999, **150**, 622–631.
112. Gunthard HF, Havlir DV, Fiscus S *et al.* Residual human immunodeficiency virus (HIV) Type 1 RNA and DNA in lymph nodes and HIV RNA in genital secretions and in cerebrospinal fluid after suppression of viremia for 2 years. *J Infect Dis*, 2001, **183**, 1318–1327.
113. Bourlet T, Cazorla C, Berthelot P *et al.* Compartmentalization of HIV-1 according to antiretroviral therapy: viral loads are correlated in blood and semen but poorly in blood and saliva. *AIDS*, 2001, **15**, 284–285.
114. Celum CL, Buchbinder SP, Donnell D *et al.* Early human immunodeficiency virus (HIV) infection in the HIV Network for Prevention Trials Vaccine Preparedness Cohort: risk behaviors, symptoms, and early plasma and genital tract virus load. *J Infect Dis*, 2001, **183**, 23–35.
115. Nunnari G, Otero M, Dornadula G *et al.* Residual HIV-1 disease in seminal cells of HIV-1-infected men on suppressive HAART: latency without on-going cellular infections. *AIDS*, 2002, **16**, 39–45.
116. Liuzzi G, D'Offizi G, Topino S *et al.* Dynamics of viral load rebound in plasma and semen after stopping effective antiretroviral therapy. *AIDS*, 2003, **17**, 1089–1092.
117. Barroso PF, Schechter M, Gupta P *et al.* Adherence to antiretroviral therapy and persistence of HIV RNA in semen. *J Acquir Immune Defic Syndr*, 2003, **32**, 435–440.
118. Loussert-Ajaka I, Mandelbrot L, Delmas MC *et al.* HIV-1 detection in cervicovaginal secretions during pregnancy. *AIDS*, 1997, **11**, 1575–1581.
119. Hart CE, Lennox JL, Pratt-Palmore M *et al.* Correlation of human immunodeficiency virus type 1 RNA levels in blood and the female genital tract. *J Infect Dis*, 1999, **179**, 871–882.
120. Cu-Uvin S, Caliendo AM, Reinert S *et al.* Effect of highly active antiretroviral therapy on cervicovaginal HIV-1 RNA. *AIDS*, 2000, **14**, 415–421.
121. Debiaggi M, Zara F, Spinillo A *et al.* Viral excretion in cervicovaginal secretions of HIV-1-infected women receiving antiretroviral therapy. *Eur J Clin Microbiol Infect Dis*, 2001, **20**, 91–96.
122. Ellerbrock TV, Lennox JL, Clancy KA *et al.* Cellular replication of human immunodeficiency virus type 1 occurs in vaginal secretions. *J Infect Dis*, 2001, **184**, 28–36.
123. Coombs RW, Wright DJ, Reichelderfer PS *et al.* Variation of human immunodeficiency virus type 1 viral RNA levels in the female genital tract: implications for applying measurements to individual women. *J Infect Dis*, 2001, **184**, 1187–1191.

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