Effect of antiretrovirals on semen quality

Sidonie Lambert-Niclot, Catherine Poirot, Roland Tubiana, Allal Houssaini, Cathia Soulié, Stephanie Dominguez, Benoit Schubert, Marie Prades, Manuela Bonmarchand, Vincent Calvez, et al.

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Keywords:	Antiretrovirals drugs, semen quality, penetration score

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Effect of antiretroviral drugs on the quality of semen

Running Title: Effect of antiretrovirals on semen quality

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26 This work was presented at the 12th European Aids Conference/EACS (November 11-24,
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Abstract

The aim of this cross-sectional study was to determine which antiretroviral drugs (ARVs) are associated with changes in the characteristics of semen and the impact of these ARVs according to their score penetration into the male genital compartment. Data from 144 men infected with HIV-1 enrolled in an Assisted Reproductive Technology program were analyzed retrospectively. A seminal penetration score of ARV was based on the available literature. The nonparametric Kruskal-Wallis test and Khi-2 test were used. There was no difference on sperm parameters between NRTI, NNRTI or PI regimen. In patients receiving NRTIs or PIs no differences were observed between antiretrovirals of these classes. However in patients receiving NNRTIs, nevirapine (n=22) was associated with a higher percentage of progressively motile spermatozoa ($p<0.0001$) versus efavirenz (n=38) as well as vitality ($p=0.0004$). No relationship was observed between semen quality and the penetration score. NRTIs and PIs were not associated with any semen changes. Nevirapine was associated with a better quality of semen versus efavirenz. It would be of interest to validate, improve and test our penetration score in a prospective study.

Key Words: Antiretrovirals drugs, semen quality, penetration score

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Introduction

Potent combination antiretroviral therapy has increased the life expectancy and quality of life of patients infected with HIV-1. Currently, some couples request medical assistance to achieve conception while reducing to a minimum the risk of HIV-1 transmission. This technical requirement for Assisted Reproductive Technology program raises the question of semen characteristics in men infected with HIV-1 under antiretroviral treatment. Several studies have assessed semen quality in men infected with HIV-1 [Bujan et al., 2007; Dulioust et al., 2002; Nicopoullos et al., 2004]. However the use of antiretroviral drugs (ARVs) did not appear to be correlated with any sperm characteristics while most of the patients were receiving antiretroviral therapy. A practical method was developed for quantifying the penetration in genital tract of the many different ARV drug regimens currently prescribed, as estimated by the penetration score. This method uses publicly available information about ARV drug characteristics, measured semen concentrations, and effectiveness in the genital tract to rank drugs relative to one another. Antiretroviral drugs showed different penetration score in the male genital tract and may therefore influence differently spermatogenesis, or semen quality which is a key factor for reproductive success.

The aim of this cross-sectional cohort study was undertaken to determine which ARVs were associated with changes in semen characteristics and the impact of these ARVs according to their penetration score into the male genital compartment.

75 Patients and methods

76 One hundred and forty four men infected with HIV-1 attending the Pitié-Salpêtrière Hospital
77 in the multidisciplinary assisted reproductive technology program between January 2002 and
78 September 2007 were studied retrospectively. HIV-1 RNA quantitation was determined by
79 using the Amplicor Monitor assay (Cobas 1.5; Roche Diagnostics, Basel, Switzerland), which
80 has a detection limit of 200 copies/ml in seminal plasma.

81 All semen samples were tested in the Unit of Reproductive Biology according to standardized
82 methods throughout the study period and according to the World Health Organization (WHO)
83 guidelines [WHO, 1992].

84 Semen samples were collected in the laboratory by masturbation into a sterile graduated
85 container after 3-5 days of sexual abstinence. After 30 minutes at 35°C, each sample was
86 examined. The volume of the seminal fluid, semen pH and viscosity were determined.
87 Motility was graded (a) rapid progressive motility; (b) slow progressive motility, (c) non-
88 progressive motility and (d) no motility according the WHO guidelines. The percentage of
89 motile spermatozoa was determined at T0 (30-45 min after semen collection) and at T1 (4-5
90 hours after semen collection). Sperm and round cell concentrations were determined using a
91 Kova Slide (HYCOR Biomedical, Garden Grove, California, USA). Sperm vitality was
92 assessed after staining with Eosin-Nigrosin. The multiple abnormalities index represents the
93 ratio of the number of sperm abnormalities, over the number of abnormal spermatozoa.
94 Teratospermia is the opposite of the percentage of morphologically normal spermatozoa.

95 The present study was carried out in accordance with the declaration of Helsinki and was
96 approved by ANRS AC11 Resistance Study Group scientific committee. All patients gave
97 written informed consent.

98 Penetration of antiretroviral drugs was characterized using a hierarchical approach based on
99 the best available evidence. Data on chemical and pharmacokinetic characteristics were

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reviewed for antiretroviral drugs approved by national agencies using package inserts, drug references, published papers, and international conference abstracts [Pereira et al., 2002; Solas et al., 2003; Taylor et al., 2001]. Using this approach, antiretroviral drugs were classified into three categories. Individual antiretroviral drugs were assigned a rank based on penetration category (0=lowest, 0.5=intermediate and 1=highest penetration) (table2). The final rank was then determined by adding the individual penetration scores for each ARV drug in a regimen. Continuous variables were compared using the Kruskal-Wallis test while categorical variables were compared using Khi-2 test. All analyses used a 2-sided α of 0.05. Statistical analyses were performed with SAS release 9.1 for Windows XP Pro.

Results

The main characteristics of the study population are described in Table 1. At the time of semen collection, 124 men (86%) were receiving ARVs (dual therapy: 3 men; triple therapy: 109 men; ≥ 4 drugs: 12 men). The median number of drugs used was 2 nucleosides reverse transcriptase inhibitors NRTIS (range, 0-4), 1 protease inhibitor PI (range, 0-1) and 0 non nucleoside reverse transcriptase inhibitors NNRTI (range, 0-1).

Semen characteristics are shown in Table 1. The characteristics of seminal fluid ranged from 0.13-8.50 mL (mean 3.5) for volume and 7.4-9.0 (mean 8.0) for pH. Majority of ejaculates 83.9% (115/137) had a normal viscosity. The concentration of spermatozoa ranged from 2.61-676 million per millilitre (mean 116). The concentration of round cells ranged from 0-36 10^6 /mL (mean 2.79). The total sperm count was ranged from 1.3-2000 10^6 per ejaculate (mean 385.3). The mean percentage of rapid progressively motile spermatozoa was 16.6 (range 0-60) and the mean percentage of slow progressively motile spermatozoa was 27.3 (range 5-75). The mean percentage of vitality was 78.6 (range 35-98). The mean of multiple abnormality index was 1.70 (range 1.17-2.86). The percentage of abnormal sperm morphology was ranged from 30 to 99 (mean 69). Twenty four % of patient (35 out of 144) had all their sperm characteristics strictly normal according to WHO standards.

Studying the different variables of the quality of semen, no relationship between semen quality and the penetration score was found. There was no difference in the characteristics of sperm between patients receiving NRTI, NNRTI or PI regimen. In patients receiving NRTIs or PIs no differences were observed between the antiretroviral drugs of these classes. No association was shown between thymidine analogues (D4T or AZT) or other NRTIs and semen characteristics. However, considering only the NNRTI containing regimen, it was shown that nevirapine was associated with a higher percentage of both progressively motile spermatozoa (at T0 (immediately after liquefaction) and at T1 (4 hours after liquefaction)) and

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134 vitality versus efavirenz. Regarding the mean percentage of progressively motile spermatozoa
135 both at T0 and T1, nevirapine (22.05 and 15.24) and PI (17.31 and 12.71) provided similar
136 values while efavirenz (7.92 and 3.81) led to smaller values. The same trend was observed in
137 the percentage of vitality with a mean of 85.3 for nevirapine, 72.2 for efavirenz and 80.1 for
138 PI. The difference observed between nevirapine and efavirenz does not seem to be related to
139 the NRTI associated treatment. Indeed thymidine analogues were distributed equally between
140 nevirapine and efavirenz groups. The known characteristics of patients were similar between
141 nevirapine and efavirenz groups.

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Discussion

In the present study, the characteristics of sperm were the same as those found in other studies [Bujan et al., 2007; Dulioust et al., 2002; Nicopoullos et al., 2004; van Leeuwen et al., 2008b] except for sperm concentration which was higher. The same trend was observed for the total sperm count. Several studies have assessed semen quality in men infected with HIV-1 in comparison to HIV-1 seronegative men [Bujan et al., 2007; Dulioust et al., 2002; Nicopoullos et al., 2004]. The study by Bujan et al (2007) demonstrated decreases in the semen volume, spermatozoa motility, and total motile sperm count and increases in the pH values and multiple anomaly indices of patients infected with HIV. In the study by Dulioust et al, (2002) the most significant semen alterations were a decrease of the rapidly progressive motile spermatozoa (motility a) and an increase of less rapidly progressive spermatozoa (motility b) the patients infected with HIV. In the study by Nicopoullos et al (2004), ejaculate volume, sperm concentration, total count, progressive motility and normal morphology were decreased. However, none of these studies related these semen alterations to HIV infection or ARV therapy. Another study explored the characteristics of semen before and after the start of ARV with different results and demonstrated a statistically significant reduction in the percentage of progressively motile spermatozoa in patients with HIV-1 infection during treatment with ARV therapy [van Leeuwen et al., 2008b]. They demonstrated previously that there was no detectable change in semen quality in patients during a period of untreated asymptomatic HIV-1 infection with similar proportion of progressively motile spermatozoa [van Leeuwen et al., 2008a]. These observations suggest that the reduction in progressively motile spermatozoa, which occurred in the current study during treatment, was related to the use of ARV and not to HIV infection. In these studies, it is not clear if some semen alterations could be the result of antiretroviral treatment or the result of HIV infection. Indeed various studies have demonstrated a relationship between mitochondria and sperm motility [Donnelly

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et al., 2000; May-Panloup et al., 2003]. Since several ARVs have mitochondrial toxicity, the observed changes in motility could be due to the ARV itself. However no association was found in the current study between quality of semen and the thymidine analogue containing treatment.

However it was found that nevirapine was associated with a higher percentage of progressively motile spermatozoa at T0 and at T1 and a better vitality in comparaison with efavirenz. The proportion of progressively motile spermatozoa under nevirapine or PI were normal according to WHO standards whereas in the efavirenz group this percentage was decreased. Concerning vitality, nevirapine, PI or efavirenz remained according to WHO standards although the efavirenz group presented vitality significantly inferior to nevirapine or the PIs groups. The percentage of progressively motile spermatozoa and vitality are related measures of semen quality. Nevirapine has a better penetration score than efavirenz and is a priori more efficient in this compartment, a better control of the virus may explain a better quality of semen. Another explanation could be a direct toxicity of efavirenz on cells producing spermatozoa. Indeed there is no study on sperm characteristics before and after infection. Despite no relationship with the penetration score it would be interesting to improve this score and to try to validate it in a prospective study.

In conclusion, NRTIs and PIs were not associated with any semen changes. However, in patients receiving NNRTIs, nevirapine was associated with a better quality of semen versus efavirenz. This study confirms the fact that semen alterations are frequent in patients infected with HIV and suggests that these alterations could be related to ARV treatment.

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Ethics

The present study was carried out in accordance with the declaration of Helsinki and was approved by the ANRS AC11 Resistance Study Group scientific committee. All patients gave written informed consent.

Conflict of interest

There are no conflicts of interest.

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208 **Table 1 Characteristics of patients and semen.**

Variable of patients	n	Mean (SD)
Age	144	39.00 (5.75)
Viral load, mean log ₁₀ copies/mL	103	2.48 (0.89)
CD4 ⁺ cell count, means cells/mm ³	93	497.00 (211.71)
Semen characteristics		
Ejaculate volume	143	3.50 (1.70)
pH	144	8.00 (0.30)
Viscosity	137	
Low (%)	7 (5.10)	
Normal (%)	115 (83.90)	
High (%)	15 (10.90)	
Concentration of spermatozoa (cell per 10 ⁶ /mL)	144	116.00 (109.74)
Round cells (10 ⁶ /mL)	144	2.79 (4.67)
Total sperm count (10 ⁶ per ejaculate)	144	385.30 (371.10)
Rapid progressively motile spermatozoa at T0 (%)	144	16.60 (12.80)
Slow progressively motile spermatozoa at T0 (%)	144	27.30 (12.80)
Progressively motile spermatozoa at T1 (%)	135	11.40 (9.70)
Slowly motile spermatozoa at T1 (%)	135	22.30 (10.00)
Vitality (%)	143	78.60 (14.11)
Multiple abnormality index	143	1.70 (0.29)
Teratospermia (%)	144	69.00 (15.00)

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Table 2 Penetration score of antiretroviral drugs.

Penetration score of antiretroviral drugs			
	1 (>50%)	0.5 (10-50%)	1 (<10%)
NRTI ¹	Tenofovir Zidovudine Abacavir Emtricitabine Lamivudine Didanosine		
NNRTI ²	Nevirapine		Efavirenz Etravirine
PI ³	Indinavir/r	Amprenavir/r Atazanavir/r Darunavir/r	Nelfinavir Ritonavir Lopinavir/r Saquinavir/r Tipranavir/r
FI ⁴	Enfuvirtide		
CCR5 inhibitor	Maraviroc		
INSTI	Raltegravir		

NRTI: nucleoside reverse transcriptase inhibitor; NNRTI: non nucleoside reverse transcriptase inhibitor; PI: protease inhibitor; FI: fusion inhibitor; CCR5: chemokine coreceptor 5 ; INSTI: integrase strand transfer inhibitor. 1: [Dumond et al., 2006; Kashuba et al., 1999; Pereira et al., 2002; Reddy et al., 2003] ; 2: [Dumond et al., 2006; Ghosn et al., 2004; Reddy et al., 2003]; 3: [Dumond et al., 2006; Ghosn et al., 2004; Kashuba et al., 1999; Pereira et al., 2002; Reddy et al., 2003; Solas et al., 2003; Taylor et al., 2001; van Leeuwen et al., 2007; van Praag et al., 2000]; 4: [Ghosn et al., 2004]

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