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Semen parameters of a semen donor before and after infection with human immunodeficiency virus type 1: Case report

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Abstract

Semen samples from a donor who seroconverted for human immunodeficiency virus type 1 (HIV-1) during the period that he was donating at our clinic were stored before and after infection. Semen analysis was done on all of these samples before cryopreservation. Retrospectively, both qualitative and quantitative HIV-1 testing was performed on the cryopreserved semen samples to determine the time of primary HIV-1 infection. After HIV-1 infection, semen volume, sperm motility and the percentage of spermatozoa with normal morphology were reduced compared with the same parameters before HIV-1 infection. HIV-1 RNA was intermittently detectable in semen. HIV-1 infection led to a reduction in semen volume, sperm motility and normal sperm morphology in this donor. However, the clinical significance of these findings is unclear. A longitudinal cohort study on the effects of HIV-1 infection on semen quality is necessary to confirm these findings.

Introduction

During the last decade, the effect of a human immunodeficiency virus type 1 (HIV-1) infection on semen quality has been evaluated in several studies [1-5]. Most data suggest that semen quality is not altered in early HIV-1 infection [1,3,4]. A drawback of these studies, however, is that all men were already HIV-1 infected when the first semen analysis was performed. Therefore, no comparison can be made between semen parameters before and after infection with HIV-1.

Here we report a unique case of one man who participated in a semen donation programme and became infected with HIV-1, thereby allowing for, what we believe to be, the first time the comparison of semen parameters before and after infection in the same individual.

Case Report

Our patient was a Caucasian man, participating in a semen donation programme of the semen bank of the Center for Reproductive Medicine of the Academic Medical Center from 1994 until 1998. He was 44 years old when he entered the programme in 1994. His history revealed a hepatitis A and a gonorrhoea infection in 1981 and a hepatitis B infection in 1982. He denied having homosexual contacts at that time. After an interval of 2 years, he started donating semen again in September 2000, because of requests for second children from this donor by successfully treated couples. At this time, he was found to be HIV negative, but urine ligase chain reaction (LCR) revealed a chlamydia infection that was treated with azithromycin. Subsequently, chlamydial DNA was undetectable in November 2000.

In total, 12 semen samples were collected between September 2000 and June 2001. According to protocol, the donor was screened approximately every 6 months for HIV, hepatitis B/C and chlamydia. Semen was cryopreserved and quarantined, which implies that it is not used for any fertility treatment until a negative test, at least 6 months after the donation, is available. In June 2001, the HIV-1 test was positive. For this reason, semen donation was stopped. For research purposes, he was asked to donate semen again in January 2003.

The donor had acquired the HIV-1 infection through unsafe homosexual contact. The HIV-1 infection was classified as category A1, according to the Centers for Disease Control and Prevention (CDC) classification system of 1993 [6]. Therefore, he did not receive any antiretroviral therapy and was still therapy naive at the end of July 2004. Blood HIV-1 viral load increased

from 2192 copies/ml in July 2001 to 97 057 copies/ml in November 2003. CD4 counts were stable over this period, between 540 and 940 cells/mm^3. His blood measurements showed antibodies against hepatitis A, hepatitis B, cytomegalovirus (CMV), Epstein–Barr virus (EBV) and toxoplasmosis, with no signs of recent infections. Physical and genital examination revealed no abnormalities.

Table I shows the results of the retrospective HIV-1 testing of the 12 cryopreserved semen samples, which were collected between the last negative HIV-1 test in September 2000 and the first positive HIV-1 test in June 2001.

Qualitative HIV-1 RNA screening was done by nested PCR on the cryopreserved semen. The lower detection limit of this test was determined by spiking 2 x 10^6 spermatozoa from the September 2000 sample with 1 – 100 000 HIV particles using HIV reference virus stock [7]. After isolation and amplification, 10 HIV-1 RNA molecules could be detected in the background of 400 000 spermatozoa. The whole isolation and amplification process was repeated once, with the same results. All samples were tested in duplicate.

The qualitative HIV-1 test was positive for the first time in the semen sample of November 9, 2000. The test remained positive until January 2001, but from February 2001 until April 2001, HIV-1 RNA could not be detected. In May 2001, one of the duplicate tests became positive and both tests were positive again in June 2001.

Table I. Qualitative HIV-1 RNA detection by nested PCR and quantitative HIV-1 RNA detection on the cryopreserved semen between the last negative and first positive HIV test

	2000						2001					
	28-9	2-10	26-10	9-11	23-11	21-12	18-1	22-2	22-3	19-4	17-5	27-6
Flu like symptoms				+	+	+						
HIV serology	-	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	+
Spermatozoa (x 10^5) per straw	195	100	83	110	223	170	163	163	175	170	190	113
NSCs (x 10^5) per straw	4	8	12	6	9	10	7	3	4	6	9	4
HIV testing	-	-	-	+	+	+	+	-	-	-	+	+
HIV-1 RNA copies per 10^5 spermatozoa	<5	<5	<5	5	315	5	<5	<5	<5	<5	<5	<5

ND = not done

A quantitative real-time HIV-1 RNA assay, developed on an ABI Prism® 7000, was then used to determine the amount of HIV-1 RNA. The lower detection limit of this assay was five copies of HIV-1 RNA molecules per 100 000 spermatozoa. At most time points, undetectable viral loads were observed. Very low viral loads were observed from November until December 2000. During the same period, the donor reported flu-like symptoms. In view of the data summarized in Table I, we conclude that the primary HIV-1 infection occurred at the beginning of November 2000.

In Table II, semen variables before and after HIV-1 infection are presented.

According to our protocol for semen donors, all semen samples were produced after 2–5 days of sexual abstinence. After HIV-1 infection, semen volume, sperm motility and the percentage of spermatozoa with normal morphology were reduced compared with before HIV-1 infection. Although spermatozoa concentration increased after HIV-1 infection, semen total count (TC) and total motile count (TMC) did not change.

Discussion

In the donor described in this case report, semen volume, sperm motility and the percentage of spermatozoa with normal morphology were reduced after acquisition of HIV-1. Since no statistical test exists for repeated measurements in one person, these findings cannot be tested statistically. Whether the described changes truly reflect an effect of HIV-1 on

Table II. Semen variables before and after HIV-1 infection

Mean semen variables	Before HIV-1 infection June 1994-October 2000 (*n*=64)		After HIV-1 infection November 2000-November 2003 (*n*=13)	
Volume (ml)	3,5	(0,12)	2,5	(0,13)
Concentration (x 10^6/ml)	74	(3,5)	110	(11,1)
% Motility	49	(1,1)	41	(2,3)
% Normal morphology	55	(1,5)	46	(2,6)
TC (x 10^6)	263	(15,0)	264	(24,8)
TMC (x 10^6)	131	(8,1)	105	(9,4)
NSCs (x 10^6/ml)	3	(0,3)	4	(0,6)

Results are expressed as mean (SE).
TC = total count; TMC = total motile count; NSCs = non-sperm cells.

testicular function remains unclear, since only one person was involved. Semen parameters are known to be variable on repeated sampling in one man, and the effects of HIV-infection on testicular function might differ between individuals.

Nevertheless, the decrease in semen volume could be indicative of dysfunction of the accessory glands; the prostate and seminal vesicles. Dysfunction of the accessory glands could also be an explanation for the more viscous sperm that is often found in HIV-1 infected subjects [2,3]. A reduction in sperm motility in HIV-1-infected men has been described before [2,5], although an explanation for this finding is lacking. A decrease in the percentage of spermatozoa with normal morphology has been reported in one other study [8], while others reported normal values for sperm morphology [1,4,5]. In this donor, the semen TC and TMC remained stable. The observed increase in semen concentration appeared to be caused by a decrease in semen volume in this donor.

Consistent with most other studies on semen quality of asymptomatic HIV-1-infected men, nearly all semen parameters matched the World Health Organization criteria for normal human semen at all time points in this donor [1-3,9].

On PCR testing, the amount of virus was expressed per 10^5 spermatozoa. The spermatozoa were counted and a constant input of 10^5 spermatozoa was used for PCR testing. Each straw also contained a low variable number of non-sperm cells (NSCs). The NSCs were calculated using the original concentration of NSCs and a known dilution factor (Table I). The small number of NSCs in the sample most probably had no influence on the sensitivity of the PCR test since the spermatozoa were always by far the majority of cells in the test.

HIV-1 is most probably present as cell-free virus in seminal plasma and as cell-associated virus in seminal NSCs, such as lymphocytes, monocytes and macrophages [10]. It seems unlikely that spermatozoa themselves can become infected with HIV-1, since there are no HIV-1 CD4 receptors or co-receptors present on spermatozoa and vasectomy does not influence the amount of cell-free virus in semen [11,12].

In retrospect, HIV-1 was detectable in semen at the same time the patient suffered from flu-like symptoms, indicative of a primary HIV-1 infection. This is consistent with two other case reports where HIV-1 RNA was present in semen during primary HIV-1 infection [13,14].

On qualitative screening, HIV-1 RNA was intermittently detectable in the semen samples. This pattern of intermittent shedding is often found in HIV-1-positive men [15-17]. It is hypothesized that a fluctuation of viral transcription from infected cells in the prostate is the cause of this intermittent shedding of HIV-1 in semen [15]. Also local factors such as inflammation of the male genital tract can increase the amount of HIV-1 RNA in semen, probably by an increase of lymphocytes and macrophages [18].

Quantitative testing of HIV-1 RNA revealed a very low copy number of HIV-1 RNA during primary infection and an undetectable viral load in semen at all other time points. Because the qualitative test is more sensitive than the quantitative test, it was not always possible to quantify HIV-1 RNA precisely, despite a positive qualitative test. In these samples the quantity is presumably < 5 copies per 100 000 spermatozoa. The highest amount of HIV-1 RNA in semen was detected during primary HIV-1 infection, when blood viral load was probably very high. This observation supports our recently published hypothesis that HIV-1 RNA in semen could be spill-over from blood [19].

To our knowledge, we are the first to describe semen parameters before and after acquiring HIV-1 in the same individual. In all other studies published, men were already HIV-1 infected by the time the first semen analysis was performed. However, our results show that HIV-1 infection may lead to a reduction in semen volume, sperm motility and the percentage of spermatozoa with normal morphology. The clinical significance of these findings is not clear. Larger longitudinal studies on the effects of HIV-1 on semen quality will have to be carried out to confirm our findings.

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