Impaired sperm motility in HIV-infected men: an unexpected adverse effect of efavirenz?

C. Frapsauce1,*, S. Grabar2, M. Leruez-ville3, O. Launay4, P. Sogni5, V. Gayet6, J.P. Viard7, M. De Almeida1, P. Jouannet1, and E. Dulioust1,*


*Correspondence address. Laboratoire de Biologie de la Reproduction, Centre Hospitalier Cochin Port Royal, 123 Boulevard du Port Royal, 75014, Paris, France (E.D.)/ Service de Médecine de la Reproduction, CHU Bretonneau, 2 Boulevard Tonnelle, 37044 Tours cedex 9, France (C.F.). Tel: +33-1-58-41-37-38 (E.D.)/ +33-2-47-47-88-97 (C.F.); E-mail: emmanuel.dulioust@cch.aphp.fr (E.D.)/ cynthia.frapsauce@gmail.com (C.F.)

Submitted on December 26, 2014; resubmitted on May 11, 2015; accepted on May 26, 2015

STUDY QUESTION: Are antiretroviral therapies associated with semen alterations in HIV-infected men?

SUMMARY ANSWER: Antiretroviral regimens that included the non-nucleosidic reverse transcriptase inhibitor efavirenz were associated with a significant impairment of sperm motility, whereas regimens without efavirenz were not associated with significant semen changes.

WHAT IS KNOWN ALREADY: Semen alterations including decreased ejaculate volume and sperm motility have been reported in HIV-infected men. The hypothesis ascribing reduced sperm motility to damages induced in sperm mitochondria by nucleosidic (or nucleotidic) reverse transcriptase inhibitors (NRTIs) has not been confirmed in HIV-infected patients and the effects of antiretroviral treatments on semen parameters remain unclear.

STUDY DESIGN, SIZE, DURATION: This case–control study compared semen characteristics across 378 HIV-1 infected patients receiving different antiretroviral regimens or never treated by antiretroviral drugs, in whom an initial semen analysis was done between 2001 and 2007.

PARTICIPANTS/MATERIALS, SETTING, METHODS: The patients were partners from serodiscordant couples requesting medical assistance to procreate safely. Their status with regard to antiretroviral therapy at the time of semen analysis was categorized as follows: 1/ never treated patients (n = 66); 2/ patients receiving NRTIs only (n = 49); 3/ patients receiving a NRTIs + protease inhibitor (PI) regimen (n = 144); 4/ patients receiving a NRTIs + non-nucleosidic reverse transcriptase inhibitor (NNRTI) regimen (n = 119). Semen parameters were assessed through standard semen analysis. Additional analyses included measurement of sperm motion parameters using computer-assisted semen analysis, seminal bacteriological analysis, seminal biochemical markers and testosterone plasmatic levels. All analyses were performed in the Cochin academic hospital. The data were analyzed through multivariate analysis.

MAIN RESULTS AND THE ROLE OF CHANCE: Sperm motility was the only semen parameter which significantly varied according to treatment status. The median percentage of rapid spermatozoa was 5% in the group of patients receiving a regimen including efavirenz versus 20% in the other groups (P < 0.0001). Accordingly, sperm velocity was reduced by about 30% in this group (P < 0.0001). The role of chance was minimized by the strict definition and the size of the study population, which included a large enough group of never treated patients, the controlled conditions of semen collection and analysis, the multivariate analysis, the specificity and the high significance level of the observed differences.

LIMITATIONS, REASONS FOR CAUTION: The design of the study did not allow demonstrating a causal link between exposure to efavirenz and sperm motility.

© The Author 2015. Published by Oxford University Press on behalf of the European Society of Human Reproduction and Embryology. All rights reserved. For Permissions, please email: journals.permissions@oup.com
Introduction

The efficacy of combined antiretroviral therapies (cART) now allows human immunodeficiency virus (HIV) to be considered as a chronic disease. Moreover, in patients receiving cART where plasma HIV-RNA is repeatedly undetectable, the levels of seminal HIV-RNA are very low, especially in male partners of heterosexual couples requesting medical assistance to procreate (MAP) (Dulioius et al., 2010; Halfon et al., 2010; Lambert-Nicot et al., 2012). Several studies have shown that cART can strongly reduce the risk of virus transmission (Cohen et al., 2011; Anglemyer et al., 2013). Thus, natural procreation in HIV-discordant couples could be considered as safe under defined conditions. These hopes underline the importance of fertility issues in HIV-infected men.

Several studies have reported semen alterations in HIV-infected men but with heterogeneous conclusions. According to three studies comparing semen parameters between large numbers of asymptomatic HIV-infected patients seeking MAP for safe procreation and appropriate controls (Dulioius et al., 2002; Nicopoullos et al., 2004; Bujan et al., 2007), decreased ejaculate volume and sperm motility were the most frequently observed alterations. Since sperm motion needs ATP and considering the mitochondrial toxicity of several nucleosidic (or nucleotidic) reverse transcriptase inhibitors (NRTIs) used in cART (Brinkman et al., 1998; Côté et al., 2002; Lewis et al., 2003), it seemed pertinent to ascribe the reduced sperm motility observed in these men to damage induced in sperm mitochondria by NRTIs. Although some results seemed to support this hypothesis (White et al., 2001; Leandri et al., 2003; Pavili et al., 2010), the links between antiretroviral treatments and semen parameters remain unclear. In the present study, we addressed this issue in a population of HIV-1 infected male patients who were seeking MAP to procreate safely, by comparing the semen characteristics between the patients receiving various cART regimens and those who had never received any antiretroviral therapy for their HIV infection.

Methods

Patients

The study population consisted of asymptomatic HIV-1 infected men, partners of an uninfected woman and requesting medical assistance to procreate safely, in whom an initial semen analysis was done between January 2001 and December 2007 in the Laboratory of Reproductive Biology of the Cochin University Hospital, Paris, France. In addition to the usual information relevant to fertility (previous fertility, genito-urinary diseases, life mode) and to medical history, the following data relevant to HIV infection were retrieved from medical records at the beginning of the couple’s clinical care: date of HIV-1 infection diagnosis, contamination mode, hepatitis B virus (HBV) or C (HCV) co-infection, nadir of CD4 cell count, date of the first antiretroviral therapy, current cART regimen with date of onset, last plasma viral load and CD4 cell count. Patients with a medical cause of semen alteration or with symptomatic genital infection were excluded. Patient’s status with regard to antiretroviral therapy within the 3 months before the first semen analysis was categorized as follows: 1/ never treated; 2/ receiving nucleosidic (or nucleotidic) reverse transcriptase inhibitors (NRTIs) only; 3/ receiving a NRTIs + protease inhibitor (PI) regimen; 4/ receiving a NRTIs + non-nucleosidic reverse transcriptase inhibitor (NNRTI) regimen; 5/ other regimens. We excluded patients whose cART regimen had started or changed within the 3 months before the first semen analysis, patients with poor adherence to treatment or who had discontinued their treatment and patients for whom the data concerning the current cART were insufficient. The last category (27/405 patients) consisted of patients receiving heterogeneous regimens, so we only included the 378 men belonging to the four other categories. The semen characteristics analyzed in the study were those of the first ejaculate collected in our center. The study was approved by the Ethics Review Committee ‘Comité de Protection des Personnes Ile de France 3’ (AC 017).

Semen standard analysis

All semen samples were collected in the laboratory by self-masturbation into a sterile container after micturition and washing hands and penis with chlorhexidine. Time since last ejaculation was recorded. Ejaculate volume, pH, sperm concentration, non-sperm cells concentration, sperm vitality and motility were evaluated according to World Health Organization (WHO) guidelines as previously described (World Health Organization, 1999). Sperm motility was categorized as rapid progressive (a-type), slow progressive (b-type), non-progressive (c-type) and absent (d-type). Sperm morphology studied under × 1000 magnification on Schorr-stained semen smears was categorized according to modified David’s classification (Auger and Eustache, 2000). All semen analyses were performed by trained observers who were unaware of the patient’s status regarding antiretroviral therapy.

Sperm motion analysis

In a subset of 221/378 unselected patients, an automated analysis of sperm motion at 37 °C using a computer-assisted semen analysis (CASE) device (Hamilton Thorne IVOS) was performed on the first ejaculate in parallel with the standard analysis. CASE measurements were made with counting chamber with a depth of 20 μm and with a semen sample of 3 μL (Microcell17)). Sperm velocity parameters (straight line velocity (VSL), average path velocity (VAP) and curvilinear velocity (VCL)) were compared according to the NNRTI (efavirenz or nevirapine) used in patients receiving an NNRTI-containing regimen.

Additional biological analyses

Semenal bacteriology was studied through standard bacteriological culture, mycoplasma culture and chlamydial detection by direct immunofluorescence on the first ejaculate in 335/378 unselected patients; genital tract and accessory glands secretions were investigated on the first ejaculate in 242/378...
unselected patients by quantifying fructose (semen vesicles), acid phosphatase, zinc, and citrate (prostate), and α 1-4 glucosidase (epididymis). Blood levels of total testosterone, free testosterone and testosterone binding globulin (TeBG) were evaluated on a blood sample collected concomitantly with the first semen sample in 155/378 unselected patients. There were no significant differences between the treatment groups in the proportions of patients subjected to the additional biological analyses.

Statistical analyses

Patients’ characteristics and semen parameters were first compared according to the four categories of patients defined above: never treated, receiving NRTIs only, or NRTIs+PI, or NRTIs+NNRTI. In a second step, the same data were compared considering together all regimens without a NNRTI (NRTIs only and NRTIs+Pi) and according to each of the two NNRTIs that were used, or nevirapine (NVP). Complementary analyses compared sperm motility and velocity between the patients who were receiving EFV or NVP according to the other antiretroviral drugs associated with the NNRTI.

Continuous variables were compared using Kruskal–Wallis tests and categorical variables with Chi-square or Fisher exact tests when necessary. Multiple ANCOVA were used to identify the independent factors associated with semen characteristics after adjustment for age, CD4 cell count, plasma viral load, known duration of seropositivity, c-ART regimens, duration of sexual abstinence. C-ART regimens were modeled using the different categorizations cited above. Multiple testing was corrected by Bonferroni method and a P-value < 0.01 was considered statistically significant. All statistical analyses were performed using SAS 9.2 (SAS Institute, Cary, NC, USA).

Results

Patients’ characteristics

Patients’ distribution across the four studied groups and their characteristics are described in Table I. The four groups were similar with regard to age, contamination route and frequencies of HCV or HBV co-infection. Never treated patients significantly differed from the others with shorter time since HIV-infection diagnosis and higher nadir of CD4 cell count and blood viral load. Among the three groups of treated patients, the only significant differences concerned CD4 cell count and current cART duration, which were lower in the patients treated by NRTIs+Pi than in the two other groups.

Semen characteristics

Semen characteristics according to current treatment status are described in Table II. Sperm motility parameters significantly differed between groups. In particular, the median percentage of rapid progressive (a-type) sperms in the group of patients receiving a NNRTI regimen was half the value observed in the three other groups (10 versus 20%, P < 0.0001), whereas no difference in sperm motility was found between the never treated patients and those receiving a NRTIs only or a NRTIs+Pi regimen. Seminal pH showed variations reaching the significance threshold, although the median values were identical and in the normal range. This was due to higher frequencies of pH values over 8 in the never treated and NRTIs-Pi groups (13.6 and 12.8%, respectively) than in the NRTIs only and NRTIs-NNRTI groups (7.5 and 7.4%, respectively). These differences were not significant. The other semen parameters did not significantly differ between the four groups. After adjustment for age, sexual abstinence delay, CD4 cell count, plasma HIV-RNA, time since HIV diagnosis and, in the treated groups, duration of current cART, a-type motility was found independently associated with the type of cART (P = 0.00025).

No difference in semen characteristics was found between the groups of patients receiving either the NRTIs only or NRTIs+Pi regimens, and only two NNRTIs (NVP and EFV) were used in our patients during this study. Therefore, we performed a second analysis in which all patients receiving a cART regimen without a NNRTI were considered together and patients receiving EFV or NVP were distinguished, so that semen characteristics were compared across the following four groups: never treated (n = 66), cART without NNRTI (n = 193), NVP-NRTIs regimen (n = 53) and EFV-NRTIs regimen (n = 66). Semen characteristics of the patients receiving NVP-containing regimens did not differ from those of never treated patients or from those of patients receiving cART regimens without NNRTI. In contrast, sperm motility was selectively impaired in the group of patients receiving EFV, where the median value of a-type motility was 5 versus 20% in the three other groups (P < 0.0001, Fig. 1). Again, multivariate analysis showed that this decrease of motility was independently associated with the type of cART (data not shown). No other significant semen alteration was found in the EFV-treated patients. More especially, median vitality was identical in NVP- and EFV-treated patients (64%). This value was lower than those found in the patients receiving regimens without NNRTI (68%) and in the untreated patients, but the differences were not significant.

Additional biological analyses

The prevalence of positive semen culture (treatment-naive: 11%, NRTIs only: 4%, NRTI+IP: 13%, NRTI+NNRTI: 13%) did not significantly differ between groups. No significant variations were observed as regards to seminal markers or to plasma levels of testosterone and TeBG (data not shown).

Comparisons between NVP and EFV-treated patients

In order to precise the association between EFV-containing regimens and altered sperm motility, and to quantify the reduction of sperm velocity, further comparisons were done between patients receiving NVP or EFV-containing regimens.

Sperm motility according to the antiretroviral drugs associated with NVP or EFV

All the patients receiving NVP or EFV were also treated with two NRTIs. In order to investigate possible variations of sperm motility according to these associated NRTIs, we divided the NNRTIs regimens into the following three subsets: 1/ regimens including either didanosine or stavudine or both molecules, according to their higher mitochondrial toxicity (Birkus et al., 2002; Venhoff et al., 2007); 2/ regimens including lamivudine and zidovudine, because this association was the most frequently used; 3/ other associations of two NRTIs. Comparison of a-type motility across these subsets of patients showed that this parameter did not change according to the NRTIs associated with either NVP or EFV but was severely reduced (median value decreased by about 60%) in the three subsets receiving EFV when compared with the three corresponding NVP subsets (Fig. 2). No parallel differences were observed for pH or vitality (data not shown). These results confirmed the association between the exposure to EFV and the impairment of sperm motility.
Table 1 Characteristics of the 378 HIV-1-infected patients according to treatment status (initial analysis). P-value < 0.01 was considered statistically significant.

<table>
<thead>
<tr>
<th></th>
<th>Never treated</th>
<th>Nucleosidic reverse transcriptase inhibitors (NRTI)*</th>
<th>P-value across all groups</th>
<th>P-value across treated groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Alone + protease inhibitor + non-nucleosidic RTI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number (%)</td>
<td>66 (17.5)</td>
<td>49 (12.9) 144 (38.1) 119 (31.5)</td>
<td>0.24</td>
<td>0.28</td>
</tr>
<tr>
<td>Age (years)</td>
<td>36.4 (32.9–42.0)</td>
<td>37.9 (34.4–41.6) 37.9 (33.9–41.5) 39.2 (34.8–42.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infection route [No. (%)]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>12 (18.2)</td>
<td>10 (20.4) 20 (13.9) 17 (14.3)</td>
<td>0.54</td>
<td>0.40</td>
</tr>
<tr>
<td>IV drug use</td>
<td>12 (18.2)</td>
<td>14 (28.6) 40 (27.8) 23 (19.3)</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>Blood</td>
<td>11 (16.7)</td>
<td>6 (12.2) 29 (20.1) 22 (18.5)</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>Sex</td>
<td>31 (46.9)</td>
<td>19 (38.8) 55 (38.2) 57 (47.9)</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>Hepatitis C co-infection [No. (%)]</td>
<td>17 (25.8) 10 (20.4) 37 (31.1)</td>
<td>0.10</td>
<td>0.22</td>
<td></td>
</tr>
<tr>
<td>Hepatitis B co-infection [No. (%)]</td>
<td>6 (9.1) 4 (8.2) 4 (3.4)</td>
<td>0.23</td>
<td>0.13</td>
<td></td>
</tr>
<tr>
<td>Time since HIV diagnosis (years)</td>
<td>3.6 (1.7–13.9)</td>
<td>10.9 (3.6–13.3) 10.9 (4.1–16.2) 8.5 (5.5–12.8)</td>
<td>0.0016</td>
<td>0.12</td>
</tr>
<tr>
<td>Nadir CD4 cell count (cells/μl)</td>
<td>390 (285–505)</td>
<td>233 (169–317) 164 (56–284) 205 (108–329)</td>
<td>&lt; 0.0001</td>
<td>0.03</td>
</tr>
<tr>
<td>CD4 cell count (cells/μl)</td>
<td>476 (381–665)</td>
<td>470 (382–636) 439 (309–603) 534 (400–707)</td>
<td>0.006</td>
<td>0.0025</td>
</tr>
<tr>
<td>Plasma HIV RNA (copies/ml)</td>
<td>14050 (2730–310 000)</td>
<td>25 (25–280) 25 (25–100) 25 (25–25)</td>
<td>&lt; 0.0001</td>
<td>0.02</td>
</tr>
<tr>
<td>Detectable plasma HIV RNA§ (% patients)</td>
<td>93.9 40.8 36.1 17.6</td>
<td>/</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>(copies/ml)</td>
<td>15313 (153 000)</td>
<td>560 (29–5.9) 560 (2.4–8.7) 560 (3.7–7.9)</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td>Time since first antiretroviral treatment (years)</td>
<td>/ 4.6</td>
<td>5.9 (2.9–5.9) 5.9 (2.4–8.7) 5.9 (3.7–7.9)</td>
<td>/</td>
<td>0.11</td>
</tr>
<tr>
<td>Current combined antiretroviral therapies duration (years)</td>
<td>/ 2.1</td>
<td>1.3 (1.2–3.7) 1.3 (0.8–2.9) 1.3 (0.9–3.6)</td>
<td>/</td>
<td>0.0038</td>
</tr>
</tbody>
</table>

HIV: Human immunodeficiency virus.
*NRTIs: include nucleosidic reverse transcriptase inhibitors.
§ > 50 copies/ml.
Sperm velocity parameters

To confirm by a quantitative evaluation of individual sperm velocity the impairment of sperm motility evidenced through standard semen analysis, we compared sperm velocity parameters between the 29 NVP-treated patients and 31 EFV-treated patients in whom CASA data were available on the first ejaculate. A significant shift toward low values was found for all three velocity parameters in EFV-treated patients (median values: VSL: 30 versus 43 μm/s; P = 0.0001; VAP: 38 versus 51 μm/s, P < 0.0001; VCL: 64 versus 77 μm/s, P < 0.0008). A detailed analysis of the distribution of VSL values showed that the EFV-treated patients had higher percentages of sperms with VSL under 40 μm/s and lower percentages with VSL above 40 μm/s (Fig. 3).

Discussion

The causes of the semen alterations observed in HIV-infected men and especially the respective contributions of HIV infection and antiretroviral treatments remain unclear. Van Leeuwen et al. reported a significant decrease in the percentage of progressive motile spermatozoa after starting cART in 34 men (van Leeuwen et al., 2008a). The same team observed no change in semen parameters over a median 77 weeks follow-up of 55 untreated HIV-infected men (van Leeuwen et al., 2008b). Another study comparing semen characteristics across 33 HIV-infected patients without treatment, 100 patients on cART and 93 uninfected men suggested that semen alterations were more important in the patients on cART (Kehl et al., 2011). Lambert-Niclot et al. analyzed semen parameters of 124 HIV-infected patients seeking MAP for safe procreation according to their cART regimens (NRTIs alone or in association with PI or NNRTI). Although no difference was detected between these groups, comparisons between the patients receiving EFV versus those receiving NVP showed better sperm motility and vitality in the latter (Lambert-Niclot et al., 2011). Recent correlation was found between cART duration or number of antiretroviral drugs and any semen parameter in HIV patients under stable cART (Pilatza et al., 2014).

The present work was carried out on a cohort of 378 HIV-infected patients including 66 (17.5%) patients who had never received any antiretroviral therapy. semen characteristics were similar in the never
**Figure 1** A-type motility according to the addition of the non-nucleosidic reverse transcriptase inhibitors (NNRTIs), nevirapine (NVP) or efavirenz (EFV) to combined anti-retroviral therapy (cART). P-values were obtained by Kruskal–Wallis test. Box-plot: the bar in the box indicates the median, the + symbol the mean and the lower and upper hinge the interquartile range. The whisker extends to the most extreme data point, which is no more than 1.5 times the interquartile range. Squares represent values outside the fences.

**Figure 2** The association between EFV and reduced A-type motility is not affected by the combination of nucleosidic or nucleotidic reverse transcriptase inhibitors (NRTIs) included in the treatment regimen. P-values were obtained by Kruskal–Wallis test. Treatment groups were defined as follows: EFV1: EFV + Stavudine or Didanoside or both, EFV2: EFV + Zidovudine + Lamivudine, EFV3: EFV + other NRTIs, NVP1: NVP + Stavudine or Didanoside or both, NVP2: NVP + Zidovudine + Lamivudine, NVP3: NVP + other NRTIs.
treated patients and in those receiving NRTIs alone or in combination with PI or NVP. In contrast, a marked drop (about 60%) of the percentage of rapid spermatozoa was found in the group of patients receiving an EFV-containing regimen, whatever the associated NRTIs, together with significant decreases of total progressive motility (about 25%) and of sperm velocity assessed by CASA (about 30% for VSL). We observed no other semen alteration. More especially, vitality values were similar in NVP- and EFV-treated patients. There were no concomitant variations of seminal markers or testosterone plasmatic levels. These results confirm in a more extensive analysis the difference in sperm motility reported between NVP- versus EFV-treated patients from a similar population to ours (Lambert-Niclot et al., 2011).

The present results do not demonstrate a causal link between the exposure to EFV and impaired sperm motility, as one cannot exclude that the patients receiving EFV were more exposed to other factors impairing sperm motility. Another limitation is that only one semen sample per patient was analyzed. Although more than two semen analyzes were performed in a majority of our patients, we chose to use only the first one because the delay between the first and the second semen analysis was highly variable (up to >1 year), and because changes in treatment status occurred in some patients during this time. However, no significant difference was found when comparing first and second semen analyzes in the EFV- and NVP-treated patients for whom this delay was <6 months, and comparing the results of the second semen analysis between these two groups (data not shown) led to the same conclusions as the main analysis (Supplementary Table S1). Moreover, our study has several strengths. The size of the population allowed the comparison of semen parameters by multivariate analyses across large groups of patients, including never treated ones. The cohort included only asymptomatic men from heterosexual HIV-discordant couples who had never tried to conceive naturally and requested MAP, not because of infertility but to avoid HIV transmission. No patient displayed clinical genital infection at the time of semen analysis and the prevalence of positive semen bacterial culture was low in all groups. Therefore, biases associated with infertility or with other diseases were minimized. Furthermore, semen samples were all collected in the laboratory and were analyzed using the same technical procedures by trained technicians who were blind to the patients’ status regarding antiretroviral therapy. Lastly, the measurement of individual sperm velocity through CASA provided an objective confirmation of the reduced percentage of rapid spermatozoa that was observed in standard semen analysis.

How could the decrease of sperm motility observed in EFV-treated patients be explained? Semen viscosity was not higher (data not shown) in these patients. Moreover, neither ejaculate volume nor seminal markers showed significant alterations. Moderate variations of seminal pH were observed in some patients but not especially in those receiving EFV. Thus we think that the impairment of sperm motility was not due to pH or to a higher mechanical resistance against sperm progression linked to abnormal seminal plasma composition. Impaired sperm motility could also result from flagellar dysfunction, and several hypotheses can be considered. Flagellar beating involves the axoneme and the peri-axonemal structures, depends on several metabolic pathways and is controlled by multiple regulation factors. The energy required for flagellar beating comes from the hydrolysis of ATP produced by the mitochondrial oxidative phosphorylation and by glycolysis. Mitochondria are localized in the flagellar midpiece, while glycolytic enzymes are closely associated with periaxonemal structures (Krisfalusi et al., 2006).

As most cART regimens include one NRTI or more, and according to the mitochondrial toxicity of some of these molecules, a dysfunction of sperm mitochondrial respiration induced by NRTIs has been proposed to explain the impaired motility observed in HIV-infected men (White et al., 2001; Leandri et al., 2003; Pavili et al., 2010). However, in the present study, no decrease of sperm motility was observed in the

Figure 3 Distribution of straight line velocity (VSL) values in the spermatozoa of patients treated with efavirenz (EFV, 31 patients) and nevirapine (NVP, 29 patients). Data are mean ± SD.
groups of patients receiving NRTIs alone or combined with other drugs than EFV. Moreover, when analyzing sperm motility in the NVP- and EFV-treated patients according to the associated NRTIs, we observed that in both groups, a-motility values were similar in the patients receiving the NRTIs considered as the most toxic for mitochondria (D4T and /or DDI) and in the patients receiving other NRTIs (Fig. 2). On the other hand, the possibility of mitochondrial dysfunction induced by EFV itself could also be considered, although mitochondrial toxicity is less documented for EFV than for NRTIs. Mitochondrial dysfunctions induced in vitro by EFV or 8-OH EFV have been evidenced in hepatocytes (Blas-Garcia et al., 2010; Bumpus, 2011) and neurons (Blas-Garcia et al., 2014; Funes et al., 2014). However, similar effects on brain tissues from mice exposed to NVP have been reported (Streck et al., 2011). Moreover, mitochondrial respiration does not seem to influence significantly the motility of human spermatozoa in seminal plasma, where their main energy source is glycolysis (Nascimento et al., 2008; Piomboni et al., 2012). Considering these different observations and our results, we think that mitochondrial dysfunction could not alone explain the decreased sperm motility observed here in EFV-treated patients.

Other hypotheses can be proposed from clinical and pharmacological data. The first one involves vitamin D (1,25(OH)2D3). Several studies have demonstrated the expression of the 1,25(OH)2D3 receptor and of vitamin D metabolizing enzymes in human spermatids, spermatozoa and male genital tract (Corbett et al., 2006; Aquila et al., 2009; Blomberg Jensen et al., 2010), indicating the involvement of vitamin D in epididymal sperm maturation. Moreover, a positive correlation was observed between serum vitamin D level and sperm motility (Blomberg Jensen et al., 2011) and, an association between cART regimens containing EFV and vitamin D deficiency has been reported by several authors (Dao et al., 2011; Allavena et al., 2012; Childs et al., 2012). These data suggest that exposure to EFV and impaired sperm motility might be linked through alterations of vitamin D-dependent processes occurring during epididymal sperm maturation. Another hypothesis relies upon the high accumulation rate of EFV in cells and its high affinity for protein binding (Almond et al., 2005a), which are both much higher than those of NVP (Almond et al., 2005b). These data indicate that the majority of EFV molecules inside cells could be bound to cellular constituents (Almond et al., 2005a). This could especially be the case for the sperm flagellum, which is extremely rich in proteins: those constituting the axonemal and periaxonemal structures and, as mentioned above, numerous others with enzymatic and transport functions. A massive binding of EFV molecules to these tightly assembled and regulated proteic elements might impact their functions, impairing motility. Moreover, deleterious effects of exposure to EFV could also be mediated by certain hydroxylated EFV metabolites, for which an accumulation in seminal plasma has been reported (Avery et al., 2013).

It is noteworthy that cART regimens including EFV are also associated with more frequent neuropsychiatric symptoms (Shubber et al., 2013). Actually, spermatozoa and neurons display similarities like long cellular extensions containing abundant and complex cytoskeletal elements, important energy requirements and also common signaling processes such as the endocannabinoid system, which seems to modulate sperm and neuron functions (Schuel and Burkman, 2005; Amoako et al., 2013). These observations suggest that impaired sperm motility and neuropsychiatric symptoms could be two cell-specific functional consequences of common intracellular effects of EFV.

Conclusion

The aim of the present study was to investigate in HIV-infected men the association between semen alterations and antiretroviral treatments. Our results indicate that NRTIs, PIs and NVP have probably little or no influence on semen parameters. By contrast, we evidenced an association between the exposure to EFV and a significant impairment of sperm motility. Antiretroviral therapies have evolved since the period of our study but EFV is widely used in current regimens, so these results may concern many HIV-infected men. Further studies are necessary to clarify the relations between exposure to EFV and impairment of sperm motility, to precise the underlying mechanisms and to evaluate the impact of this impairment on natural fertility and MAP outcome. If a causal link with EFV was to be demonstrated, elucidating the molecular mechanisms of this adverse effect could also help to understand better the neuropsychiatric side effects of EFV.

Supplementary data

Supplementary data are available at http://humrep.oxfordjournals.org/.

Acknowledgements

We thank Dr Aminata Touré for reading the manuscript and for her useful remarks.

Authors’ roles

C.F.: data collection, file processing, manuscript writing and submission. S.G.: statistical analyses, figures, manuscript writing. M.L.V.: patients management and manuscript reviewing. O.L.: patients management and manuscript reviewing. P.S.: patients management and manuscript reviewing. V.G.: patients management and manuscript reviewing. J.P.V.: patients management and manuscript reviewing. M.D.A.: patients management, study initiation and manuscript reviewing. E.D.: patients management, study designing, data collection and analysis, manuscript writing.

Funding

No external funding was used for this study.

Conflict of interest

None declared.

References


Blas-García A, Apostolova N, Ballesteros D, Monleón D, Morales JM, Rocha M et al. Inhibition of mitochondrial function by efavirenz increases lipid content in hepatic cells. *Hepatology* 2010; **52**:115–125.


Krisfalusi M, Miki K, Magyar PL, O’Brien DA. Multiple glycolytic enzymes are tightly bound to the fibrous sheath of mouse spermatozoa. *Biol Reprod* 2006; **75**:270–278.


Nascimento JM, Shi LZ, Tam J, Chandrasanghawu C, Durrant B, Botvinick EL, Berns MW. Comparison of gycolysis and oxidative phosphorylation as energy sources for mammalian sperm motility, using the combination of fluorescence imaging, laser tweezers, and real-time automated tracking and trapping. *J Cell Physiol* 2008; **217**:745–751.


