Semen and hormonal parameters in men with chronic hepatitis C infection

Male patients with chronic hepatitis C virus (HCV) infection (n = 57) demonstrated a statistically significant decrease in semen volume, sperm count, and progressive sperm motility and a statistically significant increase in abnormal sperm morphology compared with healthy controls (n = 40). The duration of the HCV infection was negatively correlated with semen volume and sperm motility where the HCV RNA viral load was negatively correlated with sperm count and sperm motility. Chronic HCV patients had statistically significantly lower total serum testosterone and higher serum E_2 and prolactin levels compared with healthy controls. (Fertil Steril® 2011;95:2557–9. ©2011 by American Society for Reproductive Medicine.)

Key Words: Hepatitis, HCV, semen, hormones, viral load

Infection with hepatitis C virus (HCV) is a health problem that affects millions worldwide (1). In addition to being a cause of liver-related mortality, chronic HCV infection causes substantial morbidity due to disabling symptoms such as fatigue and depression; during the infection's course, 40% to 74% of HCV cases develop at least one extrahepatic manifestation (2, 3). An increasing number of young men of reproductive age are associated with the high prevalence of HCV, raising the issue of their basic fertility evaluation.

Most studies concerned with the reproductive aspect of HCV-infected men have focused on the risk of viral transmission and the presence or absence of viral genome in semen and/or spermatozoa, especially the studies of couples considering assisted reproductive technologies (4). It has been suggested that chronic HCV infection can alter seminal parameters and reproductive hormone profiles as part of its extrahepatic manifestation, with a possible negative effect on spermatogenesis and/or semen parameters (5). Moretti et al. (4) reported that some HCV patients show reduced sperm motility, despite having normal sperm concentration. Lorusso et al. (6) demonstrated that men with chronic HCV infection have a significantly impaired sperm quality compared with healthy fertile controls. We assessed the seminal parameters and reproductive hormone levels of men with chronic HCV.

Fifty-seven men with positive anti-HCV and serum HCV RNA of at least 1 year's duration were enrolled in the study. They were recruited from the university hospital after institutional review board approval, and informed consent was obtained. The study's inclusion criteria were persistent viral infection, positive serum HCV antibodies, positive HCV RNA, seronegative human immunodeficiency virus (HIV) status, and maintained hepatic function. The exclusion criteria were features of cirrhosis, portal hypertension, varicocele, cryptorchidism, testicular atrophy, hypogonadism, systemic diseases, taking cytotoxics or interferon, or current excessive alcohol intake. The study controls were 40 anti-HCV-negative, healthy, fertile men with normal liver function and sonographic findings, who were free of medical or genital conditions that could adversely affect their reproductive capacity.

Semen analysis was carried out twice separated by 2 weeks according to World Health Organization guidelines (7). Blood samples were obtained to assay serum follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin (by enzyme-linked immunosorbent assay), total serum testosterone, and estradiol (by enzyme immunoassay) (Diagnostic System Laboratories), as well as serum HCV RNA viral load (8). All sera were immediately stored at −20°C, aliquoted, and thawed only once before the HCV RNA testing assay. Quantitative HCV RNA detection was performed with the Amplicor HCV assay (Roche Diagnostics).

In brief, 0.1 mL of serum was added to 0.4 mL of lysis reagent and incubated at 60°C for 10 minutes. The RNA was precipitated by adding 0.5 mL of isopropyl alcohol and then pelleting by

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centrifuge at 13,000 × *g* for 15 minutes; it then was washed with 70% ethanol and resuspended in 1 mL of specimen diluent. We added 0.05 mL of this suspension to 0.05 mL of PCR Master mix. Reverse transcription and PCR were performed on the Roche Amplicor instrument. The primers used were noncoding region (NCR) (5′-TGC GGA ACC GGT GAG TAC A; positions −193 to −175) and NCR-as (5′-CTT AAG GTT TAG GAT TCG TGC TCA T; positions 24 to 1). Hybridization probes were HCV-NCR-LR (5′-LC Red640-TGC CTG ATA GGG TGC TTG CGA GT-p; positions −52 to −30) and HCV-NCR-FL (5′-GGT CGC GAA AGG CCT TGT GGT A-FL; positions −75 to −54). Primer and probe sequences were selected for optimal conservation between the HCV isolates. High-level samples (>800,000 IU/mL) that fell outside the linear range of the assay were diluted with normal human serum to produce an accurate concentration within the assay's linear range.

The mean age of the men was 37.35 ± 7.35 years (HCV group) and 36.72 ± 7.72 years (controls). Fifty-three of HCV patients were married; 17 (32.1%) cases demonstrated primary infertility with oligoasthenoteratozoospermia, and 36 (67.9%) cases were fertile. The mean duration of proven HCV infection was 3.54 ± 2.8 years (range: 1.0 to 12.0 years), and the mean HCV RNA viral load was 338,920 ± 273,440 IU/mL (range: 66,000–1,252,000 IU/mL). The chronic HCV patients demonstrated a statistically significant decrease in mean sperm count and sperm motility, and a statistically significant increase in sperm abnormal morphology percentage compared with the healthy controls. Also, chronic HCV patients demonstrated a statistically significant decrease in the mean total serum testosterone, a statistically significantly higher serum E_2 level, and a nonsignificant decrease in the mean serum FSH or LH levels compared with the healthy controls (Table 1).

The duration of the HCV infection showed a statistically significant negative correlation with semen volume (r = −0.358, *P*=.006) and sperm motility percentage (r = −0.533, *P*=.001); however, the correlation with sperm count (r = 0.192, *P*=.152) and sperm abnormal morphology percentage (r = −0.229, *P*=.087) was not statistically significant. The HCV viral load was statistically significantly negatively correlated with sperm count (r = −0.373, *P*=.046) and sperm motility percentage (r = −0.661, *P*=.001); however, the correlation with sperm abnormal forms percentage (r = 0.205, *P*=.285) and semen volume (r = −0.248, *P*=.194) was not statistically significant.

In our study, the mean sperm count in the HCV patients—among whom 32.1% of the infertility cases had oligoasthenoteratozoospermia—was statistically significantly lower than in the healthy controls. Durazzo et al. (5) reported that the sperm concentration between controls and HCV patients was different, suggesting a possible negative influence of HCV on spermatogenesis. Moretti et al. (4) added that sperm concentration was reduced in 5 (38.5%) out of 13 of their HCV patients in their study; Hofer et al. (9) and Lorusso et al. (6) also reported low sperm concentration in most or all of their chronic HCV patients compared with fertile controls. Safarinejad et al. (10) also showed that in HCV patients the mean total sperm count, motility, and normal morphology were significantly lower than in controls. However, Garrido et al. (11) reported that semen analysis of HCV-infected patients showed no differences when compared with uninfected men.

We found a statistically significantly reduced semen volume and statistically significantly lower total serum testosterone in the chronic HCV patients compared with the healthy controls. Swerdolff and Wang (12) explained the significantly reduced semen volume of HCV patients by noting their reduced serum testosterone, which is important for the function of male sex glands.

Statistically significant reduced sperm motility and normal sperm morphology were detected in the chronic HCV patients compared with the healthy controls. Zignego and Brechot (13) and Hofer et al. (9) also reported a significant impairment in progressive sperm motility and normal sperm morphology. Durazzo et al. (5) associated reduced sperm motility and sperm normal morphology in these patients with significantly lower levels of free serum testosterone and inhibin B. Levy et al. (14) suggested that HCV RNA or virions present in the seminal plasma may interfere with sperm motility by passive adsorption into the cell membrane. Machida et al. (15) found that HCV stimulates oxidative stress, which causes impaired spermatogenesis in general and impaired sperm motility in particular either directly by the virus or by the host-immune response as a potentially important pathogenic mechanism in chronic liver diseases. These oxidants also overwhelm cellular antioxidant defenses, producing a typical form of DNA oxidative damage in the form of 8-oxo-7,8-dihydro-2'-deoxyguanosine (16). Pal et al. (17) showed that HCV induction of reactive oxygen species is involved in the

TABLE 1

Comparison between raw semen in patients with chronic hepatitis C infection and healthy controls.

	HCV cases (n = 57)	Healthy controls (n = 40)	*P* value
Semen volume (mL)	2.33 ± 0.8	2.15 ± 0.7	<.05[a]
Sperm count (10^6/mL)	40.1 ± 34.6	75.4 ± 23.9	<.01[a]
Sperm abnormal forms (%)	40.35 ± 17.83	12.6 ± 2.3	<.01[a]
Sperm motility (%)	39.6 ± 15.1	58.1 ± 7.1	<.01[a]
FSH (mIU/mL)	2.7 ± 1.03	3.08 ± 1.14	>.05
LH (mIU/mL)	1.9 ± 0.8	2.7 ± 0.8	>.05
Prolactin (ng/mL)	10.0 ± 1.4	6.7 ± 0.9	<.05[a]
Estradiol (pg/mL)	18.24 ± 2.50	8.6 ± 4.45	<.01[a]
Testosterone (ng/mL)	1.43 ± 0.91	3.51 ± 0.8	<.01[a]

Note: Values presented as mean +/− SD. FSH = follicle-stimulating hormone; HCV = hepatitis C virus; LH = luteinizing hormone.
[a] Statistically significant difference (Mann-Whitney test).

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progression of liver disease, suggesting that antioxidant and antiviral therapy can reverse these effects in part by restoring function of the DNA repair enzymes.

Safarinejad et al. (10) reported a significantly greater frequency of disomy in men with chronic HCV than in controls for chromosomes 18, X, and Y. These results were supported with the significant negative correlation of sperm motility with HCV duration and HCV RNA viral load, where the sperm count demonstrated a significant negative correlation with the viral load. However, Garrido et al. (11) reported that a long evolution of HCV disease did not negatively affect sperm motility. Bourlet et al. (18) found that, although 20% of the semen plasma were positive for HCV in their cases, there was no change of semen parameters. Also, Vicari et al. (19) showed that sperm parameters had nonsignificant correlation with the duration of either infertility or viral infection.

Compared with the healthy controls, chronic HCV patients had significantly lower total serum testosterone and higher serum estradiol and prolactin levels but nonsignificant decrease in serum FSH or LH levels. Shimizu (20) demonstrated that variant estrogen receptors were expressed to a greater extent in male patients with chronic liver disease than in healthy female subjects. Nguyen et al. (21) added that more severe liver disease was associated with lower free testosterone and higher sex hormone–binding globulin levels. Durazzo et al. (5) reported lower serum inhibin B and free testosterone levels but nonsignificant difference in FSH, LH or prolactin levels between HCV patients and healthy controls. Safarinejad et al. (10) also found lower LH and FSH levels in these cases.

Semen abnormalities are not uncommon among chronic HCV patients. These abnormalities may impact the fertility of affected patients during their reproductive life, especially in borderline cases.

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