

Decreased Semen Volume and Spermatozoa Motility in HIV-1-Infected Patients Under Antiretroviral Treatment

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ABSTRACT: Inconsistent results have been reported for the semen quality in HIV-infected men, due to the biases inherent in some studies. The objective of the present study was to investigate the semen parameters in HIV-1-infected patients and to compare their sperm characteristics with those of a control group of fertile, noninfected men. Factors implicated in semen alterations in HIV-1 patients were also analyzed. HIV-infected men (n = 190), of whom 91% were undergoing antiretroviral therapy, and 218 fertile men were studied. Infertility risk factors were recorded and clinical examinations were performed for both groups. Records of history of HIV infection, antiretroviral treatment, and HIV-1 RNA detection in the blood as well as HIV-1 genome detection in the semen were obtained for the infected patients. Semen volumes, percentages of progressive motile spermatozoa, total sperm counts, and poly-

morphonuclear cell counts were decreased, while the pH values and spermatozoa multiple anomaly indices were increased in HIV-infected patients. Even after adjustment for possible sources of bias, the decreases in semen volume and progressive motility and the increase in pH remained significant. The present study demonstrates sperm motility and ejaculate volume alterations in HIV-1-infected patients, most of whom were receiving antiretroviral therapy. In HIV-1 patients, further longitudinal studies are required to analyze the impact of treatment regimen on sperm parameter alterations.

Key words: HIV-1, infectious disease, mitochondrial DNA, semen quality.

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HIV-1 infection is a sexually transmitted disease that affects at least 40 million individuals worldwide (UNAIDS and World Health Organization, 2006). HIV-1 may be present in the human male genital tract (Dejuq and Jegou, 2001). Its transmissibility depends on the type of sexual exposure, frequency of sexual intercourse, level of infectivity of the infected partner, and the susceptibility of the noninfected partner (Mayer and Anderson, 1995; Royce et al, 1997). Sexual transmission is also related to blood viral load and to

immunologic and virologic statuses (Lee et al, 1996; Quinn et al, 2000).

Several studies have reported the presence of HIV-1 genomes or infectious viruses in the seminal fluids or sperm cells of HIV-1-infected men (Anderson et al, 1992; Vernazza et al, 1994; Tachet et al, 1999; Pasquier et al, 2000). Moreover, evidence for compartmentalization of HIV-1 between semen and blood has been reported (Byrn and Kiessling, 1998; Coombs et al, 1998; Kiessling et al, 1998; Ghosn et al, 2004), although this evidence is debated (Lowe et al, 2004).

Currently, and particularly following the improved prognosis of HIV-1 infection thanks to highly active antiretroviral therapy (HAART), couples request medical assistance to achieve conception while reducing to a minimum the risk of HIV-1 transmission.

Several teams have developed medically assisted procreation (MAP) programs for serodiscordant couples with an HIV-1-infected male partner, which allow these couples to have children without infection of the female partner (Semprini, 1993; Marina et al, 1998; Gilling-Smith, 2000; Guibert et al, 2001; Loutradis et al, 2001; Pena et al, 2002; Sauer and Chang, 2002; Marina et al, 2003; Ohl et al, 2003; Pena et al, 2003; Bujan et al, 2004; Garrido et al, 2004; Nicopoullos et al, 2004;

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Mencaglia et al, 2005). In these programs, spermatozoa are isolated from semen and usually tested for HIV-1 nucleic acid before use in MAP. Good semen parameters facilitate the different steps of MAP in these couples.

Several studies have assessed semen quality in HIV-1-infected men, with inconsistent results. Krieger et al (1991) have reported no difference in semen parameters in 24 HIV-1-infected men compared with 40 HIV-negative controls. Crittenden et al (1992) have shown reduced motility and increased frequency of round cells in infected men. Other studies have reported decreases in motility, sperm concentration or total sperm count (Dondero et al, 1996; Muller et al, 1998; Dulioust et al, 2002; Nicopoullos et al, 2004). Three studies have reported ejaculate volume decreases (Muller et al, 1998; Dulioust et al, 2002; Nicopoullos et al, 2004). Moreover, a reduced percentage of morphologically normal spermatozoa has been reported in several studies (Dondero et al, 1996; Muller et al, 1998; Nicopoullos et al, 2004) but not in the study of Dulioust et al (2002). Correlations between blood CD4 counts or viral loads and sperm motilities or sperm counts have been reported in some studies but not in others (Gupta et al, 1997; Vernazza et al, 1997; Tachet et al, 1999; Pasquier et al, 2000; Bujan et al, 2004).

The discrepancies between these studies may result from differences between infected populations or from the low numbers of patients and controls, with the exceptions of the two more recent studies (Dulioust et al, 2002; Nicopoullos et al, 2004), and could also result from the choice of control group. Moreover, andrologic and disease histories (such as cryptorchidism, varicocele or genital infection) were not taken into account in these studies.

The present study of 190 HIV-1-infected men compares their sperm characteristics with those of a control group of 218 fertile men and analyzes the factors implicated in semen alterations in HIV-infected men.

Materials and Methods

Patients

HIV-1-infected men (n = 190) who attended the Centre d'Etudes et de Conservation des Oeufs et du Sperme Humain (CECOS) Midi-Pyrenees, Hôpital Paule de Viguier, Toulouse provided 190 semen samples between April 1998 and April 2004. All of the subjects were clinically asymptomatic.

Control Group

Men of proven fertility (n = 218; having fathered at least one child or having a pregnant partner) were included in the control group. The control subjects included candidates for

sperm donation (n = 58), volunteers in a research study of male reproductive health in France (n = 74), and men who were requesting vasectomy (n = 86). All of the men provided semen samples and gave their informed consent. The present study was carried out in accordance with the Declaration of Helsinki and was approved by the institutional review board.

Clinical Investigations

Medical and andrologic histories, including past genital (urethritis, orchiepididymitis, prostatitis) or urinary infections, cryptorchidism, varicocele, and tobacco habits, were obtained from the patients and the control group. In addition, the patients underwent a clinical examination in which the position and trophicity of the epididymis and scrotal vas deferens were assessed. The length (L) and width (W) of each testis were measured with calipers according to our previously published method (Mieusset et al, 1989). Testicular volume was calculated according to the following formula: volume = $0.71 \times L \times W^2$. The presence of a varicocele, abnormal position or trophicity of the epididymis, and deferens absence were recorded as andrologic abnormalities.

Semen Samples

Semen samples from patients and control subjects were collected at the CECOS laboratory by masturbation into sterile containers after a recommended 3-day period of sexual abstinence. Samples were processed at the laboratory within 2 hours of ejaculation according to World Health Organization (WHO) recommendations (WHO, 1999) and our previously published method (Bujan et al, 2004). Semen pH was measured using pH-indicator strips (pH ranges 6.5–10.0 and 4.0–7.0; Merck, Darmstadt, Germany). Motility was classified according to the WHO criteria as follows: a, rapid progressive spermatozoa; b, slow progressive spermatozoa; c, nonprogressive spermatozoa; and d, immotile spermatozoa. Round cells in the semen were assessed according the WHO recommendations. Sperm vitality was assessed after eosin Y-nigrosin staining and is expressed as the percentage of viable spermatozoa. The total sperm count was calculated as: sperm count ($10^6/\text{mL}$) \times ejaculate volume (in mL). The total motile sperm count was calculated as: total sperm count \times percentage of motility a + b. Sperm morphology was analyzed according to David classification, as modified by Jouannet et al (1988). This classification allows calculation of the multiple anomaly index (MAI), which is the mean number of anomalies per abnormal spermatozoon. Semen polymorphonuclear granulocyte counting was performed using the peroxidase staining method recommended by the WHO.

HIV-1 Genome Detection

HIV-1 RNA was assessed in blood and seminal plasma. HIV-1 RNA and DNA were assessed in native (ie, total ejaculated cells) semen cells according to previously published methods (Pasquier et al, 2000; Bujan et al, 2004). Briefly, blood plasma HIV-1 RNA was quantified with the Amplicor HIV-1 Monitor v1.5 assay (Roche Diagnostic Systems, Meylan, France) using the ultrasensitive protocol (detection limit of 20 copies/mL).

HIV-1 genomes were extracted from seminal plasma or cells using the Nuclisens extraction kit (Organon Teknika SA, Fresnes, France). For HIV-1 RNA quantification in seminal plasma, we used the modified Amplicor HIV-1 Monitor v1.5 assay (Roche Diagnostic Systems). The detection limit of the assay in seminal plasma was 100 copies/mL. We used the same assay to detect HIV-1 RNA or both HIV RNA and DNA in native semen cells. The assay detection limit in seminal cells was 20 HIV-1 genome copies/ 10^6 cells.

Statistical Analysis

All statistical analysis required $(1-\beta) = 80\%$ and a level of 5% as assessed by the Stata 6.0 software (Stata Corp, College Station, Tex). Sperm counts, total sperm counts, and total motile sperm count distributions were normalized by logarithmic transformation. The nonparametric Mann-Whitney test was used to compare all of the quantitative data. Fisher exact test was used to compare qualitative data between subgroups. Logistic regression analyses were performed to compare differences between patients and controls. These analyses were adjusted for known confounder variables (ie, age of patient and abstinence delay) and subsequently for medical status (ie, medical andrologic history and presence or absence of clinical anomalies).

Results

In the 190 HIV-1-infected men, all of whom were clinically asymptomatic, the mode of virus transmission was intravenous drug use for 34%, blood transfusion for 12%, sexual intercourse for 44%, and unknown for 10% of the infected men. The mean duration of HIV-1 infection was 10.2 ± 4.9 years (range: 1–20 years). Of the infected men, 49.7% were coinfecting with hepatitis virus. Ninety-one percent of the patients were undergoing antiretroviral therapy (ART), with 20 patients receiving two nucleoside inhibitors and 152 patients receiving three or more drugs.

The mean CD4 cell count was $581.9 \pm 274.9 \times 10^6/L$ (range 165–2246 $\times 10^6/L$) and 129 patients had detectable HIV-1 RNA levels in the blood plasma (range 3–400,000 copies/mL). Twenty-seven patients had detectable HIV-1 RNA levels in seminal plasma (mean 813.1 ± 1364.0 copies/mL, range 3–5600 copies/mL). Thirty-two percent of the HIV-1-infected men had positive andrologic medical histories (see the Methods section) vs 20.1% of the control subjects ($P < .005$). A history of urinary or genital infection was more frequent in the HIV patients than in the control men (21.8% vs 10%, $P < .01$). The frequency of clinical andrologic abnormalities did not differ between the groups (24.8% vs 19.5% for the HIV-infected man vs control men, $P > .05$). The mean testis volumes did not differ between the two groups (36.4 ± 10.7 mL vs 35.8 ± 11.7 mL for the right

testis and 33.8 ± 10.3 mL vs 33.6 ± 11.4 mL for the left testis, for the HIV-infected men vs control men, respectively).

The semen characteristics of the HIV-infected men and control men are presented in Table 1. The ejaculate volumes, percentages of progressive spermatozoa (motility a, motility a + b), total sperm motile counts, and polymorphonuclear cell counts were significantly decreased compared with the control group values. In contrast, the pH values and MAI were increased in the HIV-infected men. Since patient age and length of abstinence could influence the results, we performed further analysis after adjustment for these variables. The differences persisted after adjustment.

Since andrologic history or clinical abnormalities influence sperm parameters, we conducted a second analysis after adjustment for these variables. Reduced ejaculate volumes, decreased percentages of progressive motile spermatozoa, reduced total numbers of motile spermatozoa, and increased pH values were observed for the HIV-1-infected men compared with the control group.

Considering, only the HIV-1 patients group no differences in sperm parameters were found according to the CD4 cell counts below or above $500 \times 10^6/mL$ (data not shown). On the other hand, detectable blood viral load was associated with reduced progressive motility b ($7.3 \pm 4.2\%$ and $6.1 \pm 4.6\%$ ($P < .05$) for the undetectable and detectable viral loads, respectively). Detectable seminal viral loads ($n = 27$) were not associated with sperm parameter modifications. However, when the HIV-1 genome was present in native semen cells ($n = 22$), there were decreases in sperm count ($P < .05$), total sperm count ($P < .05$), and total motile sperm count ($P < .05$), which were accompanied by an increase in the number of polymorphonuclear cells ($P < .05$).

The mean duration of treatment was 5.7 ± 3.2 years. No statistically significant correlation was found between the sperm parameters and treatment duration. All of the treated patients take at least 1 d-drug, 128 take 2 d-drugs, 27 take 3 d-drugs, and 1 patient takes 4 d-drugs. The semen parameters and particularly motility did not differ according to the number of d-drugs. In relation to treatment presence or absence, no statistically significant differences were found in the sperm parameters, although the number of patients without treatment in the present study was low (data not shown).

Discussion

The present study is the first to compare sperm parameters in 190 HIV-1-infected men with those of

Table 1. Comparisons of age, body mass index, testis volume, and semen characteristics between the HIV group and the control group

	HIV	Control	HIV	Control	P	P**
	n		Mean (SD)			
Age (y)	190	216	37.2 (5.8)	37.9 (6.2)	NS	NS
BMI	128	160	22.9 (3.0)	24.7 (2.8)	< .01*	< .01
Testis characteristics						
Right testis volume (mL)	137	124	36.4 (10.7)	35.8 (11.7)	NS	NS
Left testis volume (mL)	137	124	33.8 (10.3)	33.6 (11.4)	NS	NS
Length of abstinence (days)	189	206	5.1 (3.2)	6.2 (7.8)	NS	NS
Semen characteristics						
Ejaculate volume (mL)	190	218	3.3 (1.6)	3.9 (1.9)	< .01*	< .01
pH	190	217	8.2 (0.3)	7.9 (0.3)	< .01*	< .01
Motility a (%)	190	218	32.8 (17.2)	37.4 (14.1)	< .05*	< .05
Motility b (%)	190	218	6.4 (4.5)	6.2 (3.6)	NS	NS
Progressive motility a + b (%)	190	218	39.2 (16.2)	43.6 (13.8)	< .05*	< .01
Motility c (%)	190	218	7.0 (3.2)	6.3 (2.9)	< .05*	NS
Motility d (%)	190	218	53.8 (15.6)	50.1 (13.4)	< .05*	< .05
Vitality (%)	189	218	68.8 (15.3)	69.7 (13.3)	NS	NS
Sperm count (10 ⁶ /mL)	190	218	108.3 (96.8)	96.7 (88.2)	NS	NS
Total sperm count (10 ⁶ per ejaculate)	190	218	330.9 (287.7)	353.8 (317.9)	NS	NS
Total motile sperm count (10 ⁶ per ejaculate)	190	218	127.9 (120.7)	150.7 (127.1)	< .05	< .05
Morphologically normal spz (%)	97	107	27.0 (11.4)	28.6 (14.3)	NS	NS
Multiple anomaly index	97	107	1.8 (0.2)	1.7 (0.2)	< .05*	NS
Round cells (10 ⁶ /mL)	188	218	2.3 (3.2)	2.0 (2.3)	NS	NS
Polymorphonuclear cells (10 ⁶ /mL)	139	30	0.1 (0.4)	0.8 (2.4)	< .01*	NS

* Significant difference persisted after adjustment for age and length of abstinence

** P-value with adjustment for age, length of abstinence, medical andrology history and result of andrological examination.
NS, Not statistically significant.

a large group of healthy men of proven fertility. Moreover, the present study analyses sperm parameters according to the results of clinical examination and andrologic history, both of which can represent bias in sperm parameter studies. The present study demonstrates decreases in the semen volume, spermatozoa motility, and total motile sperm count and increases in the pH values and multiple anomaly indices of HIV-infected patients.

Earlier studies were performed with low numbers of patients (Krieger et al, 1991; Crittenden et al, 1992; Dondero et al, 1996) or with low numbers of control men (Dulouist et al, 2002; Muller et al, 1998). The first study, which compared 24 HIV-infected men with 40 healthy men who provided semen for other investigations during the study period, found no differences in sperm parameters between the 2 groups (Krieger et al, 1991). In that study, 3 patients with AIDS had abnormal semen. Crittenden et al (1992) found a decreased percentage of motile sperm and an increased proportion of round cells in the sperm of HIV-1-infected men (n = 39) compared with the sperm men without HIV (n = 51). Similar sperm alterations have been reported in another study of 21 HIV-infected men (76%

under ART) and 30 control men (Dondero et al, 1996). Moreover, a decreased percentage of sperm with normal morphology (Dondero et al, 1996; Nicopoullou et al, 2004) and a decrease in total sperm count (Dondero et al, 1996) have been reported in infected patients. In another study that included a large HIV-infected population (n = 250) but with a low number of fertile men (n = 38), the same sperm alterations were observed, with in addition, decreased ejaculate volume in the HIV-infected men (Muller et al, 1998).

Two recent studies have reported the sperm characteristics of 105 and 189 HIV-infected men compared with 234 and 79 control men, respectively (Dulouist et al, 2002; Nicopoullou et al, 2004). It is noteworthy that in these studies, the control groups were composed of men whose female partners were undergoing in vitro fertilization (IVF) due to tubal infertility. However, the male partners of women with tubal infertility due to a past or present genital infection could themselves have sperm or genital infection, either past or present, which could have resulted in sperm alterations. In the studies of Nicopoullou et al (2004) and Dulouist et al (2002), no information has been provided on the andrologic history or the results of clinical examinations of the control

men. In the study of Dulioust et al (2002), in which 94% of the HIV patients were undergoing ART, a decrease of the rapidly progressive motile spermatozoa (motility a) and an increase of less rapidly progressive spermatozoa (motility b) have been reported for the HIV patients. An increase in the number of round cells and a decrease in the total sperm count have been reported. However, the decrease in total sperm count could be explained by the decrease in ejaculate volume, since a sperm count decrease was not observed.

In the study of Nicopoulos et al (2004), in which 56% of the HIV patients were undergoing ART, a decrease of progressive motility (a + b), decreases in sperm count and total sperm count, and a reduction of ejaculate volume have been reported. Furthermore, the percentage of sperm with normal morphology was reduced in the HIV-infected men. In contrast to these findings, no differences were reported for ejaculate volume, sperm concentration or sperm motility in another study of 70 HIV-infected patients (70% of whom were undergoing ART) and 73 healthy seronegative male partners of women with tubal infertility (Garrido et al, 2005).

Differences in the results of published studies (Table 2) could be due to differences in: 1) recruitment of the HIV-infected men and the fertility status of the men in the control group, 2) methodological variations in semen analysis, and 3) the proportion of men undergoing ART. Moreover, andrologic history (ie, genital or urinary infections, cryptorchidism, varicocele) and the results of clinical andrologic examinations were not reported in these studies. In the present study, we took into consideration several issues. First, we had a similar number of men in the HIV-infected and control groups. Second, our control group was composed of fertile men who were recruited by three different methods. Third, andrologic history was evaluated and clinical andrologic examinations were performed for the men in both groups. Fourth, all semen samples were collected and analyzed in our laboratory according to the same procedure. Fifth, age and length of abstinence (Auger et al, 1995; Chen et al, 2003) and andrologic data (Comhaire, 2000; Schlegel and Hardy, 2006) were integrated in the analyses, since they can influence semen parameters.

In the HIV-infected men, we observed semen alterations, such as a decrease in ejaculate volume, which is in agreement with the three largest studies of HIV-infected men (Muller et al, 1998; Dulioust et al, 2002; Nicopoulos et al, 2004), as well as an increase in pH value. A decrease in the number of progressive motile spermatozoa was also found, in agreement with other studies (Crittenden et al, 1992; Dondero et al, 1996; Muller et al, 1998; Dulioust et al, 2002; Nicopoulos et al, 2004). In contrast with three previous studies, we did

not observe decreased sperm counts. A decrease in the total sperm count of the ejaculate has been reported in two published studies (Dulioust et al, 2002; Nicopoulos et al, 2004), whereas, in the present study, this decrease did not persist after adjustment for andrologic history and the results of the andrologic evaluations. It is noteworthy that the most frequently altered semen parameters in the largest studies of HIV-infected men (Muller et al, 1998; Dulioust et al, 2002; Nicopoulos et al, 2004) were ejaculate volume and the percentage of progressive motility.

The mechanism underlying the semen alterations observed in HIV-infected men remains unclear, although several hypotheses have been put forward. Men with advanced HIV infection, and particularly those with AIDS status, have abnormal sperm (Krieger et al, 1991) or abnormal spermatogenesis (Dejucq-Rainsford and Jegou, 2004), whereas all the patients in the present study were asymptomatic.

The CD4 cell count, which reflects HIV infection immune status, was positively correlated with both the percentage of motile spermatozoa (Crittenden et al, 1992; Dondero et al, 1996; Lasheeb et al, 1997; Muller et al, 1998; Nicopoulos et al, 2004) and sperm count (Politch et al, 1994; Lasheeb et al, 1997; Nicopoulos et al, 2004), and negatively correlated with ejaculate volume (Dulioust et al, 2002). The duration of HIV infection was negatively correlated with ejaculate volume in a previous study (Dulioust et al, 2002). In the latter study, blood viral load was negatively correlated with motility, in agreement with our findings, and positively correlated with the percentage of morphologically normal spermatozoa, in contrast to the results reported by Nicopoulos et al (2004).

It is very difficult to reach a conclusion concerning the relationship between immunologic and virologic HIV status and sperm parameters in asymptomatic HIV-infected patients. No study has examined the sperm characteristics of several patients before and after HIV contamination. Nevertheless, it is interesting to note that a case report has shown decreases in sperm motility and morphologically normal spermatozoa and an increase in sperm concentration after HIV-1 contamination in a man who participated in a semen donation program (van Leeuwen et al, 2004).

Decreases in ejaculate volume and increases in semen pH may be due to reduced accessory gland secretions or ejaculatory dysfunction. Dysfunction of the prostate and seminal vesicles, which are responsible for about 90–95% of the ejaculate volume, could be due to past or silent inflammation or infection, virus cell gland colonization or to the effects of ART drugs present in the genital tract (Henry et al, 1988; Eron et al, 1998; Taylor et al, 2001).

Table 2. Published studies comparing sperm parameters between HIV patients and control groups*

Study	Krieger, 1991	Crittenden, 1992	Dondero, 1996	Muller, 1998	Dulioust, 2002	Nicopoulos, 2004	Garrido, 2004	Present Study
HIV patients, n (%under ART)	24 (50)	39 (48)	21 (76)	250† (?)	189 (94)	105 (55)	73 (70)	190 (91)
Control group	Healthy men	Sperm donor or hemophiliac men	Fertile or pre-marriage men	Fertile men	PWTI	PWTI	PWTI	Fertile men‡
N	40	51	30	38	79	234	73	218
Adjustment for								
Age	no	no	no	no	yes	no	yes	yes
Abstinence delay	no	no	no	no	yes	no	yes	yes
Andrologic history	no	no	no	no	no	no	no	yes
Andrologic examination	no	no	nd§	no	no	no	no	yes
Significant changes in HIV-patients compared with control group								
Volume (mL)	no	no	no	reduced	reduced	reduced	no	reduced
pH	no	nd	nd	no	no	nd	no	increased
Motility a (%)	no	nd	nd	reduced	reduced	no	no	reduced
Motility a + b (%)	no	reduced	reduced	reduced	no	reduced	no	reduced
Sperm count 10 ⁶ /mL	no	no	reduced	reduced	no	reduced	no	no
Total sperm count 10 ⁶ /ejaculate	no	no	nd	nd	reduced	reduced	no	no
Normal sperm morphology (%)	no	nd	reduced	no	no	reduced	nd	no, but MAI increased

* ART, antiretroviral treatment; PWTI, partner of women with tubal infertility included in IVF program; MAI, multiple anomalies index; nd, not done; no, no significant changes between HIV group and control group.

† Sperm samples were analyzed outside the laboratory.

‡ From 3 different modes of recruitment (see Methods section).

§ Men with andrologic diseases were excluded.

Decreased motility may be due to viral effects on spermatozoa, abnormal seminal plasma composition, and changes in spermatozoal metabolism due to ART. HIV RNA has been detected in several genitourinary secretions (Coombs et al, 2006). Whether HIV has impacts on the gland functions and biochemical seminal parameters of asymptomatic patients is not known.

Various studies have demonstrated a relationship between mitochondria and sperm motility (Folgero et al, 1993; Ruiz-Pesini et al, 1998; Donnelly et al, 2000; May-Panloup et al, 2003). As several ARTs have mitochondrial toxicity (Johns, 1995; Lewis and Dalakas, 1995; Brinkman et al, 1998), the observed changes in motility could be due to the ART itself. A report of increased frequency of multiple DNA deletions in the sperm of patients receiving HAART for more than 12 months supports this hypothesis (White et al, 2001). In a small study of treated HIV patients, a negative correlation was reported between duration of treatment with drugs known to be strong inhibitors of mtDNA replication and the mtDNA content of spermatozoa, without any difference in sperm count, motility or mtDNA according to the type of antiretroviral drug (Diehl et al, 2003). On the other hand, Robbins et al

(2001) did not find sperm or lymphocyte chromosomal changes after the start of nucleoside-containing antiretroviral therapy. They observed improved sperm motility for men with CD4 cell counts of up to 200 cells/mm³ at study entry, and no improvement for men with fewer than 200 cells/mm³, although this study was performed with a small number of patients and the authors consider that chronic long-term effects could have been missed by the sampling method used. Unfortunately, as in our present study, in which 91% of the HIV-patients were undergoing ART, there were no statistical differences between the treated and untreated patients and no correlations between d-drug usage and sperm motility.

In conclusion, our findings demonstrate sperm alterations in HIV-infected men. Long-term prospective studies in men starting antiretroviral therapy are needed to evaluate the impact of ART on semen parameters, and more particularly, on the male gamete genome. Indeed, considering the mitochondrial toxicities of several antiretroviral drugs and the possible incorporation of these molecules into lymphocytic or testis DNA of men and monkeys (Olivero et al, 1999, 2000, 2001, 2002), it is of paramount importance to answer these questions.

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