# Seminal parameters before and during combined antiviral (pegylated interferon $\alpha$ -2a and ribavirin) treatment in chronic hepatitis C virus patients in upper Egypt

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#### Background

Some reports suggest that chronic hepatitis C virus (HCV) infection and its combined antiviral therapy could alter seminal parameters, and so chronic HCV infection may influence male fertility. **Aim** 

The aim of this study was to evaluate seminal parameters before and during combined antiviral (pegylated interferon  $\alpha$ -2a+ribavirin) treatment in chronic HCV patients. Because of the possible teratogenic effect of ribavirin, contraception is mandatory during therapy.

This study was conducted on 40 male chronic HCV patients (PCR based), age 20–58 years: 30 patients were given combined therapy (group 1) and 10 were not given treatment (group 2); 10 normal controls were included (group 3).

The seminal fluid (volume, concentration, motility, and morphology) was analyzed. Parameters were determined at the beginning, and in group 1, they were reassessed after 12 weeks of therapy. **Results** 

Semen abnormalities were common at baseline with further impairment during antiviral therapy in group 1: oligoasthenoteratozoospermia was detected in 15 patients, asthenozoospermia in six, and athenoteratozoospermia in six (sperm density: BL,  $59.2 \pm 40.7 \times 106$ /ml; week 12,  $26.7 \pm 22.4 \times 106$ /ml; progressive motility: BL,  $44.5 \pm 15.2\%$ ; week 12,  $31.2 \pm 12.5\%$ ). The proportion of sperm without motility reached its peak after 12 weeks of therapy. The percentage of abnormal forms was BL  $14 \pm 0.04\%$  and week 12  $16.8 \pm 5.2\%$ , with further increase during therapy. In group 2, oligoasthenoteratozoospermia in two. The density was  $46.7 \pm 32.4 \times 106$ /ml, and the progressive motility  $40 \pm 10.7\%$ . The percentage of abnormal forms was  $13.5 \pm 1.6\%$ . In group 3, there was no oligoasthenoteratozoospermia, one patient had asthenozoospermia, and one had athenoteratozoospermia. The density was  $95.2 \pm 28.7 \times 106$ /ml, and the progressive motility  $57.2 \pm 18.2\%$ . The percentage of abnormal forms was  $9.5 \pm 2.8\%$ .

#### Conclusion

Semen abnormalities were common in chronic HCV patients, with further impairment during combined antiviral therapy.

#### Keywords:

combined antiviral therapy, hepatitis C virus, seminal fluid

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#### Introduction

Hepatitis C virus (HCV) is widespread worldwide (150–180 million carriers) [1]. HCV is a small RNA encased liner virus. Eighty percent of patients have a history of parenteral exposure. Combined pegylated interferon plus ribavirin is the treatment of choice (45–80% eradication) [2]. HCV is involved in extrahepatic conditions [3]: cryoglobulinemia (most frequent) [4], glomerulonephritis, thyroid diseases, Sjögren syndrome, and diabetes mellitus [5]. The presence of the virus in the semen is controversial due to the presence of PCR inhibitors [6]. Levy *et al.* [7] demonstrated that 30% of the studied men have semen abnormalities and confirmed the presence of the virus in the semen.

#### **Patients and methods**

This work was carried out on 40 male patients with chronic HCV (PCR based) referred from the Hepatology Department of the Tropical Medicine and Interferon therapy unit in Beni-Suef University Hospital: 30 of them were given pegylated interferon- $\alpha$ and ribavirin and reassessed after 12 weeks (group 1), 10 patients were not given any treatment (group 2), and 10 normal control individuals were included (group 3). Full semen analysis was performed for all patients according to the scheme of WHO [8]. The seminal fluid (sperm concentration, motility, and morphology) was analyzed morphologically. For group 1, examination of the seminal fluid was carried out at baseline and at week 12 of antiviral combination therapy. Exclusions criteria: azoospermia, varicocele, cryptoorchidism, drugs or occupational exposure to agents that are known to affect spermatogenesis, liver cirrhosis, pancytopenia, and renal failure.

All patients were subjected to the following.

#### History

Personal history including name, age, residence, occupation, special habits, and duration and regularity of marriage. Medical history: systemic and endemic diseases, previous operations, or drug intake.

#### Clinical examination

#### General examination

Examination of secondary sexual characteristics and body build was performed.

#### Local examination

Examination of the penis, the scrotum, the testes, the epididymis, the vas, and the cord was performed.

#### Semen analysis

Patients were instructed to wash their hands and genital region before masturbation. Each ejaculate was collected by masturbation after an abstinence period of 3–5 days. The sample was delivered immediately to the laboratory and was allowed to liquefy in the incubator at 37°C; the sample was then analyzed macroscopically and microscopically for volume, viscosity, pH, the total sperm concentration, the proportion of progressively motile sperms, the proportion of normal and abnormal sperm forms, and pus cells.

#### **Physical examination**

- (1) Semen volume: a value of 1.5 ml was considered normal [8].
- (2) Viscosity: viscosity was reported as normal when the length of the thread did not exceed 2 cm [8].

#### **Microscopic examination**

#### Sperm motility

Motility is classified into the percent of progressive motile (PR) sperms, nonprogressive motile (NP) sperms, and immotile sperms. The New WHO manual 2010 [8] shows that the lower reference limit for the total motility (PR+NP) is 40% [95% confidence interval (CI) 38–42%] and the PR is 32% (95% CI 31–34%).

#### Sperm concentration

The lower reference value for the sperm concentration is 15 × 106 spermatozoa/ml (5th centile, 95% CI  $12-16 \times 10^6$ ) [8]. The lower reference value for the total sperm concentration is  $39 \times 10^6$  spermatozoa/ ejaculate (5th centile, 95% CI 33-46 × 106) [8]. The concentration of spermatozoa was determined using the hemocytometer method. In this procedure, a 1:20 dilution was prepared from each well-mixed sample by diluting 50  $\mu$ l of liquefied semen with 950  $\mu$ l of water or diluent. When the preliminary examination of the semen showed that the concentration of spermatozoa present is either excessively high or low, the extent of dilution was adjusted accordingly: 1:10 dilution for concentrations less than  $20 \times 10^6$  spermatozoa/ml and 1:50 dilution for concentrations more than  $100 \times 10^6$ spermatozoa/ml. Only spermatozoa (morphologically mature germinal cells with tails) were counted; pinheads or tailless heads were not counted.

#### Abnormal forms

The percentage of abnormal forms was determined regardless of the type of abnormality. The lower reference value for normal forms is 4% [8].

#### Leukocytes

The concentration of leukocytes was estimated roughly per visual field in a wet preparation during estimation of the number of spermatozoa per visual field.

#### Statistical analysis

The statistical package for social sciences (SPSS Inc. Released 2007. SPSS for Windows, Version 16.0. Chicago, SPSS Inc.) was used. Descriptive statistics were applied to compare results of different groups; tests of significance were applied: the unpaired Student's *t*-test was used to compare mean values between two groups. The  $\chi^2$ -test was used to compare discrete variables in different groups. Significant level was considered at *P*-value less than 0.05.

#### Results Descriptive data

Forty chronic HCV-infected male patients were enrolled in this study from the Hepatology department and the Interferon therapy unit in Beni-Suef General Hospital during 2013–2014. Thirty patients were under treatment with pegylated interferon- $\alpha$  and ribavirin. Ten normal male individuals were included as the control group. The seminal fluid (sperm concentration, motility, and morphology) was analyzed morphologically. For group 1, examination of the seminal fluid was carried out at baseline and at week 12 of antiviral combination therapy.

Patients' age ranged from 20 to 58 years (mean 32.9+12.5). Among the studied groups, 42 patients (84%) were married and eight (16%) were single. The duration of marriage ranged from 1 to 35 years (mean 11.8+10.7) (Figs. 1–4 and Tables 1–6).

#### Discussion

The mainly affects the liver, but there are many other conditions that are associated with hepatitis C. Extrahepatic manifestation refers to diseases or conditions that affect organs other than the liver. Several extrahepatic manifestations have been reported in the natural history of HCV infection. Up to 40–74% of patients infected with HCV might develop at least one extrahepatic manifestation during the course of their disease. It may affect the skin, eyes, joints, the immune system, the nervous system, kidneys, and even male germinal cells [9].

It has been shown that HCV could stimulate the production of reactive oxygen species (ROS) through

#### Figure 1



The volume of the ejaculate (ml) in the studied groups.

#### Figure 3



The percentage of abnormal forms in the studied groups.

## Table 1 Semen parameters in group 1 at baseline and after12 weeks of therapy

Semen parameters	Group 1 baseline		Group 1 week 12		P-value	Significance
(microscopic)	Mean %	SD	Mean %	SD		
Volume of ejaculate (ml)	2.5	0.44	2.4	0.44	0.3	NS
Sperm concentration (million/ml)	59.2	40.7	26.7	22.4	<0.001	HS
Abnormal forms	14	0.04	16.8	5.2	<0.001	HS

Although there was no statistically significant difference between the volume of the ejaculate before and after 12 weeks of treatment in group 1 patients, there were statistically significant differences regarding the sperm concentrations and abnormal forms; HS, highly significant.

## Table 2 Comparison of semen parameters between group 1 at baseline and group 2

Semen parameters	Group 1 baseline		Group 2		<i>P</i> - value	Significance
(microscopic)	Mean %	SD	Mean %	SD		
Volume of ejaculate (ml)	2.5	0.44	2.35	0.69	0.4	NS
Sperm concentration (million/ml)	59.2	40.7	46.7	32.4	0.3	NS
Abnormal forms	14	0.04	13.5	1.6	0.9	NS

There were statistically insignificant differences between group 1 before treatment and group 2 with regard to the sperm concentration, the volume of ejaculate, and abnormal forms.



Sperm concentration (million/ml).



Figure 2



The percentage of progressive motility in the studied groups.

Table 3 Comparison of semen parameters between group 1 at baseline and group 3

		-				
Semen parameters	Group 1 baseline		Group 3		<i>P</i> - value	Significance
(microscopic)	Mean %	SD	Mean %	SD		
Volume of ejaculate (ml)	2.5	0.44	2.75	1.43	0.4	NS
Sperm concentration (million/ml)	59.2	40.7	95.2	28.7	0.015	S
Abnormal forms	14	0.04	9.5	2.8	0.00	HS

There was an insignificant difference between group 1 before treatment and group 3 with regard to the volume of ejaculate, whereas statistically significant differences were found with regard to the sperm concentration and abnormal forms; HS, highly significant; S, significant.

Table 4 Comparison of motility characteristics of semen analysis in group 1 at baseline and after 12 weeks of antiviral therapy

Semen	Group 1		Group 1	week	P-value	Significance
parameters	Mean (%)	SD	Mean (%)	SD		
Progressive motility (PR)	44.5	15.2	31.2	12.5	<0.001	HS
Nonprogressive motility (NP)	11.8	5.8	15.7	5.7	<0.001	HS
Immotile (IM)	21.2	10.6	37.2	9.3	<0.001	HS

Significant differences were found between baseline and after 12 weeks' analyses with regard to progressive motility, nonprogressive motility, and immotile percentages; HS, highly significant.

 Table 5 Comparison of motility characteristics of semen

 analysis between group 1 at baseline and group 2

Semen parameters	Group 1 baseline		Group 2		P-value	Significance
(microscopic)	Mean (%)	SD	Mean (%)	SD		
Progressive motility (PR)	44.5	15.2	40	10.7	0.3	NS
Nonprogressive motility (NP)	11.8	5.8	13.1	6.7	0.5	NS
Immotile (IM)	21.2	10.6	25.9	8.4	0.2	NS

Insignificant differences were found between group 1 at baseline and group 2 analyses with regard to progressive motility, nonprogressive motility, and immotile percentages.

 Table 6 Comparison of motility characteristics of semen

 analysis between group 1 at baseline and group 3

Semen	Baseline		Group 3		P-value	Significance
parameters (microscopic)	Mean (%)	SD	Mean (%)	SD		
Progressive motility (PR)	44.5	15.2	57.2	18.2	0.03	S
Nonprogressive motility (NP)	11.8	5.8	7.8	2.9	0.04	S
Immotile (IM)	21.2	10.6	12.9	8.7	0.03	S

Significant differences were found between group 1 at baseline and group 3 analyses with regard to progressive motility, nonprogressive motility, and immotile percentages; S, significant.

the expression of the core protein with resulting invitro and in-vivo mitochondrial injury, which might explain its hepatic damage, at least in part. There are some evidences now that suggest that ROS-mediated damage to the sperm is a significant contributing pathology in 30–80% of cases [10].

ROS, including oxygen ions, free radicals, and peroxides, cause infertility by two principal mechanisms. First, ROS damage the sperm membrane, which in turn reduces the sperm's motility and ability to fuse with the oocyte. Second, ROS directly damage sperm DNA, compromising the paternal genomic contribution to the embryo [11].

The presence of HCV-RNA in the semen is controversial. In Hofer et al. [11], HCV-RNA was detected in the seminal fluid only in a minority of HCV 'carriers'. The fact that HCV-RNA is detectable in the seminal fluid in only a small proportion of HCV-monoinfected patients is supported by the low prevalence of sexual transmission of HCV. In contrast, methodological issues with assaying HCV may explain the divergent results. Taq polymerase inhibitors present in the seminal fluid could interfere with the results obtained by the assay (TaqMan). To exclude such interference, the seminal fluid was diluted before HCV measurement to reduce the effect of Taq inhibitors. Interestingly, patients in whom a substantial amount of virus could be detected were the patients with the highest viral load in the serum, suggesting a correlation between the viral load in the serum and the HCV concentration in the semen. This might also be of practical utility in counseling patients with the frequently asked question of sexual transmission of HCV.

This study suggested that chronic HCV infection could affect semen parameters in the form of count, motility, viability, and even morphology.

Our results were in agreement with Hofer *et al.* [11] who found that chronic infections such as HCV had a significantly impaired sperm quality. Other researchers found that the treatment of HCV with antiviral drugs led to worsening of semen parameters and advised the wives to use contraception methods during treatment.

In our study, we found that the sperm concentration of HCV patients decreased during combined antiviral therapy, and this is in agreement with Hofer *et al.* [11] who found that the sperm concentration was significantly lowered during combined antiviral therapy.

Regarding the sperm motility, we found that progressive sperm motility in HCV patients was significantly impaired during antiviral combination therapy, and these results were in agreement with those of Hofer *et al.* [11], who studied 15 male patients with chronic HCV who were treated with pegylated interferon  $\alpha$ -2a in combination with ribavirin and found that the sperm motility was impaired during antiviral therapy. Some reports had found a negative influence of HCV infection on spermatogenesis, which augments our results, but improvement in the sperm morphology was found after treatment in contrast to our study [12].

Our study did not address whether these alterations are reversible or not. Hofer *et al.* [11] tested a group of patients after the end of antiviral therapy, and a marked improvement in the sperm parameters was observed compared with the parameters during the treatment period. Also, Pecou *et al.* [13] found that ribavirin and pegylated interferon treatment was associated not only with semen alteration but also with sperm deoxyribonucleic acid fragmentation, and the DNA fragmentation index (measured by the sperm chromatin structure assay) increased markedly during treatment and remained elevated 8 months later. Alterations that persisted 8 months after treatment indicate the need for a longer contraception period after treatment discontinuation in men.

This study concluded that HCV infections and their line of treatment had a bad effect on the male fertility and semen parameters. Further studies with large numbers of patients are necessary for further confirmation and to determine the exact action on seminal parameters to establish the proper method to deal with infertile male patients with HCV infection.

#### Summary and conclusion

This work was performed to evaluate the effect of antiviral combination therapy on seminal fluid parameters in patients with chronic HCV at baseline and at week 12 of treatment. This work was carried on 40 chronic HCV patients (PCR based) referred from the Hepatology Department of Tropical Medicine and the Interferon therapy unit in Beni-Suef University Hospital during 2013–2014. Thirty of them (group 1) were given pegylated interferon- $\alpha$  and ribavirin, and 10 were not given treatment (group 2); 10 normal male individuals were included as the control group (group 3). Full semen analysis was performed for all patients according to the scheme of WHO [8]. The seminal fluid (sperm concentration, motility, and morphology) was analyzed morphologically. For group 1, examination of the seminal fluid was carried out at baseline and at week 12 of antiviral combination therapy. The patients' age ranged from 20 to 58 years (mean 32.9+12.5). Among the studied groups, 42 (84%) were married and eight (16%) were single. The duration of marriage ranged from 1 to 35 years (mean 11.8 + 10.7).

This is the first study to investigate the effect of antiviral therapy on semen parameters in chronic HCV patients.

The following observations were made regarding the semen parameters:

- (1) The mean semen volume showed no significant difference before and during antiviral therapy.
- (2) Progressive sperm motility was significantly reduced during combined antiviral therapy.
- (3) Abnormal sperm morphology showed a significant difference before and during treatment.

### Conclusion

From the present study, the following conclusions could be made:

- (1) Chronic HCV patients treated with combined antiviral therapy show a worse sperm concentration and sperm motility in comparison with baseline data. The negative influence of pegylated interferon- $\alpha$  with ribavirin on semen parameters can be explained by the explanation of Hofer *et al.* [11].
- (2) The semen volume was not significantly different before and during treatment.

#### Recommendations

There are only a few reports about seminal changes in patients with chronic HCV, and these preliminary data show a negative influence of combined antiviral therapy on the sperm concentration, the PR, and even the morphology.

Further studies with larger numbers of patients are necessary for further confirmation and to determine the exact action of antiviral combination therapy on the seminal pattern to establish the proper method to deal with infertile male patients with HCV infection.

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Conflicts of interest There are no conflicts of interest.

#### References

- Englert YB, Lesage JP, Van Vooren C, Liesnard I, Place AS, Vannin S, et al. Medically assisted reproduction in the presence of chronic viral diseases. Hum Reprod Update 2004; 10:149–162.
- 2 Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, *et al.* Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomized trial. Lancet 2001; **358**:958–965.
- **3** Zignego AL, Brechot C. Extrahepatic manifestations of HCV infection: facts and controversies. J Hepatol 1999; **31**:369–376.
- 4 Cacoub P, Poynard T, Ghillani P, Charlotte F, Olivi M, Piette JC, et al. Extrahepatic manifestations of chronic hepatitis C. Arthritis Rheum 1999; 42:2204–2212.

- 5 Petit JM, Bour JB, Galland-Jos C, Minello A, Verges B, Guiguet M, et al. Risk factors for diabetes mellitus and early insulin resistance in chronic hepatitis C. J Hepatol 2001; 35:279–283.
- 6 Devaux A, Soula V, Sifer C, Branger M, Naouri M, Porcher R et al. Hepatitis C virus detection in follicular fluid and culture media from HCV+ women, and viral risk during IVF procedures. Hum Reprod 2003; 18:2342–2349.
- 7 Levy R, Bourlet T, Maertens A, Salle B, Lornage J, Laurent JL *et al.* Pregnancy after safe IVF with hepatitis C virus RNA-positive sperm. Hum Reprod 2002; 17:2650–2653.
- 8 World Health Organization. WHO laboratory manual for the examination and processing of human semen. 5th ed. 2010. Geneva, Switzerland: WHO Press; Available at: http://whqlibdoc.who.int/publications/2010/9789241547789\_eng.pdf [Last accessed on 2012 Jan 11].
- 9 Aitken RJ, Baker MA, Sawyer D. Oxidative stress in the male germ line and its role in the etiology of male infertility and genetic disease. Reprod Biomed Online 2003; 7:65–70.

- 10 Agarwal A, Sharma RK, Nallella KP, Thomas AJ Jr, Alvarez JG, Sikka SC. Reactive oxygen species as an independent marker of male factor infertility. Fertil Steril 2006; 86:878–885.
- 11 Hofer H, Donnerer J, Sator K, Staufer K, Scherzer TM, Dejaco C, *et al.* Seminal fluid ribavirin level and functional semen parameters in patients with chronic hepatitis C on antiviral combination therapy. J Hepatol 2010; 52:812–816.
- 12 Durazzo M, Premoli A, Di Bisceglie C, Bertagna A, Faga E, Biroli G. Alterations of seminal and hormonal parameters: an extrahepatic manifestation of HCV infection?. World J Gastroenterol 2006; 12:3073–3076.
- 13 Pecou S, Moinard N, Walschaerts M, Pasquier C, Daudin M, Bujan L. Ribavirin and pegylated Interferon treatment for hepatitis C was associated not only with semen alterations but also with sperm deoxyribonucleic acid fragmentation in humans. Fertil Steril 2009; 91:17–22.