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EDITORIAL

Viral Hepatitis and Assisted Reproduction

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Abstract Infertility treatment in couples where one or both parents are infected with hepatitis raises many concerns about transmission of the infection to the baby, laboratory technicians, and medical staff, and contamination of other gametes/embryos that are from virus-free parents in the same laboratory. Exposure to the other partner is only a risk when the couple's hepatitis status is discordant. The management of infertility, in association with HBV or HCV, has sparked debates about the potential risk of spread of infection to virus-free individuals, embryos, and/or semen. This risk can only be minimized or eliminated by the use of SOPs for safety in fertility clinics and by the use of proper initial detection and segregation of potentially hazardous materials. HBV may interfere with the development of embryos and even result in abortion and other adverse outcomes. Although sexual transmission of HCV is very low, in subfertile or infertile couples sperm washing should be used to treat HCV-positive semen before ART. Testing for HBsAg and HCV should be offered to high-risk infertile couples seeking fertility therapy to reduce the potential risk of transmission to an uninfected partner, baby, staff members, and disease-free gametes and embryos in the same laboratory. Testing for HIV, HBsAg,

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and HCV status should be performed on the couple prior to cryopreservation of semen or embryos.

Keywords Viral hepatitis · IVF · ART · Assisted reproduction

Introduction

Viral hepatitis is a term commonly used for several clinically similar, yet etiologically and epidemiologically distinct, diseases. Seven human hepatitis viruses have been identified. Hepatitis A, B, C, and E are endemic in India; Hepatitis D is rarely reported in India; Hepatitis F has not been confirmed as a distinct genotype; and Hepatitis G is a newly described flavivirus. HBeAg positivity is associated with a higher rate of transmitting infection in chronic HBsAg carriers. HCV is a blood-borne RNA virus that is transmitted through percutaneous exposures to blood (transfusions, transplants), needle sticks, or the contamination of supplies shared among hemodialysis patients or IV drug abusers. HCV RNA has also been detected in saliva, urine, breast milk, semen, and menstrual fluid. Therefore, both sexual and vertical transmissions have been suggested as alternative modes for transmission of HCV [1]. Infertility treatment in couples where one or both parents are infected with hepatitis raises many concerns about transmission of the infection to the baby, laboratory technicians, and medical staff, and contamination of other gametes/embryos that are from virus-free parents in the same laboratory. Exposure to the other partner is only a risk when the couple's hepatitis status is discordant. For couples where one partner is HBsAg positive, the best option is HBV vaccination to prevent transmission. Since 95 % of patients will seroconvert after vaccination, physicians will rarely see a patient in which the patient and partner are at risk of transmission. With HCV, however, the risks are different, as no vaccine for HCV is currently available.

The risk of viral transmission in assisted reproduction is still a much-debated issue, especially for hepatitis C virus (HCV). HCV is a common causative agent for parenterally transmitted viral hepatitis. In addition, it has been incriminated in other routes of transmission, including sexual transmission and nosocomial infections. The management of infertility, in association with HCV, has sparked debates about the potential risk of spread of infection to virus-free individuals, embryos, and/or semen. Ignorance about worldwide-accepted screening policies has helped to fuel this debate. Today, it is evident that there is a potential risk of spread of HCV through biological fluids, including semen, to other non-infected individuals. This risk can only be minimized or eliminated by the use of SOPs for safety in fertility clinics and by the use of proper initial detection and segregation of potentially hazardous materials. Techniques and protocols have been established to help the andrologist and embryologist to safeguard patients against such dangers and should be followed stringently in all centers [2]. Chronic viral infections can infect sperm and are considered a risk factor in male infertility. Recent studies have shown that the presence of HIV, HBV, or HCV in semen impairs sperm parameters, DNA integrity and in particular reduces forward motility [3]. Besides the risk of horizontal or vertical transmission, the negative impact of any viral sperm infection on male reproductive function seems to be dramatic. In addition, treatment with antiviral and antiretroviral therapies may further affect sperm parameters.

Discussion

Data are both limited and controversial on the transmission of hepatitis virus during assisted reproduction. Transmission of HBV and HCV is the main areas of research, and limited information is now available on HDV and HGV. Concerns over laboratory/nosocomial infection in assisted reproduction clinics have been of great concern since the publication of a case report that described the transmission of HCV from an infected patient undergoing IVF to two non-infected patients undergoing IVF within the same clinic during the same time period [4–6]. These cases led to the development of additional regulations for ART in hepatitis patients in 2001 and the emergence of "viral risk" laboratories in France where serodiscordant couples (HCV-positive men and HCV-negative women) undergo ART. In addition, the transmission of HBV from HBV-contaminated cryopreserved bone marrow samples to HBV-negative cryopreserved bone marrow samples has raised significant concerns for transmission of HBV in cryopreserved semen samples and embryos [7].

Whether hepatitis B virus (HBV) infection impairs human infertility is unclear. Gong et al. [8] set up a study to investigate the malondialdehyde (MDA) level and paraoxonase-1 (PON-1) activity in the serum and seminal plasma of infertile men with chronic viral hepatitis and their influence on the semen parameters of the patients. The MDA level was significantly higher, but the PON-1 activity remarkably lower in the serum and seminal plasma of the infertile males with chronic viral hepatitis than in the healthy controls and infertile patients ($P < 0.01$ or $P < 0.05$). Total sperm motility and sperm survival rate were significantly lower, while the sperm DFI markedly higher in the former than in the latter two groups ($P < 0.01$ or $P < 0.05$). No statistically significant difference was found among the three groups in sperm concentration

($P > 0.05$). The WBC counts in the semen of the infertile and infertile with chronic viral hepatitis groups were significantly higher than that in the health controls ($P < 0.05$). The MDA level and PON-1 activity in the seminal plasma were positively correlated with those in the serum in the infertile males with chronic viral hepatitis ($r = 0.57$ or 0.48, $P < 0.01$). Virus-induced chronic active hepatitis enhances oxidative stress in the reproductive system, aggravates sperm damage, and affects sperm quality parameters [8]. In Su et al.'s paper [9], the incidence of infertility was 1.59 times higher in patients with HBV infection than in those without HBV infection (2.21 vs. 1.39 per 1000 person-years). The risk of developing infertility remained significant among patients with HBV infection (hazard ratio 1.52, 95 % CI 1.20–1.92) after adjusting for covariates in a multivariate Cox proportional hazards model. Their data showed an increased incidence and risk of infertility among men with HBV infection compared with men without HBV [9].

Savasi et al. [10] studied the results of reproductive assistance with sperm washing in HCV-discordant couples, all treated in a single center, including the serological status of mothers and babies, and the outcome of the pregnancies. Thirty-five HCV-serodiscordant infertile couples with an HCV viremic positive male partner were enrolled. All of them completed the immuno-virological and fertility triage and were treated according to our clinical protocols. Forty-seven superovulation and IUI and 38 second-level ART procedures are reported. The pregnancy rates for IUI and ICSI are similar to those reported by the Italian ART register. All the 85 sperm samples were treated with sperm washing technique to reduce HCV in semen and the possible risk of transmission. They did not observe any preterm delivery or negative perinatal outcome. No mothers or babies were infected by HCV. This was the biggest prospective study conducted in a single center involving HCV-discordant infertile couples in an ART program. Although sexual transmission of HCV is very low, in subfertile or infertile couples sperm washing should be used to treat HCV-positive semen before ART. The authors suggested that it is not necessary to perform nested PCR to detect HCV RNA in the final swim-up [10].

In a retrospective case-controlled study, Shi et al. [11] investigated the impact of HBV on sperm parameters, ovarian stimulation, and outcomes of in vitro fertilization (IVF) and embryo transfer. A total of 224 couples with at least one partner being HBsAg-seropositive undergoing their first IVF and embryo transfer cycle were identified, which included 77 couples with female partners being HBsAg-seropositive, 136 couples with male partners being HBsAg-seropositive, and 11 couples with both partners being HBsAg-seropositive. A total of 448 both HBsAg-seronegative couples served as controls. The percentage of normal sperm morphology was significantly lower in HBsAg-seropositive male partners than that in HBsAg-seronegative male partners (11.9 ± 9.4 % vs. 19.0 ± 11.9 %, $P < 0.01$). The duration of infertility was significantly prolonged in HBV-seropositive patients compared with HBV-seronegative patients (4.9 vs. 4.1 years, $P < 0.01$). Couples with female partners being HBsAg-seropositive had significantly lower top-quality embryo rate than control group (22.4 vs. 31.6 %, $P < 0.01$). In addition, the fertilization rates in groups with male or female partners being HBsAg-seropositive were both significantly lower than the matched controls (80.2 vs. 82.8 %, $P < 0.05$; 76.6 vs. 84.3 %, $P < 0.01$, respectively). HBV infection was also found to be associated negatively with fertilization rate by logistic regression analysis (ORs 0.410, 95 % CI 0.186–0.906, $P < 0.05$). However, there was no significant difference in clinical pregnancy rates between HBsAg-seropositive and HBsAg-seronegative group. These results suggest that chronic HBV infection is likely to represent a significant cause of infertility [11].

Absence of ovarian response was statistically significantly higher for HCV-seropositive women compared with controls (10/42 vs. 5/84 cycles, respectively) [12]. For cycles with oocyte retrieval, HCV-seropositive women required more gonadotropin units compared with controls. The maximum estradiol levels and number of collected oocytes were similar, but HCV-seropositive women had statistically significantly fewer embryos available compared with controls. Embryo morphologic features, number of transferred embryos, and rates of implantation and pregnancy were similar for HCV-seropositive women and controls. The study summarized that compared with matched uninfected controls, HCV-seropositive women display a decreased ovarian response [12].

There has been controversy about the effect of hepatitis B virus (HBV) infection on pregnancy outcome after IVF treatment. A total of 1676 couples undergoing their first IVF cycle were included in Lee et al.'s study [13]. This study was the first report on the live-birth rate of hepatitis B (HBV)-seropositive couples, revealing the effect on the pregnancy outcome of IVF.

The prevalence of HBV infection in the female partners of the subfertile population seeking treatment with assisted reproductive technology was 7.8–9.6 % during the study period. The ongoing pregnancy rate was not significantly different between couples with HBV-seropositive wives and seronegative ones (26.7 vs. 30.2 %). The ongoing pregnancy rate and the live-birth rate of couples with both partners being HBV surface antigen positive was not significantly different from couples with discordant HBV serostatus and those couples with both partners being HBV surface antigen negative (23 vs. 29 vs. 30 %, respectively;

23 vs. 27 vs. 27 %, respectively). The percentage of normal sperm morphology in HBV-seropositive husbands was significantly lower than that of seronegative counterparts (5.0 vs. 10.0 %, $P = 0.009$). The study concluded that there was no adverse effect of HBV infection on the assisted reproduction outcomes [13].

The aim of Papaxanthos–Roche's study [14] was to investigate the susceptibility of human oocytes from hepatitis C virus (HCV) RNA-positive women to HCV contamination during assisted reproductive technology (ART). A reverse transcriptase PCR assay was used to test for the presence of HCV RNA associated with 24 unfertilized oocytes 48 h after follicular fluid aspiration in 10 IVF attempts (seven conventional IVF and three ICSI). Negative and positive controls (10 unfertilized oocytes from HCV-negative women and 20 unfertilized oocytes artificially contaminated with HCV RNA-positive plasma; HCV RNA was also quantified in plasma and follicular fluid) were included. HCV RNA was associated with 17/24 (70.8 %) oocytes (6/7 after ICSI and 11/17 after conventional IVF) and was found in 19/20 (95 %) follicular fluid samples. A weak correlation was found between plasma and follicular fluid HCV RNA loads ($r = 0.73$, $P < 0.001$). HCV associated with unfertilized oocytes surrounded by their intact zona pellucida from anti-HCV antibody-positive and viraemic women undergoing ART raises questions concerning the safe management of medically assisted procreation for these women and good practice of oocyte/embryo cryopreservation and donation [14].

The aim of Devaux et al.'s study [15] was to: (a) search for HCV RNA in FF and in culture media at each step of IVF undergone by HCV(+) women; (b) investigate the impact of blood contamination of FF induced by vascular injury associated with transvaginal ovarian puncture; (c) assess risk of the embryo and the impact on the contamination rate of the newborn; and (d) determine the viral risk presented by these fluids in order to define guidelines for the laboratory. FF from 22 IVF procedures performed in 17 HCV(+) women were classified as either clear, lightly bloody or bloody FF. Oocytes from each FF were washed and incubated in separated fertilization media. At 20 h after puncture (day 1), the fertilized oocytes were washed and transferred to fresh media until embryo transfer. HCV RNA was detected and quantified in FF and media using Cobas Amplicor and Cobas Monitor HCV RNA kits. HCV RNA was positive in 39 of 44 FF samples, and viral loads increased with blood contamination. At day 1, after rinsing of oocyte–cumulus complexes, only 8 of 44 media were still positive. Viral loads were quantified in 5 of 8 positive media and were below the test sensitivity threshold in 4 of 5 HCV RNA-positive media and just above it in the fifth medium. The day of transfer HCV RNA was undetectable in all media. HCV RNA was detected in 89 % of FF irrespective of the degree of blood contamination and in 25 % of the media at day 1. FF must be considered as potentially infected. Blood contamination increases HCV load in the FF. Rinsing oocytes seems significantly to discard the HCV RNA. After counseling, attempting IVF in HCV(+) women is justified. Universal guidelines prevent nosocomial infection, and IVF does not specifically increase the professional risk [15].

Chu et al. [16] assessed the reproductive performance of men co-infected with hepatitis C virus (HCV) and human immunodeficiency virus (HIV-1) undergoing assisted reproduction. They reviewed 217 consecutive ART cycles performed on 106 HIV-1 serodiscordant couples between August 1997 and March 2004, in which 28 men (26 %) were seropositive for HCV and HIV-1 (group 1). Co-infected men and their partners were of similar age as men infected only with HIV-1. Comparing group 1 to group 2, like values were noted for HIV-1 viral loads [1993 ± 1140 copies/ml (mean ± SE) vs. 1659 ± 487 copies/ml]; CD4 counts (520 ± 98 vs. 604 ± 38 mm^{-3}); and semen parameters. IVF performance and outcomes were similar, with fertilization rate (0.68 ± 0.03 vs. 0.71 ± 0.02); number of normally cleaving embryos (6.0 ± 0.5 vs. 5.3 ± 0.3); embryo implantation rate (0.27 ± 0.04 vs. 0.2 ± 0.02); and clinical pregnancy rate (40 vs. 29 %). Although the male mortality rate was low in both groups, morbidity among co-infected men was significantly higher. Seven of 28 men (25 %) had detectable HCV viral loads, and 14 (50 %) had elevated liver function tests. Men co-infected with HCV and HIV-1 do reasonably well undergoing ART to prevent transmission of viruses to their partners and children [16].

Ali et al. [17] set up a study to detect the expression of hepatitis B virus (HBV) genes (HB S and C genes) in early embryonic cells after introducing motile human sperm-carrying HBV DNA into zona-free hamster oocytes via the in vitro fertilization (IVF) technique (XX). Polymerase chain reaction (PCR) was used to detect HBS and pre-core/core (pre-C/C) coding genes both in one- and two-cell embryos. Reverse transcription PCR (RT-PCR) analysis was used to study the expression of the two genes. Fluorescence in situ hybridization (FISH) analysis using the full-length HBV DNA as the hybridization probe was performed to confirm the integration of viral DNA in the host embryonic genome. Both HB S and pre-C/C coding genes were present and transcribed in one- and two-cell embryos originating from hamster ova IVF with human spermatozoa-carrying HBV DNA sequences. The study concluded that sperm-mediated HBV genes are able to replicate and express themselves in early embryonic cells [17]. These results provide direct evidence that HBV DNA could transmit vertically to the next generation via the male germ line.

Ye et al. [18] investigated father-to-infant transmission of hepatitis B virus (HBV) by detecting HBV mRNA in the IVF embryos with paternal HBV infection. They collected 18 discarded IVF embryos (nine cases) with paternal chronic HBV infection and detected HBV mRNA in the embryos by single-cell RT-PCR. HBV mRNA-positive signals were found in 1 of the 18 embryos with paternal serum HBV-positive markers (5.6 %), but no specific HBV mRNA signals were observed in the 84 embryos of the negative control group. Follow-up visits revealed no significant difference between the experimental and negative control groups either in the rate of clinical pregnancy ($P > 0.05$) or in that of early abortion ($P > 0.05$). The IVF embryo with paternal HBV mRNA-positive signals was successfully implanted, but early abortion occurred. HBV infection was not transmitted to progeny in either of the two groups. The positive results of HBV mRNA indicate that HBV can get into early cleavage embryos through sperm and replicate there, which may be the main channel of father-to-infant transmission. HBV may interfere with the development of embryos and even result in abortion and other adverse outcomes [18].

A retrospective cohort study of 25 IVF-ET cycles in HBV- and HCV-discordant couples was published by Pirwany et al. [19]. Thirteen patients in the study cohort were discordant for HBV (10 males and 3 females) and 12 (9 males and 3 females) for HCV. Twenty-seven consecutive age-matched patients comprised the control group. All patients underwent controlled ovarian hyperstimulation using the long down-regulation protocol followed by IVF or ICSI. Despite comparable response to COH, and similar fertilization, and cleavage rates in the three groups, couples discordant for HBV or HCV had significantly poorer implantation and pregnancy rates (7.7, 0 % respectively) compared with controls (41 %) [19].

Leruez-Ville et al. [20] evaluated the viral contamination of sperm obtained after testicular sperm extraction (TESE) and microsurgical epididymal sperm aspiration (MESA) in men with azoospermia and human immunodeficiency virus (HIV) or hepatitis C virus infection. This prospective study included six men with azoospermia: two HIV-1 infected with undetectable blood viral load and four HCV infected with detectable blood viral load. Gradient supernatants and testis tissues tested HCV RNA-positive in all cases, while processed spermatozoa always tested negative. Gradient supernatants, testis tissues, and processed spermatozoa tested HIV-1 RNA negative. HIV-1 DNA was detectable in one testis tissue. All female partners tested HCV or HIV negative after ICSI. Density gradient and washing suppressed virus detection in final suspensions of testicular and epididymal spermatozoa. ICSI after MESA or TESE appears to be feasible and could be offered in azoospermic men infected by HCV or HIV [20].

Over a 25-month period, six multiply transfused patients undergoing cytotoxic treatment for hematological or other malignant disorders developed icteric acute hepatitis B virus (HBV) infection [7]. Bone marrow or peripheral blood stem cells had been harvested from all six patients and stored in the same cryopreservation tank for possible future transplantation. Human DNA, HBsAg, and HBV DNA with sequences identical to those from four patients with related infections were subsequently found in the liquid nitrogen. Leakage of the cryopreservation bags used to store bone marrow harvested from the first patient when acutely infected with HBV led to contamination of the tank and its contents with HBV and subsequent transmission to patients after transplantation [7]. This incident reinforces the requirement for primary containers used to cryopreserve human tissue to be sealed in a way that prevents exchange of material between the specimen and the liquid nitrogen.

Cobo's study assessed the presence of viral RNA or DNA sequences in spent culture media used after ovum pickup (OPU) or embryo culture and in liquid nitrogen (LN) used for oocyte or embryo vitrification in patients seropositive for human immunodeficiency virus (HIV), hepatitis C virus (HCV), and hepatitis B virus (HBV) undergoing IVF cycles [21]. The study included 24 women who underwent controlled ovarian stimulation for oocyte vitrification or IVF/ET. A total of 6, 11, and 6 patients were seropositive for HIV, HCV, and HBV, respectively, whereas 1 patient showed a co-infection with HCV and HBV. Seven patients presented positive blood viral load (HIV, $n = 1$; HBV, $n = 1$; HCV, $n = 5$). Sixty-three samples were analyzed: follicular fluid, $n = 3$; spent culture media, $n = 33$ (20 after OPU and 13 after embryo culture); and LN, $n = 27$ (14 and 10 after oocyte and embryo vitrification; and 3 LN storage tank samples). All the samples analyzed tested negative for the detection of viral RNA or DNA sequences. The authors have not detected viral sequences after culture and vitrification of oocytes/embryos from HIV-, HBV-, and HCV-seropositive patients. These findings represent good evidence of the lack of risk of cross-contamination among seropositive patients, even using an open device for vitrification [21].

Recent Advances

Hepatitis delta virus (HDV) is a satellite of HBV and needs the latter's envelope for its morphogenesis and propagation. An estimated 5–20 % of HBV-infected patients are also infected with HDV. Mansour et al. [22] investigated

the presence of HDV markers in the FF or in the semen of two HDV-infected patients. Two unrelated HDV-infected patients, a woman and a man pursuing in vitro fertilization (IVF), participated in this study. FF was collected after analysis of oocyte retrieval. The supernatant of seminal plasma (SP) and the final pellet (FP) were fractionated from freshly ejaculated semen. Serological and molecular markers of HDV infection were searched for in these different samples. The woman was infected with an HDV-7 genotype strain, and her HDV plasma viral load (VL) was 6 log copies/mL. HDV antibodies and RNA were also detected in the FF; however, the RNA VL value there was lower by more than 4 log. The man was infected with an HDV-1 strain, and his plasma VL was 6.7 log copies/ml. Total anti-HDV antibodies were positive in the serum, in the SP and in the FP, while IgM were detected only in the serum. However, HDV RNA was negative in the SP and in the FP. The study summarized that HDV markers can be found in the follicular fluid or in the semen of infected patients [22].

Conclusions

Transmission of viral hepatitis in assisted reproduction is possible, but the magnitude of the risk is unknown. HBV is of particular interest to the obstetrician–gynecologist because of the risk of transmission to the partner and the fetus. Healthcare workers, including nurses, are well recognized as being at occupational risk of HBV infection. Further studies are needed to better define the risk of transmission of hepatitis in cryopreserved semen samples, cryopreserved embryos, or gradient-washed semen samples prepared for IUI. Ideally, semen and embryos from HCV and HBV patients should be stored in HCV- or HBV-designated storage tanks. Methods suggested for reducing the potential risk of virus transmission among cryopreserved sperm and embryos include: storage of sample in the nitrogen vapor state instead of the liquid state; use of sperm washing techniques to reduce viral load prior to freezing semen samples; and use of a double-sealing technique for cryocontainers.

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