

Hepatitis and reproduction

The Practice Committee of the American Society for Reproductive Medicine

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This Educational Bulletin will review the various viral etiologies of hepatitis, their mode of transmission, and implications for infertile couples, pregnant women, and health care workers. (Fertil Steril® 2008;90:S226–35. ©2008 by American Society for Reproductive Medicine.)

HEPATITIS AND REPRODUCTION

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 D are endemic in the United States; Hepatitis E is rarely reported in the United States; Hepatitis F has not been confirmed as a distinct genotype; and Hepatitis G is a newly described flavivirus. 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. This bulletin will review the various viral etiologies of hepatitis, their mode of transmission, and implications for infertile couples, pregnant women, and health care workers.

Hepatitis A Virus

Hepatitis A virus (HAV) is an RNA virus and a major cause of acute hepatitis in the United States (1). The incubation period is 15–50 days and is inversely related to age. Symptoms include fever, malaise, anorexia, nausea, abdominal discomfort, and jaundice. Hepatitis A is primarily transmitted from person to person through fecal–oral contamination and is facilitated by poor personal hygiene, poor sanitation, and intimate contact. Most cases of Hepatitis A occur in community-wide outbreaks, with the highest rates of disease in children, adolescents, and young adults. HAV infection is viewed as a rather benign condition. Only 1 in 10,000 patients has a severe or aggressive course (2).

Serologic assays are necessary for the diagnosis of HAV because the symptoms associated with HAV infection are not distinguishable from hepatitis caused by other agents. Two serologic assays are commercially available: total antibody to HAV indicates past infection, and immunoglobulin M (IgM) antibody to HAV indicates recent infection, usually within the past 6 months. There is no chronic infection with Hepatitis A, but approximately 15% of persons experience relapsing Hepatitis A, with recurring symptoms lasting as

long as 6 months. Health care workers are at minimal risk for transmission of HAV (Table 1).

Fetal transmission of HAV is extremely rare. The incidence of HAV in pregnancy is 1 in 1,000 (Table 2) (3). For short-term protection, immune globulin, a preparation of antibodies, is used prophylactically and after exposure to HAV. Hepatitis A vaccine is the best protection and is recommended for persons at high risk for HAV infection (4). The HAV vaccination may be given during pregnancy (Table 3).

Hepatitis B Virus (HBV)

Hepatitis B, caused by the double-stranded DNA Hepatitis B virus (HBV), is a well-documented cause of acute and chronic hepatitis. Chronic HBV infection develops in 2% to 6% of adults, 30% to 60% of young children, and as many as 90% of infants (<1 year) exposed to HBV. The incubation period for acute Hepatitis B is 45–160 days. It usually has an insidious onset, with clinical symptoms similar to those of other types of viral hepatitis (2). HBV is of particular interest to the obstetrician–gynecologist because of the risk of transmission to the partner and the fetus.

Hepatitis B virus can be transmitted parenterally, through sexual contact, or via mucosal exposure to infectious body fluids from persons who have either acute or chronic HBV infection. Approximately 25% of regular sexual contacts of HBV infected persons will become seropositive for HBV (Table 4). Health care workers, including nurses, are well recognized as being at occupational risk for HBV infection (Table 1). Serologic studies conducted before the implementation of recommendations to prevent transmission of blood-borne pathogens showed that health care workers had a prevalence of HBV infection three to five times higher than that of the general population. The risk of transmission from a percutaneous exposure to HBV ranges from 6% to 30% (5).

Strict adherence to standard precautions and vaccination with Hepatitis B vaccine, commercially available since 1982, is the most effective way to prevent HBV infection. Hepatitis B vaccine is safe and produces a protective antibody response in 95% of young, healthy adults. It may be administered to pregnant women and those contemplating pregnancy. The Occupational Safety and Health Administration (OSHA) enacted a rule in 1991 that required employers

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TABLE 1	
Risk of transmission to and from health care workers.	
Disease	Risk of transmission to and from health care workers
Hepatitis A	Minimal risk of transmission; use standard precautions.
Hepatitis B	The risk of transmission from a percutaneous exposure ranges from 6% to 30% (5). OSHA requires employers to offer HBV vaccine to employees at risk of exposure.
Hepatitis C	3%–10% risk of transmission from an infected health care worker to a patient (22).

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HBV in Pregnancy and Newborns Acute HBV occurs in one to two of every 1,000 pregnancies, with 1.5% of pregnant women being chronic carriers of HBV. There is no evidence that HBV infection is any more common in pregnancy. The incidence of spontaneous abortion during the first trimester in patients with acute viral hepatitis is increased. Similarly, when viral hepatitis occurs during the third trimester, there is an increased incidence of preterm labor. The increased incidence of spontaneous abortion and preterm delivery is probably no higher than that seen with other febrile illnesses (6–8). Perinatal transmission of HBV occurs quite frequently (Table 2).

Transmission can occur as a consequence of intrapartum exposure, transplacental transmission, and breastfeeding. Approximately 20%–30% of patients who are seropositive for HBsAg will transmit the virus to their neonates in the absence of immunoprophylaxis. In women who are HBsAg and HBeAg positive, the frequency of transmission increases to 90% (9). In addition, the frequency of transmission also depends on the time during gestation that the infection occurs. If the acute maternal infection occurs in the first trimester, up to 10% of infants will be HBsAg positive. If the mother has an acute infection during the third trimester, 90% of neonates will be positive without prophylaxis. Infants born to mothers who are HBsAg positive should receive both Hepatitis B immune globulin (HBIG) and Hepatitis B vaccine within 12 hours of birth followed by two more injections of HBV vaccine in the first 6 months of life (10–12). The combined use of passive (HBIG) and active immunization is 85% to 95% effective in preventing neonatal HBV infection (13). Breastfeeding is not contraindicated in women with chronic HBV infection if their newborn has undergone immunoprophylaxis (10).

Hepatitis C Virus

More than 50 years ago, it was known that a viral infection different from Hepatitis A and Hepatitis B was responsible

to provide Hepatitis B vaccine free of charge for all employees at risk of exposure to blood.

The most serious health consequence of HBV infection occurs in persons who develop chronic infection. A chronic carrier of HBV is defined as an individual with HBsAg detectable in serum for more than 6 months. The carrier state occurs equally in both men and women, and carriers can transmit the infection to others. HBeAg positivity is associated with a higher rate of transmitting infection in chronic HBsAg carriers (Table 5). These chronically infected persons are not only a major reservoir for transmission of HBV, but also are at risk for serious health consequences. The association between HBV infection and liver cancer is very strong and it is second only to tobacco as a known human carcinogen.

TABLE 2	
Risk of transmission of hepatitis to the fetus.	
Disease	Risk of transmission to the fetus
Hepatitis A Hepatitis B	Fetal transmission of HAV occurs with extreme rarity. Can occur as a consequence of intrapartum exposure, transplacental transmission, and breastfeeding. 20%–30% of HBsAg-positive/HBeAg-negative women will transmit virus to their infants. 90% of HBsAg- and HBeAg-positive women will transmit virus to their infants. Immunoprophylaxis at birth with both HBIG and Hepatitis B vaccine within 12 hours of birth decreases the risk of transmission. Passive (HBIG) and active immunization is 85%–95% effective in preventing neonatal HBV infection.
Hepatitis C	Among infants born to HCV PCR-positive and HIV-negative mothers, the risk of transmission was 36% when HCV RNA titers were $\geq 10^6$ copies/mL and 0% with titers $\leq 10^4$ copies/mL. The overall risk of transmission is approximately 5%–10% with unknown viral titers. All pregnant women with HCV should have viral titers performed.

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TABLE 3**Hepatitis A vaccine is recommended for the following.**

1. Travelers to areas with high rates of Hepatitis A.
2. Children in communities with high rates of Hepatitis A.
3. Men who have sex with men.
4. Patients with chronic liver disease.
5. Persons with clotting factor disorders.
6. The only occupational groups for whom vaccine is recommended are persons who work with HAV-infected primates or HAV in a research setting. In general, health care workers, including nurses, are not at increased risk for Hepatitis A. Seroprevalence studies show that the prevalence of HAV infection in health care workers is comparable to that in the general population. If a patient with Hepatitis A is admitted to the hospital, standard precautions will prevent transmission to the hospital staff (4).

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for causing hepatitis. In 1974 this agent was given the name Non-A Non-B hepatitis virus and was found to be responsible for 90% of post-blood transfusion hepatitis cases. In 1989, the cause of most cases of the Non-A, Non-B hepatitis cases was identified and given the designation of Hepatitis C virus (HCV) (14). 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 (13–19). HCV affects more than 1% of the world population (2). It affects 20% of prostitutes, 60% of hemophiliacs, 12% of homosexuals, 30% of sexual partners of HCV infected persons, and 60% of IV drug abusers (15–20).

The HCV is the most common cause of post-transfusion and chronic viral hepatitis in the Western world and ranks only slightly below chronic alcoholism as the leading cause of cirrhosis and end-stage liver disease. HCV can be transmitted vertically to infants, making HCV of particular significance to the obstetrician–gynecologist. Since most patients (80%) with acute HCV will develop chronic liver disease (persistently elevated alanine aminotransferase levels [ALT] for more than 6 months after illness onset), it is very important to identify patients at risk. Indications for screening for HCV antibodies are listed in Table 4 (2, 20).

Currently there are no recommendations regarding restriction of health care workers with HCV. The risk of transmission from an infected health care worker to a patient

appears to be lower (3%) than the risk of transmitting HBV but higher than the risk of transmitting HIV (21–23). Newer assays to detect HCV antibodies have reduced the risk of transmission. Current estimates place the risk of HCV transmission to 1 in 100,000 per blood unit transfused; this risk is higher than the current estimate for the risk of transmission of HIV from a blood transfusion (1 in 450,000) (21–23).

Acute HCV has an incubation period of 14–180 days; 75% of the acute cases are asymptomatic. However, current data suggest 80% of patients infected with HCV will develop chronic liver disease manifested as chronic hepatitis, 35% will develop cirrhosis, and 5% will progress to hepatocellular carcinoma (23, 24). This is in contrast to HBV in which only 5%–10% of infected patients will develop chronic liver disease. Progression of HCV is insidious in most patients. With HCV the average time to clinically significant hepatitis is 10 years after initial exposure, 20 years to cirrhosis, and 30 years to hepatocellular carcinoma. Recent evidence suggests that co-infection with HIV modifies the severity of the liver disease and accelerates the disease process.

Several tests are available for HCV screening. An enzyme-linked immunosorbent assay (ELISA) was initially used to detect antibody to a single antigen, the c100-3 antigen in the NS-3 region of the HCV (24–27). The first generation ELISA lacked sensitivity in the early diagnosis of HCV and had a 50%–70% false-positive rate (26). This led to the development of the second and third generation ELISA, which detects three or four antigens from both the structural (c200, c33c, c22-3) and nonstructural regions (NS-5) of the HCV. The sensitivity of these newer tests exceeds 95% and decreases the seronegative “window” to 8 weeks, during which patients with acute HCV may have a negative test result (26, 27). Because

TABLE 4**Risk factors for Hepatitis B (15–17) and Hepatitis C (27–30).**

1. Persons of Asian or Sub-Saharan African descent.
2. History of IV drug use.
3. History of sexually transmitted diseases.
4. Multiple sexual partners.
5. History of hemodialysis.
6. Health care worker.
7. History of solid organ transplant.
8. History of transfusion of blood or blood products.
9. Prisoner.
10. Infant that delivers to an HBsAg or HCV Ab-positive mother.
11. Sexual partner of HBV or HCV infected person.
12. Sexual partner of an IV drug user.
13. Tattoos or body piercing.

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TABLE 5**Interpretation of serologic testing in patients with HBV infection.**

HBsAg	HBsAb	HBcAb	HBeAg	HBeAb	Possible interpretation
+	-	IgM	+	-	Acute HBV infection, highly infectious
+	-	IgG	+	-	Chronic HBV infection, highly infectious
+	-	IgG	-	+	Late acute or chronic HBV infection, low infectivity
+	+	IgG/IgM	+/-	+/-	1 HBsAg of one subtype and heterotypic anti-HBs (common) or, 2 Process of seroconversion from HBsAg to anti-HBs (rare)
-	-	IgM	+/-	+/-	1 Acute HBV infection 2 Anti-HBc window
-	-	IgG	-	+/-	1 Low-level HBsAg carrier 2 Remote past infection 3 False positive
-	+	IgG	-	+/-	Recovery from HBV infection
-	+	-	-	-	1 Immunization 2 Possible remote infection 3 False positive

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of the problem of nonspecificity of the ELISA, particularly among populations with a low (<10%) prevalence of HCV infection, false-positive results still range from 15% to 60% in asymptomatic persons. It is critical, therefore, that the diagnosis of HCV be confirmed with an independent supplemental test that has high specificity (26).

The most widely used method is the recombinant immunosorbent assay (RIBA) in which antibodies are sought to three or four recombinant antigens of HCV. Samples are considered positive if antibodies to two or more of the HCV proteins are present and indeterminate if antibody to only one antigen is found. The most sensitive way to detect HCV RNA is by the polymerase chain reaction (PCR). The presence of viral RNA is the gold standard to make the diagnosis of Hepatitis C infection but this method should not be used as a primary screening test. Ninety percent of patients with a positive RIBA will have a positive HCV-PCR. Quantitative detection of HCV is possible using PCR and is useful for monitoring patients receiving therapy (Table 6).

There are very few options for the treatment of HCV. The most widely studied drug for the treatment of HCV is interferon. Interferon alpha (IFN- α) has been used to successfully treat patients with chronic HCV. However, relapse is common after treatment. Interferons appear to interfere with viral protein synthesis. Interferon for HCV is given at a dose of 3 million units three times a week for 6 months (10). At this dose, 50% of the treatment groups had a decline in viral load and normalization of ALT levels compared to 9% of controls. However, follow-up studies indicate that the sustained suppression rate is less than 25% at 1 year (27-30). These studies indicate that patients who normalize their ALT but do not completely clear HCV RNA are more likely to relapse.

More recently, combination therapy, IFN- α plus ribavirin, has resulted in a better response rate compared with monotherapy and is now considered first-line therapy (3); 31% of patients who received 24 weeks of combination therapy responded compared to 6% of patients receiving monotherapy for 24 weeks. Both of these drugs are currently contraindicated in pregnancy. However, a review of the literature showed that IFN use in early pregnancy revealed no teratogenic effects in 23 cases. It is believed that the lack of teratogenicity is due to the simple fact that IFN does not cross the placenta. Currently, IFN- α is considered a Category C drug and IFN use in pregnancy is not advised. Ribavirin, in contrast, is a Category X drug and should not be used by pregnant women, women attempting pregnancy, or their male partners (31, 32). Patients with progressive liver damage who do not respond to medical therapy are candidates for liver transplant. However, 40% of HCV-positive patients who get a liver

TABLE 6**Clinical indication for HCV-PCR testing.**

Indeterminate RIBA results
Chronic hepatitis with negative HCV antibody
Normal ALT levels with positive HCV antibodies
Acute HCV infection
Fulminant hepatitis C
Patients refusing liver biopsy
Pregnant women who have HCV antibodies
Newborns exposed to HCV
Monitor response to antiviral therapy

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transplant will develop hepatitis within 3 years of their transplant, making this therapy controversial (27, 28, 30).

HCV in Pregnancy and Newborns The seroprevalence of HCV among prenatal populations in the United States has been reported to range from 2.3% to 4.5% using second generation assays. Among HIV-infected pregnant patients, the seroprevalence rate is as high as 33%. Both of these rates are considerably higher than the rate observed in the general population (1.4%). This stresses the important role the obstetrician–gynecologist plays in the identification and management of women with HCV.

Very little is known about the effect of HCV on pregnancy; however, it appears that most women are asymptomatic, and fewer than 10% will have elevated transaminases. Although few women have been studied, there does not appear to be an increase in adverse pregnancy outcome in HCV-infected pregnant women. A correlation between the risk of vertical transmission and maternal viral burden was recently reported (29). Among all infants born to HIV-negative mothers, the risk of HCV transmission was 10%; however, the risk increased to 36% among infants born to women with HCV RNA titers of $\geq 10^6$ copies/mL. No woman whose titer was less than 10^4 copies/mL transmitted the virus to her infant. Similarly, two other studies of HIV-negative mothers have confirmed the strong association between vertical transmission of HCV and maternal viral titers greater than 10^6 (29).

Recent data suggest that the perinatal transmission of HCV is higher in women co-infected with HIV. Based on these results, it was postulated that women with HIV infection who transmitted HCV more frequently had higher maternal HCV viremia. Unfortunately, there is currently no prospective study in HIV seropositive and seronegative women that can definitively answer that question. A cohort of HCV and HIV co-infected women from the Women's and Infants' Transmission study showed that the median plasma concentration of HCV RNA was higher among HIV-positive women who transmitted HCV to their infants (1.9×10^6 copies/mL) than among those women with HCV-negative infants (3.5×10^5 copies/mL). There was no association between mother-to-infant HCV transmission and gestational age, duration of ruptured membranes, type of delivery, use of fetal scalp electrode, or detection of chorioamnionitis (18–20, 29, 30). Most infants delivered to HCV-infected mothers ($>10^6$ copies/mL) progress to chronic hepatitis.

The role of breastfeeding in the vertical transmission of HCV has been heavily debated. HCV RNA has been isolated from the colostrum of patients who were viremic, albeit in much smaller quantities (10^2 – 10^4 copies/mL) than was found in their serum (10^4 – 10^8 copies/mL). Although HCV RNA can be found in breast milk; infection through breastfeeding has not been demonstrated. These findings may be explained by several factors. First, the amount of HCV RNA present in breast milk may be so low as to be unable to infect the newborn. Alternatively, the tiny amount of HCV present in milk may be easily inactivated by gastric juices. Finally, the integ-

riety of the oral and gastrointestinal mucosa may effectively preclude HCV infection by the oral route. Therefore, at the present time, breastfeeding is not contraindicated in patients with HCV by either the CDC or the American Academy of Pediatrics, but this issue is debated among experts (19, 20).

The physician specializing in obstetrics and gynecology has an integral role in reducing the morbidity and mortality associated with chronic hepatitis C. Routine screening of pregnant women is not currently recommended. Screening occurs on the basis of high-risk factors (Table 6). In an infertility practice, routine screening for HCV may be offered to couples to help identify asymptomatic women/men who may need therapy before conceiving. Patients should be advised that the course of HCV may be adversely affected by co-infection with HIV, use of illicit drugs, and the consumption of alcohol. Additionally, hepatitis A and B vaccination is recommended for all persons with HCV if they are seronegative for either of these.

Specific questions often are asked about the transmissibility of HCV. Individuals positive for HCV should refrain from donating blood or organs. Safe sexual practices should be strongly encouraged. The physician should recommend that sexual partners of infected patients be tested for HCV antibodies.

In households with a member positive for HCV, sharing razors, toothbrushes, and manicuring tools should be avoided. Covering open wounds is recommended. It is not necessary to avoid close contact with family members or to avoid sharing meals or eating utensils. Transmission through intercourse is controversial so the use of condoms is recommended for those not trying to conceive until this issue is clearly resolved.

Hepatitis D Virus or Delta Hepatitis (HDV)

Hepatitis D virus (HDV, also called delta virus) is a circular RNA virus. HDV replication is defective and therefore cannot propagate in the absence of another virus. In humans, HDV infection only occurs in the presence of HBV, since HDV requires the presence of HBV for replication and expression. Therefore only persons who are infected with HBV will test positive for HDV.

A patient can acquire HDV at the same time as he/she is infected with HBV (co-infection). A patient with HBV can also be infected with HDV at any time after acute HBV infection (super-infection). Approximately 25% of chronic HBV carriers will be co-infected or super-infected with HDV. Approximately 70%–80% of patients infected with HDV will develop cirrhosis and portal hypertension. This is in sharp comparison to those with HBV only, where only 15% will develop cirrhosis. Transmission of HDV to the fetus has been documented. Since HBV is needed for HDV replication, active measures to prevent transmission of HBV will prevent transmission of HDV.

HDV infection is transmitted by blood and blood products. The risk factors for infection are similar to those for HBV infection. HDV is most common in intravenous drug users.

The HDV super-infection should be suspected in a patient with chronic hepatitis B whose condition suddenly worsens. There will usually be an obvious history of continued exposure to blood or blood products. A particularly aggressive acute HBV infection could suggest HDV co-infection. Co-infection or super-infection with HDV in a patient with HBV is diagnosed by the presence of antibodies against the HDV. Serum tests for HDV total antibody, HDV antigen, and IgG and IgM antibody are available. HDV antigen will persist in patients with chronic disease (30). IgM antibodies indicate acute infection.

Hepatitis E Virus (HEV)

Hepatitis E is a single-stranded RNA virus. Hepatitis E virus (HEV) is transmitted by the fecal–oral route, with contaminated water being the most common mode of transmission. HEV is rarely reported in the United States, but it is endemic in Mexico, Asia, and Africa. HEV is usually self-limited and mild. However, in underdeveloped countries, 20% of women infected during their third trimester of pregnancy will die of fulminant hepatitis (30). There is documented transmission of HEV from mother to infant. No Hepatitis E carrier state has been recognized. Serologic tests for HEV are only available in research laboratories. There is no vaccine available, and commercially available immunoglobulin preparations in the United States do not contain antibody against HEV. As with HAV, standard precautions will prevent transmission to hospital staff. Sexual contact has not been identified as a risk factor for transmission.

Hepatitis F Virus (HFV)

Hepatitis F has not been confirmed to be a separate genotype and may be a variant of HBV.

Hepatitis G Virus (HGV)

Hepatitis G is a newly described single-stranded RNA flavivirus that causes chronic infection. In the late 1960s a surgeon with the initials “GB” developed hepatitis. Serum taken from “GB” was passaged serially in primates and HGV was one of the first hepatitis viruses to be transmitted through blood. Subsequent study found GB’s hepatitis to be distinct from Hepatitis A, B, C, D, and E. This virus was then called Hepatitis GB or G after the surgeon “GB.” Hepatitis G virus (HGV) is found in 1% of volunteer blood donors in the United States of America, 15% of intravenous drug users, and approximately 20% of patients with HCV. HGV has been found in 24% of drug users with chronic HCV infection and 47% of patients receiving multiple blood transfusions, observations that suggest parenteral transmission. There is no data on transmission through sexual contact, but a few reported cases have documented vertical transmission.

The majority of patients infected with HGV alone do not get any liver disease. HGV accounts for only 0.3% of community-acquired acute viral hepatitis in the United States of America. Persistent viremia is common, but clinical disease

and chronic hepatitis rarely occur. Dual infection with HCV and HGV compared to HCV infection alone does not increase the severity of the disease. More data about HGV/HCV are needed to determine the clinical significance of HGV (30).

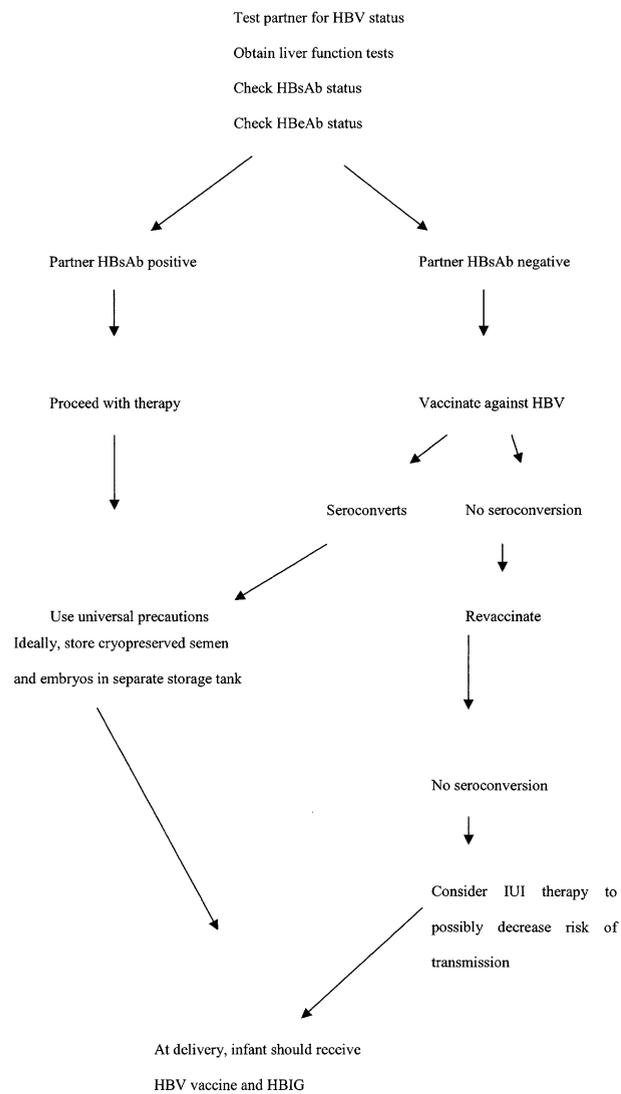
Viral Hepatitis and Assisted Reproduction

Data are both limited and controversial on the transmission of hepatitis virus during assisted reproduction. Transmission of HBV and HCV are the main areas of research, and limited information is now available on HDV and HGV. Concerns over laboratory/nosocomial infection in assisted reproduction clinics has 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 noninfected patients undergoing IVF within the same clinic during the same time period (33–35). 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.

Hepatitis and Semen/Embryo Cryopreservation

The occurrence of cross-contamination of HBV during liquid nitrogen storage of biological material and subsequent cross-infection of patients has been demonstrated in a few studies (35). Other viruses have also been found to survive direct exposure to liquid nitrogen, including herpes simplex virus, adenovirus, and papilloma virus. There is also evidence to suggest that liquid nitrogen can be contaminated by many micro-organisms, including a wide range of bacterial and fungal species. This information has raised the concern for ART labs of cross-contamination of samples. A recent study reported that the most likely source of contamination was during the cryostorage process, probably from the contaminated liquid nitrogen (35). HBV and HCV can survive direct exposure to liquid nitrogen, and under certain conditions result in cross-infection (36, 37). Given the strength of the evidence of liquid nitrogen contamination by microbes and the cross-infection that occurred with bone marrow samples, the possibility of contamination or cross-contamination during semen cryopreservation should be taken seriously.

Risks for Semen and Embryos Stored in Liquid Nitrogen Potential sources of liquid nitrogen contamination include cracking of straws during freezing or leaking vials, which may then expose uninfected specimens to virus. To date, however, there have been no documented cases of transmission of HBV or HCV among cryopreserved embryos or semen. Consideration of the evidence presented for the transmission of HBV in bone marrow samples leads to the conclusion that cross-infection via the clinical use of cryopreserved

FIGURE 1**Counseling guidelines for infertile women who are HBsAg positive or HBcAb-IgM positive.**

Note: In instances in which the male is HBsAg positive or HBcAb-IgM positive and the woman is seronegative, the woman should be vaccinated against HBV.

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3. The use of a “double bagging” or sealing technique to prevent direct contact of cryocontainers with liquid nitrogen.
4. The storage of samples in the nitrogen vapor state instead of the liquid phase.
5. The use of sperm “washing” techniques to decrease the viral load before freezing semen samples.

Currently there is no definitive experimental evidence to demonstrate the effectiveness of any of these procedures. Until these issues are examined carefully, it is difficult to make specific recommendations. That notwithstanding, unless there is the ability to store specimens from infected patients separately from noninfected patients, the option of not cryopreserving sperm or embryos from HBV- or HCV-infected patients should be considered.

Precautions Before Cryopreservation of Semen or Embryos

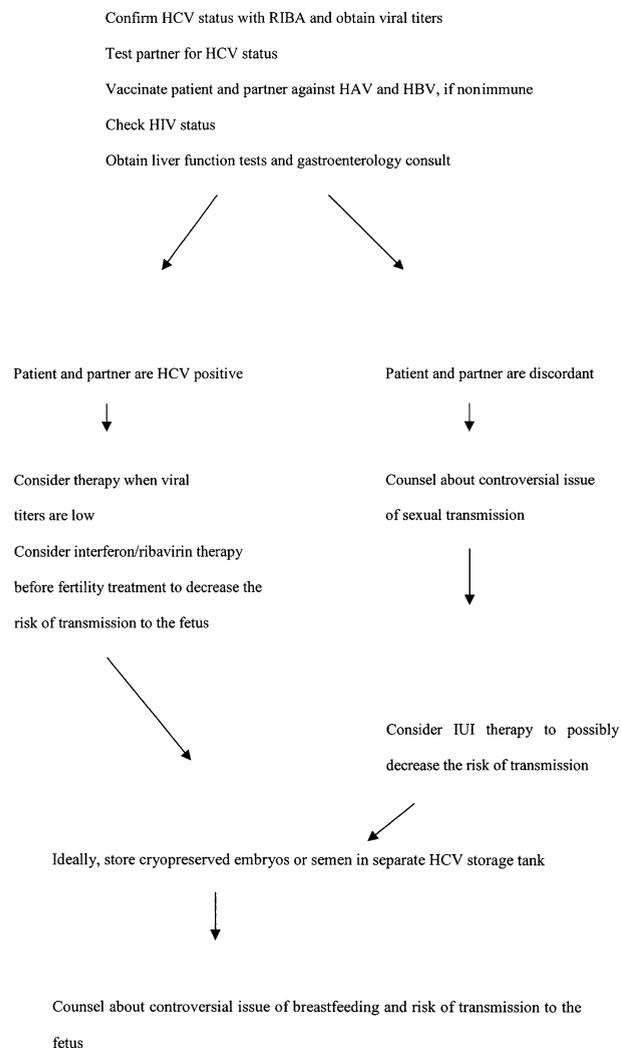
1. Screening tests for hepatitis that should be performed prior to cryopreservation of semen or embryos are HBsAg and HCV antibody. Additional testing that may be considered includes HBcAb-IgM to identify that anti-HBcAb window (Table 5).
2. Testing for these infections should be offered to high-risk infertile couples prior to fertility therapy.
3. All hepatitis patients need to be counseled about the risks of transmission to their partner, children, and staff (Table 1).
4. All office and clinical staff who are at risk for exposure should be offered vaccination for HBV.
5. Universal precautions should be utilized when handling blood and body fluids for all patients.
6. If cryopreservation in liquid nitrogen is going to be used, separate storage tanks for HBV and HCV patients are preferable as an additional safety measure until there is a better understanding of the risk of transmission in cryopreserved semen samples and embryos. Ideally, separate storage tanks for HBV and HCV patients should be used (Fig. 1 and 2).
7. Ideally, cryopreserved semen specimens with an unknown hepatitis status are individually stored in separate quarantine tanks until the results of infectious disease testing are known.
8. Laboratories should follow manufacturers’ guidelines for sealing straws and vials to limit potential for leakage of viral agents into their liquid nitrogen storage tanks.

semen/embryos is a realistic possibility. To protect cryopreserved semen/embryos from potential cross-infection, separate storage tanks for HBV and HCV patients are advised. In addition, there is topical interest in cryopreservation techniques that may provide additional safety for gametes and embryos. Options that have been proposed to reduce risks include:

1. Separate or off-site storage of embryos or sperm from couples infected with hepatitis B and C.
2. The use of containers guaranteed by the manufacturer to withstand freezing temperatures and thawing cycles.

Hepatitis and Fresh Insemination Procedures

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 (Fig. 1). With HCV, however, the risks are different, as no vaccine for HCV is currently available. Research studies have evaluated methods of semen preparation to decrease the amount of hepatitis virus in the inseminated sample. Information on

FIGURE 2**Counseling infertile couples in which one partner is HCV positive.**

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transmission of HBV and HCV in IUI samples is limited, and separation and removal of the infective fraction of the ejaculates has not been well studied (Fig. 2). One study evaluated HCV RNA in sera, semen, and in sperm fraction after density gradient centrifugation. HCV RNA was detected in 5% (2/39) of the semen samples but 0% of the samples after density gradient preparation (34). This suggests that the risk of transmission is low, but this has never been defined in humans. Additionally, data suggest that nested PCR techniques are more sensitive than commercially available one-round PCR, suggesting that sperm wash followed by nested PCR is the more appropriate method to test semen samples used when ARTs are offered to sero-discordant couples (38).

Couples need to be educated and counseled regarding the potential risk of transmission to the mother or child. It is

also necessary to consider the risks to the employees preparing the sample and laboratory contamination of other noninfected couples' gametes through cryopreservation and manipulation before deciding to offer care to HCV-positive patients (33–37).

Summary and Conclusions

- Transmission of viral hepatitis in assisted reproduction is possible, but the magnitude of the risk is unknown.
- Testing for HBsAg and HCV should be offered to high-risk infertile couples seeking fertility therapy to reduce the potential risk for transmission to an uninfected partner, baby, staff members, and disease-free gametes and embryos in the same laboratory.
- Patients positive for HCV or HBV should be tested for HIV and other sexually transmitted diseases.
- Testing for HIV, HBsAg, and HCV status should be performed on the couple prior to cryopreservation of semen or embryos.
- 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; use of a double-sealing technique for cryocontainers.
- Infants born to mothers who are HBsAg positive should receive both HBIG and the hepatitis B vaccine within 12 hours of birth. Breastfeeding of neonates is not contraindicated after immunoprophylaxis.
- Women who are HCV positive should be counseled about the risk of transmission of HCV to their fetus with increasing viral loads and positive HIV status. Additionally, sexually active women who are HCV positive should be counseled to use condoms when not actively trying to achieve pregnancy. Breastfeeding is not contraindicated.
- HCV and HBsAg-positive patients should be referred for evaluation of liver disease.
- HCV patients should be vaccinated for HAV and HBV. Partners of HBsAg-positive patients should be vaccinated against HBV.

Acknowledgments: This report was developed under the direction of the Practice Committee of the American Society for Reproductive Medicine as a service to its members and other practicing clinicians. While this document reflects appropriate management of a problem encountered in the practice of reproductive medicine, it is not intended to be the only approved standard of practice or to dictate an exclusive course of treatment. Other plans of management may be appropriate, taking into account the needs of the individual patient, available resources, and institutional or clinical practice limitations.

GLOSSARY OF HEPATITIS B TERMS

HBV: HBV is a spherical particle with a diameter of 42 nm. Its outer shell (or envelope) is composed of several proteins known collectively as HBs or surface proteins (indicated by “s”). The outer shell, frequently referred to as the surface coat, surrounds an inner protein shell, composed of HBc protein (indicated by “c”). The inner shell is referred to as the core particle or capsid. Finally, the core particle surrounds the viral DNA and the enzyme DNA polymerase.

HBsAg (Hepatitis B Surface Proteins): The outer surface coat composed of Hepatitis B surface proteins is produced in larger quantities than required for the virus to reproduce. The excess surface proteins clump together into spherical particles of between 17 and 25 nm in diameter but also form rods of variable length. In some cases, these particles encapsulate a core particle and produce a complete and infectious virus particle that enters the bloodstream and can infect other liver cells. The excess spheres, rods, and also complete viral particles enter the bloodstream in large numbers and are easily detectable. Hepatitis B surface protein is detectable in the bloodstream before the patient mounts an antibody response. HBsAg clears from the bloodstream as the infection resolves (Table 2).

HBeAg (Hepatitis B e Protein): HBeAg is a peptide and is normally detectable in the bloodstream when HBV is actively reproducing. This, in turn, leads to the person being much more infectious and at a greater risk of progression to liver disease. The exact function of this nonstructural protein is unknown. HBeAg is usually detectable at the same time as HBsAg and disappears before HBsAg disappears. The presence of HBeAg in chronic infection indicates that HBV is actively reproducing and there is a higher probability of liver damage. Hepatitis B e protein levels parallel HBsAg levels and indicate active viral replication (Table 2).

HBcAb (Hepatitis B core Antibody): The first detectable antibody to appear around 8 weeks after infection with HBV is antibody to the HBV core protein. HBcAbs persist in serum after an infection with HBV has been defeated, and testing for this antibody has been used to detect previous exposure to the live virus. The presence of both HBcAb IgM and HBcAg in the bloodstream is diagnostic of acute infection.

HBsAb (Hepatitis B surface Antibody): These are generally the last antibodies to appear. HBsAb can neutralize the Hepatitis B virus and their appearance can be taken as an indicator that an initial infection has been defeated. HBsAb can also be induced to appear by vaccination to provide protection against Hepatitis B.

HBeAb (Hepatitis B e Antibody): Antibodies to the “e” antigen (HBeAb) normally appear a few weeks after HBeAg is no longer detectable. The presence of HBeAb generally indicates a favorable prognosis.

Anti-HBc window: The period of time after the appearance of HBcAb IgM and after the disappearance of HBsAg, but prior to the appearance of HBsAb. In this case, the presence of HBcAb IgM is the only serologic marker for acute infection.

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