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-
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Table 1. Demographic data and details of ovarian stimulation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male age at cycle start (years)</td>
<td>32.78±5.46</td>
<td>33.00±4.93</td>
<td>33.73±5.71</td>
<td>0.190</td>
</tr>
<tr>
<td>Female age at cycle start (years)</td>
<td>31.24±4.81</td>
<td>31.54±4.54</td>
<td>32.39±10.80</td>
<td>0.487</td>
</tr>
<tr>
<td>Duration of gonadotropin administration (days)</td>
<td>10 (8-11)</td>
<td>10 (9-12)</td>
<td>11 (9-12)</td>
<td>0.181</td>
</tr>
<tr>
<td>Total dose of gonadotropin used (IU)</td>
<td>2025 (1575-3262.5)</td>
<td>2362.50 (1931.25-3300)</td>
<td>2625 (1950-3675)</td>
<td>0.269</td>
</tr>
<tr>
<td>Endometrial thickness on the day of hCG administration (mm)</td>
<td>10.7 (9.85-13.1)</td>
<td>11.35 (10.1-12.63)</td>
<td>11.2 (9.7-12.9)</td>
<td>0.841</td>
</tr>
<tr>
<td>Endometrial thickness on the day of ET (mm)</td>
<td>11.75±2.30</td>
<td>10.96±2.34</td>
<td>11.35±2.78</td>
<td>0.185</td>
</tr>
<tr>
<td>Percentage of oocytes retrieved (%) P_{25}–P_{75}</td>
<td>84 (70-95.37)</td>
<td>89.2 (76.44-100)</td>
<td>86.42 (70-100)</td>
<td>0.205</td>
</tr>
</tbody>
</table>

Values are presented as mean ± S.D. or median (25-75 percentile). Abbreviations: hCG = human chorionic gonadotropin; ET = embryo transfer. There were no statistically significant differences between the 3 groups.

Table 2. Comparison of IVF indications

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

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

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 hyper-stimulation 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-
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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 P25-P75 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...
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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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