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Original Article

Effect of hepatitis C virus infection on the outcomes of in vitro fertilization

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Abstract: Objective: To investigate the impact of Hepatitis C Virus (HCV) infection on in vitro fertilization (IVF) outcomes. Methods: A retrospective analysis was conducted using IVF cases occurring at the Centre for Reproductive Medicine, General Hospital of Tianjin Medical University between January 2008 and December 2013. A total of 1424 couples undergoing IVF cycles were included: Ninety couples where the female was HCV positive, 78 couples where the male was HCV positive, and 1256 control couples where both the male and female were HCV negative by for the presence of HCV RNA and antibodies. Results: All experimental and control groups (HCV-positive men, HCV-positive women, and controls) had similar sperm parameters, ovarian stimulation, fertilization and pregnancy results. Conclusion: HCV infection has no affection on IVF treatment outcomes.

Keywords: Hepatitis C virus, infertility, *in vitro* fertilization, sperm

Introduction

Hepatitis C virus (HCV) is a common liver pathogen that is a global public health problem affecting over 185 million individuals worldwide (approximately 3% of the world population) [1, 2]. There are approximately 35,000 individuals newly infected with HCV each year [3]. Most HCV cases are subclinical, leading to eventual chronic liver injury that can lead to chronic inflammation of the liver and progressive fibrosis. Further, 25% of patients can develop liver cirrhosis and hepatocellular carcinoma (HCC) [4]. American Association for Study of Liver Diseases [5] predicts HCV associated mortality will continue to increase over the next 20 years causing liver failure and HCC deaths to increase.

In vitro fertilization (IVF) is a common and effective assisted reproductive technology (ART) for infertility treatment. The worldwide increase in HCV infection has in large part contributed to an increase in HCV-seropositive subfertile couples opting for ART treatment. While data exists related to the effect of HBV infection on IVF outcomes [6], this data often overshadows that of HCV infection. Some data exist regarding HCV positive individuals attempting IVF including the HCV infection rate of infants born to HCV positive mothers who had undergone intracytoplasmic sperm injection (ICSI) [7], pregnancy outcomes after IVF with HCV positive men [8], and IVF outcomes between men co-infected with HCV and HIV-1 and men infected only with HIV-1 [9]. Therefore, a need for a more comprehensive body of evidence to assess whether HCV infection is related to IVF outcomes is necessary.

In this study, we analyzed 1424 individuals, a larger samples size compared to previous studies, for the presence of HCV-specific antibodies (HCV-Ab). The subjects were separated into male and female groups and the effect of HCV infection on IVF outcomes was evaluated.

Materials and methods

Subjects

Couples opting for IVF between January 2008 and December 2013 were included in this study. Three groups were established: 90 cou-

Table 1. Demographic data and details of ovarian stimulation

Parameter	Group A	Group B	Group C	*P* value
Male age at cycle start (years)	32.78±5.46	33.00±4.93	33.73±5.71	0.190
Female age at cycle start (years)	31.24±4.81	31.54±4.54	32.39±10.80	0.487
Duration of gonadotropin administration (days)	10 (8-11)	10 (9-12)	11 (9-12)	0.181
Total dose of gonadotropin used (IU)	2025 (1575-3262.5)	2362.50 (1931.25-3300)	2625 (1950-3675)	0.269
Endometrial thickness on the day of hCG administration (mm)	10.7 (9.85-13.1)	11.35 (10.1-12.63)	11.2 (9.7-12.9)	0.841
Endometrial thickness on the day of ET (mm)	11.75±2.30	10.96±2.34	11.35±2.78	0.185
Percentage of oocytes retrieved (%) P_{50} (P_{25}-P_{75})	84 (70.49-95.37)	89.2 (76.44-100)	84.62 (70-100)	0.205

Values are presented as mean ± S.D. or median (25-75 percentile). Abbreviations: hCG = human chorionic gonadotropin; ET = embryo transfer. There were nostatistically significant differences between the 3 groups.

Table 2. Comparison of IVF indications

Causes of subfertility	Group A	Group B	Group C	*P* value
Male	17/90 (18.9%)	19/74 (25.7%)	377/1256 (30.0%)	0.061
Tubal	18/90 (20.0%)	11/74 (14.9%)	257/1256 (20.5%)	
Ovulation disorder	13/90 (14.4%)	8/74 (10.8%)	203/1256 (16.2%)	
Mixed causes	30/90 (33.3%)	25/74 (33.8%)	301/1256 (24.0%)	
Unexplained	12/90 (13.3%)	11/74 (14.9%)	118/1256 (9.4%)	

Values are presented as number/total (%). There were no statistically significant differences between the 3 groups.

ples where the female was HCV positive (group A), 78 couples where the male was HCV positive (group B), and 1256 control couples where both the male and female were HCV negative by seroanalysis and the presence of HCV RNA (group C). Control couples were matched for both age and based on the IVF ovarian hyperstimulation protocol. Any patients with abnormal liver function, chronic hepatitis, or those undergoing antiviral treatment were excluded.

Semen analysis

Semen samples were obtained via masturbation after a minimum of 2 days and a maximum of 7 days of sexual abstinence in accordance with the Laboratory Manual for the Examination and Processing of Human Semen, 5th edition [10]. The sperm concentration, progressive motility (PR) percentage, sperm volume, Normal Sperm Morphology (NSM) percentage and TZI (teratozoospermia index) were assessed. HCV-Ab were detected by ELISA. Briefly, solid-phase antigen was made by purified human HCV-Ab, the sample was added to the enzyme label plate and incubated at 37°C for 30 minutes, and each well was washed 5 times followed by the addition of enzyme, color developing agent and termination liquid. The optical density was then measured at 450 nm. A positive result was only recognized if the OD value was less than the cut off (cut off calculation: average value of negative-control well + 0.15). Four milliliters of venous blood was then added to the anticoagulant blood vessels and serum was separated by centrifugation at 2500 rpm for 10 minutes, FSH was measured by a Roche Cobas41I automatic electrochemiluminescence device.

In vitro fertilization

Indications for IVF included polycystic ovary syndrome (PCOS) and unexplained/mixed factors. Follicle stimulating hormone (FSH) was tested on days 2-4 of the menstrual cycle within 6 months of undergoing IVF. Ovarian hyperstimulation was conducted using the OC + long protocol and final oocyte maturation was triggered with a single intramuscular injection of human chorionic gonadotropin. Transvaginal oocyte retrieval was performed approximately 36 hours later. Embryos were evaluated based on the following parameters: blastomere number, blastomere size, fragment rate and presence of multinucleated blastomeres on day 2. Embryos were considered high quality if they had 4 regular blastomeres and <25% fragmentation. Two embryos were transferred 3 days after oocyte retrieval. The luteal phase was supported by oral progesterone from the day of oocyte retrieval to the day of the pregnancy test. Pregnancies were initially diagnosed by increasing serum hCG levels, tested 14 days after ET (embryo transfer). A clinically recognized pregnancy was confirmed by the pres-

Table 3. Semen parameters from male participants.

Semen parameter	Group A	Group B	Group C	*P* value
Sperm concentration (10^6/mL)	36.10 (20.10-56.70)	28.55 (21.40-85.30)	41.50 (19.50-64.50)	0.380
PR percentage (%)	30.95 (22.05-37.30)	18.85 (7.80-39.85)	29.25 (19.15-37.00)	0.142
Volume (mL)	3.50 (3.00-4.00)	2.80 (2.50-3.50)	3.30 (2.40-4.40)	0.195
NSM percentage (%)	15.00 (11.00-20.00)	12.00 (10.00-15.50)	12.00 (8.00-16.50)	0.640
TZI	1.29 (1.34-1.41)	1.33 (1.29-1.48)	1.33 (1.27-1.40)	0.970

Values are presented as median (25-75 percentile). Abbreviations: PR = progressive motility; NSM = Normal Sperm Morphology; TZI = teratozoospermia index. There were no statistically significant differences between the 3 groups.

Table 4. IVF outcomes

Parameter	Group A	Group B	Group C	*P* value
Fertility rate (%)	76.93±19.18	80.99±19.95	78.41±19.73	0.413
2pn rate (%)	63.65±20.32	69.68±19.13	67.26±32.33	0.438
Cleavage rate (%)	92.79±17.09	92.86±18.59	95.21±12.20	0.093
High-quality embryo rate (%)	66.67 (36.93-100.00)	73.23 (44.01-100.00)	70.7 (50.00-100.00)	0.649
Clinical pregnancy rate (%)	41/90 (45.6%)	36/74 (48.6%)	691/1256 (55.0%)	0.138
Miscarriage rate %	21/90 (23.3%)	14/74 (18.9%)	239/1256 (19.0%)	0.605

Values are presented as mean ± S.D. or median (25-75 percentile) or number/total (%). Abbreviations: 2pn = two-pronuclear. The data in the high-quality embryo rate was significantly lower in seropositive women group with the *P* value of 0.039

ence of a gestational sac on abdominal ultrasound examination during week.

Common Calculations

IVF fertilization rate = no. of oocytes fertilized/no. of oocytes obtained ×100%; IVF 2pn rate = no. of 2pn oocytes/no. of oocytes obtained ×100%; cleavage rate = no. of zygote cleavages/no. of normal fertilized oocytes ×100%; high-quality embryo rate = no. of high-quality embryos/no. of embryo ×100%; pregnancy rate = no. of pregnancies/no. of periodicities; miscarriage rate = no. of miscarriage/no. of periodicities.

Statistical analysis

Statistical calculations were performed using SPSS 17.0 statistical package for Windows. The Kolmogorov Smirnov test was used to analyze the normal distribution of continuous variables. Continuous variables were given as mean ± standard deviation if normally distributed and as median and range *P*25-*P*75 if not normally distributed. One-way ANOVA was applied to compare data between 3 groups. Non-parametric analysis was performed using Wilcoxon rank sum (Mann-Whitney) test and all categorical variables were analyzed using chi-squared tests as appropriate. Binary logistic regression was performed to assess the contributions of HCV infection status predicting variables on clinical pregnancy and abortion rates.

Results

During the study period, 1424 couples undergoing IVF treatments were included in the analysis. The demographic data and comparison of IVF indications from participants in all three groups are shown in **Tables 1** and **2**. Several similarities were noted among the positive groups and the negative groups, including age, ovarian responses (total gonadotropin used), the duration of gonadotropin administration, endometrial thickness on the day of hCG administration and ET. When comparing the group with seropositive men versus the control, no differences were observed in sperm parameters including concentration (none had azoospermia in either group), PR differences, volume, NSM percentage and TZI (**Table 3**). We next investigated the effect of HCV infection on pregnancy outcomes and found no differences on pregnancy rates per cycle (**Table 4**).

Discussion

HCV infection is characterized by its chronic nature, resulting in not only chronic liver disease, but also extra-hepatic manifestations including HCC [3]. Any chronic condition poses

a concern for couples planning pregnancy, and his is especially a concern among clinicians facilitating assisted reproduction procedures. It was initially shown that no significant differences were recorded in fertilization, pregnancy rates, obstetric or neonatal results for HCV seropositive patients undergoing IVF [11]. Further, Prisant et al. found clinical pregnancy rates for HCV infertile seropositive couples was not significantly different from that for seronegative controls [12]. Our study showed there were no differences in fertilization rate, 2pn rate, cleavage rate and pregnancy rates. In contrast, Pirwany et al. showed that HCV positive couples had lower implantation and pregnancy rates [13]. However, the samples size of this study was very small and there was no indication to whether the male or the female contributed to the HCV seropositivity of the couple. In a retrospective study, Li et al., found no differences in high-quality embryo rate but an increase in early abortion rate and total abortion rate [14], while Hanafi et al [15] showed there were no differences in embryo cleavage or morphology but did find a significantly reduced pregnancy rate between the in HCV infected couples. However, both of these studies included only limited control subjects.

HCV receptors are found not on the oocyte itself, but rather on granulosa cells surrounding the oocytes [16], and since HCV is an RNA virus, it cannot integrate into the host genome to potentially cause chromosomal instability like HBV [17, 18]. Some studies suggest HBV infection has no effect on IVF outcomes [19]. Additionally, for HCV, many studies have denied the possible risk of sperm/oocyte-HCV transmission during ART [20, 21]. Further supporting this point, spent culture media used after ovum pickup or embryo culture and in liquid nitrogen used for oocyte or embryo vitrification in HCV positive IVF patients could detect no HBV, HCV, or HIV-1 transcripts [22]. The HCV RNA in those samples would likely to be a result of blood contamination during the surgical procedure, like ovarian puncture or testicular sperm extraction (TESE) and microsurgical epididymal sperm aspiration (MESA) [23]. Furthermore, it seems widely accepted that the washing protocol was effective for HCV risk reduction and concerns about the transmission of virus to the newborn through gametes or embryos seemed unfounded [11, 24].

HCV causes abnormal liver function, which can induce improper hormone levels and altered metabolism [5], some studies noted these hormonal disturbances may lead to poor ovarian response to stimulation and in turn have a negative impact on ART outcomes [25]. Nevertheless, IVF patients with abnormal liver function are taking a risk in ovarian aspiration and pregnant procedures and should always be pre-treated with anti-viral therapy before IVF cycles. Our data show no statistical difference between gonadotropin used between the HCV positive and negative groups. However, all female subjects in the study did have regular liver function. It is then possible that HCV positive women with regular liver function were in early liver disease stages and therefore had normal hormone metabolism levels and ovarian responses.

HCV is a blood-borne viral pathogen whose subtypes vary in different parts of the world [26]. HCV sexual transmission is more common in patients also infected by HIV and studies have focused on the influence of HCV/HIV coinfection on IVF outcomes [27, 28]. In China, fewer individuals are co-infected with HCV and HIV. It is not clear now if different HCV genotypes or route of infection have same effect on gamete or IVF outcomes, but there are inconsistencies among genotypes in their pathogenesis and clinical manifestations. For example, genotypes 1b and 2a are the most common in China and 1b has less pathogenic outcomes, but a higher correlation with liver cancer [2]. There is closer relationship between 1b genotype and liver cancer. Furthermore, the presence of HIV virus in semen could be a bias for the influence of HCV [7] and none of the former studies included a control group.

Our study demonstrating that HCV infection has no effect on the pregnancy outcomes of IVF cycles. While observing the embryo morphology could be considered subjective, we took every step to ensure consistency in our study. In addition, the number of infected women in our study was more robust than previous studies but this area still requires further study with a larger sample size. The correlation between HCV infection and the embryo quality is interesting, but the mechanism remains unclear. Further studies are urgently needed to confirm these findings and to understand the molecular

mechanisms responsible for the effect of HCV infection on reproductive performance and pregnancy outcomes.

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Disclosure of conflict of interest

None.

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References

[1] Yip B, Wantuck JM, Kim LH, Wong RJ, Ahmed A, Garcia G, Nguyen MH. Clinical presentation and survival of Asian and non-Asian patients with HCV-related hepatocellular carcinoma. Dig Dis Sci 2014; 59: 192-200.

[2] Piao HX, Yang AT, Sun YM, Kong YY, Wu XN, Zhang YZ, Ding B, Wang BE, Jia JD, You H. Increasing newly diagnosed rate and changing risk factors of HCV in Yanbian Prefecture, a high endemic area in China. PLoS One 2014; 9: e86190.

[3] Lin H, Ha NB, Ahmed A, Ayoub W, Daugherty TJ, Lutchman GA, Garcia G, Nguyen MH. Both HCV and HBV are major causes of liver cancer in Southeast Asians. J Immigr Minor Health 2013; 15: 1023-1029.

[4] Ahmad J, Eng FJ, Branch AD. HCV and HCC: clinical update and a review of HCC-associated viral mutations in the core gene. Semin Liver Dis 2011; 31: 347-355.

[5] Ghany MG, Nelson DR, Strader DB, Thomas DL, Seeff LB; American Association for Study of Liver Diseases. An update on treatment of genotype 1 chronic hepatitis C virus infection: 2011 practice guideline by the American Association for the Study of Liver Diseases. Hepatology 2011; 54: 1433-1444.

[6] Shi L, Liu S, Zhao W, Zhou H, Ren W, Shi J. Hepatitis B virus infection reduces fertilization ability during in vitro fertilization and embryo transfer. J Med Virol 2014; 86: 1099-1104.

[7] Chu MC, Pena JE, Nakhuda GS, Thornton MH 2nd, Sauer MV. Assessing the reproductive performance of men co-infected with HIV-1 and hepatitis C undergoing assisted reproduction. Arch Gynecol Obstet 2006; 274: 155-159.

[8] Juniastuti, Utsumi T, Nasronudin, Alimsardjono L, Amin M, Adianti M, Yano Y, Soetjipto, Hayashi Y, Hotta H, Lusida MI. High rate of seronegative HCV infection in HIV-positive patients. Biomed Rep 2014; 2: 79-84.

[9] Operskalski EA, Kovacs A. HIV/HCV coinfection: pathogenesis, clinical complications, treatment, and new therapeutic technologies. Curr HIV/AIDS Rep 2011; 8: 12-22.

[10] World Health Organization. Chapter 2: Standard procedures. In WHO Laboratory Manual for the Examination and Processing of Human Semen. 5th editon. Geneva: World Health Organization; 2010. pp. 45-86.

[11] Molina I, Carmen De Gonzalvo M, Clavero A, Angel López-Ruz M, Mozas J, Pasquau J, Sampedro A, Martínez L, Castilla JA. Assisted reproductive technology and obstetric outcome in couples when the male partner has a chronic viral disease. Int J Fertil Steril 2014; 7: 291-300.

[12] Prisant N, Tubiana R, Lefebvre G, Lebray P, Marcelin AG, Thibault V, Rosenblum O, Bonmarchand M,Vauthier-Brouzes D, Golmard JL, Katlama C, Poirot C. HIV-1 or hepatitis C chronic infection in serodiscordant infertile couples has no impact on infertility treatment outcome. Fertil Steril 2010; 93: 1020-1023.

[13] Pirwany IR, Phillips S, Kelly S, Buckett W, Tan SL. Reproductive performance of couples discordant for hepatitis B and C following IVF treatment. J Assist Reprod Genet 2004; 21: 157-161.

[14] Li J, Xu LN, Zhou CQ. Effect of C hepatitis on outcome of in vitro fertilization and embryo transfer. Chin J Clinicians (electronic version) 2008; 2: 1365.

[15] Hanafi NF, Abo Ali AH, Abo el kheir HF. ICSI outcome in women who have positive PCR result for hepatitis C virus. Hum Reprod 2011; 26: 143-147.

[16] LaVoie HA, Garmey JC, Day RN, Veldhuis JD. Concerted regulation of low density lipoprotein receptor gene expressionby follicle-stimulating hormone and insulin-like growth factor I in porcinegranulosa cells: promoter activation, messenger ribonucleic acid stability, and sterol feedback. Endocrinology 1999; 140: 178-186.

[17] Zhou XP, Hu XL, Zhu YM, Qu F, Sun SJ, Qian YL. Comparison of semen quality and outcome of assisted reproductivetechniques in Chinese men with and without hepatitis B. Asian J Androl 2011; 13: 465-469.

[18] Huang JM, Huang TH, Qiu HY, Fang XW, Zhuang TG, Liu HX, Wang YH, Deng LZ, Qiu JW. Effects of hepatitis B virus infection on human sperm chromosomes. World J Gastroenterol 2003; 9: 736-740.

[19] Chen H, Ge HS, Lv JQ, Wu XM, Xi HT, Huang JY, Zhu CF. Chronic hepatitis B virus infection in women is not associated with IVF/ICSI outcomes. Arch Gynecol Obstet 2014; 289: 213-217.

[20] Briat A, Dulioust E, Galimand J, Fontaine H, Chaix ML, Letur-Könirsch H, Pol S, Jouannet P, Rouzioux C,Leruez-Ville M. Hepatitis C virus in the semen of men coinfected with HIV-1: prevalenceand origin. AIDS 2005; 19: 1827-1835.

[21] Savasi V, Oneta M, Parrilla B, Cetin I. Should HCV discordant couples with a seropositive male partner be treatedwith assisted reproduction techniques (ART)? Eur J Obstet Gynecol Reprod Biol 2013; 167: 181-184.

[22] Cobo A, Bellver J, de los Santos MJ, Remohí J. Viral screening of spent culture media and liquid nitrogen samples of oocytes and embryos from hepatitis B, hepatitis C, and human immunodeficiency virus chronically infected women undergoing *in vitro* fertilization cycles. Fertil Steril 2012; 97: 74-78.

[23] Leruez-Ville M, Thiounn N, Poirot C, Launay O, Sogni P, Grabar S, Dulioust E. Intracytoplasmic sperm injection with microsurgically retrieved spermatozoa in azoospermic men infected with human immunodeficiencyvirus 1 or hepatitis C virus: the EP43 AZONECO ANRS study. Fertil Steril 2013; 99: 713-717.

[24] Pasquier C, Souyris C, Moinard N, Bujan L, Izopet J. Validation of an automated real-time PCR protocol for detection and quantitation of HIV and HCV genomes in semen. J Virol Methods 2006; 137: 156-159.

[25] Englert Y, Moens E, Vannin AS, Liesnard C, Emiliani S, Delbaere A, Devreker F. Impaired ovarian stimulation during *in vitro* fertilization in women who areseropositive for hepatitis C virus and seronegative for humanimmunodeficiency virus. Fertil Steril 200; 88: 607-611.

[26] Nie HM, Chen JJ, Wang R, Wang CB, Dong HL, Chen YY. Genotypes distribution of hepatitis C virus through multi-center, largesample studies among chronic hepatitis C patients in Chinese Hanpopulation. Zhonghua Liu Xing Bing Xue Za Zhi 2012; 33: 501-504.

[27] Nesrine F, Saleh H. Hepatitis C virus (HCV) status in newborns born to HCV positive women performing intracytoplasmic sperm injection. Afr Health Sci 2012; 12: 58-62.

[28] Levy R, Bourlet T, Maertens A, Salle B, Lornage J, Laurent JL, Pozzetto B, Guerin JF. Pregnancy after safe IVF with hepatitis C virus RNA-positive sperm. Hum Reprod 2002; 17: 2650-2653.