Research Article
Viral Hepatitis

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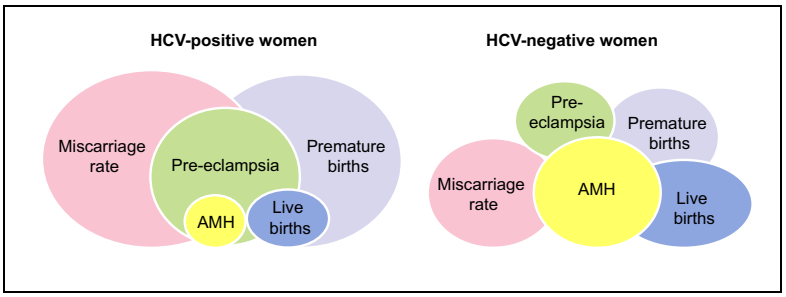
Premature ovarian senescence and a high miscarriage rate impair fertility in women with HCV

Graphical abstract



Highlights

- Women of child-bearing age who are HCV positive undergo premature ovarian senescence.
- Such women have fewer live births, and higher rates of miscarriage and gestational diabetes.
- Total fertility rate in women who are HCV positive *vs.* the general population is 0.7 *vs.* 1.37.
- Miscarriage rate is significantly reduced by successful HCV treatment.
- Antivirals should be tested for their effects on other adverse pregnancy outcomes.

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Lay summary

Most new cases of HCV infection are among people who inject drugs, many of whom are young women in their child-bearing years. Women of reproductive age who are HCV+ display markers of ovarian senescence. This is associated with an increased burden in terms of infertility and adverse pregnancy outcomes, including stillbirth, miscarriage, fewer live births, and gestational diabetes. Early viral suppression with therapy is likely to mitigate these risks.

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Premature ovarian senescence and a high miscarriage rate impair fertility in women with HCV

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Background & Aims: Premenopausal women who are HCV positive (HCV+) have failing ovarian function, which is likely to impact their fertility. Thus, we investigated the reproductive history, risk of infertility, and pregnancy outcomes in women of childbearing age who were HCV+.

Methods: Three different groups were studied: (1) Clinical cohort: 100 women who were HCV+ and also had chronic liver disease (CLD), age matched with 50 women who were HBV+ with CLD and with 100 healthy women; all women were consecutively observed in three gastroenterology units in hospitals in Italy; (2) 1,998 women who were HCV+ and enrolled in the Italian Platform for the Study of Viral Hepatitis Therapies (PITER); (3) 6,085 women, who were mono-infected with HCV, and 20,415 women, who were HCV−, from a large de-identified insurance database from the USA. Measurements: total fertility rate (TFR) defined as the average number of children that a woman would bear during her lifetime. To define the reproductive stage of each participant, levels of anti-Müllerian hormone (AMH) and 17β-estradiol were measured.

Results: Clinical cohort: women who were either HCV+ or HBV+ had similar CLD severity and age at first pregnancy. Based on a multivariate analysis, women who were HCV+ had a higher risk of miscarriage than those who were HBV+ (odds ratio [OR] 6,905; 95% CI 1.771–26.926). Among women who were HCV+, incidence of miscarriage was correlated with median AMH level (1.0 ng/ml). Achieving a sustained virologic response (SVR) after antiviral treatment reduced the risk of miscarriage (OR 0.255; 95% CI 0.090–0.723). In the PITER-HCV cohort, miscarriage occurred in 42.0% of women (44.6% had multiple miscarriages). TFR for women who were HCV+ and between 15 and 49 years of age was 0.7 *vs.* 1.37 of Italian population of the same age range. In the US cohort: compared with women who were HCV−, women who were HCV+ positive were significantly more likely to have infertility (OR 2.439; 95% CI 2.130–2.794), premature birth (OR 1.34; 95% CI 1.060–1.690), gestational diabetes (OR 1.24; 95% CI 1.020–1.510), and pre-eclampsia (OR 1.206; 95% CI 0.935–1.556), and were less likely to have a live birth (OR 0.754; 95% CI 0.622–0.913).

Conclusions: Ovarian senescence in women of childbearing age who are HCV+ is associated with a lower chance of live birth, greater risk of infertility, gestational diabetes, pre-eclampsia and miscarriage. Such risks could be positively influenced by successful HCV cure.

Keywords: Anti-Müllerian hormone; Antiviral therapy; Sustained viral response; HBV; HCV.
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Lay summary: Most new cases of HCV infection are among people who inject drugs, many of whom are young women in their childbearing years. Women of reproductive age who are HCV+ display markers of ovarian senescence. This is associated with an increased burden in terms of infertility and adverse pregnancy outcomes, including stillbirth, miscarriage, fewer live births, and gestational diabetes. Early viral suppression with therapy is likely to mitigate these risks.

Introduction

Menopause represents a critical event in a woman's life. Rapid loss of oestrogen and of its protective anti-inflammatory effects leads to a series of biological events characterised by a shift from a non-inflamed to an inflammation-prone environment. These changes translate into systemic symptoms, such as joint pain, metabolic syndrome, and others. In women with HCV, menopause has direct effects on the liver, which include faster progression of fibrosis and marked resistance to interferon (IFN)-based antiviral therapy.[1–3] Prolonged periods of hormone replacement therapy (HRT) appear to be associated with a lower stage of fibrosis[1,4] and improved response to antiviral therapy, at least in the Japanese population.[5]

Previous data indicated that, at premenopause, women who were HCV+ already had menopausal levels of anti-Müllerian hormone (AMH), an accurate marker of ovarian reserve, suggesting possible consequences for reproductive function.[3,6–8]

There was an initial decline in, followed by stabilisation of, the prevalence of HCV from 2006 onwards.[9] However, in some, mostly resource-rich, settings, its prevalence is now increasing.[10] Most new HCV cases are among people who inject drugs, many of whom are young women in their childbearing years.[11] Although it appears that women clear HCV infection more easily than men, they undergo reinfection at a similar rate.[12] Given that HCV infection does not impact a woman's desire to bear a child,[13] it is important to evaluate the effects of this disease on reproductive function. Although there are reports of several different health issues (such as low birth weight, prematurity, and need for intensive care at birth) in infants born to women who are HCV positive,[14,15] only a few studies have evaluated the risk of miscarriage or of stillbirth in such women, with contrasting results.[16–18] While a study from Iraq suggested the possibility of higher miscarriage rate in women who were HCV+ *vs.* those who were HCV−,[16] other studies did not report such a correlation.[17,18]

Therefore, the objective of the present study was to investigate ovarian function in women of childbearing age who were HCV+ and to relate these findings with the women's reproductive history, risk of infertility, and pregnancy outcomes, including premature birth, live birth, still birth, gestational diabetes, pre-eclampsia, and miscarriage.

Materials and methods

Patients

From July 2011 to March 2014, women of childbearing age with chronic liver disease (CLD) were prospectively enrolled at the Gastroenterology Units of the University Hospitals of Modena, Turin, and Naples (Table 1). Follow-up was continued until December 2015. In total, 100 women with HCV+ CLD were matched by age in a 2:1 ratio with 50 women with HBV+ CLD, as well as in a 1:1 ratio with 100 healthy women without liver disease (control group). None of the women enrolled had a history of drug abuse or HIV co-infection. All patients were regularly followed up as outpatients. Coverage by the Italian National Health System was active for all women, regardless of whether they were employed. Women were defined as being of reproductive age if they had regular menses.

As external control groups to assess the effect of HCV infection on infertility and pregnancy outcomes, data from the Italian Platform for the Study of Viral Hepatitis Therapies (PITER) and from a large de-identified insurance database in the USA were used. The PITER-HCV cohort is a structured collaboration between the Italian National Institute of Public Health, the Italian Society for the Study of the Liver, the Italian Society for Infectious Diseases, and their affiliated clinical centres.[19] The *ad hoc* web-based platform, which is certified to international standards, includes demographic, biochemical, clinical, and virological data, as well as specific items regarding a woman's reproductive history (*i.e.* age of menarche, gravity, parity, and miscarriage). The proportion of women in the study who could have had drug involvement was evaluated by determining the number of women with an opioid-dependency code, which was found to be <6%. The US de-identified insurance database contains patient-level medical, prescription, and laboratory data from between 2000 and 2015. Women aged 18–45 years who had had one or more medical claims with an ICD-9 diagnosis code for HCV were matched with women who were HCV− in a 1:3 ratio for the analysis of infertility and in a 1:10 ratio for the analysis of pregnancy outcomes on the basis of age, census region, and index year.

In the infertility analysis, women co-infected with HIV/HCV were added as a third comparison arm.

The study was approved by the Ethical Committee of the Azienda Ospedaliero-Universitaria di Modena and conducted in accordance with provisions of the Declaration of Helsinki and Good Clinical Practice guidelines (ClinicalTrials.gov identifier: NCT01402583; NCT01945008).

Clinical and laboratory assessments

Age, reproductive status, and serum levels of AMH were collected for the three clinical cohorts. For the HCV+ and HBV+ groups, virus genotype, presumed duration of infection, body mass index (BMI), oestrogen level, insulin-like growth factor-1 (IGF-1) level, presence of steatosis on ultrasound, histologic features, antiviral treatment and response, lifestyle habits (*e.g.* smoking and alcohol consumption before and during pregnancy), parity, age at first full-term pregnancy, and occurrence and number of spontaneous abortions were also registered. Women with a history of hormonal manipulation, including hormone treatment for pregnancy support, were excluded from the study.

Liver biopsy and assessment of liver stiffness were performed for women who were HCV+ or HBV+ at enrolment in the study. A single pathologist (LM) reviewed all biopsies according to previously defined criteria.[20] Steatosis was classified as absent, mild (<5%), moderate (5–20%), or severe (>20%). Liver stiffness was measured by transient elastography. HCV RNA and HBV DNA were quantified by the Abbott RealTime HCV and HBV assay, respectively (Abbott Molecular Inc., Des Plaines, IL, USA). HCV genotyping was performed by the INNO-LiPA II assay (Innogenetics, Gent, Belgium).

Table 1. Baseline characteristics of study participants with liver disease.

Characteristic	HCV+ group (n = 100)	HBV+ group (n = 50)	*p* value
Age (yr)[a]	37.2 ± 8.5	35.1 ± 8.0	0.209
Duration of HCV infection (yr)[a]	12 ± 7	12 ± 8	0.972
Maternal smoking habits [n (%)][b]			
Never smoked	55 (55)	26 (52)	0.932
Former smoker	35 (35)	19 (38)	
Current smoker	10 (10)	5 (10)	
Alcohol consumption [n (%)][b]			
No	90 (90)	44 (88)	0.708
Yes	10 (10)	6 (12)	
Employment status [n (%)][b]			
Employed	92 (92)	47 (94)	0.657
Unemployed	8 (8)	3 (6)	
Platelet count ($\times 10^3/mm^3$)[a]	219 ± 70	202 ± 65	0.144
Alanine aminotransferase (IU/L)[a]	79 ± 30	89 ± 65	0.305
Gamma-glutamyl transferase (IU/L)[a]	35 ± 21	29 ± 22	0.113
Cholesterol (mg/dl)[a]	179 ± 50	189 ± 45	0.219
Triglyceride (mg/dl)[a]	99 ± 35	85 ± 55	0.105
Ferritin (ng/ml)[a]	89 ± 30	77 ± 54	0.148
Blood glucose (mg/dl)[a]	75 ± 22	80 ± 25	0.233
HOMA[a]	1.7 ± 1.5	1.5 ± 1.0	0.715
Mean BMI (kg/m^2)[a]	23.6 ± 5.0	23.2 ± 4.6	0.637
HCV genotype 1/2/3/4 (n)[b]	56/15/21/8	n.a.	-
HCV viral load ($IU/ml \times 10^3$)	1.992 ± 3.900	n.a.	-
HBeAg/anti-HBe status	n.a.	0/50	-
HBV DNA (IU/ml)	n.a.	196.000 ± 565.060	-
Liver histology[a]			
Grade of inflammation	3.6 ± 1.5	5.2 ± 0.5	0.001
Stage	1.8 ± 1.3	2.4 ± 1.1	0.043
Presence of steatosis [n (%)][b]	40 (40)	10 (20)	0.014
Stiffness (kPa)[a]	6.0 ± 3.0	5.7 ± 2.0	0.468

Data are reported as the mean ± SD, unless otherwise noted.
NA, not available; BMI, body mass index.
[a] Student's *t* test for independent samples.
[b] Chi-square test.

Hormone assays

Serum AMH levels were assessed by an ELISA (AMH Gen II ELISA; Beckman Coulter, Inc., Brea, CA, USA). The sensitivity of the assay was 0.08 ng/ml, with linearity up to 22.5 ng/ml. Intra- and interassay coefficients of variation were <5% and <7%, respectively. 17β-Oestradiol levels were measured by chemiluminescent microparticle immunoassay (CMIA) using commercially available kits and the c4000 Architect system (Abbott Diagnostic Division, Abbott Laboratories, Abbott Park, IL, USA). Oestradiol was additionally quantified by the Abbott Architect Estradiol Assay 200 (2004 revision) one-step CMIA. The assay sensitivity was <10 pg/ml, with intra- and interassay coefficients of variation of 5.6% and 4.9%, respectively. Serum IGF-1 levels were measured by immunoassay (Quantikinine; R&D Systems, Minneapolis, MN, USA). The sensitivity of the assay was 0.007–0.056 ng/ml. Assay procedures were performed in accordance with the manufacturers' instructions.

Statistical analysis

Continuous variables (mean ± SD) were compared using either the Student's *t* test for independent samples or the nonparametric Mann–Whitney *U* test as appropriate. Categorical variables were summarised as frequency and percentage and compared using the Chi-square test. The Pearson correlation test was used to assess the relationship between hormonal levels and selected clinical parameters. Odds ratios (ORs) and 95% CIs were calculated as appropriate. Multiple logistic regression models were used to assess the relationship between risk of miscarriage and the demographic, metabolic, and histological characteristics of a combined group of women with HBV or HCV and controls, and of only those women who were HCV+. The following baseline variables were considered for univariate analysis: age, etiology, grade and/or stage of disease, liver stiffness, steatosis (absent *vs.* present), and AMH levels. In the HCV cohort, genotype and viral load were also considered in the model. In the US cohort, adjusted ORs for rates of infertility associated with HCV or HIV/HCV co-infection were estimated using logistic regressions adjusting for age, region, year of index date, types of health plan, and co-morbidities. Similarly, ORs were calculated for the association of HCV infection with pregnancy outcomes, including rates of premature birth, live birth, still birth, gestational diabetes, pre-eclampsia, and miscarriage.

Total fertility rate (TFR) was defined as the average number of children that a woman would bear during her lifetime. In the PITER-HCV cohort, TFR was calculated by dividing the general fertility rate by 1,000. For comparison, data from the database of National Institute of Statistics of Italy (Istat) for the year 2014 were used.[21]

Variables with p <0.10 in univariate analysis were included in the final multivariate model. In the statistical models, dependent variables were coded as 1 (present) or 0 (absent). The IBM SPSS Statistics, version 20 (IBM Corp., Armonk, NY, USA) was used for analysis.

Table 2. Hormonal and reproductive characteristics of study participants.

Characteristic	HCV+ group (n = 100)	HBV+ group (n = 50)	Control group (n = 100)	*p*, I *vs.* II	*p*, I *vs.* III	*p*, II *vs.* III
AMH, all women (ng/ml)[a]	1.8 ± 2.0	1.9 ± 0.9	2.5 ± 1.9	0.673	0.011	0.009
AMH, 20–30 yr (ng/ml)[a]	2.3 ± 2.1	2.4 ± 0.9	4.4 ± 3.2	0.684	<0.0001	<0.0001
AMH <0.16 ng/ml [n (%)][a]	34 (34)	2 (4)	4 (4)	<0.0001	<0.0001	0.981
Estradiol (ng/ml)[a]	77 ± 58	87 ± 33	65 ± 50	0.219	0.410	0.882
IGF-1 (ng/ml)[a]	110 ± 58	126 ± 60	n.a.	0.145	--	–
Parity, n (%)[a]	52 (52)	21 (42)	n.a.	0.248	–	–
Age at first pregnancy (yr)[a]	30 ± 6	27 ± 7	n.a.	0.235	–	–
Occurrence of miscarriage [n (%)][b]	46 (46)	8 (24)	32 (32)	0.0005	0.042	0.020
N°. women with multiple miscarriages, n (%)[b]	8 (8)	1 (2)	7 (7)	0.144	0.788	0.196

Data are reported as the mean ± SD, unless otherwise noted.
NA, not applicable. AMH, anti-Müllerian hormone; IGF, insulin-like growth hormone; I *vs.* II, I *vs.* III, and II *vs.* III indicate the *p* values for HCV+ *vs.* HBV+ group, HCV+ *vs.* control group, and HBV+ *vs.* control group, respectively.
[a] Mann-Whitney *U* test.
[b] Chi-square test.

For further details regarding the materials used, please refer to the CTAT table and supplementary information.

Results

Demographic characteristics of the HCV+ and HBV+ groups are summarized in Table 1. No significant difference in age was observed among the HCV+, HBV+, and control groups (mean age of control group: 38.4 ± 5.9 yr). No significant difference was present in lifestyle habits (cigarette smoking or alcohol consumption) or in rate of employment between women who were HCV+ or HBV+. Grade and stage were slightly higher in the HBV + compared with the HCV+ group ($p = 0.043$). Women who were HCV+ had a higher prevalence of steatosis. Liver stiffness was not significantly different between the two groups (Table 1).

Hormone assays

Serum levels of hormones in the HCV+, HBV+, and control groups are reported in Table 2. The HCV+ and HBV+ groups had similar AMH levels ($p = 0.673$, Table 2 and Fig. 1A), which were lower than those of the control group ($p = 0.011$ and $p = 0.009$, respectively). This was particularly evident in the subgroup of women of full reproductive age (20–30 yr; Table 2). Moreover, women who were HCV+ were more likely to have menopausal AMH levels (<0.16 ng/ml) than women who were HBV+ (OR 11.625; 95% CI 2.651–50.970; p <0.0001) or controls (OR 5.26; 95% CI 2.169–12.820; p <0.0001). No significant difference in menopausal AMH levels was present between women who were HBV+ and controls (OR 0.812; 95% CI 0.090–2.267; $p = 0.981$; Fig. 1B). There was no significant difference in 17β-oestradiol levels among the three groups.

A significant correlation was found between AMH level and hepatic grade ($p = 0.041$) or stage ($p = 0.038$) in women who were HCV+ but not in those who were HBV+ ($p = 0.640$ and $p = 0.974$, respectively).

Reproductive characteristics

No significant differences in parity or age at first pregnancy were observed between women who were HCV+ and those who were HBV+ (Table 2).

In women who were HCV+, occurrence of miscarriages was significantly related with the median AMH level (1.0 ng/ml; OR 2.333; 95% CI 1.029–5.292; $p = 0.043$). No significant relationship between occurrence of miscarriage and HCV genotype was found ($p = 0.947$). Eight women who were HCV + reported multiple miscarriages. Among those who were HBV+, miscarriages were rare and not significantly related with median AMH level (OR 1.55; 95% CI 0.200–12.053; $p = 0.672$). Only one woman who was HBV+ had had multiple miscarriages. No relationship was found in controls between AMH levels and risk of miscarriage (OR 1.71; 95% CI 0.746–3.961; $p = 0.203$).

In the cohort of women who were HCV+, at univariate analysis, age (OR 1.084; 95% CI 1.021–1.151; $p = 0.008$) and AMH levels (OR 2.414; 95% CI 1.020–5.713; $p = 0.045$) were related with miscarriage risk. At multivariate analysis, only age was independently related with miscarriage risk (OR 1.082; 95% CI 1.019–1.149; $p = 0.010$).

Univariate regression analysis of the HCV and HBV cohorts showed that HCV infection (OR 10.580; 95% CI 3.531–31.700; p <0.0001), age (OR 1.071; 95% CI 1.018–1.127; $p = 0.008$), grade at histology (OR 0.694; 95% CI 0.492–0.977; $p = 0.037$), and median AMH levels (OR 4.485; 95% CI 2.141–9.394; p <0.0001)

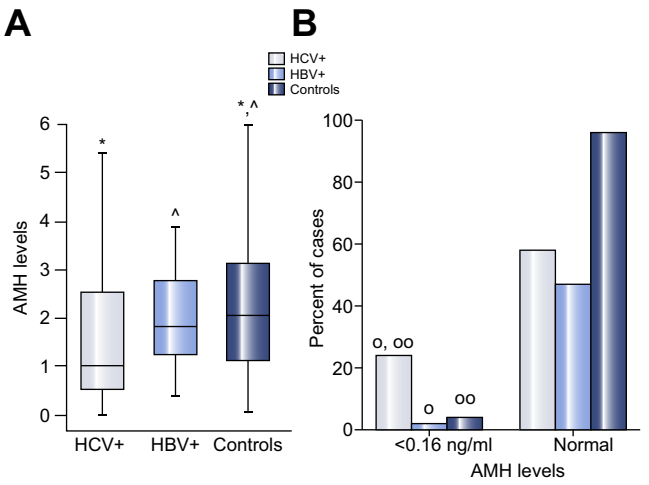

Fig. 1. Levels of AMH in the serum of women who were HCV+, HBV+, or controls. (A) Mean serum levels (bars represent SD and bold lines inside the box plot median levels). Levels of significance: *$p = 0.011$; ^$p = 0.009$ (Mann–Whitney U-test). (B) Distributions of normal and menopausal serum levels of AMH among women in the HCV+, HBV+, and control groups. A significant difference was present between women who were HCV+ *vs.* those who were HBV+ (°) or controls (°°). Levels of significance: °,°°: p <0.0001 (Chi-square test). AMH, anti-Müllerian hormone.

were significantly related with risk of miscarriage. At multivariate analysis, only HCV etiology was independently associated with risk of miscarriage (OR 6.905; 95% CI 1.771–26.926; p = 0.005). When controls were tested against women with HCV or HBV, at univariate analysis, only the presence of HCV infection (OR 7.360; 95% CI 2.416–22.420; p <0.0001) and lower AMH levels (OR 2.242; 95% CI 0.981–5.127; p = 0.056) were related with risk of miscarriage. At multivariate analysis, HCV infection alone (OR 9.363; 95% CI 2.569–34.123; p <0.001) was significantly associated with miscarriage.

AMH levels and response to antiviral therapy

After a diagnosis of HCV infection, 75 women received antiviral treatment with pegylated (IFN) plus ribavirin, combined in some cases with telaprevir (two patients) or boceprevir (six patients). Mean age at therapy was 36 ± 8 years. Sustained virologic response (SVR) was achieved in 53 women (70.6%). The rate of SVR was lower in women with menopausal AMH levels (10/19, 52.6%) compared with those with normal levels (44/56, 78.6%) (OR 3.300; 95% CI 1.094–9.952, p = 0.030). Regression analysis revealed that independent factors for non-response to treatment were HCV genotype 1 (OR 2.309; 95% CI 1.119–1.190; p = 0.023) and lower AMH levels (OR 3.649; 95% CI 1.123–11.904; p = 0.031).

AMH levels remained stable in women who achieved SVR (before therapy: 2.8 ± 2.6 ng/ml; after therapy: 1.8 ± 1.7 ng/ml; p = 0.103). In women who experienced failure of antiviral therapy, AMH levels continued to decrease after failure (baseline: 3.4 ± 2.7 ng/ml; after therapy: 2.0 ± 1.8 ng/ml; p <0.0001). Women who achieved SVR had a lower miscarriage rate (17 miscarriages in 53 pregnancies 32.07%) than women who failed antiviral therapy (14 miscarriages in 22 pregnancies, 63.6%) (OR 0.255; 95% CI 0.090–0.723; p = 0.010).

IGF-1 levels

Mean IGF-1 levels were similar in women who were either HCV + or HBV + women (110 ± 58 ng/ml *vs.* 118 ± 57 ng/ml; p = 0.493). No significant correlation was observed with hepatic grade (p = 0.461), fibrosis stage (p = 0.697), or liver stiffness (p = 0.666) (Fig. 2). A positive correlation between AMH and IGF-1 levels was observed in women who were HCV+ (p = 0.004), but not in women who were HBV+ (p = 0.127) (Fig. 2).

Data from the PITER-HCV cohort

The PITER-HCV cohort was recruited in 2014. Since then, a total of 7,496 patients who are HCV+ (3,317 women) have been enrolled (Appendix Table 1). Data on parity were available in 1,998 women. Overall, 1,667 pregnancies had been registered. TFR for the 590 women who were HCV+ and between 18 and 49 years of age was 0.7, compared with 1.37 for the whole Italian population of the same age range (data from Istat for the year 2014).[11] Among the 650 women who were HCV+ and for whom miscarriage data were available, 273 (42.0%) had a history of miscarriage; 122 of whom (44.6%) had a history of multiple miscarriages [71 women (26.0%) had had two miscarriages, 26 women (0.9%) had had three miscarriages, five women (1.8%) had had four miscarriages, four women (1.4%) had had five miscarriages, two women (0.7%) had had six miscarriages, two women (0.7%) had had seven miscarriages, and one woman (0.36%) had had eight miscarriages].

Carriers of HCV genotype 1 had a lower risk of miscarriage compared with carriers of all other genotypes (OR 0.62; 95% CI 0.438–0.882, p = 0.007). When HCV genotypes 1 and 2 were combined, they had a lower OR of miscarriage than the combination of genotypes 3 and 4 (OR 0.464; 95% CI 0.271–0.794; p = 0.005; Table 3). History of miscarriage was not associated with fibrosis stage (F3–F4 *vs.* F0–F2; OR 0.969; p = 0.855), BMI (<25 kg/m^2 *vs.* >25 kg/m^2; OR 0.827; p = 0.145), presence of cirrhosis (present *vs.* absent, OR 0.961; p = 0.766), diabetes (present *vs.* absent; OR 1.20; p = 0.392), hypertension (present *vs.* absent; OR 0.852; p = 0.333), history of past and/or present drug addiction (present *vs.* absent; OR 0.784; p = 0.382) or employment status (employed *vs.* unemployed; OR 0.752; p = 0.142).

Analysis of US sample

A total of 27,525 women were identified in the analysis of infertility (20,415 HCV− and HIV−negative; 6,805 HCV+; and 305 HCV+/HIV+). Women with HCV had a significantly higher probability of infertility compared with those who were HCV free (OR 2.439; 95% CI 2.130–2.794). Moreover, this risk was even higher among women co-infected with HIV/HCV compared to

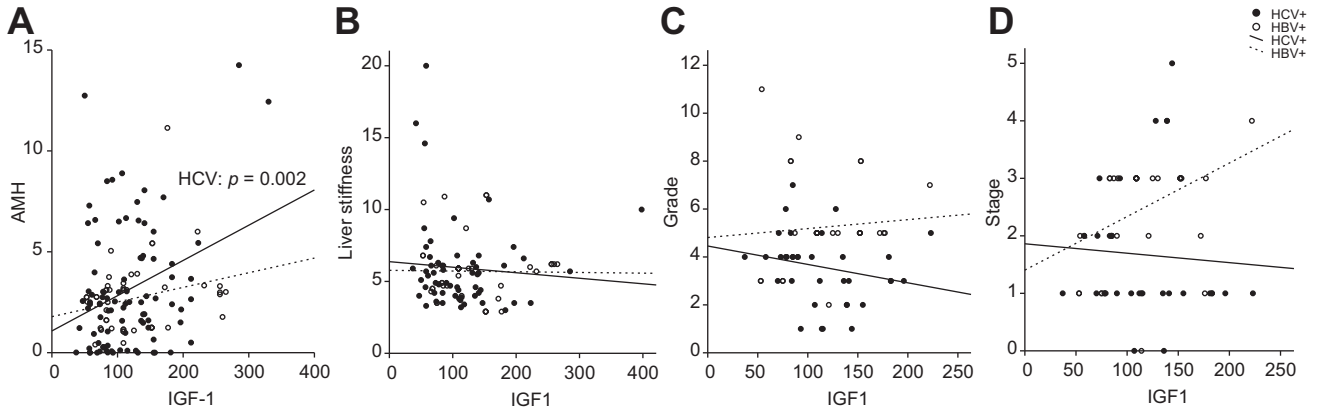

Fig. 2. Relationship between IGF-1 and AMH levels in the serum of women who were HCV+ or HBV+. Different panels report the correlation between IGF-1 and (A) AMH, (B) liver stiffness, (C) grade of inflammation, and (D) stage. Lines represent the best-fit regression line for each subgroup. Data were analysed by Pearson correlation test. AMH, anti-Müllerian hormone; IGF, insulin-like growth factor.

Table 3. Risk of miscarriage for women with different HCV genotypes in the PITER-HCV cohort.[a]

Comparison	OR (95% CI)	*p*
Genotype 1 *vs.* all others	0.6225 (0.4389–0.8828)	**0.007**
Genotype 1 + 2 *vs.* 3 + 4	0.4647 (0.2719–0.7941	**0.005**
Genotype 1 *vs.* 2	1.2688 (0.8347–1.9284)	0.265
Genotype 1 *vs.* 3	0.6163 (0.3136–1.2108)	0.160
Genotype 1 *vs.* 4	0.3412 (0.1440–0.8084)	**0.014**
Genotype 2 *vs.* 3	0.4857 (0.2305–1.0233)	0.057
Genotype 2 *vs.* 4	0.2689 (0.1073–0.6737)	**0.005**
Genotype 3 *vs.* 4	0.5536 (0.1918–1.598)	0.274

Data in bold indicate statistically significant *p* value (<0.05).
[a] Adjusted odds ratios (ORs) and 95% CIs were estimated using logistic regressions.

those who were HCV/HIV– (OR 3.635; 95% CI 2.467–5.357). Among the 13,475 women included in the analysis of pregnancy outcomes, women with HCV were significantly more likely to report premature birth (OR 1.336; 95% CI 1.059–1.685) and gestational diabetes (OR 1.240; 95% CI 1.019–1.510), and significantly less likely to report live birth (OR 0.754; 95% CI 0.622–0.913). Risks of stillbirth (OR 1.252; 95% CI 0.491–3.188), pre-eclampsia (OR 1.206; 95% CI 0.935–1.556) and miscarriage (OR 1.106; 95% CI 0.885–1.383) were also greater among women with HCV; however, the ORs were not statistically different (Table 4).

Discussion

Several recent studies have reported a significant relationship between HCV infection and female reproductive status, highlighting the protective effect of a fertile hormonal status on the progression of fibrosis[1,3] and the response to antiviral therapy.[2,5] Conversely, during the period around or immediately after menopause, rapid and unfavourable evolution of both conditions is observed.[2,3] The data reported here highlight that the relationship between HCV infection and reproductive status in women is deeper and broader than previously thought, with profound consequences for reproductive function confirmed in cohorts from different countries. Specifically, we found that Italian women who were HCV+ had significantly lower levels of AMH than age-matched uninfected controls or women with HBV+ and CLD, and this was significantly associated with a higher miscarriage rate. In a different cohort of women with HCV in the USA, infertility and premature birth were also significantly higher and the rate of live birth without complications significantly lower than among women who were HCV–.

Levels of AMH, which is produced by cells of the developing ovarian follicles, begin to decline approximately one year before follicle-stimulating hormone levels increase.[6–8] Thus, the AMH level represents an early and sensitive marker of a woman's reproductive potential, with declining levels being a reliable indicator of ovarian senescence.[7] We found that AMH levels were significantly lower in women who were HCV+ than in age-matched healthy controls. Such a difference persisted even when we restricted the analysis to the age range of greatest fertility (20–30 years; Table 2). In addition, women who were HBV + had AMH levels that were lower than controls and similar to those of women who were HCV+. However, the latter had AMH levels in the menopausal range (<0.16 ng/ml) more often than age-matched women with HBV+ and CLD of similar (although moderate) severity, or controls, indicating more-impaired overall ovarian function.

A key result of our study concerns the association between HCV infection and risk of miscarriage. In Italy, women with HCV+ enrolled in PITER had an excessively high miscarriage rate. Regardless of age, compared with the general Italian population,[16] women who were HCV+ and enrolled in our study had an excessively high miscarriage rate and an unfavourable ratio between pregnancy attempts and completed pregnancies compared with uninfected controls or women with HBV. A higher rate of miscarriage in women with HBV was recently reported in a prospective Chinese cohort (513 asymptomatic HBV carriers and 20,491 non-HBV controls[22]). However, the percentage reported (9.36% *vs.* 5.70%; $p < 0.001$) was lower than the reported rates in the PITER cohort analysed in this study. These results point to a specific relationship between HCV infection, ovarian function, and reproductive efficiency, suggesting that the premature ovarian senescence observed in women who are HCV+, as indicated by the early and significant AMH decline, has a profound effect on reproductive function. No apparent relationship was found with a lower desire for pregnancy among women with HCV. Accordingly, a recent study showed that female patients with chronic viral illnesses desire children at rates similar to the general population.[23]

The mechanisms underlying this unfavourable outcome are not clear. Possible, although not confirmed, maternal risk factors for miscarriage, such as cigarette smoking or alcohol consumption[24–27] were rare in these women and similarly distributed between those who were HCV+ or HBV+. Furthermore, after being diagnosed with hepatitis virus infection, most women who smoked or drank alcohol before their diagnosis stopped completely. Other possible sociodemographic

Table 4. Risk of infertility and pregnancy outcomes for women in the US sample.[a]

Outcomes	OR	95% CI
Adjusted odds ratio of infertility for women with HCV, HIV/HCV *vs.* HCV/HIV free		
Infertility (HCV *vs.* no HCV)	2.439[b]	(2.130–2.794)
Infertility (HIV/HCV *vs.* no HCV/HIV)	3.635[b]	(2.467–5.357)
Adjusted odds ratio of pregnancy outcomes for women with HCV *vs.* HCV free		
Premature birth	1.336[b]	(1.059–1.685)
Live birth	0.754[b]	(0.624–0.913)
Stillbirth	1.252	(0.491–3.188)
Gestational diabetes	1.240[b]	(1.019–1.510)
Pre-eclampsia	1.206	(0.935–1.556)
Miscarriage	1.106	(0.885–1.383)

[a] Adjusted odds ratios (ORs) and 95% confidence intervals (CIs) were estimated using logistic regressions.
[b] $p < 0.05$.

factors, such as history of drug addiction, unemployment, or lack of health coverage, were not independently related with miscarriage risk in the Italian cohorts and were rare or not represented in the US cohort (the latter is derived from a database of commercially insured patients, who are highly unlikely to be unemployed, to have unstable housing, or to use drugs, because a drug screen is frequently a prerequisite for employment). Hence, although we agree that factors such as a history of drug abuse, unstable housing, or lack of access to healthcare can be important determinants of fertility and pregnancy outcomes, our analysis showed that HCV impacts these outcomes even in a commercially insured population, where such factors are unlikely to occur, or in women with universal health coverage.

CLD could be another possible cause of impaired ovarian function. The liver synthesises IGF-1, which is the most potent trophic ovarian factor, and abnormal liver function could affect its synthesis.[28–30] However, in our prospective clinical cohort, liver damage, assessed by histology and liver stiffness, was generally mild and, therefore, would not affect liver synthetic function. Moreover, in the large PITER-HCV cohort, which included subjects with all stages of liver disease, no relationship was observed between severity of CLD (as evaluated by histology and/or liver stiffness) and history of miscarriages.

Several published data, especially from *in vitro* fertilisation (IVF) studies, support the central role of AMH as a predictor of fertility. Very low AMH levels correlate with a poor response to stimulation by exogenous gonadotropins and with a lower implant success rate in IVF.[31–36] Some studies indicate that women who are HCV+ exhibit poor ovarian response to exogenous stimulation and have high rates of complication, potentially favouring miscarriage.[34,35] In particular, women who are HCV+ display reduced rates of ovarian follicle development after stimulation, higher incidence of apoptosis, and reduced pregnancy rates compared with controls.[35–37] These findings indirectly confirm our results. Although in our study, women who were HBV+ had similar mean AMH levels compared with those who were HCV+, the latter had AMH levels in the menopausal range significantly more often compared with the former. Furthermore, a significant direct correlation between IGF-1 and AMH levels and an increased occurrence of miscarriages was observed only in women who were HCV+. Taken together, these findings suggest that the reduced reproductive capacity of women who are HCV+ is related to failing ovarian function and subsequent follicular depletion occurring in the context of a more generalised dysregulation of other fertility-related factors. Among them, the direct involvement of reproductive tissues in HCV infection should not be overlooked. HCV infects and alters the cellular ultrastructure of trophoblasts, the main cell type in placenta. The resulting functional impairment of the trophoblasts and placenta could, in turn, lead to miscarriage.[38] There are also reports of structural abnormalities of the reproductive tissues in women with HCV+, which could contribute to the high risk of miscarriage.[39,40]

Pathogenic mechanisms could involve both a direct effect of HCV infection, because of the infection of reproductive tissues,[39] and an indirect effect, because of the oxidative stress induced by HCV infection.[41] Data from the PITER-HCV cohort showed that women infected with HCV genotypes 3 and 4 (which are associated with higher levels of oxidative stress compared with other genotypes) were at higher risk of miscarriage than women carrying other genotypes. A recent meta-analysis, including 5,218 pregnant women with HCV, suggested that HCV infection is significantly associated with a higher risk of preterm birth,[42] which is consistent with the premature birth risk observed in our US sample. These findings suggest that the reproductive system is another extrahepatic target for HCV and is involved in the profound and broad physiological dysregulation induced by HCV itself.[43] Indeed, existing data indicate that HCV infection is also associated with male seminal abnormalities, such as altered sperm morphology and decreased semen volume, sperm count, and progressive sperm motility.[44,45]

A limitation of this study was that the women were not enrolled in a predefined treatment protocol but were treated according to physicians' choice and the therapies used were all IFN based. However, women who achieved SVR had a significantly lower miscarriage rate than women who failed antiviral therapy. Furthermore, AMH continued to decline in non-responders and remained stable over time in women achieving SVR.

In conclusion, our observation of decreased fertility and poorer pregnancy outcomes among women who are HCV+ allows us to conclude that having HCV infection significantly and negatively affects many aspects of fertility. It remains to be assessed whether antiviral therapy at a very early age can positively influence the occurrence of miscarriages and can prevent ovarian senescence, because the latter has broader health implications than simply preserving fertility. Therefore, the effect of treatment with new-generation antiviral drugs could be assessed prospectively with this dual purpose.

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Conflict of interest

The authors have no conflict of interest to declare in relation to this manuscript.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

EV is the guarantor and confirms that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that no discrepancies from the study as planned occurred. EV developed the concept for the study and its design; AK, XH, AC, FD'A, LB, LK, FM, VB, SS, MG, and LT collected the data; EV, AK, LM, GT, SV, TT, and YSG analysed and interpreted the data; RMC, EB, and ST carried out the laboratory tests and interpreted the results; EV, GT, SB, and YSG drafted the manuscript; GT, FM, LK, SM, SB, ASG, and YB critically revised the manuscript to ensure its intellectual content: EV and SR performed the statistical analyses; EV obtained the funding for the study; all authors had access to the study data and reviewed and approved the final manuscript.

Ethics approval

The study was approved by the Ethical Committee of the Azienda Ospedaliero-Universitaria di Modena and was conducted in accordance with provisions of the Declaration of Helsinki and Good Clinical Practice guidelines (ClinicalTrials.gov identifier: NCT01402583; NCT01945008).

Trial registration: ClinicalTrials.gov identifier: NCT01402583; NCT01945008.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jhep.2017.08.019.

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