

CASE REPORT

Pregnancy after safe IVF with hepatitis C virus RNA-positive sperm

R.Levy^{1,4}, T.Bourlet², A.Maertens², B.Salle³, J.Lornage³, J.L.Laurent¹, B.Pozzetto² and J.F.Guerin³

¹Laboratoire de Biologie de la Reproduction and ²Laboratoire de Virologie, Hôpital Nord, Saint-Etienne, 42055 and ³Laboratoire de Biologie de la Reproduction, 8, Avenue Rockefeller, 69373 Lyon Cedex 08, France

⁴To whom correspondence should be addressed. E-mail: rachel.levy@chu-st-etienne.fr

In France, assisted reproductive technology (ART) for hepatitis C virus (HCV)-infected patients is now subject to strict control after the publication of recent guidelines. Infertile serodiscordant couples (HCV-viraemic men and their seronegative female partners) require special care to be carried out in designated ‘viral risk’ laboratories. Twelve sequential semen samples taken from an HCV chronically infected patient were analysed within 22 months. HCV RNA was detected in all the seminal plasma sampled before antiviral treatment with relatively high viral loads, and in two of the corresponding fractions of motile sperm obtained after a gradient selection, suggesting that a contamination risk by HCV through ART cannot be excluded. When the selection of sperm on a discontinuous gradient was followed by an additional swim-up step, HCV RNA was never detected in the motile sperm suspension that was frozen in highly secure straws. IVF was performed using cryopreserved sperm that tested negative for HCV RNA, resulting in a pregnancy. One month after embryo transfer, testing for HCV RNA and antibodies in the woman gave negative results.

Key words: ART/HCV RNA/semen/sperm

Introduction

The risk of transmission of hepatitis C virus (HCV) via semen is still much debated, especially in assisted reproductive technology (ART) (Dore and Kalder, 2000). In France, ART for HCV-infected patients is now subject to strict control after the publication of recent guidelines. Previous studies have reported the presence (Fiore *et al.*, 1995; McKee *et al.*, 1996; Leruez-Ville *et al.*, 2000; Levy *et al.*, 2000; Pasquier *et al.*, 2000), or absence (Fried *et al.*, 1992; Semprini *et al.*, 1998) of HCV RNA in seminal plasma of chronically HCV-infected males. Moreover, the presence of HCV in motile sperm has been poorly investigated. We report here the first pregnancy after controlled IVF with HCV RNA-positive seminal plasma but HCV RNA-negative cryopreserved selected sperm from an infertile man, chronically infected with HCV and a candidate for ART.

Case report

Patient

A 44 year-old male patient, entering a programme of ART for female factor infertility, gave his fully informed written consent for using surplus semen for research or diagnostic purposes.

The woman was 32 years old and both of her Fallopian tubes were obstructed. The couple was included in a national research programme concerning management of IVF for HCV-infected male patients. This programme was approved by a national ethical committee and authorized by the Health Ministry. Detailed information concerning ART and the related risks of transmitting HCV to the wife and her children was given. A fully informed written consent for the study was then obtained from the wife.

The patient tested positive for the presence of HCV RNA in blood and negative for HIV antibodies and hepatitis B virus (HBs) antigen. He was non-symptomatic with active chronic hepatitis according to biochemical and histological data. The risk factor for HCV contamination was from i.v. drug addiction >10 years ago. Semen parameters were normal according to the criteria of the World Health Organization (World Health Organization, 1992)

Samples

A total of 12 consecutive samples was tested. Semen characteristics are reported in Table I. The patient had not been taking any antiviral medication during the period in which the first nine semen samples were obtained, due to the possible severe

Table I. Chronology and characteristics of semen and blood samples taken from the case report patient.

Date	Semen characteristics				HCV RNA copies/ml in		Detection of HCV RNA in the sperm fraction
	Volume (ml)	Count/ml (x10 ⁶)	% motility (type a + b)	% normal morphology	Semen	Blood	
06/07/2000	3	140	45	37	40–100	ND	–
07/05/2000	3	135	45	35	~40	ND	–
10/12/2000	2.5	140	45	37	40–100	1.4×10 ⁶	–
02/06/2001	3	140	45	37	1400	ND	–
03/15/2001	2	140	45	37	40–100	2×10 ⁶	–
04/03/2001	2	140	45	37	860	ND	+
05/03/2001	3	140	50	37	1000	1.8×10 ⁶	+
06/14/2001 ^a	2.8	78	50	37	1450	1.5×10 ⁶	–
11/29/2001	2	140	45	37	ND	1.4×10 ⁶	–
03/05/2002 ^b	1.5	140	45	35	40–100	14 500	–
03/28/2002	2	170	45	38	~40	ND	–
04/30/2002	2	105	45	37	<40	ND	–

ND = not done/available.

^aSpecimen used for IVF after freezing of HCV RNA negative motile sperm.

^bSpecimen taken 2 weeks after the antiviral treatment was initiated.

adverse effects of ribavirin on the germinal cell lines (Narayana *et al.*, 2002). Once IVF succeeded, three additional semen samples were obtained after a bitherapy combining alpha interferon and ribavirin had been started.

Sample processing

Plasma samples were separated from blood by centrifugation and frozen at –80°C until use. Semen samples were obtained by masturbation into a sterile container after 3 days of sexual abstinence and processed within 2 h. One ml of the semen sample was centrifuged at 800 g for 10 min, then the seminal fluid was carefully separated from the cell pellet and immediately frozen at –80°C until use. Another 1 ml was centrifuged through a three-layer (50, 70 and 90% respectively) discontinuous PureSperm gradient (300 g for 20 min). After each centrifugation and removal of the supernatant, the pellet was transferred to a new, clean tube. Subsequent washes were performed before the swim-up step for 30 min at 37°C. A total of 500 µl of fresh medium was added to the final pellet for the swim-up step.

HCV RNA detection and quantification in blood serum and seminal plasma

The qualitative and quantitative detection of HCV RNA in blood serum and seminal plasma were performed using the Cobas Amplicor® HCV assay 2.0 and the Cobas Monitor® HCV assay 2.0 (Roche Diagnostics, France) respectively. For seminal plasma, a modified protocol was performed for the RNA extraction step including a 1:2 sample dilution in PBS, a centrifugation at 24 000× g for 1 h at 4°C and the use of the QIAmp Viral RNA kit® (Qiagen, France) as previously described (Bourlet *et al.*, 2002).

HCV RNA detection in sperm fractions

An aliquot of 500 000 cells was tested for the presence of HCV RNA. The RNA extraction step was carried out using the RNA Easy Mini Kit® (Qiagen). Reverse transcription and qualitative PCR were performed using the Cobas Amplicor HCV assay 2.0

HCV genotyping

The genotypes of HCV strains were analysed in blood and semen samples by sequencing the 5' non coding region of HCV using the TruGene® HCV assay (Visible Genetics, France).

Follow-up of HCV RNA detection in blood and semen

HCV RNA in blood could be quantified for five samples, before antiviral therapy, with a mean viral load of 1.62×10⁶ copies/ml (6.20 log copies/ml) (range: 1.4–2.0×10⁶ copies/ml) (6.15–6.30 log copies/ml) (Table I). The threshold of our qualitative assay in seminal plasma was estimated at ~40 copies/ml (1.6 log copies/ml) by using serial dilutions of quantified blood plasma from an HCV-infected patient diluted in an HCV negative seminal plasma (Bourlet *et al.*, 2002). No PCR inhibitor was detected in any semen sample. All the nine sequential seminal plasma samples collected before antiviral bitherapy were found positive for the presence of HCV RNA, with viral loads ranging from <40–1450 copies/ml (<1.60–3.18 log copies/ml) (Table I). The average difference between blood and seminal viral loads was roughly 3 log 10. The HCV strains isolated from both seminal and blood samples belonged to genotype 3. During the course of the follow-up, two of the motile sperm fractions obtained after gradient selection showed the presence of HCV RNA (Table I). However, when the sperm recovered after the three-layer gradient were submitted to an additional 30 min swim-up step, no HCV RNA was detected in the final motile sperm suspension. Following the detection of HCV RNA in both the seminal plasma and the motile sperm fraction of these two semen samples, the programmed IVF attempt with fresh semen was immediately stopped. As no HCV RNA was detected in the sperm fraction obtained from a further semen sample, we decided to freeze the motile sperm selected by swim-up migration in CBS® highly secure straws (CryoBiosystem, France) which were stored in a separated cryotank.

IVF and pregnancy follow-up

IVF was carried out using a single straw after washing the thawed HCV RNA negative sperm by centrifugation at 400 g

for 10 min. The post-thawing recovery rate was 50%. After the ovarian stimulation treatment, 27 oocytes were collected by means of an ultrasound-guided vaginal follicular puncture. Twenty-four embryos resulted from the IVF. Two days post-fertilization, two grade I embryos were transferred to the woman and 14 grade I embryos were frozen in secure straws which were stored in a separate container. Two weeks after the embryo transfer, the β -hCG concentration indicated pregnancy, which was confirmed 2 weeks later by an ultrasound scan. A further ultrasound examination revealed the presence of twins, with normal fetal heart activity. The woman tested negative for HCV antibodies at the time of the transfer, and again 1 month later. She also tested negative for HCV RNA detection at 8 and 20 weeks following the embryo transfer. The woman and her children will be tested again for HCV antibodies and RNA at birth and 3 months later, according to the informed consent approved by the parents.

Patient's antiviral treatment

Antiviral treatment combining pegylated interferon and ribavirin was given to the male infected partner after the pregnancy was confirmed. Two weeks after treatment, the viral load decreased to 14 500 copies/ml in blood and to between 40–100 copies/ml in seminal plasma (see Table I). Ten weeks after the treatment was started, no HCV RNA was detectable in the seminal plasma.

Discussion

The present case report demonstrates that HCV RNA was unquestionably detected in seminal plasma with relatively high viral loads ranging from 40–1450 copies/ml through 12 sequential semen specimens sampled over a period of 22 months. Our results illustrate that HCV viral loads in seminal plasma showed dramatic and rapid variations through time. We conducted the same observation from another HCV-viraemic patient whose seminal HCV viral load varied from negative to highly positive within the same month (data not shown). This variability of HCV concentration in seminal plasma is a critical point, one of its main consequences being that each sample of seminal plasma should be tested individually for the presence of HCV RNA prior to use in ART. Viral loads seemed to correlate in blood and semen, as previously described for HIV-1 (Tchet *et al.*, 2000; Bourlet *et al.*, 2001). Unfortunately, the low number of paired values of HCV viral load in both semen and blood did not allow us to establish a statistically significant correlation. As no easy cell culture systems of HCV are currently available, it is important to consider that no conclusion can be drawn about the infectivity of the viral RNA detected in seminal plasma.

For two successive semen samples, HCV RNA was detected in both the seminal plasma and the motile sperm obtained after PureSperm centrifugation, suggesting that a risk of contamination by HCV cannot be excluded through the use of ART. The presence in these fractions of a very low number of leukocytes cannot be definitely excluded. As it is extremely unlikely that sperm support HCV replication, another valuable explanation could rely on a passive adsorption to the cell

membrane of HCV RNA or of virions present in the seminal plasma, as suggested by the association between the positivity of the sperm fractions for HCV RNA and high viral loads in seminal plasma (see Table I). This adsorption could impair the motility of the sperm, as illustrated by the absence of HCV RNA in the corresponding swim-up fraction.

Before using sperm from a patient exhibiting HCV RNA in seminal plasma for ART, the following recommendations could be given: (i) raw semen and corresponding motile sperm selected after gradient and swim-up migration must be cryopreserved in highly secure straws in liquid nitrogen until ART; (ii) the corresponding seminal plasma and motile sperm must be stored at -80°C for HCV RNA testing; (iii) only specimens corresponding to sperm tested negative for HCV RNA must be considered for ART. Furthermore, we recommend the use of highly secure straws for semen freezing, as previously suggested for potentially infectious samples (Benifla *et al.*, 2000) and the storage of these straws in a separate cryotank dedicated to samples with infectious hazard. These guidelines would seem to prevent the mother and the embryos from a potential HCV infection, as illustrated by the preliminary negative results of HCV RNA and antibody detection from the pregnant woman.

Another interesting finding of this case report is the rapid decrease in the HCV viral load in seminal plasma after introduction of an antiviral treatment combining alpha interferon and ribavirin. Unfortunately, it is not possible to recommend the use of such a treatment before starting ART, in order to reduce the risks of HCV transmission, since ribavirin has been shown to induce serious damage to spermatogenesis (Narayana *et al.*, 2002).

In view of the case reported herein, we consider IVF to be a safe method for achieving pregnancies in HCV serodiscordant infertile couples on condition that: (i) an attempt to discover the presence of HCV RNA is performed in semen and motile sperm; and (ii) strict secure measures of security are observed for the storage of infected material.

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