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Shedding of Hepatitis C Virus in Semen of HIV-infected Men

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40-word summary: HCV was shed into the semen of one-third of HIV-infected MSM with recent or chronic HCV infection. Levels were plausibly sufficient to transmit HCV during unprotected anal intercourse, therefore condoms should be worn to prevent transmission.

ABSTRACT

Background

The epidemic of sexually-transmitted hepatitis C virus (HCV) infection among HIV-infected men who have sex with men (MSM) has been documented for over a decade. Despite this, there is no consensus as to the risk factors for sexual acquisition of HCV in these men.

Methods

We obtained paired semen and blood samples at 2-week intervals from HIV-infected MSM with recent and chronic HCV infection and quantified HCV in semen.

Results

HCV was quantified in 59 semen specimens from 33 men. HCV was shed in 16 (27%) of semen specimens from 11 (33%) of the men. Median HCV viral load (VL) in semen was 1.49 $\log_{10}$ IU/mL. HCV VL in blood was significantly higher at the time of HCV shedding in semen than when HCV shedding in semen was not detected (p=0.002). Further, there was a significant correlation between the HCV VL in blood and semen overall (r_s = 0.41; p=0.001), and in the subgroup with recent HCV infection (r_s = 0.37; p=0.02), but not in the subgroup with chronic HCV infection (r_s = 0.34; p=0.1).

Conclusions

One third of HIV-infected MSM co-infected with HCV shed HCV into their semen. Based on the HCV VL in semen in this study, an average ejaculate would deliver up to 6,630 IU of virus into the rectum of the receptive partner. Our data therefore strongly support that condoms should be used during anal intercourse among MSM to prevent transmission of HCV.

The epidemic of sexually-transmitted hepatitis C virus (HCV) infection among HIV-infected men who have sex with men (MSM) has been well documented for over a decade [1]. Despite this, there is still no consensus as to what the risk factors are for sexual acquisition of HCV, probably because there are multiple routes of infection among MSM, and due to regional variability in sex-related practices [1]. We have previously shown that in New York City (NYC), the main sexual risk for HCV acquisition among HIV-infected MSM was receptive anal intercourse without a condom and *with* ejaculation of the insertive partner [2]. Witt et al [3], in a multi-site US study that did not include NYC, also found unprotected receptive anal intercourse to be a significant risk factor for HCV acquisition. In contrast, a study in Germany [4] found no association with anal intercourse, and emphasized that a subgroup of 7 (20.6%) HIV-infected MSM with recently acquired HCV reported frequent rectal trauma with bleeding, while Vanhommerig et al. [5] in Amsterdam found that rectal trauma with bleeding was not associated with acquisition of HCV. Even if rectal trauma were an important risk factor for HCV acquisition, HCV would still need to be inoculated in the rectal mucosa. Since penises rarely bleed during insertive anal intercourse, a more likely source of HCV is

semen.

That HCV is shed into the semen has been known for well over two decades [6]. The rates of shedding of HCV into semen have ranged widely among studies, between 5 and 57% [7-10]. Semen HCV viral load (VL) are significantly lower than the corresponding HCV VL in the blood [8, 11], as would be expected from a blood-semen barrier. With multiple large epidemiological studies of discordant, stable heterosexual couples showing no HCV transmissions [12-14], the general assumption has prevailed that the level of HCV in semen was insufficient to transmit HCV through sex. However, with the emerging international epidemic of HCV infection among sexually-active HIV-infected MSM, and our epidemiological study results that strongly implicated semen as the source of HCV during receptive anal intercourse [2], we revisited this assumption. In this study we enrolled HIV-infected MSM from our well-characterized cohort of recently-HCV-infected MSM and recruited additional men with chronic HCV infection to quantify HCV shedding in semen.

METHODS

HIV-infected MSM referred to the Mount Sinai Medical Center for the management of recent and chronic HCV infections were enrolled in this study. Written informed consent was obtained with approval of the Institutional Review Board of the Icahn School of Medicine at Mount Sinai ("Mount Sinai School of Medicine" at the time of enrollment of participants) in accordance with the Helsinki Declaration of 1975, as revised in 2000.

The date of clinical onset of HCV infection was defined as the date of the first-noted ALT elevation, date of HCV antibody (Ab) seroconversion, or date of first-noted HCV viremia, whichever came first. In this study, recent HCV was defined as the 1-year period after HCV seroconversion, while chronic HCV infection was defined as occurring after this 1-year period. We did not study seronegative acute HCV.

At the initial study visit, a detailed clinical history was obtained, including information about the likely route(s) of acquisition of HCV, and a physical was performed by one of us (DSF), and medical records were obtained from the referring provider to ascertain the date of clinical onset of HCV infection. Testing for sexually-transmitted infections (STI) was performed for syphilis (rapid plasma reagin [RPR], Mount Sinai Clinical Laboratory), and testing for *Chlamydia* and *N. gonorrhea* was performed on or within a week before the first semen specimen was collected, using nucleic acid testing on urine (APTIMA COMBO 2 Assay, Gen-Probe Inc, San Diego, CA).

Three paired blood and semen specimens were collected from participants at 2-week intervals. HCV VL in blood was measured using the COBAS AmpliPrep/COBAS TaqMan HCV Test (Roche Diagnostics; lower limit of quantification [LLOQ] 43 IU/mL). Collection and processing of semen was performed as previously described [15, 16]. Briefly, home collection kits were provided to the participants at the first visit, which included sterile specimen containers, aliquots of sterile transport medium (80% RPMI1640, 9% fetal bovine serum, 9% penicillin/streptomycin, and 2% nystatin, to be refrigerated at home), and printed instructions. Semen was collected at home by

masturbation without lubrication into sterile containers, to which 1.7mL of transport medium was immediately added. The specimens were brought to the clinic visit and processed within 4 hours of collection. Following liquefaction at room temperature, seminal plasma and cells were separated by centrifugation at 700 x g for 12 minutes, and the seminal plasma was removed and frozen at -80 ℃. RNA was isolated using the QIAmp Viral RNA Mini Kit (Qiagen, Courtaboeuf, France), and HCV RNA was quantified using the *m*2000 RealTi*me* System [Abbott Molecular; LLOQ 12 IU/mL]. The determination of HCV VL was made for each semen specimen individually by adjusting for the total volume of the semen plus transport medium.

Statistical data analysis was performed using SPSS Statistics v19.0. Pearson Chi-Square Test and Fisher's Exact Test were used for categorical variables as appropriate. For continuous variables the Mann-Whitney U test was used, as was Spearman's Rank-Order Correlation for two ranked continuous variables not normally distributed. Results with a p value of <0.05 were considered statistically significant.

RESULTS

Baseline characteristics

Thirty-three HIV-infected MSM with HCV co-infection were enrolled and provided at least one semen specimen between April 2013 and September 2014. Twenty-one (64%) were categorized as having recent HCV infection, 17 with primary HCV and 4 with re-infection after documented SVR (*Table 1*). The criteria for detection of HCV infection in those with primary HCV was a seroconversion interval of < 6 months and

ALT elevation > 10 times the upper limit of normal (ULN) in 12 (71%); a seroconversion interval of < 6 months and ALT elevation > 3-5 times the ULN in two; and a seroconversion interval of > 6 months but with ALT elevation > 10 times ULN in three. The 4 re-infections were detected by either ALT elevation > 10 times the ULN or detection of new HCV viremia within 6 months of a negative VL test. Twelve (36%) were categorized as having chronic HCV infection.

Men with recent HCV infection showed the expected differences of higher ALT levels and lower blood HCV VL than those with chronic HCV infection, and men with recent HCV infection were significantly younger (*Table 1*). All men in both groups reported receptive anal intercourse without a condom. Although almost half the men had also injected methamphetamine at least once, sharing of injection equipment was rare.

Blood and Semen HCV levels

A total of 63 semen specimens were collected from 33 men, 42 from men with recent HCV infection, and 21 from men with chronic HCV infection. Four (6%) specimens failed HCV VL quantification due to PCR inhibition, resulting in 59 evaluable semen specimens. Shedding of HCV into semen was detected in 11 (33%) of the men (*Table 2*), and in a total of 16 (27%) of the semen specimens (*Table 3*). Comparing men with and without shedding of HCV into semen, HCV VL in blood was significantly higher in the men with shedding both at baseline (p=0.02), and at the time of HCV shedding (p=0.002). There was no association between having a reactive RPR and detection of

shedding of HCV into semen. None of the men tested positive for urethral *Chlamydia* or *N. gonorrhea*.

The median HCV VL of the semen specimens in which HCV was detected was 1.49 $\log_{10}$ IU/mL. The median difference between blood and semen HCV VL was 5.08 $\log_{10}$ IU/ml. The relationship between the blood HCV VL and the detection of HCV shedding into semen is shown in *Figure 1* and the correlation between the magnitude of blood and semen HCV VL is shown in *Figure 2*. Interestingly, there was a significant correlation between the HCV VL in blood and semen overall (r_s = 0.41; p=0.001) and in the recent HCV subgroup (r_s = 0.37; p=0.02), but not in the chronic HCV subgroup (r_s = 0.34; p=0.1) (*Figure 2*).

In the recent HCV subgroup, the median time between clinical onset of HCV and collection of semen specimens was 8 weeks; there was no association between detection of HCV shedding into semen and time between clinical onset of HCV and the collection of the semen specimen. Comparing the recent and chronic HCV subgroups, there were no significant differences in either the proportion of semen specimens in which HCV shedding was detected (21% and 38%, respectively; p=0.2) or in the median semen HCV VL of those specimens (1.32 $\log_{10}$ IU/ml and 1.77 $\log_{10}$ IU/ml, respectively; p=0.2), although the trend was toward both a higher proportion of detection and higher VL in those with chronic HCV infection.

Eleven men with recent HCV infection and 6 men with chronic HCV infection provided multiple semen specimens. Among those with recent HCV infection who provided multiple specimens, HCV shedding was detected in at least one specimen from 4 (36%) men, and one had HCV shedding detected in all specimens. Among those with chronic HCV infection who provided multiple specimens, HCV shedding was detected in at least one specimen in 4 (67%) men, and three had HCV shedding detected in all specimens.

DISCUSSION

Despite significant epidemiological [2, 3, 5, 17] and virological evidence [7-10], the role of HCV in semen in the sexual transmission of HCV among HIV-infected MSM still remains controversial. The phrases “traumatic sex” or “anal trauma” are commonly cited as necessary elements of HCV acquisition, but these factors have not been found consistently [1]. Such discordant results could have been due to insufficient statistical power to address the multiple risk factors, not asking the same question the same way in all studies, and especially important, due to regional differences in sex and drug practices. Schmidt et al in Germany [4], found that rectal trauma with bleeding was associated with acquiring HCV in a subset of patients, yet Vanhommerig et al in Amsterdam did not [5], nor have we (Fierer DS, unpublished results). These discussions, however, have largely skirted addressing the issue of the source of the HCV transmitted during sex to the anal-receptive partners.

Interestingly, this controversy over risk factors and source for sexually-acquired HCV among MSM is reminiscent of the controversy that initially surrounded the source for

sexually-acquired hepatitis B virus (HBV) among MSM over 3 decades ago. Similar to HCV but in contrast to HIV, HBV is highly infectious through blood contact, and it does not infect cells present in anogenital tract to directly mediate infection [18]. Early epidemiological studies strongly suggested that HBV was sexually transmitted among MSM, but the source of the virus was attributed to hypothesized bleeding of the penis during rough sex. However, the demonstration of HBV shedding into semen by Karayiannis et al. suggested HBV shedding into semen as the source of HBV transmission, rather than from the unrealistic possibility of bleeding of the penis [19]. As a result, HBV from semen (and not blood) has now long been accepted as the source of sexually-transmitted HBV. This acceptance has not, however, been the case with HCV, despite that the first publication clearly demonstrating shedding of HCV into semen was published almost 25 years ago [6] and the several epidemiological studies supporting this route in HIV-infected MSM [2, 3, 5, 17].

Based on the findings of our study, an average ejaculate [20] would deliver between 50 and 6,630 IU of virus into the rectum of the receptive partner. Overall, this range of semen HCV VL is lower than those for either HBV or HIV, two other important viruses transmitted by semen [21, 22]. However, as few as 10-20 HCV particles delivered parenterally are required to establish infection [23]. While HCV in semen does not appear to mediate HCV transmission in stable heterosexual discordant couples [12-14], these seemingly low HCV levels could play a significant role in sexual transmission of HCV when deposited into a rectum whose surface epithelial layer has been disrupted through anal intercourse.

A number of questions are raised by our results. Consistent with previous work [7, 8, 11], we have found that approximately one third of HIV-infected men with HCV infection shed HCV into their semen, and that this shedding is both qualitatively (i.e. detected or not detected) and quantitatively related to the magnitude of blood HCV VL; however, less-so during chronic HCV infection. Shedding in semen was not always present when the blood HCV VL was high, nor was it always absent when the blood HCV VL was low, although these were significantly correlated, with 15 (94%) of 16 of specimens with detectable HCV shedding having a paired blood VL of > 5 $\log_{10}$ IU/mL. Shedding in semen in individual men also varied qualitatively and quantitatively over the short collection period of this study (≤4 weeks), including shedding during very low-level blood HCV viremia in two semen specimens. Taken together, these observations suggest that although generally there may be a threshold that has to be reached for passage of HCV from blood to semen, the phenomenon is more complicated than a simple barrier that needs to be breached for HCV to enter seminal plasma. We have previously shown that HIV shedding in semen is affected by concomitant shedding of human herpes viruses (HHV) [24-27], and HHV may play a similar role in HCV shedding into semen. Studies are planned to collect these specimens and characterize these interactions.

Our study has a number of weaknesses. Testing for other STI was performed in only just over half the men, but none were positive, so clearly STI are not necessary for HCV shedding to occur. Also, we were not able to obtain multiple semen specimens from all

participants, which limited our ability to determine the variability of HCV shedding over time. Blood HCV VL was obtained on the day of semen collection from fewer men with chronic HCV infection than recent HCV infection. It is unlikely that this resulted in incorrect associations between blood VL and HCV shedding into semen, however, as blood VL rarely varies by more than 1 $\log_{10}$ IU/mL during chronic HCV infection [28, 29]. Ultimately, we believe the major limitation of this study was our inability to assess HCV shedding into semen during the ramp-up period of viremia in acute HCV infection, which occurs prior to HCV seroconversion [30]. This may be the period, similar to HIV infection [22], when HCV transmission linked to higher HCV shedding into semen is highest. Additional studies are needed to investigate the relationship between the timing of HCV acquisition and shedding of HCV into semen. In addition, we do not believe seminal HCV shedding is the source of HCV in all sexually-acquired infections among MSM. For instance, in our experience, non-bloody fomite transmission (e.g. penises, fists, sex toys) is likely to occur during group sex (Fierer, unpublished observations), and we have found HCV in rectal fluid that may explain these transmissions (manuscript in preparation).

In summary, while more "traumatic" acts such as fisting or even vigorous penile insertion may increase susceptibility to HCV, we have demonstrated that semen often contains HCV and postulate that this semen contains sufficient virus to transmit infection to anal-receptive partners. The rectal mucosa changes generated by anal intercourse alone, without more significant trauma, may allow absorption of HCV from semen. The rectum in HIV-infected people in general may be more vulnerable to the

penetration of HCV into the bloodstream due to the mucosal changes accompanying the persistent depletion of rectal $CD4^+$ lymphocytes due to HIV infection [31]. With recent reports of sexual acquisition of HCV by MSM taking pre-exposure prophylaxis to prevent HIV infection [32] (Fierer DS unpublished results), however, pre-existing HIV infection is unlikely to be necessary for acquisition of HCV via the rectum. Our data therefore strongly support that condoms should be used during anal intercourse among MSM to prevent HCV acquisition, regardless of HIV serostatus.

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CONFLICT OF INTEREST

No authors report a conflict of interest.

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Table Legends:

Table 1. Baseline characteristics of 33 HIV-infected men with recent and chronic HCV infection.

Abbreviations: n, number; IQR, interquartile range; cART, combination antiretroviral therapy; STI, sexually transmitted infection; RPR, rapid plasma reagin; IDU, injection drug use; ALT, alanine aminotransferase; VL, viral load; IU, international units

[a] Nucleic acid amplification testing for *Chlamydia* and *N. gonorrhea* was performed in 14 (67%) with recent HCV and 4 (33%) with chronic HCV

Table 2. Factors associated with detection of HCV shedding into semen in 33 HIV-infected MSM with recent and chronic HCV infection.

Abbreviations: n, number; IQR, interquartile range; RPR, rapid plasma reagin; ALT, alanine aminotransferase; VL, viral load; IU, international units

[a] In men with recent HCV only

[b] Levels at baseline

Table 3. Factors associated with detection of HCV in 59 semen samples from HIV-infected men with recent and chronic HCV infection.

Abbreviations: n, number; IQR, interquartile range; ALT, alanine aminotransferase; VL, viral load; Δ, difference; IU, international units

[a] In men with recent HCV only

[b] Levels at the time of semen donation

Figure Legends:

Figure 1. Detection of HCV in semen as a function of HCV VL in blood.

Triangles represent semen specimens from HIV-infected men with recent HCV infection, and closed circles represent semen specimens from HIV-infected men with chronic HCV infection.

Figure 2. Correlation between HCV VL in blood and semen.

The recent HCV subgroup is shown in panel A; the chronic HCV subgroup in panel B; and the combined groups in panel C. Triangles represent semen specimens from men with recent HCV infection, and closed circles represent semen specimens from men with chronic HCV infection.

Table 1. Baseline characteristics of 33 HIV-infected men with recent and chronic HCV infection.

Characteristic	**Recent HCV** n=21	**Chronic HCV** n=12	**p value**
Median age (IQR), years	36 (31-46)	52 (38-55)	0.007
Race			
White (%)	12 (57)	7 (58)	0.6
Black (%)	4 (19)	2 (17)	
Hispanic (%)	5 (24)	3 (25)	
HIV parameters			
Median duration of HIV infection (IQR), years	7 (4-12)	10 (4-30)	0.1
Median CD4 count (IQR), cells/µL	691 (537-808)	710 (272-847)	0.6
Receiving cART (%)	20 (95)	12 (100)	0.4
Detectable HIV viremia (%)	7 (33)	8 (67)	0.06
STI			
Reactive RPR (%)	13 (62)	2 (17)	0.1
Urethral STI test performed (%) [a]	0/14	0/4	-

Characteristic	**Recent HCV** n=21	**Chronic HCV** n=12	**p value**
HCV status			
Primary infection (%)	17 (81)	12 (100)	0.3
Re-infection (%)	4 (19)	0	
HCV genotype			
1a (%)	20 (95)	9 (75)	
1b (%)	1 (5)	1 (8)	0.3
2b (%)	0	1 (8)	
3a (%)	0	1 (8)	
Route of HCV acquisition			
Sex only (%)	13 (62)	6 (50)	0.5
Sex + IDU (%)	8 (38)	6 (50)	
Median ALT (IQR), U/L	231 (87-492)	62 (46-105)	0.002
Median blood HCV VL (IQR), log_{10} IU/mL	5.52 (4.13-6.24)	6.62 (6.21-6.93)	0.006

Table 2. Factors associated with detection of HCV shedding into semen in 33 HIV-infected MSM with recent and chronic HCV infection.

Characteristic	**HCV detected** n=11	**HCV not detected** n=22	**p value**
Median age (IQR), years	46 (34-54)	40 (31-50)	0.2
HIV parameters			
Median duration of HIV infection (IQR), years	9 (4-12)	9 (3-14)	1.0
Median CD4 count (IQR), cells/µL	702 (469-837)	691 (501-816)	0.8
Detectable HIV viremia (%)	7 (64)	8 (36)	0.2
Receiving cART (%)	11 (100)	21 (96)	1.0
Reactive RPR (%)	6 (55)	9 (41)	0.4
HCV status			
Recent (%)	6 (29)	15 (71)	
Chronic (%)	5 (42)	7 (58)	0.5
Primary infection (%)	9 (31)	20 (69)	
Re-infection (%)	2 (50)	2 (50)	0.6

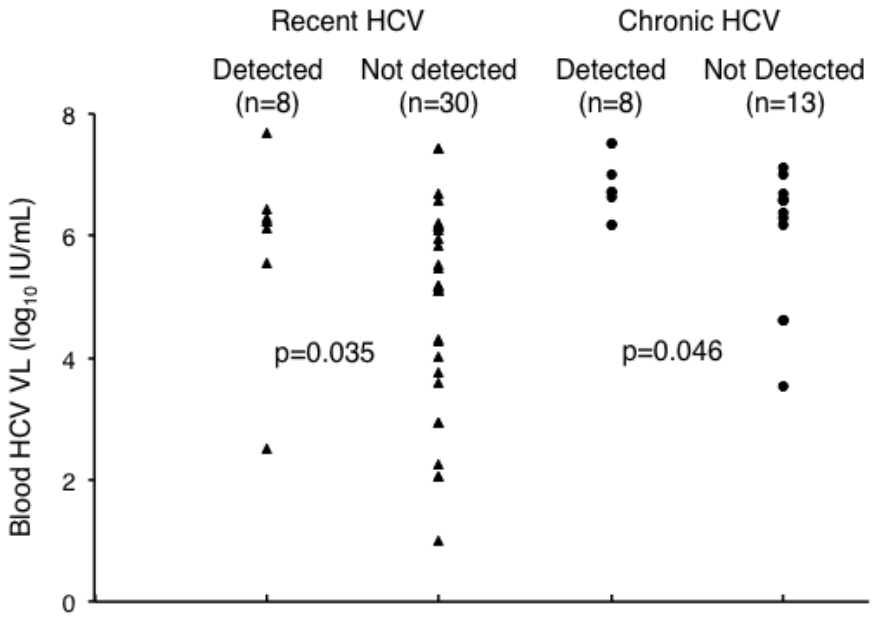
Characteristic	**HCV detected** n=11	**HCV not detected** n=22	**p value**
Time from HCV onset to first semen donation (IQR), median weeks [a]	7 (3-11)	8 (5-17)	0.3
Median ALT (IQR), U/L [b]	107 (61-523)	138 (68-249)	0.6
Median blood HCV VL (IQR), log_{10} IU/mL [b]	6.64 (6.18-7.00)	5.64 (4.20-6.35)	0.02

Table 3. Factors associated with detection of HCV in 59 semen samples from HIV-infected men with recent and chronic HCV infection.

Characteristic	**HCV detected** n=16	**HCV not detected** n=43	**p value**
HCV status			
Recent (%)	8 (21)	30 (79)	0.2
Chronic (%)	8 (38)	13 (62)	
Primary infection (%)	14 (26)	40 (74)	0.6
Re-infection (%)	2 (40)	3 (60)	
Time from HCV onset to semen donation (IQR), median weeks [a]	8 (5-11)	9 (6-17)	0.4
Median ALT (IQR), U/L [b]	107 (66-507)	99 (60-222)	0.3
Volume of semen (IQR), mL	1.5 (0.9-3.6)	2.1 (1.2-2.8)	0.8
Semen HCV VL (IQR), log_{10} IU/mL	1.49 (1.20-2.05)	-	-
Median blood HCV VL (IQR), log_{10} IU/mL [b]	6.36 (6.18-6.93)	5.47 (4.26-6.38)	0.002

Characteristic	**HCV detected** n=16	**HCV not detected** n=43	**p value**
Median paired blood and semen Δ HCV VL (IQR), log_{10} IU/mL	5.08 (4.34-5.65)	-	-

Figure 1. Detection of HCV in semen as a function of HCV VL in blood.



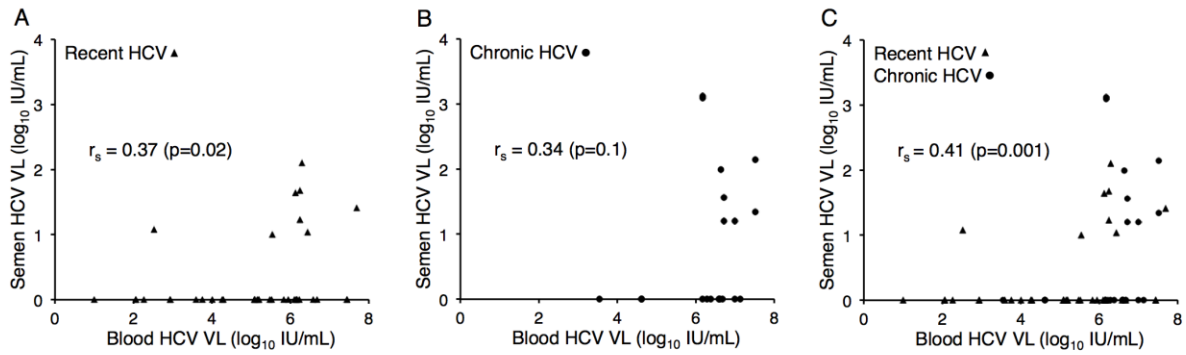


Figure 2. Correlation between HCV VL in blood and semen.