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CYTOMEGALOVIRUS AND HUMAN IMMUNODEFICIENCY VIRUS IN SEMEN TESTED DURING THE COVID19 EMERGENCY ERA

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ABSTRACT

Men seeking surrogacy in the United States must undergo Food and Drug Administration (FDA)-mandated infectious disease screening within seven days of the sperm collected for fertility treatments. Travel bans imposed during the COVID19 pandemic (April 1, 2020 to March 7, 2022) did not deter 156 men from 12 countries from pursuing fatherhood by traveling to facilities to cryopreserve and screen semen specimens for virus, along with paired FDA serology and urine tests. Heterosexual couples not needing FDA serology testing collected specimens remotely and shipped them overnight for cryopreservation and testing. A total of 464 semen specimens were evaluated for viable sperm, 453 of which were RT-PCR tested for HIV and cytomegalovirus (CMV). More specimens tested positive for CMV (136, 30%) than for HIV (52, 11%). Fifty two men (33%) had at least one CMV-positive specimen, 38 men (24%) had at least one HIV-positive specimen, and 16 men (11%) had at least one specimen test positive for both HIV and CMV. Results were similar for the 32 international clients as for domestic clients and for gay clients as for heterosexual clients. No correlations between virus-positivity and CD4+ blood cell counts, leukocytospermia, anti-HIV medications, nor age were noted. During the same time frame, 43 babies were born to gestational carriers and wives, 82 gestational carriers and wives underwent embryo transfer without consequent infection, and 32 pregnancies from nine fertility clinics were reported. Virus shedding into semen is in urgent need of better understanding to avoid the risks associated with new infections early in pregnancy.

Introduction

Given the major role of male reproductive tract tissues to protect sperm from immune response, the surprising presence of HIV and HIV-infected immune cells in semen specimens has been studied since the beginning of the HIV/AIDS pandemic[1], without success in discovering all the tissue(s) of origin[2,3]. HIV RNA is detectable in cell-free seminal plasma and infected cell provirus DNA is detectable in semen cell pellets, but rarely are both detected in the same specimen, and genetic analyses reveal the infected cells may not be the source of the seminal plasma virus[4-7]. These findings indicate the presence of multiple male reproductive tract reservoirs of HIV infection. The lag in virus clearing in semen relative to the blood of men initiating anti-viral therapy suggests semen producing tissues are important reservoirs of long term HIV infection[8,9]. The wide-spread adoption of multi-drug treatments for HIV infection, termed highly active anti-retroviral therapy (HAART), dramatically reduced deaths from Acquired Immunosuppressive Disease (AIDS), but has not stopped new infections in the United States, approximately 41,000 in 2000[10] and approximately 31,200 two decades later[11]. Therefore, the numbers of men living with HIV increases annually in the U.S. and around the globe[12].

U. S. Food and Drug Administration (FDA) guidelines for sperm and egg donation from known donors are the same as for other organ/tissue donations, and include blood serology for infectious diseases: Hepatitis viruses, Human T Cell Leukemia Virus, Cytomegalovirus (CMV), Human Immunodeficiency Virus (HIV), West Nile Virus, Treponema pallidum, and urine tests for gonorrhea and chlamydia, all of which must be obtained within seven days of the semen specimens cryo-preserved for fertility treatments. In the case of anonymous sperm donors in the U.S., the sperm are cryopreserved without direct testing of the semen specimens and the donor 's blood and urine are re-tested for the infectious disease panel six months later. If no new infection history is evident, the sperm specimens can be released for fertility procedures.

Men testing positive for sexually transmitted diseases are not eligible to be anonymous sperm donors. They are eligible, however, to be known sperm donors if the sperm or embryo recipient signs a consent form acknowledging that she is aware of the infectious disease status of the sperm donor. This also applies to gestational carriers undergoing embryo transfer with an egg fertilized by sperm from an HIV-infected man. Because financially compensated surrogacy is legal in most states in the U.S., and the FDA guidelines clarify acceptable precautions against transmitting infection to the gestational carrier, an increasing number of U.S. fertility clinics assist HIV-infected couples achieve pregnancy if the sperm are from tested semen specimens with an undetectable burden of HIV. Hence, HIV-infected homosexual men around the world are seeking surrogacy in the U.S. for their family building goals, including during COVID19-era travel and vaccination restrictions.

CMV is a member of the Herpesviridae family, also referred to as Herpes virus 5, and has the biological properties of latency and reactivation. CMV infection is wide-spread throughout the global population, infecting up to 70% of people in industrialized countries and 100% in emerging countries[13]. Initial CMV infection is detectable by IgM class antibody, which converts to IgG over time; however, reactivation of infection or a second primary infection is not always accompanied by the re-appearance of IgM[14]. Primary infection of the mother during the first trimester of pregnancy carries a 24% to 75% risk of transmission to the fetus and a non-primary infection carries around 1% to 2.2% risk of transmission to the fetus. The outcome of fetal infection may be serious, it is the leading cause of sensorineural loss and retardation in children[15,16]. Detection of CMV in seminal fluid has been demonstrated in several studies, although epidemiological information concerning the prevalence of CMV in semen and its role in transmission is limited[17,18].

A semen-screening program to assist HIV-infected men safely achieve a pregnancy in a female partner or in a gestational carrier (GC) was established in 1997[3]. The HIV-infected men and female partners or GCs are counseled that approximately 15% of semen specimens from men on successful anti-viral therapy with an undetectable burden of virus in blood test RT-PCR positive for HIV. Since a large portion of the semen specimen is used for the RT-PCR analyses, the men produce two to three specimens for testing to ensure sufficient sperm for the pregnancy attempt. To satisfy the FDA guidelines for specific blood and urine tests to be matched with the semen specimens, most HIV-infected men seeking surrogacy travelled to the lab in person to produce at least three semen specimens paired within seven days of the blood/urine tests that included CMV IgG/IgM. Travel and vaccination restrictions during the COVID19 pandemic era, April 1, 2020 through March 1, 2022, were expected to suspend family building plans until the restrictions were lifted, but 156 men (approximately half the usual number for this time period) were not deterred, necessitating some modifications to the standard lab testing protocols, including adding a SARS2 test to the FDA-required tissue donor panel, and the establishment of two semen collection centers in Europe: one in LaCoruna, Spain, and one in Munich, Germany.

A prior study of 150 semen specimens from 62 HIV-infected men[18] revealed no concordance between the presence of HIV and CMV in semen, and the presence of semen virus did not correlate with the concentration of semen leukocytes, the age of the men, or their anti-HIV medications. Fewer than 700 $CD4^+$ leukocytes/ul of peripheral blood did correlate with CMV, but not HIV, in semen specimens. Similar correlations were found in this larger study of 156 sperm donors, but the correlations between lower $CD4^+$ cells in blood and CMV in semen were not fully supported.

Methods and Materials

Men using a GC for pregnancy travelled to the lab in person to ensure compliance with FDA infectious disease blood and urine testing paired within seven days of the sperm. Men with female partners for the pregnancy submitted specimens via overnight shipping with a portion of the specimen set aside for RT-PCR testing and the remainder added to sperm transport medium (Irving Scientific). During the COVID19 restrictions, two Andrology labs in Europe, one in Spain and one in Germany, established programs of semen collection and blood/urine collection, with aliquots of the semen specimen couriered to the Bedford lab for RT-PCR testing; sperm from the remainder of the specimen were cryo-preserved in the collection laboratory pending the outcome of the PCR testing.

The portion of the semen specimen for RT-PCR testing was combined with a denaturing agent, guanidine isothiocyanate crystals, at a concentration (3.0 to 3.5 mM) designed to lyse virus and non-sperm cells (NSCs) while preserving sperm heads[4]. Following centrifugation to remove unlysed sperm heads, the lysate was run through Qiagen Purification columns and isolated nucleic acids (NA) are eluted in embryo-tested water (Sigma Aldrich) according to manufacturer's instructions.

The isoated nucleic acids were tested for HIV RNA and infected cell proviral DNA by methods standard in this laboratory[18] and by multiplex PCR for the presence of CMV and an internal control gene, HapB, using a sensitive assay.[19] The genes targeted for amplification are the highly conserved *gag* protein and the highly conserved CMV immediate early transcriptional regulator (UL123). Briefly, the RT step for HIV RNA detection was 20 µL, consisting of 10ul semen nucleic acids, 0.5 µg of Random Primers, 40U of RNase Inhibitor Murine, 5 mM MgCl2, 8U of BST 3.0 DNA Polymerase (New England BioLabs), and PCR Buffer with a final concentration of 20 mM TRIS pH 8.3, 25°C, 20 mM KCl, 0.1 mg/mL Bovine Serum Albumin, 0.05% NP-40, 3.5% ethylene glycol, and 20 µM each of dATP, dCTP, dGTP, and dTTP (Sigma), incubated at 72oC for 5 minutes, 4°C for 30 sec, 42°C for 60 min, 95°C for 3 min, 4°C for 5

min. RT DNA products, and HIV provirus in infected cells, were amplified in a bracket reaction by addition of 80 ul of a master mix to the 20 ul RT reaction to yield final concentrations of 10 picomoles each of H341F (5'CCTGCTATGTCACTTCCCCT) and H341R (5' CCTTGTCTTATGTCCAGAATGC 3') in the same buffer as the RT step with final concentration of 1.8 mM $MgCl_2$ and 2.5 U Taq DNA Polymerase (New England Biolabs) with cycling strategy of 95°C for 4 minutes, 20 cycles of 95°C, 55°C and 72°C for 30 sec each, followed by final cycle of 72°C for 7 min and 4°C for at least 5 min. Two ul of the bracket reaction were added to 40 ul of a nested reaction containing the same PCR reaction buffer plus 20 pmoles each of H143F (5'AGTGGGGGGACATCAAGCAGC-CATGCAAAT) and H138R (5' CCTGCTATGTCACTTCCCCT) and the same cycling strategy as the bracket amplification. Two ul of the nested PCR were added to an internal nest assay containing the same PCR reaction buffer plus 50 picomoles each H105F (5' GAGACTATCAATGAGGAAGC 3') and H105R (5'TGCTATGTCAGTTCCCCTTGGT) plus 100 pmoles of probe (5'-/56-FAM/TGGGATAGA-/ZEN/GTGCATCCAGTGCAT/3IABkFQ), IDT, for amplification in a BioRad CFX96 using the same cycling strategy. Threshold detection of 200 to 1000 copies of HIV RNA was obtained by serial dilution of HIV controls (Accumetrix).

Semen CMV was detected by multiplex bracket round of PCR utilizing CMV primers CMVQBr-1 (5' CCGTGTACGAGGTCCGTGTGG 3') and CMVQBr-2 (5' CAAGTCGACGCGCGACCCTGCTG 3') as well as HapB primers, HapBBr- F (5' GAGCTGCAGTGGCCCGTTCCAG 3') and HapBBr-R (5' GGTATGTGAA-GGTCAGGAACTTG 3'); the nested round of PCR employed HAPBF (5' TGAAGGTGGAGGACATTCCTCTA) and HAPBR (5' CTGGAATTGCGATTTCTGGTAA) plus CMVQF (5' GCTGACGCGTTTGGTCATC) and CMVQR (5' ACGATTCACGGAGCACCAG) and corresponding fluorescent probes (CPOL-FAM, 5'TCGGCGGATCACCACGTTCG; HAPB, 5'CGAGAATCACCCTGCCAG-ACTTCCGT) for each DNA amplicon. PCR products for HIV, CMV and HapB were detected in a BioRad real time qPCR CFX96. Upper limit of positive detection for all probes was 39 cycles. Cryopreserved sperm from specimens testing RT-PCR positive for HIV, or repeatedly negative for the internal control, HapB, were discarded. Threshold detection of 100 to 1000 copies of CMV DNA was obtained by serial dilution of positive controls (Accumetrix).

Consults for U.S. clients were conducted by Zoom early in the pandemic, but in person once travel restrictions were relaxed in June, 2020. Consults for clients using European sperm collection laboratories were conducted by Zoom. GC counseling was unchanged, described the FDA requirements, and the requirements for HIV-antibody testing following the embryo transfer whether or not pregnancy was achieved.

This chart review study was approved by the Human Subjects Review Board of the Bedford Research Foundation. Men signed a consent form allowing results of their tests to be included anonymously in research studies. Chart data were compiled into a Filemaker database and exported into PRISM or Numbers for statistical analyses and graphical representation. Student's T-test and ANOVA analyses were used to assess statistical significance; p values less than 0.02 were taken as the cut-off for significant differences.

Results

Demographics. One hundred fifty-six men, 154 HIV-infected, sought semen testing for fertility treatment during the 23 months of SARS2/COVID19 pandemic travel restriction, April 1, 2020 to March 1, 2022, approximately half the usual number of men (Table 1). Twenty six of the HIV-infected men, average age of 43, were seeking fertility treatment with their female partner, the remainder were homosexual men, average age 44, seeking parenthood via surrogacy. All but one HIV-infected man was on HAART with

undetectable HIV RNA in blood. The men were from 12 countries including the U.S.: Germany, Spain, Italy, France, Israel, China, Argentina, The Netherlands, Japan, Taiwan and England. Twenty four men visited the laboratory in Spain and five visited the laboratory in Munich, designated "s" and "m" in Table 1.

HIV Testing. Semen specimens from three men were not tested due to azoospermia (Table 1). Thirty four (27%) of the remaining 127 HIV-infected gay men produced at least one HIV+ semen specimen. All three of the semen specimens from one homosexual man not on anti-viral therapy, HIV-positive for 15 years, 576 CD4+ cells/ul of blood, tested positive for HIV. Four (15%) of the 24 men with female partners had at least one HIV+ semen specimen. Overall, 50 (11%) of the 453 semen specimens tested positive for HIV, six (11%) of the 53 specimens from men with female partners and 44 (11%) of the 402 specimens from homosexual men. Eight men (2 (8%) with female partners, 6 (5%) using surrogacy) had more than one HIV-positive specimen. Testing results were comparable between domestic and international men.

CMV testing. Paired blood samples of the 129 homosexual men tested positive for CMV-IgG, none tested positive for CMV IgM. Forty-two (32%) of the homosexual men produced at least one semen specimen positive for CMV DNA and 112 (28%) of the homosexual men's semen specimens were CMV-DNA positive. CMV-antibody results were not available for the twenty six men with female partners, ten (38%) of whom produced at least one CMV-positive semen specimen with 19 (34%) of the 56 semen specimens in this group testing CMV-positive. Neither of the two HIV-negative homosexual men had detectable CMV DNA in their semen specimens, and all three of the semen specimens from the HIV-infected man not on HAART tested positive for CMV-DNA. In contrast to the singlet pattern of HIV-positive specimens, 35 (67%) of 52 men produced uniformly CMV+ specimens (Table 1). Overall, 136 (30%) of the 453 semen specimens tested positive for CMV DNA.

There was little concordance between HIV-positive and CMV-positive semen specimens. Thirteen (11%) of the homosexual men produced at least one specimen positive for both with only 17 (4.6%) of total specimens testing positive for both HIV and CMV. Three (12%) of the 24 men with female partners produced at least one specimen positive for both viruses with only 4 (7.5%) of total specimens from this group testing positive for both HIV and CMV. Results were comparable for specimens from international and domestic men.

CD4+ counts. One hundred and thirty eight of the HIV-infected men had recent blood tests for CD4+ lymphocytes/ul of blood, one of the clinical measures of treatment efficacy (Table 1). The range of reported CD4+ lymphocytes/ul of blood was an order of magnitude, 130 to 1713 CD4+ cells/ul, average was 744. All the samples from 78 men tested undetectable for both HIV and CMV; their CD4+ cells/ul blood averaged 757, range from 130 to 1554, not statistically significantly different ($p > 0.1$) from the cohort as a whole. The 38 men with at least one specimen testing positive for HIV had average CD4+ cells/ul blood of 742, range from 378 to 1713, not statistically significantly different from the men with all negative specimens. The 52 men producing specimens with at least one CMV positive had average CD4+cells/ul blood of 767, range from 331 to 1713, not statistically significantly different ($p>0.2$) from the men with all CMV-negative specimens. The average CD4+ count for the 16 men producing specimens containing both viruses was 792, range 378 to 1713, not statistically significantly different ($p>0.2$) from the virus negative semen specimen group.

Table 1. Characteristics of Semen Specimens Analyzed

Patient ID	Age	MSM / MSW	Years on HAART	CD4+ cells/ ul	total semen speci	Specimen #1				Specimen #2 mens				Specimen #3				Specimen #4				Specimen #5			
						SPM	NSC	HIV	CMV	SPM	NSC	HIV	CMV	SPM	NSC	HIV	CMV	SPM	NSC	HIV	CMV	SPM	NSC	HIV	CMV
1s	64	M	28	800	3	44.0	11.0		+	43.0	13.0		+	39	6.7										
2	62	M	19	1141	3	34.0	1.1		+	46.0	2.5		+	61	2.7		+								
3	61	M	15	1092	3	91.0	14.5			58.0	3.4			15	1.2										
4	59	M	20	701	5	23.0	2.2	+	+	54.0	1.7			3	1.3			10.0	0.9			19.0	2.1	+	
5s	58	M	26		4	42.0	5.6		+	14.0	2.8		+	28	2.2		+	15.0	2.8		+				
6s	58	M	28	788	3	97.0	9.9		+	185.0	23.0		+	96	8.8										
7	58	M	16	786	3	71.0	3.0			83.0	2.3			43	1.1										
8	58	M	18	1167	3	68.0	1.8			42.0	1.9			16	0.6										
9	57	M	4	506	1	0.3	0.4	NT	NT																
10s	57	M	28	130	3	42.0	2.0			27.0	0.7			12	0.5										
11	57	W	24	490	1	3.2	0.5																		
12	56	M	0	576	3	78.0	9.0	+	+	110.0	4.9	+	+	47	3.6	+	+								
13	56	W	10	400	1	0.0	0.5	NT	NT																
14s	56	M	29	800	3	72.0	9.5		+	39.0	3.7		+	11	0.8		+								
15	54	M	4	331	3	117.0	1.7		+	60.0	0.9		+	74	1.1		+								
16s	53	M	19	687	3	54.0	4.3			38.0	4.0			13	7.1										
17	53	W	5	598	3	35.0	0.3			75.0	1.1			63	1.3										
18	52	M	8	813	3	160.0	6.6			61.0	0.3			18	0.1										
19	52	M	5	1314	3	20.0	1.7			5.0	0.7			22	1.2										
20m	52	M	21	700	3	0.8	0.0			4.9	0.3			3.2	0.1										
21	52	M			3	33.0	2.3			83.0	3.4			24	2.1										
22	51	M	12	472	3	98.0	3.1			39.0	2.3			66	1.9										
23	51	M	13	686	3	103.0	1.5	+		25.0	0.3			50	4.2										
24	51	M	26	455	3	6.0	1.6			4.0	6.0			15	2.9	+									
25s	51	M	10	2268	3	87.0	6.3			19.0	2.3			3	0.1	+									
26s*	50	M	12	850	6	79.0	19.0			26.0	2.2			72	8.3			61.0	2.6			100.0	9.0		

Patient ID	Age	MSM / MSW	Years on HAART	CD4+ cells/ ul	total semen speci	Specimen #1				Specimen #2 mens				Specimen #3				Specimen #4				Specimen #5			
						SPM	NSC	HIV	CMV	SPM	NSC	HIV	CMV	SPM	NSC	HIV	CMV	SPM	NSC	HIV	CMV	SPM	NSC	HIV	CMV
27	50	M	9	471	3	26.0	7.2			30.0	6.6			54	4.8	+									
28	50	W	11	674	1	0.3		NT	NT																
29	50	W	19		2	131.0	2.3			45.0	0.3														
30	50	M	19	445	3	17.0	0.2			24.0	0.7	+		19	0.3										
31m*	50	M	13	688	6		0.9					+				+		21.0	5.4		+	24.0	7.6		
32	50	W	5	929	4	37.0	4.8	+		21.0	1.5			11	8.6	+		11.0	8.0						
33	50	W	5	378	3	56.0	2.1	NT	NT	62.0	1.1	+	+	68	0.9		+								
34	49	M	21	452	3	86.0	2.0			72.0	1.4			77	0.4										
35	49	W	3		2	18.0				82.0	1.4														
36	49	M	12	460	3	28.0	3.2		+	15.0	1.2		+	35	2.9		+								
37s	48	M		550	3	12.0	1.4		+	6.6	2.6		+	2.7	0.5	+	+								
38	48	M		1350	3	50.0	4.4			62.0	2.9			24	1.5										
39	48	M	4	511	3	58.0	1.4			42.0	0.6			59	1.9										
40	48	M	17	436	3	42.0	0.5			38.0	0.4			22	0.0	+									
41	48	M	10	947	3	48.0	1.1			52.0	0.8			39	0.6	+									
42	47	M	13	620	3	22.0	0.4			36.0	0.7	+		19	0.4										
43	47	M	12	427	3	14.0	10.3		+	24.0	16.5		+	28	15.9		+								
44	46	M	12	479	3	42.0	2.3			37.0	1.9			34	1.5										
45	46	M		493	2	2.0	0.4			3.4	0.1	NT	NT												
46	46	M	9	650	3	98.0	2.6	+	+	158.0	0.6	+	+	139	0.3		+								
47	46	M	6	756	3	135.0	2.1			160.0	1.0			109	2.3	+									
48	46	M	12	1490	3	70.0	1.5			32.0	1.3			32	1.0										
49	46	M			2	74.0	1.1			64.0	1.1														
50s	45	M	13	631	3	70.0	4.9			45.0	2.5					+									
51	45	M	10	1038	3	36.0	1.2		+	9.0	0.1		+	24	1.0		+								

Patient ID	Age	MSM / MSW	Years on HAART	CD4+ cells/ ul	total semen specimens	Specimen #1				Specimen #2				Specimen #3				Specimen #4				Specimen #5			
						SPM	NSC	HIV	CMV	SPM	NSC	HIV	CMV	SPM	NSC	HIV	CMV	SPM	NSC	HIV	CMV	SPM	NSC	HIV	CMV
52	45	M	13	957	3	74.0	2.2			52.0	3.7			51	1.5										
53*	45	M	6	760	6	34.0	1.8	+		29.0	1.1	+		35	1.0	+		24.0	6.0	+		27.0	5.5	+	
54	45	W	7	500	1	29.0	0.8		+																
55	44	M	8		3	19.0	3.7			15.0	1.3			19	3.7										
56	44	W	13	544	1	44.0	1.5																		
57m	44	M	9	672	3	9.3	0.1	+	+	56.0	2.3			36	1.2										
58	44	M	12	1336	3	13.0	0.5	+		26.0	1.0			27	0.6										
59s	44	M	12	717	3	14.0	2.0			45.0	1.5			61	1.9										
60	44	M	7	748	3	36.0	1.5		+	99.0	2.5		+	17	0.5		+								
61	44	M	20	1000	3	19.0	1.1			40.0	0.7			44	1.1										
62	44	M	13	1190	3	8.8	0.8		+	0.9	0.1		+	20	1.0		+								
63s	44	M	11	937	3	45.0	0.5			3.0	0.0			71	0.0										
64	44	M	18	626	3	13.0	1.6			36.0	2.6		+	56	4.8	+	+								
65	44	M			4	23.0	1.1			42.0	1.9			38	1.8			23.0	1.3						
66	43	M	5	900	3	74.0	2.2			16.0	1.5			54	0.8										
67	43	M	12	522	3	88.0	1.6			38.0	2.2			60	2.5										
68s	43	M	5		3	78.0	4.0		+	25.0	1.0		+	41	1.6		+								
69*	43	M	5	841	5	21.0	2.8			16.0	4.5			23	2.9			3.0	0.5			4.0	2.7		
70	43	M	7	694	4	23.0	0.5	NT	NT	67.0	2.1			57	1.6			48.0	1.2	+					
71s	43	M	5	1713	3	13.0	0.6		+	4.0	0.4		+	6.5	0.3	+	+								
72	43	M		647	3	56.0	1.9			101.0	2.9			29	1.1										
73	43	M	10	505	3	164.0	2.6			94.0	1.3			56	1.4										
74	43	M	7	653	3	8.0	0.9		+	14.0	0.6		+	8	0.3		+								
75	43	M	19	707	3	140.0	1.3		+	61.0	1.4		+	105	1.9		+								
76m	43	M	9	707	3	5.0	2.0	+		40.0	2.0			44	6.7										
77	43	M	12	1100	3	2.0	0.2	+	+	81.0	0.7		+	96	0.0		+								
78HK	42	W	3	390	2	45.0	3.0		+	29.0	1.6		+	25	0.4										
79	42	W	23	542	3	19.0	6.4	NT	NT	23.0	3.4		+	32	8.0										
80	42	M	5		3	56.0	0.9		+	88.0	1.0		+	64	1.1		+								

Patient ID	Age	MSM / MSW	Years on HAART	CD4+ cells/ ul	total semen specimens	Specimen #1				Specimen #2				Specimen #3				Specimen #4				Specimen #5			
						SPM	NSC	HIV	CMV	SPM	NSC	HIV	CMV	SPM	NSC	HIV	CMV	SPM	NSC	HIV	CMV	SPM	NSC	HIV	CMV
81	42	W	1	885	2	20.0	0.0			15.0	0.8		+												
82	42	M	13	480	4	160.0	2.1	+		152.0	1.8			72	0.6			88.0	0.3						
83	42	M	4	436	3	65.0	0.8			93.0	0.8			68	0.9										
84s	42	M	8	1109	3	5.0	0.0			184.0	66.0			113	33.0										
85	42	M	18	672	3	25.0	0.3		+	32.0	0.4		+	54	0.5		+								
86s	41	M	5	890	3	21.0	0.8			15.0	0.7			11	0.4	+									
87	41	M	9	688	3	54.0	1.3	+	+	52.0	0.7	+	+	35	0.5	+									
88	41	M	12	1554	3	70.0	12.0			97.0	4.7			73	2.9										
89	41	M	9		3	24.0	1.9			27.0	0.9		+	26	0.5										
90	41	M	13	550	3	24.0	2.5			36.0	0.5			57	1.2										
91	40	M	12	843	3	91.0	1.5			73.0	1.2			41	1.7										
92	40	M	12	961	3	102.0	1.4			23.0	1.4			60	3.5										
93	40	M	13	1250	2	33.0	2.4			21.0	0.3														
94	40	M	8	953	3	0.4	0.3		+	1.5	0.4		+	4.7	0.7		+								
95	40	M	6	925	3	28.0	3.5		+	30.0	2.6		+	23	2.2		+								
96	40	M	8	770	3	33.0	1.2			23.0	1.1			23	1.2										
97	40	W	2	173	1	134.0	0.3																		
98	40	W	1	585	2	21.0	0.6			7.7	0.3	NT	NT												
99s	39	M	8	800	3	73.0	8.7		+	132.0	9.9		+	165	10.3		+								
100	39	M	6	290	3	3.7	0.3			1.3	0.1			1.1	0.0										
101*	39	M	12	1000	5	1.0	0.1			2.0	0.9	+	+	3	0.9		+	2.0	1.0		+	4.8	0.2	NT	NT
102	39	M	10	591	3	80.0	5.0			69.0	8.5			50	4.4	+									
103	39	M	6	692	3	11.0	0.6			38.0	2.0			20	0.6	+									
104	39	M	10	898	3	36.0	0.9			39.0	0.4			31	0.3										
105	38	M	11	650	3	41.0	2.2			16.0	1.0			21	1.4										
106	38	M	9	1400	3	104.0	1.0			67.0	0.2			83	1.1										
107	38	M	15	1014	3	30.0	2.2		+	20.0	2.1		+	39	3.4	+	+								
108	38	W	6		1	5.6	0.4																		
109*	38	W	14	1056	5	11.0	0.0			4.7	0.4			9	0.3			11.0	0.1			3.8	0.3	NT	NT
110	38	M	13	604	3	72.0	3.4			68.0	3.4			62	1.9										

Patient ID	Age	MSM / MSW	Years on HAART	CD4+ cells/ul	total semen specimens	Specimen #1				Specimen #2				Specimen #3				Specimen #4				Specimen #5			
						SPM	NSC	HIV	CMV	SPM	NSC	HIV	CMV	SPM	NSC	HIV	CMV	SPM	NSC	HIV	CMV	SPM	NSC	HIV	CMV
111s	54	M	15	486	3	40.0	3.8			14.0	2.4			38	58.0										
112	38	M	11	823	3	40.0	0.2	+		43.0	0.5			54	0.3										
113s	38	M	11	1237	3	62.0	4.3			31.0	2.7			81	7.9										
114	38	M	11	600	3	27.0	1.0			62.0	1.1			39	1.2										
115	38	M	14	1098	3	30.0	2.5			20.0	1.5			39	2.8										
116	37	M	10	1311	3	46.0	2.1			35.0	0.9			17	0.9										
117	37	W	13	656	2	30.0	2.3		+	31.0	2.6	+	+												
118s	37	M	5	702	3	21.0	2.9			31.0	4.7			23	3.4										
119	37	W	10	643	3	60.0	0.8			45.0	0.8			31	0.8										
120	37	M	7	800	3	63.0	2.1		+	47.0	2.6		+	50	2.3		+								
121	37	M	12	975	3	38.0	1.8		+	22.0	1.7		+	47	2.9		+								
122	37	M	13		3	25.0	0.3		+	21.0	0.0			4.4	0.0										
123	37	M	9	1080	3	20.0	7.7			20.0	3.3			29	1.8										
124s	36	M	13	748	3	34.0	2.3		+	25.0	1.2		+	15	1.1		+								
125*	36	W	9	707	5	50.0	1.6		+	43.0	0.2		+	34	0.4		+	54.0	0.7		+	43.0	0.3		+
126	36	M	8	587	3	14.0	2.5			19.0	3.8			16	2.2										
127m	36	M	4	1152	3	48.0	1.8		+	89.0	4.0		+	30	0.6	+	+								
128	35	W	13	693	3	29.0	1.0	+	+	27.0	0.9	+	+	39	2.2		+								
129	35	M	12	792	3	60.0	0.8		+	22.0	0.9		+	44	1.0		+								
130	35	W	14	798	3	33.0	2.2			25.0	1.8			17	3.7										
131	35	M			2	78.0	1.6			101.0	2.7														
132	35	M	11	533	3	59.0	1.5			65.0	1.0			62	0.8										
133	35	M	9	516	3	5.0	0.1			10.0	0.1			12	0.2										
134	35	M	5	850	3	28.0	0.4		+	37.0	0.2		+	25	1.2		+								
135	35	M			2	14.0	0.9			20.0	1.9														
136	34	M	6	504	4	40.0	2.3		+	44.0	3.0		+	5	2.5				1.1	+	+				
137s	34	M	9	501	3	320.0	25.0			167.0	12.6			144	13.1										
138s	34	M	10		3	15.0	0.9			7.3	0.2			9.4	0.7	+									

Patient ID	Age	MSM / MSW	Years on HAART	CD4+ cells/ ul	total semen speci mens	Specimen #1				Specimen #2				Specimen #3				Specimen #4				Specimen #5			
						SPM	NSC	HIV	CMV	SPM	NSC	HIV	CMV	SPM	NSC	HIV	CMV	SPM	NSC	HIV	CMV	SPM	NSC	HIV	CMV
139	34	W	2	566	2	13.0	0.1			39.0	0.3														
140	33	M	6	646	3	9.1	2.6			10.0	2.5			10	2.4										
141	33	M	9	1319	3	33.0	0.9		+	32.0	1.9		+	34	1.1										
142	39	W	5	559	2	3.9	0.5		+	4.2	1.0		+												
143	40	M	7	712	3	18.0	1.4			12.0	0.7			19	1.4										
144	44	M	1	290	3	14.0	1.6			17.0	1.2			14	1.6										
145	43	M	12	479	3	7.5	8.4			4.4	7.0			6.2	5.3										
146s	49	M	10	575	3	6.6	0.2		+	53.0	3.9		+	20	1.5		+								
147*	58	M	14	786	6	64.0	3.1		+	50.0	2.2		+	47	2.4		+	43.0	1.1			71.0	3.0		
148	50	M	9	440	3	51.0	1.9			48.0	1.8			48	1.7										
149	47	M	11	666	3	27.0	1.6			19.0	0.6			21	0.9										
150	44	M	4	605	3	17.0	1.4			18.0	1.0			18	0.3										
151	44	W	1		1	43.0	1.2																		
152	43	M	12	507	3	103.0	1.4			67.0	0.2			60	1.0										
153	40	M	8	660	3	25.0	1.2			10.0	0.5			18	0.3										
154	38	W			1	102.0	2.8		+																
155	37	M	3	873	3	50.0	0.8			48.0	0.4			37	0.6										
156	34	M	5	1022	3	21.0	1.8			19.0	1.2			15	1.3										

"M" is "men who have sex with men" and "W" is "men who have sex with women"; "s" is samples collected in Spain, "m" samples collected in Munich; * is men who submitted a total of 6 specimens, the sixth having characteristics similar to #5.

Although the average CD4+ cells/ul of blood was not statistically different among the virus positive groups, the distribution of positive semen specimens (Figures 1 and 2) suggests lower CD4+ cells/ul blood is associated with a higher incidence of virus positive semen specimens. Of 191 specimens from men with CD4+ cells/ul of blood less than 700, 28 (15%) tested positive for HIV, 49 (26%) tested positive for CMV and 15 (8%) tested positive for both; of 217 specimens from men with CD4+ cells/ul of blood greater than 700, 20 (9%) tested positive for HIV, 73 (34%) tested positive for CMV and 6 (3%) tested positive for both; the trends were not statistically significant ($p>0.2$).

The 154 HIV-infected men were taking 26 different HAART regimens. Forty five men were taking Biktarvy, the most frequently prescribed medication in this patient cohort, average CD4+ cells/ul blood was 787, not significantly different from the cohort as a whole, 12 of whom (22%) had at least one HIV-positive semen specimen. Genvoya and Odefsey

were the next most frequent medications, 14 and 16 men, average CD4+ cells/ul blood 690 and 757, respectively. One man taking Genvoya (7%) and eight men taking Odefsey (47%) had at least one HIV-positive semen specimen, but the sample sizes are too small for statistical significance relative to the Biktarvy group or the patient cohort as a whole.

Semen Cells. Neither sperm concentration, nor non-sperm cell concentrations varied between virus-positive specimens and virus-negative specimens, Table 2.

Table 2. Average Semen Cell counts

Virus status	Average sperm/ml semen	Standard Deviation	Average non-sperm cells/ml semen	Standard Deviation
CMV neg	42	± 37	2.5	±6
CMV pos	42	±36	2.4	±3
HIV neg	43	±36	2.5	±6
HIV pos	39	±37	2.0	±2

Reproductive outcomes. During the 23 months of SARS2/COVID19, 43 babies were born to wives and gestational carriers of HIV-infected men whose semen had been screened for virus, 101 gestational carriers and female partners were counseled about FDA guidelines and post embryo-transfer testing requirements, 82 female partners and gestational carriers underwent embryo transfer, none of which resulted in either HIV or CMV infection of the women or babies, and 32 pregnancies were reported by nine fertility clinics.

Discussion

Tissue factors allowing the persistence of HIV infection in the male reproductive tract of men on antiviral therapy successful in suppressing blood virus is urgently needed information. Equally urgent is a better understanding of CMV shedding into semen, which is more common in HIV-infected men than sperm bank donors[20-23]. The present study extends an earlier report describing HIV and CMV in semen from a smaller cohort of men that suggested no relationship of immune status on HIV in semen but a possible effect of immune status on CMV in semen[18]. The present report suggests the opposite, a possible relationship between HIV in semen and fewer than 700 CD4+ cells/ul blood, but no relationship between CMV in semen and immune blood cell count. As with prior studies[18,24], there was no concordance between HIV and CMV in semen specimens, but it is noteworthy that those specimens testing positive for both were produced by a few men with fewer than 700 CD4+ cells/ul of blood.

Effective strategies to prevent HIV infection during gestation have been in place for decades, but similarly effective strategies are not in place to prevent fetal CMV infection. For many years it was assumed that CMV-antibody positive women would be immune-protected from a new CMV infection, but that assumption was dispelled by a report revealing CMV-induced fetal encephalitis in a cohort of CMV-seropositive women prior to pregnancy[25]. CMV infection is the most common congenital

infection worldwide with up to 25% of those children exhibiting neurodevelopmental impairments[26]. Improved strategies to prevent new CMV infections and re-emergent virus replication during pregnancy and to screen for placental CMV are urgently needed. Anti-CMV viral medications are effective[27] but studies on treatment of CMV in semen are lacking.

Conclusions

Current anti-viral therapies do not eliminate HIV in semen of HIV-infected men. Although CMV is known to be present in semen of HIV-infected men, the tissue factors leading to shedding of each virus are unknown. Studies to determine the efficacy of adding anti-CMV treatment strategies to HAART on lowering the semen burden of CMV, and thus the risk of transmission to sexual partners, are urgently needed.

Conflict of Interest Statement:

The authors declare no conflicts of interest.

Acknowledgements Statement:

All authors participated in data gathering; AH, MA, MH, MS, AAK conducted data analysis; AH, MA and AAK prepared the manuscript.

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