Male reproduction and HIV-1 infection

van Leeuwen, E.

Publication date
2009
Document Version
Final published version

Semen quality remains stable during 96 weeks of untreated human immunodeficiency virus-1 infection

4

Liesbeth van Leeuwen
Ferdinand W. Wit
Jan M. Prins
Peter Reiss
Fulco van der Veen
Sjoerd Repping

Fertility Sterility 2008;90:636-641

Abstract

Objective: To evaluate semen parameters during the natural course of asymptomatic human immunodeficiency virus-1 (HIV-1) infection.

Design: A longitudinal cohort study.

Setting: HIV outpatient clinic of the Academic Medical Center in Amsterdam, the Netherlands.

Patient(s): 55 men infected with HIV-1, with infection of variable duration but without previous or current antiretroviral therapy.

Intervention(s): Biannual blood and semen analyses.

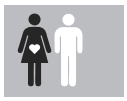
Main Outcome Measure(s): We examined the changes in semen parameters over time using a repeated measurements mixed-effects model.

Result(s): The mean follow-up period was 77 weeks (interquartile range: 39 to 111 weeks). The mean CD4 cell count showed a statistically significant decline from 480 to 400 cells/mm3, and the mean blood plasma HIV-1 RNA concentration showed a statistically significant increase from 4.1 to 4.3 $\log_{10}$ copies/mL. None of the semen parameters showed any statistically significant change over time.

Conclusion(s): Prolonged exposure to asymptomatic, untreated HIV-1 infection does not affect semen quality. These findings should be reassuring for untreated men infected with HIV-1 who wish to father a child, and they also provide relevant background information for studies investigating the potential effect of antiretroviral therapy on semen quality.

Introduction

The introduction of highly active antiretroviral therapy (HAART) in the mid-1990s has resulted in a spectacular increase in life expectancy in the industrialized world for men and women infected with human immunodeficiency virus-1 (HIV-1) [1]. With this increased life expectancy, more couples with HIV-1 infection now wish to have children, as do other couples in which one partner has a chronic illness [2,3]. In general, HIV-1 RNA concentrations in blood and seminal plasma decrease in response to HAART [4-6], but occasionally HIV-1 RNA can be detected in seminal plasma despite adequate suppression of HIV-1 RNA in blood [7-12]. As a result assisted reproductive technology (ART) involving semen processing is always necessary to allow serodiscordant couples with an HIV-1-infected man to have offspring while minimizing the risk of infecting the seronegative woman [13].



During the natural course of HIV-1 infection, CD4-positive lymphocytes (CD4 cells) decline, and the blood plasma HIV-1 RNA concentration increases [14]. At present, the moment of starting HAART is often postponed until the CD4 cell count has decreased to a level between 200 and 350 cells/mm^3. Usually, HAART is not started in patients with earlier disease because of concerns regarding the long-term side effects of the treatment, which is a combination of antiretroviral drugs that needs to be taken for life [15]. Due to considerable interperson variability in the rate of disease progression, the duration of untreated HIV-1 infection is variable, and many men remain off therapy for a lengthy period.

This raises the question whether the ongoing HIV-1 infection in these men will negatively affect their semen quality. However, to date, no longitudinal data on semen quality in HIV-1-infected men are available. Thus, it is unknown whether prolonged exposure to HIV-1 compromises a man's chance to start a family.
Thus, we performed a longitudinal cohort study to describe semen parameters over time during the natural course of untreated HIV-1 infection to determine whether chronic HIV-1 infection is associated with lower semen quality.

Materials and Methods

Patients
Between February 2003 and October 2005, asymptomatic HIV-1-positive men were recruited from the HIV outpatient clinic of the Academic Medical Center in Amsterdam, the Netherlands. Exclusion criteria were current and prior use of antiretroviral therapy and known

causes of male infertility, including a vasectomy, a history of mumps orchitis, or a history of chemotherapy or radiotherapy. The study was approved by the institutional review board of the Academic Medical Center, and all patients gave written informed consent.

Study Procedures

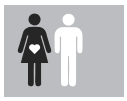
Baseline was considered to be the time of study entry. Follow-up visits were scheduled 24, 48, 72, 96 and 120 weeks after the first visit, allowing for a window of up to 12 weeks before and 12 weeks after the scheduled date. Men who entered the study at a later time point had a shorter duration of follow-up evaluation.

At the first visit and at each follow-up visit, a semen analysis was performed, and blood samples were obtained to determine CD4 cell counts, CD8 cell counts, and blood plasma HIV-1 RNA concentration. If no blood sample was drawn on the day of semen analysis, CD4 cell counts and blood plasma HIV-1 RNA concentration measured within a 2-week window period were used. During the first visit, a standardized study questionnaire was completed, and an andrologic examination, hormonal screening, urine analysis for *Chlamydia trachomatis* infection, and serological screening for active viral hepatitis were performed. The study questionnaire covered demographic data, reproductive history, reproduction wishes, sexual preference, date of the first known positive HIV test, history of sexually transmitted diseases, and history of andrologic disorders such as cryptorchidism or inguinal hernia. Data were also collected on other diseases, medication use, smoking history and alcohol intake. Testicular volume was estimated using Prader's orchidometer. Abnormalities such as bilateral congenital absence of the vas deferens, varicocele, hydrocele or epididymal cysts were recorded. The hormone screening consisted of measurement of plasma concentrations of luteinizing hormone (LH), follicular stimulating hormone (FSH), prolactin, sex hormone-binding globulin (SHBG), testosterone, and D4 androstendione. A ligase chain reaction assay (LCR; Abbott Diagnostics, Abbott Park, IL) was performed on first-void urine to detect *Chlamydia trachomatis* infection [16]. Data concerning the presence of hepatitis B virus surface and e-antigen in serum and hepatitis B and C viral antibodies were also recorded. If these suggested an active infection, hepatitis B DNA and/or hepatitis C RNA concentration was determined in blood plasma by polymerase chain reaction (PCR).

Semen Analysis

All semen analyses were performed by a single trained person (EvL) according to the World Health Organization (WHO) criteria for routine semen analysis [17]. All men were instructed to have at least 2 days of sexual abstinence, and the 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 1 hour of ejaculation. After liquefaction at 37°C, the semen volume was measured. A semen sample was considered hyperviscous if a 4-cm semen thread could be drawn [17]. The concentration of spermatozoa and motility of spermatozoa were assessed using a disposable counting chamber (Léjà Products B.V., Nieuw Vennep, the Netherlands). 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 normal morphologic characteristics was determined on a semen smear by counting 100 spermatozoa stained with Diff Quick® (Dade Behring, Dudingen, Switzerland).



Statistical Analysis

We examined the effect of time on semen parameters by a repeated measurements procedure using mixed-effects models (SAS PROC MIXED 8.02; SAS Institute, Cary, NC). Mixed-effects models allow for analyses of longitudinal data in which there are correlations between observations, and they provide a valid statistical estimate of the mean effect. In this 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 concentration were entered into the models as time-updated variables. Age, smoking, number of days of sexual abstinence, semen hyperviscosity, and baseline FSH levels, parameters that are known to correlate with semen parameters, were evaluated as covariables. The outcomes of semen parameters were adjusted for the covariables that significantly correlated with the semen parameters studied. $P<.05$ (two-sided level) was considered statistically significant. If a patient had to start HAART during the study period, only data obtained before the start of HAART were used for statistical analysis.

Results

Sixty-nine patients were included of whom 14 provided only a single semen sample and had no follow-up evaluation. Fifty-five patients provided at least two semen samples. During the entire study period, 16 men started HAART because their CD4 cells reached values of around 350 cells/mm^3. None of these men had developed symptomatic HIV-1 infection or AIDS before starting treatment.

The baseline characteristics of all 55 patients are shown in Table 1. One man had a chronic hepatitis B virus infection, as indicated by the detectable blood plasma DNA; three men had chronic hepatitis C virus infection by virtue of having the detectable blood plasma RNA. None of the patients had an active *Chlamydia trachomatis* infection.

Mean CD4 cell counts decreased statistically significantly from 480 to 400 cells/mm^3 (*P*=.003), and the mean blood plasma log_{10} HIV-1 RNA concentrations increased statistically significantly from 4.1 to 4.3 log_{10} copies/mL during the follow-up period (*P*=.021).

In total, 196 semen samples were analyzed. The median follow-up period was 77 weeks (inter quartile range [IQR] 39 to 111 weeks). Only the follow-up visits up to 96 weeks were used in this analysis because only five samples for 120 weeks were available.

The effect of time and covariables on semen parameters is shown in Table 2. Time, reflecting 96 weeks of untreated HIV-1 infection, had no effect on any of the semen parameters studied. The model revealed that, for instance, smokers had 9.3% less progressively motile spermatozoa than nonsmokers, and every unit increase in FSH decreased total motile sperm count, with 5.2 cells x 10^6/mL (see Table 2). Hyperviscosity of the semen was not statistically significantly correlated with any of the parameters studied. The CD4 count was not statistically significantly associated with any of the investigated semen parameters. Blood plasma HIV-1 RNA levels were found to be positively associated with the concentration of spermatozoa.

The observed semen parameters during the 96-week follow-up period are shown in Figure 1. The lines were adjusted for the covariables that statistically significantly correlated with the semen parameters in the mixed-effects model. All semen parameters were in the lower-normal to normal range according to WHO criteria [17].

Discussion

We demonstrated that there was no detectable change in semen quality in 55 men during a period of 96 weeks of untreated asymptomatic HIV-1 infection, in which there was a statistically significant decrease in CD4 cell counts and a statistically significant increase in blood plasma HIV-1 RNA.

Table 1. Baseline characteristics

Variable	Outcome	
Number of evaluable patients	55	
Age (years)	38	(33-42)
Desire to conceive	19	(35)
Children	7	(13)
Sexual orientation		
Homosexual	39	(71)
Heterosexual	8	(15)
Bisexual	8	(15)
Acquisition of HIV		
Homosexual	48	(87)
Heterosexual	7	(13)
Years (known) HIV positive	1.8	(0.7-3.9)
STD history		
Gonorrhea	26	(47)
Chlamydia	22	(40)
Syphilis	14	(26)
Other diseases		
Kidney disease	1	(2)
Urinary tract infection in past	7	(13)
Tuberculosis in past	2	(4)
Chronic medication use	17	(31)
Sleeping pills	3	
Antihypertensive drugs	3	
Selective serotonin re-uptake inhibitors	2	
Pneumocystis carinii prophylaxis	2	
Other drugs	7	
Smoking	20	(36)
Alcohol >20 units a week	6	(11)
Genital examination		
Testicular volume right (mL)	22	(17-25)
Testicular volume left (mL)	22	(18-25)
Varicocele, hydrocele	6	(11)
HIV-1		
Blood plasma HIV-1 RNA levels (log copies HIV-1 RNA/mL)	4.5	(3.8-4.7)
CD4+ T cells (cells/mm3)	480	(370-600)
CD8+ T cells (cells/mm3)	1060	(810-1330)
Endocrinology		
LH (U/L), n= 0.1-10	3.9	(2.7-5.8)
FSH (U/L), n=0.1-15	5.3	(3.7-7.2)
Prolactin (µg/L), n= 0-15	10	(9-16)
Testosterone (nmol/L), n=11-35	19	(16-24)
SHBG (nmol/L), n=12-75	34	(26-45)
D4 Androstendione (nmol/L), n=1-10	7.6	(6.6-9.7)
FAI, n=20-90	53	(42-76)
Semen parameters		
Semen volume (mL)	2.3	(1.46)
Concentration of spermatozoa (cells x 10^6/mL)	96	(89.1)
Progressively motile spermatozoa (%)	25	(16.7)
Slowly motile spermatozoa (%)	14	(7.4)
Immotile spermatozoa (%)	61	(17.4)
Normal-shaped spermatozoa (%)	44	(16.7)
Total sperm count (spermatozoa x 10^6)	193.6	(180.63)
Total motile sperm count (spermatozoa x 10^6)	56.8	(90.15)

Note: FAI, free androgen index; FSH, follicle-stimulating hormone; LH, luteinizing hormone; SHBG, sex hormone-binding globulin; STD, sexually transmitted disease.
Semen parameters are expressed as mean (SD); other variables are expressed as median (IQR) or n (%)

Our study has a number of strengths. First, the longitudinal study design allowed us to study the effect of progressive HIV-1 infection on semen quality using individual men as their own controls. Second, all semen analyses were performed by a single trained person. As a result, interobserver bias, which is common in the evaluation of semen parameters, was ruled out [18]. Third, we ruled out *Chlamydia trachomatis* infection at baseline, and urethral gonorrhea was diagnosed in none of the study participants during the study period.

Table 2. Results of the mixed-effects models

Semen Parameters	Estimate	*P* value
Semen volume (mL)		
Time	–	.37
Concentration of spermatozoa (cells x 10^6/mL)		
Time	–	.86
Baseline FSH (IU/L)	–8.1	.003
Blood viral load (log_{10} copies/mL)	28	.01
Days' sexual abstinence	9.7	.007
Progressively motile spermatozoa (%)		
Time	–	.70
Age (per year)	–0.5	.02
Smoking	–9.3	.02
Slowly motile spermatozoa (%)		
Time	–	.39
Immotile spermatozoa (%)		
Time	–	.99
Normal-shaped spermatozoa (%)		
Time	–	.50
Total count (spermatozoa x 10^6)		
Time	–	.62
Baseline FSH (IU/L)	–11	.02
Days' sexual abstinence	40	<.0001
Smoking	–80	.03
Total Motile Count (spermatozoa x 10^6)		
Time	–	.93
Baseline FSH (IU/L)	–5.2	.02
Days' sexual abstinence	11.7	.004

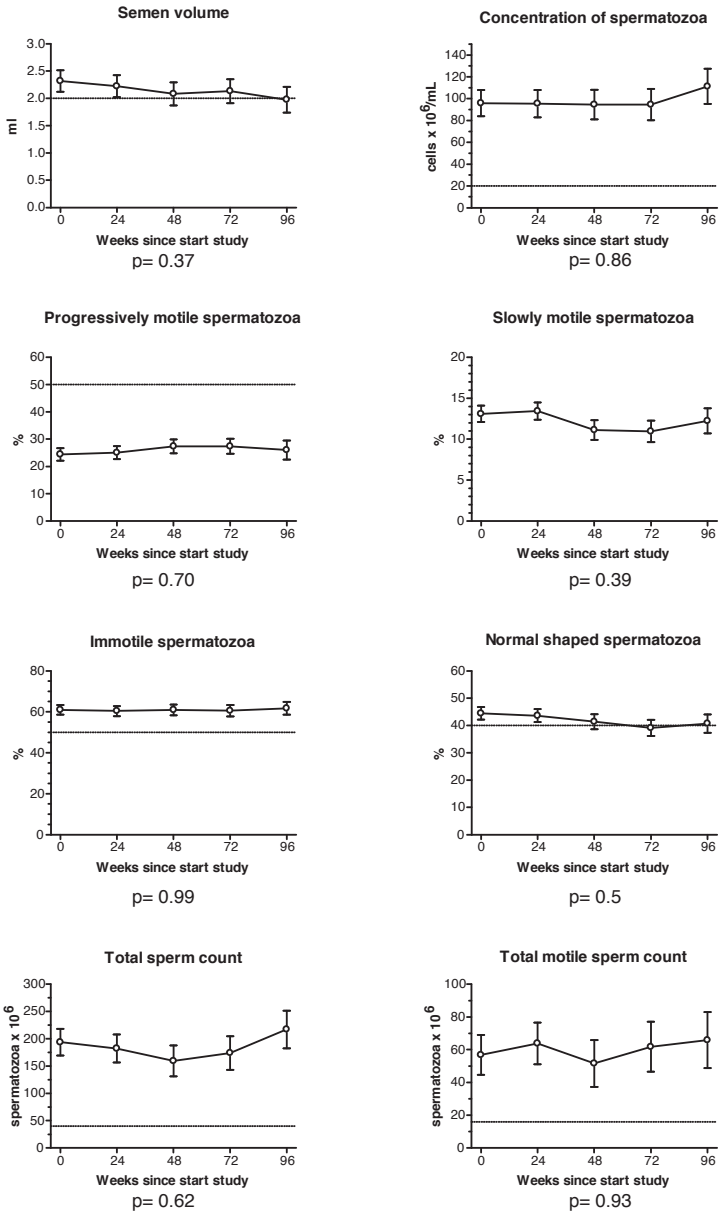


Figure 1. Semen parameters during 96 weeks of untreated HIV-1 infection. The dotted lines in the graphs display the lower normal values according to the World Health Organization criteria. The *P* values represent the change over time. For statistical analyses, repeated measurements mixed-effects models were used.

The inverse correlation of FSH with the concentration of spermatozoa, total sperm count, and total motile sperm count; the positive correlation between the duration of sexual abstinence and the concentration of spermatozoa, total sperm count, and total motile sperm count; and, finally, the finding that smoking decreased the percentage of spermatozoa with progressive motility and total sperm count are in line with previous data, and they support the validity of the obtained results [19-23].

We were unable to study any potential detrimental effects of more advanced immunodeficiency on semen quality, as none of the men reached a CD4 cell count below 200 cells/mm^3, or developed AIDS during the follow-up period. However, we cannot rule out any acute effects on semen quality resulting from acquisition of HIV-1 infection, as all of the men were already chronically infected with HIV-1 when they entered into the study, with varying durations of infection. Of note in this context is that we had previously described a decrease in semen volume and a decrease in the percentage of spermatozoa with progressive motility shortly after HIV seroconversion in a single semen donor [24]. Our observation that both semen volume and the percentage of spermatozoa with progressive motility at baseline were in the lower-normal range seems to be consistent with this finding [17]. Unexpectedly, blood plasma HIV-1 RNA concentration positively correlated with the concentration of spermatozoa; there was no correlation with any of the other semen parameters. There is no obvious biological explanation for this correlation.

Our study has also some limitations. First, our follow-up period was limited to 96 weeks, making it impossible to draw conclusions about the effects of longer exposure to HIV-1. Second, our study group consisted of only 55 men.

In 55 untreated asymptomatic HIV-1 infected men, semen quality remained stable over a period of approximately 2 years. As semen quality is one of the key factors in determining reproductive success—not only in spontaneous conception but also in the assisted reproductive techniques that are generally advised to HIV-discordant couples with an HIV-1-infected man—these findings are reassuring and may be of use when counseling patients infected with HIV-1 who wish to father a child. Moreover, these results provide relevant background information for studies investigating any potential effect of antiretroviral therapy on semen quality.

References

(1) Yeni PG, Hammer SM, Hirsch MS, Saag MS, Schechter M, Carpenter CC et al. Treatment for adult HIV infection: 2004 recommendations of the International AIDS Society-USA Panel. *JAMA.* 2004;292:251-265.

(2) Sherr L, Barry N. Fatherhood and HIV-positive heterosexual men. *HIV Med.* 2004;5:258-263.

(3) Frodsham LC, Boag F, Barton S, Gilling-Smith C. Human immunodeficiency virus infection and fertility care in the United Kingdom: demand and supply. *Fertil Steril.* 2006;85:285-289.

(4) Barroso PF, Schechter M, Gupta P, Melo MF, Vieira M, Murta FC et al. Effect of antiretroviral therapy on HIV shedding in semen. *Ann Intern Med.* 2000;133:280-284.

(5) Taylor S, Ferguson NM, Cane PA, Anderson RM, Pillay D. Dynamics of seminal plasma HIV-1 decline after antiretroviral treatment. *AIDS.* 2001;15:424-426.

(6) Leruez-Ville M, De Almeida M, Tachet A, Dulioust E, Guibert J, Mandelbrot L et al. Assisted reproduction in HIV-1-serodifferent couples: the need for viral validation of processed semen. *AIDS.* 2002;16:2267-2273.

(7) Zhang H, Dornadula G, Beumont M, Livornese L, Jr., Van Uitert B, Henning K et al. Human immunodeficiency virus type 1 in the semen of men receiving highly active antiretroviral therapy. *N Engl J Med.* 1998;339:1803-1809.

(8) Kim LU, Johnson MR, Barton S, Nelson MR, Sontag G, Smith JR et al. Evaluation of sperm washing as a potential method of reducing HIV transmission in HIV-discordant couples wishing to have children. *AIDS.* 1999;13:645-651.

(9) Gupta P, Leroux C, Patterson BK, Kingsley L, Rinaldo C, Ding M et al. Human immunodeficiency virus type 1 shedding pattern in semen correlates with the compartmentalization of viral Quasi species between blood and semen. *J Infect Dis.* 2000;182:79-87.

(10) Vernazza PL, Troiani L, Flepp MJ, Cone RW, Schock J, Roth F et al. Potent antiretroviral treatment of HIV-infection results in suppression of the seminal shedding of HIV. The Swiss HIV Cohort Study. *AIDS.* 2000;14:117-121.

(11) Bujan L, Daudin M, Alvarez M, Massip P, Puel J, Pasquier C. Intermittent human immunodeficiency type 1 virus (HIV-1) shedding in semen and efficiency of sperm processing despite high seminal HIV-1 RNA levels. *Fertil Steril.* 2002;78:1321-1323.

(12) Bujan L, Daudin M, Matsuda T, Righi L, Thauvin L, Berges L et al. Factors of intermittent HIV-1 excretion in semen and efficiency of sperm processing in obtaining spermatozoa without HIV-1 genomes. *AIDS.* 2004;18:757-766.

(13) Van Leeuwen E, Prins JM, Jurriaans S, Boer K, Reiss P, Repping S et al. Reproduction and fertility in human immunodeficiency virus type-1 infection. *Hum Reprod Update.* 2007;13:197-206.

(14) Mellors JW, Munoz A, Giorgi JV, Margolick JB, Tassoni CJ, Gupta P et al. Plasma viral load and CD4+ lymphocytes as prognostic markers of HIV-1 infection. *Ann Intern Med.* 1997;126:946-954.

(15) Yeni PG, Hammer SM, Carpenter CC, Cooper DA, Fischl MA, Gatell JM et al. Antiretroviral treatment for adult HIV infection in 2002: updated recommendations of the International AIDS Society-USA Panel. *JAMA.* 2002;288:222-235.

(16) Pannekoek Y, Westenberg SM, Eijk PP, Repping S, Van der Veen F, van der Ende A et al. Assessment of Chlamydia trachomatis infection of semen specimens by ligase chain reaction.

J Med Microbiol. 2003;52:777-779.

(17) WHO organisation. *WHO laboratory manual for the examination of Human Semen and Sperm-Cervical Mucus interaction.* 4th ed. Cambridge: The press syndicate of the University of Cambridge; 1999.

(18) Auger J, Eustache F, Ducot B, Blandin T, Daudin M, Diaz I et al. Intra- and inter-individual variability in human sperm concentration, motility and vitality assessment during a workshop involving ten laboratories. *Hum Reprod.* 2000;15:2360-2368.

(19) Rosen SW, Weintraub BD. Monotropic increase of serum FSH correlated with low sperm count in young men with idiopathic oligospermia and aspermia. *J Clin Endocrinol Metab.* 1971;32:410-416.

(20) Kjessler B, Wide L. Follicle stimulating hormone (FSH) and interstitial cell stimulating hormone (ICSH) in relation to gametic output in 643 males. *Acta Endocrinol (Copenh).* 1973;72:243-256.

(21) Vine MF, Tse CK, Hu P, Truong KY. Cigarette smoking and semen quality. *Fertil Steril.* 1996;65:835-842.

(22) Carlsen E, Petersen JH, Andersson AM, Skakkebaek NE. Effects of ejaculatory frequency and season on variations in semen quality. *Fertil Steril.* 2004;82:358-366.

(23) Levitas E, Lunenfeld E, Weiss N, Friger M, Har-Vardi I, Koifman A et al. Relationship between the duration of sexual abstinence and semen quality: analysis of 9,489 semen samples. *Fertil Steril.* 2005;83:1680-1686.

(24) 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.