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# Effects of antiretroviral therapy on semen quality

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## Abstract

**Objective:** To evaluate the effect of combination antiretroviral therapy (cART) on semen quality.

**Design:** A longitudinal cohort study.

**Setting:** The HIV outpatient clinic of the Academic Medical Centre in Amsterdam, the Netherlands.

**Subjects:** A cohort of 34 male patients with different estimated duration of HIV-1 infection, who were about to start various combinations of cART.

**Intervention(s):** Blood and semen analyses before the start of cART and 4, 12, 24, 36 and 48 weeks thereafter.

**Main outcome measure(s):** We examined the effect of cART on semen parameters by a repeated measurements procedure using a mixed-effects model.

**Results:** The median period of follow-up was 48 weeks (interquartile range 33–52 weeks). Five patients used thymidine analogue-containing cART, 23 used tenofovir-based cART, six used other regimens. At all timepoints the percentage of progressively motile spermatozoa was low according to WHO criteria, and it decreased significantly from 28 to 17% during follow-up ( $P=0.02$ ). All other semen parameters were in the normal range and remained stable.

**Conclusions:** cART negatively affected the percentage of progressively motile spermatozoa. Whether this reduced motility affects the chances of fathering a child or leads to an increased need for artificial reproductive techniques is at present unknown.



## Introduction

Potent combination antiretroviral therapy (cART) has increased the life expectancy of patients infected with HIV-1<sup>1</sup>. These drugs have to be taken lifelong, and doctors and patients are now faced with the long-term adverse effects of cART, including lipid abnormalities, insulin resistance, premature atherosclerosis, neuropathy and lipodystrophy<sup>2-5</sup>.

Most antiretroviral drugs show good penetration in the male genital tract and may therefore affect spermatogenesis<sup>6</sup>. The possible effect of cART on semen quality is of interest because semen quality is a key factor for reproductive success<sup>7,8</sup>.

Data on semen parameters before and after the start of antiretroviral therapy are limited to two studies. In one study involving five HIV-1-infected male patients semen parameters were normal according to WHO criteria<sup>9</sup>, and remained stable after zidovudine monotherapy with variable duration<sup>10</sup>. In contrast, another study reported on 20 HIV-1-infected male patients and showed that a number of semen parameters, including the percentage of progressively motile spermatozoa and the percentage of spermatozoa with a normal morphology, were low according to WHO criteria but improved after 4–12 weeks of cART<sup>11</sup>. The outcomes of those studies are not conclusive, as a result of the low numbers of patients in the first study, and the short period of follow-up in the second study, considering that spermatogenesis takes approximately 70 days.

We therefore performed a prospective longitudinal cohort study describing semen parameters before and during the first 48 weeks of cART.

## Materials and methods

### *Patients*

Between February 2003 and October 2005, HIV-1-positive male patients, in whom the decision was made to start cART, were recruited from the HIV outpatient clinic of the Academic Medical Centre in Amsterdam, the Netherlands. Exclusion criteria were current use of antiretroviral drugs and previous use of antiretroviral drugs for a period longer than 8 weeks. Other exclusion criteria were known causes of male infertility in the medical history, including vasectomy, mumps orchitis, orchidopexy and previous exposure to chemotherapy or radiotherapy. The study was approved by the Institutional Review Board of the Academic Medical Centre and all patients gave written informed consent.



### *Study procedures*

The baseline visit was defined as the last clinic visit before the start of cART. Follow-up visits were scheduled 4, 12, 24, 36 and 48 weeks after starting cART.

At baseline and at each follow-up visit a semen analysis was performed and blood was obtained to determine CD4 and CD8 cell counts and blood plasma HIV-1-RNA levels. In addition, at baseline a standardized study questionnaire was completed, and an andrological examination, hormonal screening, urine analysis for *Chlamydia trachomatis* infection, and serological screening for active viral hepatitis B and C were performed.

All semen analyses were performed by a single trained researcher (EvL) according to the WHO manual for routine semen analysis<sup>9</sup>. All participants were instructed to have at least 2 days of sexual abstinence and the exact number of days of abstinence was recorded. The ejaculate was produced by masturbation and collected in a sterile container. All semen analyses were carried out within one hour of ejaculation. After liquefaction at 37°C, semen volume was measured and semen pH was determined. The concentration of spermatozoa and motility of spermatozoa were assessed and at least 100 spermatozoa were counted for motility analysis. Motility was scored as progressive (grade a), slow (grade b plus c) or immotile (grade d). The percentage of spermatozoa with a normal morphology was determined on a semen smear by counting 100 Diff Quick (Dade Behring, Dudingon, Switzerland) stained spermatozoa. Subsequently, the total sperm count and the total motile sperm count were calculated.

### *Statistical analysis*

We examined the effect of cART on semen parameters by a repeated measurements procedure using mixed-effects models (SAS Proc Mixed 8.02; SAS Institute, Cary, North Carolina, USA). Mixed-effects models allow for analyses of longitudinal data in which there are correlations between observations, and provide a valid statistical estimate of the mean effect. In our analysis the span of data and the frequency of missing data were unbalanced, i.e. varied by individual. Mixed-effects models are robust with respect to the effects of common variation on parameter estimation.

The CD4 cell count and blood plasma HIV-1-RNA level were entered into the models as time-updated variables. Parameters that are known to correlate with semen parameters, i.e. age, smoking, number of days of sexual abstinence, semen hyperviscosity and baseline follicle-stimulating hormone levels were evaluated as covariables. The outcomes of semen



parameters were adjusted for the covariables that significantly correlated with the semen parameters studied. Statistical significance was set at a two-sided level of  $P < 0.05$ .

## Results

The baseline characteristics of the 34 patients included in the study are shown in Table 1. Two of these patients had previously used antiretroviral drugs, each during a short period of 8 weeks, 2 and 3 years before entering our study, respectively. One patient had used lamivudine, stavudine, indinavir and ritonavir because of an acute HIV-1 infection in 2000 and stopped of his own accord. The other patient had used zidovudine/lamivudine and efavirenz and stopped because of headaches. None of the patients had genital abnormalities on targeted andrological examination. Two patients had chronic hepatitis B infection as indicated by detectable blood plasma hepatitis B virus DNA, and one had chronic hepatitis C infection by virtue of having detectable blood plasma hepatitis C virus RNA. None of the patients had active *C. trachomatis* infection.



Five patients started a thymidine analogue-containing first-line cART combination, and 29 patients started cART without thymidine analogues. Within the thymidine analogue-containing cART group, all patients used zidovudine-based cART, none used stavudine. Within the group of patients without thymidine analogues, 23 used tenofovir-based cART, one used abacavir-based cART and five used nucleoside/nucleotide analogue-sparing cART. Five patients (15%) changed their antiretroviral therapy regimen within the first 14 weeks because of side effects or simplification of therapy. Two patients stopped lopinavir, one stopped nevirapine and one efavirenz. In these four patients no new antiretroviral agent was started, because they were still on effective cART. One patient switched from lamivudine, nevirapine, lopinavir and ritonavir to lamivudine, nevirapine and tenofovir after 14 weeks because of diarrhoea.

The median period of follow-up was 48 weeks (interquartile range 33–52 weeks). During follow-up mean CD4 counts increased from 276 to 428 cells/ $\mu\text{l}$  ( $P < 0.0001$ ), and mean blood plasma HIV-1-RNA levels decreased from 5.0 to 2.1  $\log_{10}$  copies/ml ( $P < 0.0001$ ). At 48 weeks, 74% of the patients had a blood plasma HIV-1-RNA concentration below the lower limit of detection of 50 copies/ml.

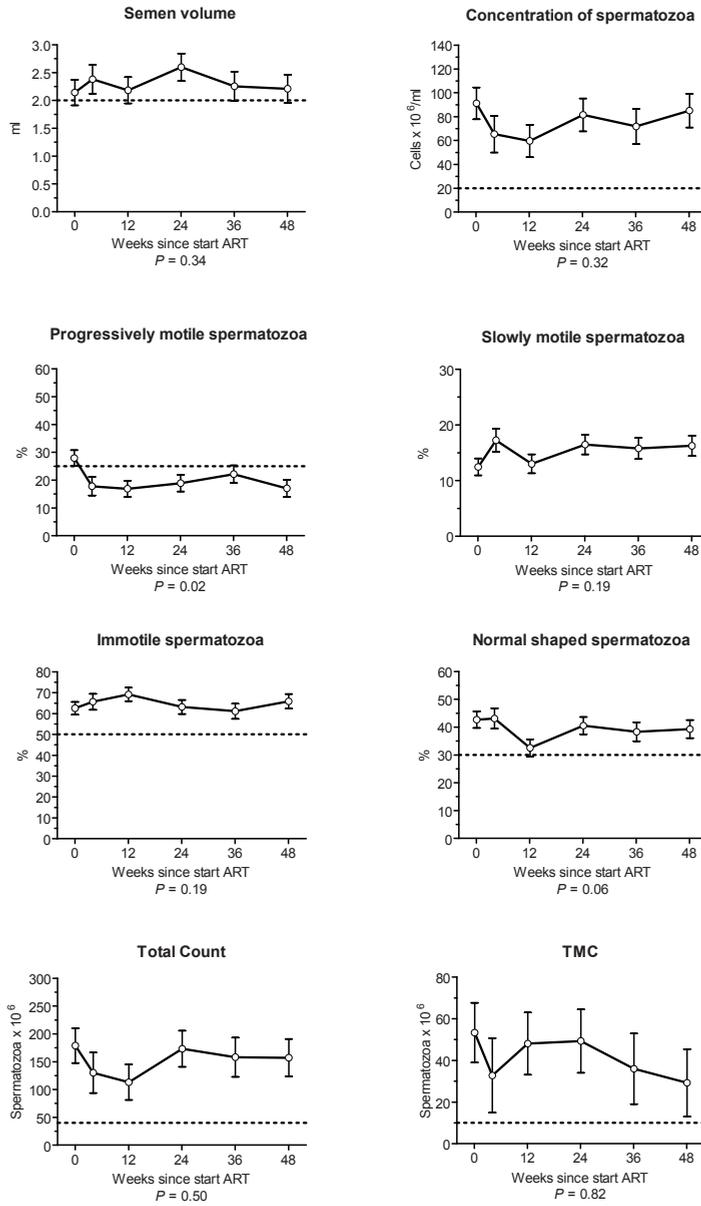
A total of 146 semen samples were analysed. The observed semen parameters during 48 weeks of cART are shown in Fig. 1. The outcomes were adjusted for the covariables that

significantly correlated with the semen parameters in the mixed-effects model. The mean percentage of progressively motile spermatozoa was low according to WHO criteria at baseline, and decreased from 28% to 17% after 48 weeks cART ( $P=0.02$ ). The percentage of immotile spermatozoa was high according to WHO criteria at all timepoints. All other semen parameters were in the normal range according to WHO criteria and remained stable during cART<sup>9</sup>.

**Table 1:** Baseline characteristics

Variable	Outcome	
No. of evaluable patients	34	
Age (years)	41	(37-47)
Duration known HIV seropositivity (years)	2.7	(1.1-3.7)
Current cigarette smoker	11	(32)
HIV-1 status before therapy		
Blood plasma HIV-1 RNA (log copies/ml)	5.0	(4.7-5.4)
CD4 T cells (cells/ $\mu$ l)	230	(190-330)
CD8 T cells (cells/ $\mu$ l)	980	(720-1740)
Endocrinology		
Luteinizing hormone (U/l) $n=0.1-10$	4.5	(3.3-6.2)
Follicle-stimulating hormone (U/l) $n=0.1-15$	6.7	(5.2-8.7)
Prolactin ( $\mu$ g/l) $n=0-15$	10	(7-15)
Testosterone (nmol/l) $n=11-35$	19	(15-22)
Sex hormone binding globulin (nmol/l) $n=12-75$	41	(35-51)
D4 Androstendione (nmol/l) $n=1-10$	7.2	(5.4-8.7)
Free androgen index $n=20-90$	44	(35-55)
Semen parameters		
Semen volume (ml) WHO 2-6	2.1	(1.3)
Concentration of spermatozoa (cells $\times 10^6$ /ml) WHO $\geq 20$	91	(77.5)
Progressively motile spermatozoa (%) WHO 'A' $\geq 20$	28	(16.5)
Slowly motile spermatozoa (%)	11	(8.9)
Immotile spermatozoa (%) WHO 'D' $\leq 50$	61	(17.7)
Normal-shaped spermatozoa (%) WHO $\geq 30$	43	(17.2)
Total count (spermatozoa $\times 10^6$ ) WHO $\geq 40$	179.2	(182.6)
Total motile count (spermatozoa $\times 10^6$ ) WHO $\geq 10$	53.4	(83.5)

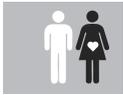
Variables are expressed as median and interquartile range (IQR) or  $n$  and percentage (%), semen parameters are expressed as mean and standard deviation (SD). For hormones normal ranges are provided as  $n$ . For semen parameters normal values according to WHO criteria are provided<sup>9</sup>.



**Figure 1.** Semen parameters during 48 weeks of first-line combination antiretroviral therapy. Total count (semen volume  $\times$  concentration of spermatozoa). Total motile count (TMC, % progressively motile spermatozoa  $\times$  total count). The dotted lines in the graphs display the lower normal values according to WHO (9). The  $P$  values represent the change during 48 weeks of combination antiretroviral therapy.

The mixed-effects model also showed correlations between sexual abstinence and the concentration of spermatozoa and total count. Inverse correlations were observed between follicle-stimulating hormone and the concentration of spermatozoa, total count and total motile count, and between age and the percentage of slowly motile spermatozoa and the percentage of normal-shaped spermatozoa. The use of thymidine analogues was not significantly associated with any of the other semen parameters, in particular not with the percentage of progressively motile spermatozoa. CD4 cell counts and blood plasma HIV-1-RNA levels were not statistically significantly associated with any of the semen parameters.

## Discussion



We demonstrated a statistically significant reduction in the percentage of progressively motile spermatozoa in 34 patients with HIV-1 infection during treatment with cART for 48 weeks. All other semen parameters remained stable.

Our study has a number of strengths. First, the longitudinal study design allowed us to study the effect of cART on semen quality using individual subjects as their own control. Second, all semen analyses were performed by a single trained individual. As a result, inter-observer bias, which is common in the evaluation of semen parameters, was ruled out<sup>12</sup>. Third, all but two of our patients were completely antiretroviral therapy naive, so our results were not biased by the effects of previous antiretroviral therapy. These two patients had only been very temporarily exposed, that is 8 weeks, 2 and 3 years before entry in the current study, making it unlikely that their previous treatment influenced our results.

We previously demonstrated that there was no detectable change in semen quality in 55 patients during a period of 96 weeks of untreated asymptomatic HIV-1 infection<sup>13</sup>. The proportion of progressively motile spermatozoa observed in that untreated group was similar to that in the current group of patients at baseline, before the start of cART. These observations suggest that the reduction in progressively motile spermatozoa, which occurred in the current study during treatment, was related to the use of cART and not to HIV-1 infection.

There are several possible explanations for the observed decrease in progressively motile spermatozoa. Mitochondria are abundant in spermatozoa and provide adenosine triphosphate, necessary to maintain progressive motility<sup>14,15</sup>.

Nucleoside analogue reverse transcriptase inhibitors, in particular the thymidine analogues zidovudine and stavudine, may affect mitochondrial function by inhibition of the mitochondrial DNA replication enzyme polymerase gamma, causing MtDNA depletion, or by other mechanisms<sup>16-18</sup>. A recent cross-sectional study was not able to detect any significant differences in mtDNA content and sperm motility between HIV patients receiving cART, HIV patients without cART, or HIV-negative individuals, but they did observe a weak negative correlation between the time on didanosine, zalcitabine and stavudine and mtDNA content<sup>19</sup>.

Protease inhibitors on the other hand are associated with inhibition of apoptosis, which may also occur in spermatozoa, leading to cell dysfunction, i.e. asthenozoospermia<sup>20-22</sup>.

Our study also has some limitations. First, albeit longer than in previously published re-ports<sup>10,11</sup>, our follow-up was still limited to one year, making it impossible to draw conclusions about the effects of longer exposure to antiretroviral therapy. Second, we cannot with certainty distinguish the effects of the different (classes of) antiretroviral drugs. Only five patients used thymidine analogues, and none of our patients were using stavudine, the agent with the strongest effect on MtDNA<sup>17</sup>. Nevertheless, the statistically significant reduction in the percentage of progressively motile spermatozoa we observed in our study may have been mediated by mitochondrial dysfunction caused by less toxic nucleoside reverse transcriptase inhibitors, such as lamivudine, which was widely used in our study, as a result of other mechanisms than MtDNA depletion<sup>23,24</sup>.

In summary, in 34 HIV-1-infected patients the percentage of progressively motile spermatozoa was reduced after a period of approximately one year of cART, whereas all other indicators of semen quality remained stable. These observations may be useful when counselling HIV-1-infected patients who wish to father a child. Whether the use of cART will result in reduced chances to father a child or an increased need for artificial reproductive techniques is at present unknown.

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*Conflicts of interest: None.*



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