ASSISTED REPRODUCTION TECHNOLOGIES

In vitro fertilization for women infected by hepatitis C virus: a matched case-control study and a systematic literature review

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Abstract

Purpose The aim of this study was to investigate whether infection of women by the hepatitis C virus (HCV) reduces the chance of conceiving after in vitro fertilization (IVF).

Methods We performed a retrospective blind matched case-control study where IVF outcomes for the first 37 cycles of HCV sero-positive women were compared to those of 107 cycles of an uninfected control group. Our results were included in a systematic literature review.

Results Out of five eligible studies, ours included, three observed an impact of HCV infection, though at various levels including response to stimulation, fertilization, implantation, and pregnancy rates. Two studies differentiated results for patients with confirmed active viral replication. Matching criteria and populations studied varied between studies.

Conclusions More and larger studies with well-defined groups are needed to clarify the eventual impact of the HCV on IVF outcomes. Data concerning the infectious status of a patient as well as her health state should be systematically recorded. A multi-disciplinary approach as well as a thorough knowledge of the patient's general health state might prove useful in the management and counseling of these patients in terms of success in conceiving.

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Introduction

Hepatitis C, an RNA-enveloped virus from the Flaviviridae family, is the first cause of chronic infectious hepatitis worldwide [1]. The World Health Organization estimates about 123 million people to be carriers of the virus [1]. Transmission occurs mainly through parenteral exposure. The virus has also been detected in other body secretions including semen, saliva, and breast milk though at lower titers. Sexual transmission is considered to be extremely low [2] but remains controversial. Vertical transmission is estimated at around 5% and doubles in case of co-infection by HIV or when HCV titers reach over 10^6 IU/ml [2]. Infection leads, in an average of 10 years, to 80% of patients developing chronic liver disease. Thereafter, 35 and 5%, respectively, will develop liver cirrhosis and hépato-carcinoma [3]. Treatment with peg-interferon Alfa and ribavirin is not efficient for all viral genotypes, is a long procedure, and can be accompanied by side effects [4]. Moreover, ribavirin has been shown to be teratogenic in animal studies. The development of new, efficient, and direct anti-viral drugs [5] is promising, though currently, not many people can afford this therapy [6]. Couples infected by HCV and who are unable to conceive naturally seek help through assisted reproductive techniques (ART). Several studies have addressed ART outcomes when the male partner is infected by HCV with some observing an adverse effect of viral infection on sperm parameters, hormonal levels, and pregnancy rates [7–10] while others saw no differences when comparing results to uninfected couples [11–13]. On the other hand, it seems that few studies have investigated ART outcomes for HCV-infected women. We therefore performed a systematic

literature review where the impact of HCV infection on women treated by IVF was evaluated. We equally performed our own matched case-control study. Ovarian stimulation, embryology, and pregnancy results were compared between HCV sero-positive and sero-negative women for their first IVF attempt. A subgroup of HCV-infected women with active viral replication was also studied.

Material and methods

Systematic literature search

A systematic literature review was registered on 6/12/16 in the Prospero registry (number CRD42016052972). The databases "pubmed" and "science direct" were searched with the keywords "HCV and in vitro fertilization" as well as "HCV and assisted reproduction." Full articles in English, where IVF outcomes for women infected by HCV were studied, were included. Studies for which there was no adequate control group or less than 10 cycles analyzed were excluded. Figure 1 shows the flowchart for article inclusion.

*1 article added by reference
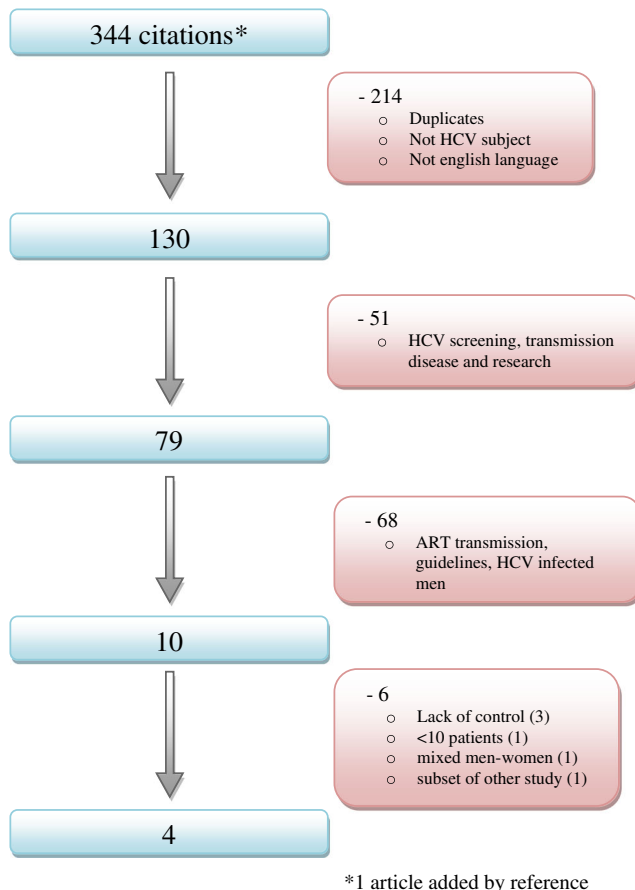
Fig. 1 Flowchart diagram of the literature search

Our matched control study

Studied population

Patients presenting HCV antibodies between 1998 and 2015 and treated for their first IVF cycle were enrolled in our study. We excluded patients who had a co-infection for HBV or HIV as well as patients whose partners were infected by HCV, HBV, or HIV. We additionally excluded patients with a false positive result or cycles involving gamete donation. The same exclusion criteria were applied for the control group of patients who all had a negative HCV antibody. The first population of HCV cases that was analyzed comprised 37 patients presenting positive antibodies to the HCV. Among these patients, three had a negative RT-PCR, 17 had active viral replication, and for the remaining 17 patients, the RT-PCR result at the time of their first IVF attempt was not available. We therefore compared clinical outcomes for the subgroup of patients with a confirmed viral replication to those of the control group of uninfected patients. Since most sero-positive patients develop chronic hepatitis, it is possible that a majority of patients with an unknown RT-PCR result had an active infection; however, we cannot exclude the possibility that some patients had been treated and had cleared the viral infection.

Database and matching of controls

Data of patients treated by ART are systematically and prospectively encoded and checked on a regular basis. A specific computer program was conceived to match HCV cases to controls following determined criteria, though without access to trial results. The matching was therefore automatized and totally blinded to the trial results. When several controls were available, the one closest in time to the IVF cycle of the HCV case was selected. Matching was performed separately for the two main studied outcomes:

1. Matching for the analysis of ovarian response to stimulation: To study the response to ovarian stimulation for HCV-infected women, we matched each case with three controls for their first IVF cycle for the following criteria (100% matching): age (±two years), period of IVF (cycle ± 6 months), and main female infertility etiology (tubal, idiopathic, hormonal, endometriosis). We then matched patients when possible for two additional criteria: primary/secondary infertility (89% matching) and ethnical origin (55% matching). The matching process was performed for HCV sero-positive patients and then for a subgroup of RT-PCR-positive patients.

2. Matching for the analysis of embryology and pregnancy results: To study embryology and pregnancy results for HCV-infected women, we then matched each case who had an embryo transfer to one control for the following

criteria (100% matching): age (±2 years), period of IVF cycle (±12 months), main female infertility etiology (tubal, idiopathic, hormonal, endometriosis), extreme male factor (sperm preparation by simple washing due to bad quality), and day of embryo transfer (days 2 and 3 versus 5). We then matched patients when possible for three additional criteria: primary/secondary infertility (96% matching), ethnical origin (48% matching), and male factor (92% matching). The matching process was performed for HCV sero-positive patients and then for a subgroup of RT-PCR-positive patients.

Ovarian stimulation and oocyte retrieval

Patients were monitored and managed according to standardized clinical protocols as previously reported [14]. Briefly, ovarian stimulation was performed with Humegon® (NV Organon, The Netherlands) or hMG (Menopur®; Ferring, Denmark), recombinant FSH (Puregon®; NV Organon, the Netherlands or Gonal-F®; Merck-Serono, Switzerland) or corifollitropine alfa (Elonva®; NV Organon, The Netherlands). The dose of gonadotropins was determined on an individual basis according to the woman's age, day 3 serum FSH value, and antral follicle count. Pituitary inhibition was obtained by GnRH analog (long or short protocol) (Suprefact®; Senofi-Avantis, Germany or Decapeptyl®; Ipsen) or GnRH antagonist (Orgalutran®; NV Organon, the Netherlands or Cetrotide®; Merck-Serono, Switzerland). When three or more leading follicles reached 17 to 18 mm, ovulation was triggered with 5000–10,000 IU of hCG (Pregnyl®; NV Organon, The Netherlands). Oocyte retrieval was performed transvaginally and ultrasound guided 34–36 h after hCG injection.

Laboratory procedures

After oocyte retrieval, oocytes were either inseminated or injected by ICSI and cultured overnight. Normally fertilized oocytes were cultured to days 2 or 3. Even though day of transfer was one of the criteria selected for the matching process, after matching, we observed that all transfers occurred at the cleavage stage. The best quality embryos were selected for transfer (number depending on the Belgian law from year 2003 onwards) and supernumerary embryos of good quality were cryopreserved. A top-quality embryo on days two and three were, respectively, defined as embryos with four–five cells/<20% fragmentation and ≥eight cells/<20% fragmentation. Infected gametes or embryos were cultured in separate incubators and cryopreserved in separate tanks.

Studied outcomes and definitions

For ovarian response to stimulation, we studied the type of stimulation, the peak estrogen value during stimulation, the number of follicles ≥15 mm, the number of retrieved oocytes, and the percentage of abandoned cycles due to poor response. For pregnancy rates, the clinical pregnancy (number of positive βHCGs with a fetal sac divided by the number of embryo transfers), biochemical (number of positive βHCGs without a fetal sac divided by the number of embryo transfers), spontaneous abortion (number of early and late miscarriages divided by the number of embryo transfers), delivery (number of births divided by the number of embryo transfers), and implantation rates (number of gestational sacs divided by the number of transferred embryos) were investigated. Additional data were collected such as fertilization rates (number of embryos obtained divided by number of inseminated or injected oocytes), embryo quality at transfer, FSH levels, insemination methods, pelvic inflammatory disease (PID), and duration of infertility. Our study covered a long period; data such as BMI, smoking habits, total amounts of administered gonadotropines, antral follicular count, or anti-mullerian hormone were not systematically recorded during the first years of the database. Since a substantial amount of data was missing, they were therefore not considered in this study.

Statistical analysis

To assess differences in continuous variables between cases and controls, the *t* test or Mann-Whitney test was used. To assess differences in categorical variables between cases and controls, the chi-square test or Fisher's exact test was used. In a subgroup analysis, we restricted to HCV-infected women with active viral replication (positive RT-PCR). *P* values <0.05 were considered statistically significant. The analyses were performed using the SAS System, SAS Institute Inc., Cary, NC, USA.

Ethical statement and informed consent

All our protocols have been approved by the local Ethics Committee and all our patients have given their informed, written consent prior to treatment.

Results

Our matched control study

Ovarian response

The principal demographic characteristics of the HCV patients and their matched control group (three controls per case) are

presented in Table 1. Most patients included in this study presented secondary tubal infertility. Response to ovarian stimulation was found to be similar for all criteria analyzed when comparing 37 HCV sero-positive and 111 HCV sero-negative patients for their first IVF attempt (Table 1). We then isolated a subgroup of 17 patients with confirmed active viral replication that were matched to 51 uninfected patients. Demographic data was similar to the sero-positive group (data not shown), and again no significant differences in terms of ovarian response to stimulation were found between cases and controls (table 7 Online Resource).

Embryology and pregnancy results

Among the 37 HCV patients who initiated an IVF cycle, 25 went on to have an embryo transfer. For these patients, we then compared embryology and pregnancy results between HCV sero-positive patients and their matched negative controls for their first IVF cycle as well as for patients with active viral replication. The demographic data for sero-positive patients (table 8 Online Resource) and RT-PCR positive patients (data not shown) were similar to those presented in Table 1. For the additional criteria matched, 80% of the couples were diagnosed with male factor infertility with 8% presenting an extreme male factor etiology. All transfers occurred at the cleavage stage. We again looked at ovarian response (except for cycle cancellation) since we matched the HCV patients to a new set of control patients and reached the same conclusions as above (data not shown). Fertilization and implantation rates were found to be significantly reduced for sero-positive patients compared to their matched negative controls (Table 2). For patients with active viral replication, implantation rates were again shown to be significantly reduced and no live births occurred for HCV-infected patients (Table 3). Calculation of clinical pregnancy rates per transfer for all IVF attempts for sero-positive and RT-PCR-positive patients were 13 (9/67) and 9 (3/33) percent, respectively, with a total of seven live births (data not shown).

Systematic literature review

Design and characteristics of the four eligible studies included after the systematic review are presented in Table 4 and compared to our own study. All five studies were retrospective matched controlled studies, though matching varied from two to ten criteria between studies. Three out of the five studies confirmed exclusion of co-infections. Only one study gave clear information on the health state of the patients included. Comparison between studies, for response to ovarian stimulation, embryology, and pregnancy results are presented in Table 5. Two studies gave results for patients with a positive RT-PCR (Table 6). Only the study from Hanafi and colleagues [15] compared IVF outcomes in relation to viral load. A

significant negative correlation was found between the number of oocytes and viral load but not between pregnancy and viral load. None of the studies analyzed results in relation to the viral genotype. We then calculated when possible, outcomes combining the results of the different studies. For patients sero-positive to HCV, the percentage of cycle cancellations compared to control cycles was, respectively, 30% (48/159) versus 11% (27/235), the mean oocytes retrieved were 6.7 ± 2.1 (150 cycles) versus 8.9 ± 2.5 (243 cycles), and the clinical pregnancy rates per transfer were 32% (71/225) versus 52% (746/1436). The same calculations were done for the two studies evaluating outcomes for RT-PCR-positive patients (Table 6). The percentage of cycle cancellations for RT-PCR positive patients compared to control cycles was, respectively, 40% (23/57) versus 13% (12/91), the mean oocytes retrieved were 5.3 ± 1.8 (34 cycles) versus 9.2 ± 4.0 (71 cycles), and the clinical pregnancy rates per transfer were 6% (2/31) versus 45% (23/51). Fertilization and implantation rates could not be combined since only percentages were available.

Discussion

We investigated the possibility that infection of women by the HCV might have a negative impact on IVF outcomes. Only five studies, ours included, addressed this issue, with three indeed observing an adverse effect of HCV infection at different levels including response to stimulation, fertilization, implantation, and pregnancy results. Our own matched controlled study showed significantly reduced fertilization and implantation rates for HCV-infected women compared to matched controls for their first IVF attempt. On the other hand, no adverse effects of the virus on ovarian response to stimulation or on the quality of transferred embryos were observed. The first population of HCV cases that was analyzed comprised patients presenting antibodies to the HCV. We observed a similar result when we isolated a subgroup of patients known to have a positive RT-PCR result at the time of their IVF attempt. Moreover, in this subgroup of patients with active viral replication, no live birth occurred. In the study by Englert and colleagues [16], 45 cycles were included with patients having a history of 3.3 previous cycles. This study equally covered a long period though with matching to control cycles at the closest calendar date. HCV sero-positive patients were shown to have reduced ovarian response to stimulation compared to sero-negative controls [16]. Higher doses of gonadotropins were required to obtain similar peak levels of estradiol and more canceled cycles due to poor response occurred when compared to cycles of uninfected women. These authors did not observe adverse effects of infection though, on fertilization, implantation, or pregnancy rates. In the second study [17], no impact of the HCV was found on IVF

Table 1 Principal demographic characteristics and response to ovarian stimulation for HCV patients and their matched control group (three controls per case)

	Controls N = 111		HCV cases N = 37		P value
Months between cycles	Median 1.9 (<0. 1 to max 5.97)				
Age mean ± std	36.0 ± 4.3		36.2 ± 4.5		0.87
Age median (min-max)	36.3 (24.7 to 44.4)		36.5 (25.4 to 44.1)		
Ethnical origin (N)	106		36		
Caucasian	58	55%	20	56%	0.36
Sub-Saharan African	36	34%	12	33%	
Maghrebian	11	10%	2	6%	
Asian	1	1%	2	6%	
Female infertility etiology					
Tubal	57	51%	19	51%	1
Hormonal	18	16%	6	16%	1
Idiopathic	30	27%	10	27%	1
Endometriosis	9	8%	3	8%	1
Tubal and hormonal	3	3%	1	3%	1
Secondary infertility (N)	111		36		
	64	58%	23	64%	0.51
Length Infertility (N)	108		29		
Median (min-max) (years)	3 (<1 to 25)		3 (1 to 14)		0.86
FSH day 3 (N)	81		21		
Median (min-max)	6.1 (1.0 to 92.0)		7.4 (3.5 to 18.0)		0.13
PID (N)	64		24		
	17	27%	6	25%	1
Type of stimulation (N)	107		37		
Long	69	64%	24	65%	0.41
Short	17	16%	3	8%	
Cetrotide®/Orgalutran®	21	20%	10	27%	
E2 max median (min-max)	1620 (5 to 15547)		1428 (5 to 6912)		0.27
Oocytes≥15 at scan (N)	109		35		
Median (min-max)	5 (0 to 31)		4 (0 to 15)		0.21
Oocytes at retrieval (N)	86		30		
Median (min-max)	6.5 (1 to 21)		6.0 (1 to 15)		0.43
Cycle cancellation					0.52
Medical (not poor response)	5	19%	3	38%	
Non-medical	1	4%	–	–	
Poor response	20	77%	5	63%	

Std standard deviation, *N* is provided when some data are missing, *FSH* follicle-stimulating hormone, *PID* pelvic inflammatory disease, *E2* estradiol, *E2 max* the peak value during stimulation

outcomes when comparing cycles of sero-positive patients to those of matched control patients. However, clinical pregnancy and implantation rates were surprisingly low for the control group (11 and 10%, respectively). In both these studies, the HCV-infected women were all sero-positive. Results were not analyzed specifically for patients with viral replication. A third and larger study from China [18] considered patients sero-positive to HCV without liver pathology or chronic hepatitis. Matching of patients and controls was limited to age and type of stimulation. No differences were found for ovarian response or pregnancy rates per cycle compared to an uninfected control population. The last study, by Hanafi and colleagues [15], found reduced response to ovarian stimulation and reduced fertilization, implantation, and pregnancy rates for RT-PCR-positive women compared to sero-negative controls. For patients with HCV antibodies without viral replication, a higher number of canceled cycles due to poor ovarian response and lower fertilization rates were observed, though no deleterious effect on pregnancy rates

Table 2 Comparison of embryology and pregnancy results between sero-positive HCV patients and their matched negative controls (one control per case)

	Controls N = 25		HCV cases N = 25		P value
N oocytes at retrieval median (min-max)	7 (1 to 14)		6 (1 to 15)		0.95
Insemination method					0.38
ICSI only	11	44%	13	52%	
IVF only	8	32%	10	40%	
ICSI and IVF	6	24%	2	8%	
Fertilization rate median (min-max)	86% (50 to 100%)		67% (14 to 100%)		0.04
N TF embryos[a] median (min-max)	2 (1 to 3)		2 (1 to 3)		0.96
Quality of TF embryos					0.13
At least one good quality	12	48%	14	56%	
No good quality, at least one average quality	12	48%	8	32%	
Only bad quality	1	4%	3	12%	
Implantation rate	11/47	23%	3/47	6%	0.04
Outcome					0.19[b]
No sac	16	64%	21	84%	
No sac, biochemical pregnancy	–	–	1	4%	
Miscarriage	3	12%	1	4%	
Delivery	6	24%	2	8%	
Clinical pregnancy	9	36%	3	12%	0.10[c]
Babies born	7		2		

Implantation rate: total N sacs/total N embryos transferred

TF transferred

[a] All transfers took place on days 2/3

[b] Overall test, considering the four categories

[c] Clinical pregnancy: yes vs no

Table 3 Comparison of embryology and pregnancy results between HCV patients with a positive RT-PCR and their matched negative controls (one control per case)

	Controls N = 13		HCV cases N = 13		P value
N oocytes at retrieval median (min-max)	7 (1 to 14)		6 (1 to 15)		0.78
Insemination method					1
ICSI only	5	38%	6	46%	
IVF only	6	46%	6	46%	
ICSI and IVF	2	15%	1	7%	
Fertilization rate median (min-max)	100% (60 to 100%)		67% (25 to 100%)		0.12
N TF embryos[a] median (min-max)	2 (1 to 3)		1 (1 to 3)		0.72
Quality of TF embryos					0.13
At least one good quality	6	46%	8	62%	
No good quality, at least one average quality	7	54%	3	23%	
Only bad quality	–	–	2	15%	
Implantation rate	6/23	26%	0/22	0%	0.02
Outcome					0.15[b]
No sac	9	69%	12	92%	
No sac, biochemical pregnancy	–	–	1	8%	
Miscarriage	1	8%	–	–	
Delivery	3	23%	–	–	
Clinical pregnancy	4	31%	–	–	0.10[c]
Babies born	4		–		

Implantation rate: total N sacs/total N embryos transferred

TF transferred

[a] All transfers took place on days 2/3

[b] Overall test, considering the four categories

[c] Clinical pregnancy: yes vs no

Table 4 Impact of HCV on IVF outcomes: comparison of study designs and matching of controls in the literature

Author and year of study	Shaw-Jackson et al. 2016[a]	Hanafi et al. [15][b]	Englert et al. [16]	Yang et al. [18]	Prisant et al. [17]
N HCV patients/N control patients	37/111	40–40/40	Not specified	90/1256	22/42
N HCV cycles/N control cycles	37/111	40–40/40	42/84	90/1256	45/45
Rank of trial	1st IVF attempt	Previous IVF attempts	Previous IVF attempts	Not specified	Previous IVF attempts
Design	Retrospective blind matched controlled	Retrospective matched controlled	Retrospective matched controlled	Retrospective matched controlled	Retrospective matched controlled
Matching criteria	Age	Age	Age	Age	Age
	Rank of trial	–	Rank of trial	–	Rank of trial
	Date of trial				–
	F infertility	Tubal condition	Tubal condition	–	F infertility
	EMF	ICSI	ICSI/IVF	–	ICSI/IVF
	M infertility	M infertility	M infertility	–	M infertility
				Stimulation	–
	Day of transfer	–	–	–	–
	Ethnical origin	–	–	–	–
	1ary infertility	–	–	–	–
	Closest trial date chosen	Closest trial date chosen	Closest trial date chosen	–	–
Exclusions	Couple co-infections Donor cycles	Not specified	Couple co-infections	Liver disease Chronic hepatitis Antiviral treatment	Couple co-infections

F infertility female etiology of infertility; *M infertility* male etiology of infertility; *EMF* extreme male factor

[a] For response to ovarian stimulation and pregnancy rates, patients were matched and outcomes were studied independently (see Material and methods). Ethnical origin, primary infertility, and male factor infertility etiology were matched when possible.

[b] Two study groups of patients were included (sero-positive and RT-PCR positive) each with 40 cases and compared to 40 controls.

Table 5 Comparison of ovarian response and laboratory and pregnancy results between studies in the literature for women sero-positive to HCV

Authors and year of study	Shaw-Jackson et al. 2016[b]			Hanafi et al. [15][c]			Englert et al. [16][d]			Yang et al. [18][e]			Prisant et al. [17][f]		
	HCV	controls	P value	HCV	controls	P value	HCV	controls	P value	HCV	controls	P value	HCV	controls	P value
Patients sero-positive for HCV with an unknown or negative RT-PCR result[a]															
Ovarian response															
Cycles	37	111	0.52	40	40	0.0001	42	84	<0.01	90	1256	—	45	45	—
Cycle cancelation	63%	77%	—	30%	5%	NS	10	5	0.03	—	—	—	—	—	—
Dose FSH	—	—	—	28.4±4.6	26.1±6.54	≤0.05	3610±1563	2968±1379	≥0.05	2025	2625	0.269	—	—	—
Length stim	—	—	—	12.5±1.1	12.0±1.5	≤0.05	11.7±2.4	11.5±2.5	≥0.05	10	11	0.181	—	—	—
Estradiol max	1428	1620	0.27	2890±1586	4670±1693	≤0.05	2496±1221	2929±1300	≥0.05	—	—	—	—	—	—
Oocytes collected	6.5	6.0	0.43	9.4±4.2	12.0±5.2	≤0.05	9.4±4.7	11.3±6.7	≥0.05	84%	85%	0.205	5.6±4.9	6.0±4.4	>0.05
P significance	<0.05			≤0.05			<0.05			NS			—		
Laboratory and pregnancy results															
Cycles	25	25	P value	28	38	0.0035	31	78	P	90	1256	P value	37	42	P value
Fertilization rate	67%	86%	0.04	32%	67%	>0.05	56%	59%	≥0.05	77%	78%	0.413	71%	70%	>0.05
Embryo quality	56%	48%	0.36	62%	61%	NS	2.9±1.3	3.1±1.0	≥0.05	67%	71%	0.649	—	—	—
Implantation rate	6%	23%	0.04	45%	52%	>0.05	19%	19%	≥0.05	—	—	—	5%	10%	>0.05
Pregnancy rate	12%	36%	0.10	33%	48%	>0.05	19%	26%	≥0.05	46%	55%	0.138	11%	13%	>0.05
Live births	2	7	—	11	15	NS	—	—	—	—	—	—	2	4	>0.05
P significance	<0.05			≤0.05			<0.05			NS			≤0.05		

Results are presented as percentages, values or mean values ± standard deviation or otherwise specified. P values are provided from individual studies with their level of significance. *Length stim* length of stimulation (days); *Estradiol max* the peak value during stimulation; *NS* not specified.

[a] All studies were for sero-positive patients with an unknown status for viral replication except for the study of Hanafi et al., where RT-PCR was negative. For the study of Yang et al., the majority of patients were also confirmed to be RT-PCR negative (personal communication).

[b] Cycle cancelation is calculated as the percentage of cancelations due to poor response. Estradiol max, oocytes collected, and fertilization rates are presented as median values without min–max due to lack of space. Embryo quality is assessed as the percentage of transferred embryos. Clinical pregnancy rates are calculated per transfer

[c] Cycle cancelation is calculated for women with no mature oocytes at retrieval. FSH dose is calculated as the number of ampoules. Clinical pregnancy rates are calculated per cycle

[d] In this study, 42 cycles are analyzed without the number of patients being specified

[e] FSH dose and stimulation length are provided as median values without min–max due to lack of space. Oocytes collected are calculated as percentage of oocytes retrieved. Clinical pregnancy rate is the ratio between the number of pregnancies and the number of periodicities

[f] 22 HCV positive women and 42 control women with 45 oocyte retrievals in both cases were included. Clinical pregnancy rates are calculated per transfer

Table 6 Comparison of ovarian response and laboratory and pregnancy results between studies in the literature for women RT-PCR positive to HCV

Authors and year of study	Shaw-Jackson et al. 2016[a]			Hanafi et al. [15][b]		
Patients sero-positive for HCV with confirmed viral replication by RT-PCR						
Ovarian response						
Cycles	17 HCV	51 controls	P value	40 HCV	40 controls	P value
Cycle cancelation	67%	83%	0.52	53%	5%	0.0001
Dose FSH	–	–	–	31.6 ± 8.3	26.1 ± 6.54	0.015
Length Stim	–	–	–	13.6 ± 1.6	12.0 ± 1.5	0.311
Estradiol max	1771	1439	0.77	1280 ± 1317	4670 ± 1693	0.012
Oocytes collected	5.5	5.5	0.93	4 ± 4.2	12.0 ± 5.2	0.001
P significance			<0.05			≤0.05
Laboratory and pregnancy results						
Cycles	13 HCV	13 controls	P-Value	19 HCV	38 controls	P value
Fertilization rate	67%	100%	0.12	28%	67%	0.0035
Embryo quality	62%	46%	0.13	60%	61%	>0.05
Implantation rate	0%	26%	0.02	33%	52%	0.038
Pregnancy rate	0%	31%	0.10	5%	48%	0.001
Live births	0	4	–	2	15	0.012
P significance			<0.05			≤0.05

Results are presented as percentages, values or mean values ± standard deviation or otherwise specified. P values are provided from individual studies with their levels of significance. *Length stim* length of stimulation (days) *Estradiol max* the peak value during stimulation

[a] Cycle cancelation is calculated as the percentage of cancelations due to poor response. Estradiol max, oocytes collected, and fertilization rates are presented as median values without min-max due to lack of space. Embryo quality is assessed for transferred embryos. Clinical pregnancy rates are calculated per transfer

[b] Cycle cancelation is calculated for women with no mature oocytes at retrieval. FSH dose is calculated as the number of ampoules. Clinical pregnancy rates are calculated per cycle

was seen when compared to uninfected controls. High cycle cancellation rates were found for both sero-positive (30%) and RT-PCR-positive (53%) patients in this study.

Heterogeneity of IVF outcomes for HCV-infected women could be explained by various factors. Matching criteria is one possibility, since important variation was found between studies. For example, we matched our patients differently according to the studied outcome and analyzed the impact of viral infection on ovarian response and pregnancy results independently. For analysis of ovarian response, apart from age, date of cycle, ethnical origin, and duration of infertility, only female etiology of sterility was considered since male factor has no influence. On the other hand, for pregnancy outcomes, day of embryo transfer [19] as well as extreme male factor was considered as criteria that might have a strong impact on results. Indeed, we hypothesize that simply matching by male etiology of infertility or by insemination method (IVF versus ICSI) as done in most studies may not be sufficient to distinguish between couples with moderate OAT infertility and extreme male factor. Nevertheless, although we matched patients for the most important criteria, one cannot exclude the possibility that other variables could have been overlooked. For example, over 50% of our studied population was diagnosed

with tubal infertility. We did not separate the various tubal etiologies even though this clearly can result in different chances of success. Prevalence of pelvic inflammatory disease was however similar between infected and uninfected patients for whom the data were available.

The type of population studied is another important variable. The general health state of each patient is probably a factor that should be considered for succeeding in conceiving. Studied HCV-infected patients could have positive antibodies with a negative RT-PCR, involving patients who spontaneously cleared the virus or treated patients. For instance, in the study by Yang and colleagues [18], no impact of viral infection on clinical outcomes was observed. Most patients were RT-PCR negative (author's personal communication) or at early stages of infection, since none of them had chronic infection or liver disease. Patients, on the other hand, could have a positive RT-PCR with various symptoms.

Infection by HCV is indeed known to be accompanied by multiple extrahepatic clinical manifestations including cryoglobulinemia, diabetes, thyroid dysfunction, fatigue, depression, and many other symptoms [20]. Treatment of women prior to IVF with antiviral therapy might therefore improve their general state of health and enhance their chances of

pregnancy by clearing hepatic as well as certain extrahepatic manifestations.

Two studies reported reduced ovarian response to stimulation [15, 16]. Altered ovarian function could vary depending on the degree of liver pathology. Modification of the function of granulosa cells is also thought to be one of the mechanisms involved in ovarian dysfunction. The latter cells carry the LDL receptor for the virus though it is not known whether the virus effectively enters and replicates in this cell type [21]. Ovary response to stimulation and possibly oocyte quality and fertilization could therefore be altered in these circumstances.

Chronic inflammation is an important factor in several extrahepatic symptoms. The study of Hanafi and colleagues [15] and ours, in which viral replication was confirmed, both observed reduced implantation despite the transfer of good quality embryos. Recent data in the literature suggest that implantation failure in IVF patients may be hindered by a deregulation in the immune profile [22].

For implantation to occur, tolerance of the fetal semi-allograft is shown to be accompanied by a local switch from the adaptive Th1 to the innate Th2 type. In patients with recurrent implantation failure, an overactivation or a low immune profile was detected [22]. Treatment to restore immune balance has shown encouraging results. Indeed, natural killer cells in the uterine environment promote angiogenesis and placental growth. Modification of the Th1/Th2 ratio and cytokine profile can however result in natural killer cells becoming cytotoxic with ensuing embryo rejection and endometrium destruction [22]. Interestingly, different species of helminthes for example were found to be associated with contrasting effects on fecundity in humans, with one species enhancing fecundity possibly by inducing an immune profile similar to the one found during implantation [23]. In the case of HCV, it would be interesting to study the uterine immune profile in patients with reduced implantation to see if they could possibly benefit from protocols involving cytokine regulation.

Our matched controlled study is hampered by some limitations. Sample size was small since we limited the study to the first IVF attempt for each patient to avoid a methodology problem during matching to controls in subsequent cycles. Additionally, we excluded patients or patients whose partners had a co-infection. Our study was equally retrospective and covered a long period. Nevertheless, we studied sero-positive as well as RT-PCR-positive patients, and matching to controls was performed carefully.

The systematic literature review shows that three out of five studies describe reduced IVF clinical outcomes for HCV-infected women, though at various levels. More and larger studies with well-defined groups are needed to clarify the eventual impact of the HCV on women treated for infertility. Data concerning the infectious status of a patient as well as her health state should be systematically recorded. A thorough knowledge of a patient's medical history as well as a multi-disciplinary approach could help to decide which patients might benefit from treatment prior to starting ART

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