



UvA-DARE (Digital Academic Repository)

Male reproduction and HIV-1 infection

van Leeuwen, E.

Publication date

2009

Document Version

Final published version

[Link to publication](#)

Citation for published version (APA):

van Leeuwen, E. (2009). *Male reproduction and HIV-1 infection*. [Thesis, fully internal, Universiteit van Amsterdam].

General rights

It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations

If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: <https://uba.uva.nl/en/contact>, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.

**Increase of spermatozoal
mtDNA copy numbers during
first twelve weeks of first-line
HAART**

**Liesbeth van Leeuwen
Sjoerd Repping
Michel P. de Baar
Ferdinand W. Wit
Jan M. Prins
Fulco van der Veen
Peter Reiss**

Submitted

6

Abstract

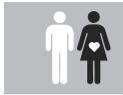
Background: The number of motile spermatozoa is decreased in patients with HIV-1 infection. In addition, the use of highly active antiretroviral therapy (HAART) has been shown to further decrease sperm motility. Spermatozoa contain numerous mitochondria that provide energy for their progressive motility. We tested whether reduction in mitochondrial DNA (mtDNA) copy number of spermatozoa underlies this decrease in motility.

Objective: To explore the effect of HAART on mtDNA copy numbers in spermatozoa.

Methods: MtDNA copy numbers were determined using a quantitative real-time duplex nucleic acid-based amplification assay in spermatozoa of a cohort of ten previously treatment-naïve HIV-1-infected patients, before and 4, 12, 24, 36 and 48 weeks after the start of various HAART regimens. In addition, mtDNA copy numbers were determined in spermatozoa from a healthy semen donor before and after documented seroconversion for HIV-1.

Results: In the cohort of ten treatment-naïve HIV-1-infected men, age and number of spermatozoa were independently associated with mtDNA copy numbers. Adjusting for these variables, the mtDNA copy numbers in spermatozoa increased significantly with $0.45 \log_{10}$ copies/cell during the first 12 weeks of HAART ($P=.001$), without significant subsequent changes from 12 to 48 weeks ($P=.34$). There was no direct association between the proportion of progressively motile spermatozoa and mtDNA copy numbers ($P=.39$). In the semen donor mtDNA copy numbers in spermatozoa declined with $0.39 \log_{10}$ copies/cell following HIV-1 seroconversion ($P=.014$).

Conclusions: MtDNA copy numbers increase during the first twelve weeks of first-line HAART, which may be preceded by a decrease in mtDNA copy number following primary HIV-1 infection.



Introduction

Highly active antiretroviral therapy (HAART) can cause serious adverse effects, including, but not limited to, lipid metabolism abnormalities, insulin resistance, premature atherosclerosis, neuropathy and lipodystrophy¹⁻⁴. Some of these adverse effects are thought to be mediated through deleterious effects of HAART on mitochondria. The thymidine analogue nucleoside reverse transcriptase inhibitor (NRTI)-containing antiretroviral regimens in particular, may negatively affect mitochondrial DNA (mtDNA) copy number, mainly through inhibition of the mtDNA replication enzyme mtDNA polymerase-gamma, causing mtDNA depletion⁵⁻⁷. Apart from HAART with thymidine analogues, HIV infection itself may result in mtDNA depletion in peripheral blood mononuclear cells (PBMC). The mechanism behind this phenomenon is so far unknown^{8,9}. Only recently it was discovered that HAART without thymidine analogues restores mtDNA copy numbers in PBMC¹⁰.

Several antiretroviral drugs show good penetration in the male genital tract and may potentially cause local adverse effects¹¹. Mitochondria are abundant in spermatozoa and provide energy to maintain their progressive motility, a key factor for reproductive success^{12,13}. We have previously reported that upon HIV-1 infection, motility was reduced in a semen donor following HIV-1 seroconversion¹⁴. In a subsequent cohort study we showed that prolonged exposure to untreated HIV-1 infection did not result in a further decline in motility¹⁵. In addition, we and others have shown that HAART can also contribute to a decline in motility^{16,17}. Whether this decline in motility after HIV-1 infection and HAART is related to mitochondrial copy numbers is unknown.

We therefore explored the effect of HAART on mtDNA copy number of spermatozoa, and longitudinally determined mtDNA copy numbers in spermatozoa of ten HIV-1-infected patients before and during the first 48 weeks of first-line HAART. In addition, we investigated mtDNA copy numbers in spermatozoa before and after primary HIV-1 infection in the same semen donor mentioned above.

Material and Methods

Patients and semen samples

Semen samples were collected from ten previously antiretroviral treatment-naïve HIV-1-infected men, in whom the decision was made to start HAART, based on having CD4 counts between 200 and 350 cells/mm³. All patients were enrolled in a study protocol on the effect



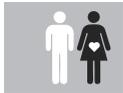
of HAART on semen quality. Purpose of this study was to examine the effect of HAART on semen parameters. As reported recently, HAART negatively affected the percentage of progressively motile spermatozoa¹⁷. The study was approved by the Institutional Review Board of the Academic Medical Center and all patients gave written informed consent.

Semen samples from the cohort were collected at baseline, defined as the last clinic visit before the start of HAART, and at 4, 12, 24, 36 and 48 weeks follow-up, allowing for a window of up to 1 week before and 1 week after the scheduled date. At baseline and at each follow-up visit blood samples were obtained for determination of CD4 cell counts, CD8 cell counts, and blood plasma HIV-1-RNA concentration. PBMC were not collected. A complete semen analysis was performed within one hour after ejaculation according to the World Health Organization (WHO) manual for routine semen analysis¹⁸.

In addition, semen samples before and after HIV-1 infection were available from a donor, participating in a semen donation program of the semen bank of the Center for Reproductive Medicine of the Academic Medical Center from 1994 until June 2001. All semen samples were cryopreserved using SpermFreeze (FertiPro, Beernum, Belgium) and stored in liquid nitrogen. Because HIV-1 blood tests were done approximately every half year, the moment of HIV-1 acquisition was retrospectively determined by the presence of HIV-1-RNA in semen in November 2000¹⁴. We previously published the changes in semen parameters during this observation period¹⁴. Eight frozen semen samples were available before acquisition of HIV-1, and eight frozen samples were available after acquisition of HIV-1. The donor provided four fresh semen samples for research purposes from March 2004 until March 2006, so in total twelve semen samples were available after acquisition of HIV-1. In March 2006, the donor was still antiretroviral treatment-naïve.

Semen preparation

The fresh semen samples were diluted 1:1 with HTF, supplemented with 15% pasteurized plasma solution (GPO; Sanquin, Amsterdam, the Netherlands) and centrifuged for 10 minutes at 400 x g at room temperature. The supernatant was discarded and the sperm pellets were resuspended in 4 ml of HTF and centrifuged at 300 x g for 10 minutes over two continuous gradients of two ml of 70% Pure Sperm (Nidacon, Göteborg, Sweden) to exclude non-spermatozoal cell contamination. The supernatant was removed and the pellets were pooled and resuspended in 4 ml of HTF, and again centrifuged at 400 x g for 10 minutes. After removing the supernatant the pellet was resuspended in 1 ml of HTF medium and transferred into a tube (Eppendorf, Hamburg, Germany). The number of spermatozoa was



counted and the spermatozoal fraction was frozen at -80°C until analysis. All semen samples were recoded and blinded before mtDNA analysis.

For the semen donor, per time point three frozen semen samples were thawed and diluted with 4 ml of human tubal fluid medium (HTF) (Lonza, Verviers, Belgium) and then handled according to the protocol for the fresh samples from the ten cohort patients. The fresh semen samples from the semen donor were processed in a similar way as the fresh samples from the ten cohort patients.

Mitochondrial DNA analysis

The assay to analyze mtDNA in spermatozoa was adapted from previous published versions^{19,20} and checked upon its performance before use on spermatozoa (unpublished data). Spermatozoa were purified from semen by a single trained person (EvL), and total DNA was extracted from an amount of 3×10^5 spermatozoa using a silica-based method²¹. An amount of total nucleic acid equivalent to 3×10^4 spermatozoa was used as input in the amplification reactions, which were always performed in duplicate. Each run included a calibration curve. MtDNA and nuclear DNA (nDNA) of each isolate were simultaneously amplified in one tube by means of real-time duplex nucleic acid sequence based amplification method, which was a modification of the Retina Mitox test (Primagen, Amsterdam, the Netherlands). Details of the original assay have been described previously²⁰. To assess the number of mtDNA molecules per spermatozoon and to express the number of mtDNA per 1 nDNA instead of mtDNA per 2 nDNA as would be relevant for diploid cells, the concentration of each of the two mtDNA primers was increased from $0.2 \mu\text{M}$ to $0.4 \mu\text{M}$. The results for each isolate were calculated as the mean value of the duplicate measurements. The lower limit of detection was 1 copy of mtDNA per spermatozoa.

Statistical Analysis

We examined the effect of 48 weeks of HAART on mtDNA by a repeated measurements procedure using a linear mixed-effects model (PROC MIXED from SAS 8.02; SAS Institute, Cary, NC). Slopes were calculated for the week 0–12 and week 12–48 periods separately. The mtDNA copy numbers were entered into the model after \log_{10} -transformation, because of considerable inter-individual variation. 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. they varied per individual. Mixed-effects models are robust with respect to the effects of common variation on parameter estimation. The concentration



of spermatozoa and the percentage of progressively motile spermatozoa were entered into the models as time-updated variables. Parameters that are known to correlate with semen parameters or parameters that possibly influence the amount of mtDNA, including age, smoking, baseline CD4 levels, baseline blood plasma HIV-1 RNA concentration, zidovudine use, protease inhibitor use, days of sexual abstinence, and baseline follicle-stimulating hormone levels were evaluated as covariables in the model. The outcomes of mtDNA copy numbers were adjusted for the covariables that significantly correlated with the semen parameters studied.

Table 1. Baseline characteristics

Variable	Outcome	
No. of patients	10	
Age (years)	40	(37–44)
CD4 cell count (cells/mm ³)	265	(200–345)
CD8 cell count (cells/mm ³)	820	(663–1295)
Blood plasma HIV-1 RNA concentration (copies/ml)	77741	(48376–161206)
Semen parameters		
Volume (ml) WHO 2-6	2.6	(2.0–3.4)
Concentration of spermatozoa (x 10 ⁶ /ml) WHO ≥20	57	(39–130)
Progressively motile spermatozoa (%) WHO 'A' ≥20	28	(17–47)
Slowly motile spermatozoa (%)	9	(9–14)
Immotile spermatozoa (%) WHO 'D' ≤50	60	(44–70)
Normal shaped spermatozoa (%) WHO ≥30	44	(40–55)
Round cells (%) ^a	1	(1–2)
Total motile sperm count (x 10 ⁶ cells) ^b WHO ≥ 10	51	(17–114)
First-line HAART		
lamivudine (emtricitabine) / tenofovir / non-nucleoside RTI	5	
lamivudine / tenofovir / protease inhibitor	3	
zidovudine / lamivudine / non-nucleoside RTI	1	
zidovudine / didanosine / protease inhibitor	1	

Variables are expressed as number (*n*) or as median and interquartile range (IQR). For semen parameters normal values according to WHO criteria are provided ¹⁸.

^a Total motile sperm count = volume x concentration of spermatozoa x progressively motile spermatozoa

^b Round cells are non-spermatozoal cells, including lymphocytes and immature spermatozoa

We compared the \log_{10} -transformed mtDNA copy numbers of the spermatozoa from the semen donor before and after HIV-1 seroconversion using a generalized linear model with time and HIV as independent variables.

Statistical significance was set at a two-sided level of $P < .05$.

Results

The baseline characteristics of the ten men commencing HAART, including the particular regimens which were started are shown in Table 1. Two men started a zidovudine-based thymidine analogue containing HAART combination, and eight patients started tenofovir-containing thymidine analogue-sparing HAART.

The ten HIV-1-infected patients on HAART provided a total of 50 semen samples (Table 2). For ten time points no semen samples were available; on eight occasions a semen sample could not be produced, and two visits were not within the prespecified window. After preparation of the semen sample, spermatozoa were by far the majority of cells in the spermatozoal fraction and nearly all other non-spermatozoal cells were removed.

The course of spermatozoal mtDNA copy numbers over 48 weeks for each individual patient is shown in Figure 1. Baseline mtDNA copy numbers ranged from 1.5 copies/cell to 269 copies/cell. The mean number of \log_{10} mtDNA copies/cell was 0.77 (SD 0.66) at baseline, peaked at week 12 with 1.29 (0.62) and was 1.03 (0.78) at 48 weeks follow-up, respectively.

In the multivariable model every year increase in age was associated with a 0.057 \log_{10} copies/cell lower mtDNA copy number ($P = .003$), and every unit ($\times 10^6$ spermatozoa/ml) increase in



Table 2. MtDNA copy numbers per cell during 48 weeks of HAART

Week	No. of samples	MtDNA \log_{10} copy number
0	10	0.77 (0.66)
4	7	0.85 (0.84)
12	9	1.29 (0.62)
24	8	0.93 (0.38)
36	9	1.09 (0.50)
48	7	1.03 (0.78)

MtDNA \log_{10} copy numbers are expressed as mean and standard deviation (SD).

the concentration of spermatozoa in the original semen sample was associated with a 0.002 \log_{10} copies/cell lower mtDNA copy number ($P=.01$).

After adjusting for age and number of spermatozoa, the mtDNA copy numbers in spermatozoa increased significantly with 0.45 \log_{10} copies/cell during the first 12 weeks of HAART ($P=.001$), without significant subsequent changes from 12 to 48 weeks ($P=.34$).

The percentage of progressively motile spermatozoa was not statistically significantly associated with mtDNA copy number ($P=.39$). Smoking, CD4 cell count, blood plasma HIV-1 RNA concentration, zidovudine use, protease inhibitor use, days of sexual abstinence, baseline follicle-stimulating hormone levels were also not statistically significantly associated with mtDNA copy number.

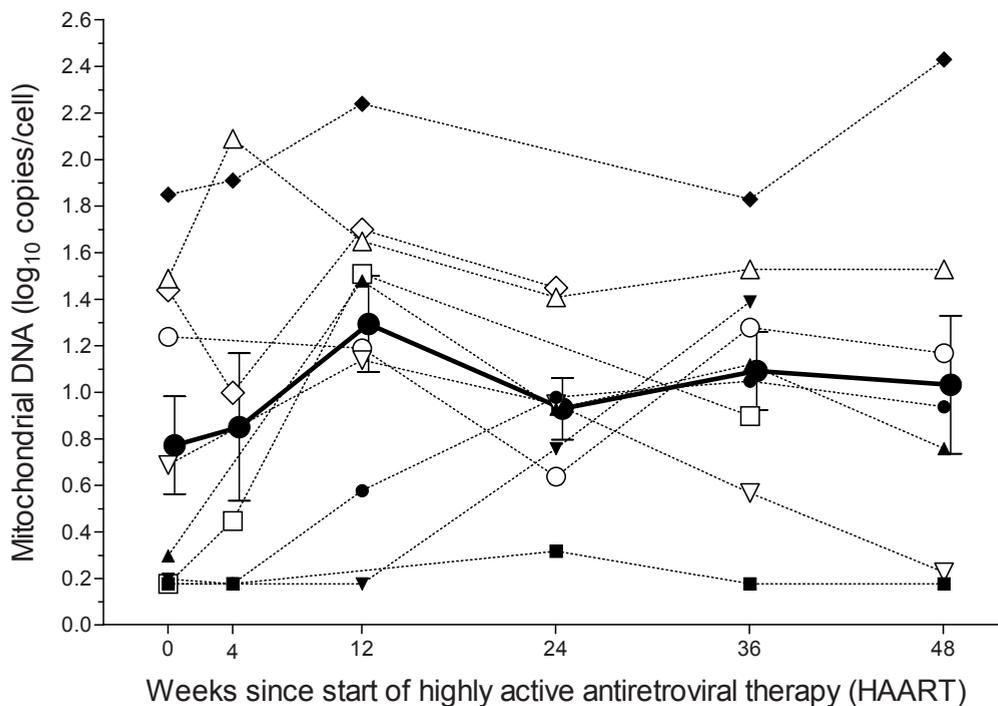


Figure 1. MtDNA copy numbers in spermatozoa during 48 weeks of highly active antiretroviral therapy (HAART) in ten patients.

The solid line reflects the mean mtDNA \log_{10} copies/cell.

Analysis of mtDNA copy number in spermatozoa from the seroconverting semen donor revealed that the mean number of \log_{10} mtDNA copies/cell was significantly lower after HIV-1 seroconversion compared to the period before seroconversion, i.e. $0.43 \log_{10}$ copies/cell versus $0.82 \log_{10}$ copies/cell, respectively ($P=.014$). Within these two periods the mtDNA copy number in spermatozoa did not change significantly ($P=.95$).

Discussion

We demonstrated an increase in the mtDNA copy number of spermatozoa in previously treatment-naïve men commencing HAART. The increase was observed during the first 12 weeks of treatment, with spermatozoal mtDNA copy numbers remaining stable during the remainder of the 48 weeks of first-line HAART. The number of progressively motile spermatozoa was not associated with mtDNA copy numbers. In addition, we demonstrated a significant decrease in mtDNA copy number of spermatozoa in a semen donor after seroconverting for HIV-1.

Our study has several limitations. First, the conclusions of our study are limited by the small sample size of our cohort and the fact that we were only able to study one HIV-1-seroconverting semen donor. Second, only two of our patients used a HAART regimen which included a thymidine analogue NRTI (i.e. zidovudine) known to have a more pronounced potential for mitochondrial toxicity. Thus, any detrimental effect of such agents as compared to other agents cannot be inferred from our study. Finally, no direct parallels can be drawn with blood as appropriate blood samples were not available from our patients.

Other studies have demonstrated an increase in peripheral blood mononuclear cell (PBMC) mtDNA copy numbers during the first year of HAART treatment in previously treatment-naïve patients^{7,10}. Earlier cross-sectional studies of mtDNA content of spermatozoa have yielded conflicting results. One study reported no difference between patients on or off HAART, compared to HIV-negative individuals²², while another study did show a statistically higher mtDNA copy number in spermatozoa from 11 HAART-treated men compared with eight HIV-1-positive men not on HAART and ten HIV-negative men²³.

Although limited to a single case, our finding concerning mtDNA copy number in spermatozoa before and after seroconversion are compatible with those reported earlier for PBMCs from individuals with documented HIV-1 seroconversion, in whom PBMC mtDNA copy number was shown to have decreased significantly one year after acquisition of infection^{24,25}. The



mechanism by which HIV-1 may affect the mtDNA copy number in spermatozoa remains elusive. Spermatozoa themselves do not seem to be susceptible to HIV-1 infection, as they do not express CD4 or any of the co-receptors required for entry of HIV-1 into host cells²⁶. It could be that Sertoli cells, the cells within the testis that nourish the developing spermatozoa, are affected by HIV, although it is likewise unknown if Sertoli cells are susceptible to HIV-1.

We observed the increase in spermatozoal mtDNA copy number only during the first twelve weeks of HAART. Levels did not increase further during the subsequent 36 weeks of observation, and in fact showed a tendency to decline. A potential explanation for this time-dependent effect may be the difference in duration of exposure of spermatozoa of different maturity to both HIV-1 infection and HAART²⁷. As spermatogenesis takes around 70 days, spermatozoa ejaculated during the first 12 weeks of HAART were mostly mature cells already present in the testis and epididymis before the start of HAART. In contrast, spermatozoa which were ejaculated during the subsequent 36 weeks were cells that were exposed to HAART during their complete maturation cycle from spermatogonial stem cell to a mature spermatozoon.



The increase in mtDNA copy number that we observed during the first 12 weeks of HAART appears not to be in line with the statistically significantly decrease in the percentage of progressively motile spermatozoa that was observed during 48 weeks of first-line HAART¹⁷. Moreover, in the current study the mtDNA copy number in spermatozoa was not directly associated with the percentage of progressively motile spermatozoa. Although the reduction in mtDNA copy number thus does not seem to be associated with a reduction in sperm motility, we cannot rule out that the decrease in the percentage of progressively motile spermatozoa may result from mechanisms involving mitochondria other than those affecting mtDNA copy numbers²⁸.

In summary, we demonstrate that commencing HAART is associated with an increase in the mtDNA copy number of spermatozoa. The data obtained from our single semen donor suggest that this possibly represents a reversal of reduced mtDNA copy numbers induced by HIV-1 infection, but clearly additional data will be needed to confirm this. Interestingly, the changes which were observed in spermatozoal mtDNA copy numbers after the start of HAART did not provide an explanation for the reduction in the percentage of progressively motile spermatozoa which we have reported previously¹⁷. Future studies should address potential other mechanisms by which HAART may reduce spermatozoal motility which may

include other toxic effects on mitochondria such as the induction of mitochondrial DNA deletions and changes in mitochondrial membrane potential.

References

- (1) Brinkman K, ter Hofstede HJ, Burger DM, Smeitink JA, Koopmans PP. Adverse effects of reverse transcriptase inhibitors: mitochondrial toxicity as common pathway. *AIDS*. 1998;12:1735-1744.
- (2) Carr A, Samaras K, Burton S, Law M, Freund J, Chisholm DJ et al. A syndrome of peripheral lipodystrophy, hyperlipidaemia and insulin resistance in patients receiving HIV protease inhibitors. *AIDS*. 1998;12:F51-F58.
- (3) van der Valk M, Bisschop PH, Romijn JA, Ackermans MT, Lange JM, Endert E et al. Lipodystrophy in HIV-1-positive patients is associated with insulin resistance in multiple metabolic pathways. *AIDS*. 2001;15:2093-2100.
- (4) Deeks SG. Antiretroviral treatment of HIV infected adults. *BMJ*. 2006;332:1489.
- (5) Brinkman K, Smeitink JA, Romijn JA, Reiss P. Mitochondrial toxicity induced by nucleoside-analogue reverse-transcriptase inhibitors is a key factor in the pathogenesis of antiretroviral-therapy-related lipodystrophy. *Lancet*. 1999;354:1112-1115.
- (6) van der Valk M, Casula M, Weverling GJ, van Kuijk K, Eck-Smit B, Hulsebosch HJ et al. Prevalence of lipodystrophy and mitochondrial DNA content of blood and subcutaneous fat in HIV-1-infected patients randomly allocated to zidovudine- or stavudine-based therapy. *Antivir Ther*. 2004;9:385-393.
- (7) Casula M, Weverling GJ, Wit FW, Timmermans EC, Stek Jr. M, Lange JM et al. Mitochondrial DNA and RNA increase in peripheral blood mononuclear cells from HIV-1-infected patients randomized to receive stavudine-containing or stavudine-sparing combination therapy. *J Infect Dis*. 2005;192:1794-1800.
- (8) Cossarizza A, Moyle G. Antiretroviral nucleoside and nucleotide analogues and mitochondria. *AIDS*. 2004;18:137-151.
- (9) Gerschenson M, Brinkman K. Mitochondrial dysfunction in AIDS and its treatment. *Mitochondrion*. 2004;4:763-777.
- (10) Saitoh A, Fenton T, Alvero C, Fletcher CV, Spector SA. Impact of nucleoside reverse transcriptase inhibitors on mitochondria in human immunodeficiency virus type 1-infected children receiving highly active antiretroviral therapy. *Antimicrob Agents Chemother*. 2007;51:4236-4242.
- (11) Lowe SH, Sankatsing SU, Repping S, van der Veen F, Reiss P, Lange JM et al. Is the male genital tract really a sanctuary site for HIV? Arguments that it is not. *AIDS*. 2004;18:1353-1362.
- (12) Evenson DP, Darzynkiewicz Z, Melamed MR. Simultaneous measurement by flow cytometry of sperm cell viability and mitochondrial membrane potential related to cell motility. *J Histochem Cytochem*. 1982;30:279-280.
- (13) Graham JK, Kunze E, Hammerstedt RH. Analysis of sperm cell viability, acrosomal integrity, and mitochondrial function using flow cytometry. *Biol Reprod*. 1990;43:55-64.
- (14) Van Leeuwen E, Cornelissen M, de Vries JW, Lowe SH, Jurriaans S, Repping S et al. Semen parameters of a semen donor before and after infection with human immunodeficiency virus type 1: Case report. *Hum Reprod*. 2004;19:2845-2848.



- (15) Van Leeuwen E, Wit FW, Prins JM, Reiss P, van der Veen F, Repping S. Semen quality remains stable during 96 weeks of untreated human immunodeficiency virus-1 infection. *Fertil Steril*. 2008;90:636-641.
- (16) Bujan L, Sergerie M, Moinard N, Martinet S, Porte L, Massip P et al. Decreased semen volume and spermatozoa motility in HIV-1-infected patients under antiretroviral treatment. *J Androl*. 2007;28:444-452.
- (17) Van Leeuwen E, Wit FW, Repping S, Eeftinck Schattenkerk JK, Reiss P, van der Veen F et al. Effects of antiretroviral therapy on semen quality. *AIDS*. 2008;22:637-642.
- (18) WHO organisation. *WHO laboratory manual for the examination of Human Semen and Sperm-Cervical Mucus interaction*. 4th edn ed. Cambridge, UK: The press syndicate of Cambridge University Press; 1999.
- (19) de Baar MP, Timmermans E, Dobbelaer-Bosboom I, van Gemen B, and de Ronde A. New tests for measurement of mitochondrial DNA and RNA relative to nuclear DNA based on high throughput real-time monitored duplex amplification. *Antivir.Ther*. L14-15. 2002.
- (20) Timmermans EC, Tebas P, Ruiter JP, Wanders RJ, de Ronde A, de Baar MP. Real-time nucleic acid sequence-based amplification assay to quantify changes in mitochondrial DNA concentrations in cell cultures and blood cells from HIV-infected patients receiving antiviral therapy. *Clin Chem*. 2006;52:979-987.
- (21) Boom R, Sol C, Beld M, Weel J, Goudsmit J, Wertheim-van Dillen P. Improved silica-guanidiniumthiocyanate DNA isolation procedure based on selective binding of bovine alpha-casein to silica particles. *J Clin Microbiol*. 1999;37:615-619.
- (22) Diehl S, Vernazza P, Trein A, Schnaitmann E, Grimbacher B, Setzer B et al. Mitochondrial DNA and sperm quality in patients under antiretroviral therapy. *AIDS*. 2003;17:450-451.
- (23) La Sala GB, Pilotti E, Nicoli A, Pinelli S, Villani MT, Ronzi P et al. Dynamics of HIV viral load in blood and semen of patients under HAART: impact of therapy in assisted reproduction procedures. *AIDS*. 2007;21:377-379.
- (24) Casula M, Bosboom-Dobbelaer I, Smolders K, Otto S, Bakker M, de Baar MP et al. Infection with HIV-1 induces a decrease in mtDNA. *J Infect Dis*. 2005;191:1468-1471.
- (25) Maagaard A, Holberg-Petersen M, Kollberg G, Oldfors A, Sandvik L, Bruun JN. Mitochondrial (mt)DNA changes in tissue may not be reflected by depletion of mtDNA in peripheral blood mononuclear cells in HIV-infected patients. *Antivir Ther*. 2006;11:601-608.
- (26) Robbins WA, Witt KL, Haseman JK, Dunson DB, Troiani L, Cohen MS et al. Antiretroviral therapy effects on genetic and morphologic end points in lymphocytes and sperm of men with human immunodeficiency virus infection. *J Infect Dis*. 2001;184:127-135.
- (27) Hafez ESE. *Human semen and fertility regulation in men*. The C.V. Mosby Company; 1976.
- (28) Sternfeld T, Schmid M, Tischleder A, Mudra S, Schlamp A, Kost BP et al. The influence of HIV infection and antiretroviral therapy on the mitochondrial membrane potential of peripheral mononuclear cells. *Antivir Ther*. 2007;12:769-778.

