Cytomegalovirus and human immunodeficiency virus in semen of homosexual men

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Objective: To assess the accuracy of serology to predict the presence of cytomegalovirus (CMV) in semen of homosexual men without and with HIV coinfection.
Design: Semen CMV was detected by electron microscopy and by polymerase chain reaction (PCR) amplification; paired serum was tested for CMV IgG/IgM. Semen HIV was detected by reverse transcription–PCR.
Setting: Licensed clinical and research laboratory.
Patient(s): Sixty-eight men.
Intervention(s): None.
Main Outcome Measure(s): Frequency of CMV and HIV in semen.
Result(s): Cytomegalovirus was detected by electron microscopy in 3 of 10 specimens examined. Forty-six (89%) of 52 HIV-infected men were seropositive for CMV by combined assay for IgG/IgM; two more (48 of 52, 92%) were seropositive for CMV IgG by separate assay; 25 (48%) of the HIV-infected men had PCR-detectable CMV DNA in at least one semen specimen, 22 of whom (42%) had CMV in all specimens. Nineteen (13%) of the 150 specimens tested positive for HIV, whereas 67 (45%) tested positive for CMV; seven specimens tested positive for both CMV and HIV. Cytomegalovirus, but not HIV, detection in semen correlated with decreased $CD4^+$ lymphocytes in peripheral blood ($<700/\mu L$) but was not accurately predicted by serology, leukocytospermia, or age.
Conclusion(s): Cytomegalovirus in semen is not accurately predicted by serology. Sperm banking needs to include direct assessment of CMV in semen specimens. Strategies to eliminate CMV from semen specimens are needed to alleviate the risk of virus transmission. (Fertil Steril® 2014;101:350–8. ©2014 by American Society for Reproductive Medicine.)
Key Words: Cytomegalovirus serology, HIV, leukocytospermia, semen, $CD4^+$ lymphocytes

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Cytomegalovirus (CMV) is a common viral infection in humans that causes severe and life-threatening conditions in neonates and immunocompromised individuals (1–7). Like other members of the *Herpesviridae* family, CMV has the biological properties of latency and reactivation (2, 8). The vertical transmission rate of CMV is approximately 40% with primary infections and 0.2%–1.8% with reactivated infections (8, 9). Approximately 2.3% of women seroconvert during pregnancy in the United States (10). Vaccine development is lagging because of the ability of different substrains of CMV to escape immune surveillance, although partial protection may be possible through vaccination (11, 12) or passive immunization (13). Cytomegalovirus is the leading cause of sensorineural hearing loss in newborns and the most frequently known viral cause of mental retardation in the United States (8, 9, 14). Approximately 1 in 750 children born in the United States have permanent problems due to congenital CMV infection (15). It is important to note that being CMV seropositive, or receiving CMV vaccination, only partially protects against new CMV infection(s) in pregnant women and their offspring (16–20), thus semen CMV poses an infection threat to both seropositive and seronegative women seeking pregnancy.

Cytomegalovirus has been isolated from most human fluids, including oropharyngeal secretions, urine, cervical

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and vaginal secretions, semen (21–25), human milk, and blood (1, 7, 26–28). Recovery of CMV from genital secretions and results from sexual partner studies support the sexual transmission of CMV (25, 29, 30). Epidemiologic information concerning the prevalence of CMV in semen and its possible role in sexual transmission is limited, although more recent polymerase chain reaction (PCR)-based studies indicate higher prevalence than previously thought (31–34) and suggest frequency of CMV shedding may depend on the population of men under study—for example, higher in Greece (62.5%) (34) than in Denmark (2.7%) (33) or Massachusetts (8.7%) (32).

Whereas serosurveillance indicates that CMV infection in the general population is 50%–80% (15), it is reported to be 95% in men who have sex with men (24). Like other herpes family viruses, the initial infection is detectable by the presence of CMV-reactive IgM class antibody, which converts to IgG class antibody over time. However, reactivation of CMV production is not usually accompanied by IgM reappearance but sometimes an elevation of IgG or IgA titer (35). Comparing sero-immunoglobulin levels (IgG, IgA, or IgM) does not distinguish between primary CMV infection and a reactivated CMV infection (35). Hence, it is not possible to rule out active CMV production in someone who is CMV-IgG antibody positive, or to know when CMV antibody is accompanied by the presence of CMV in semen. Detection of HIV in semen before seroconversion (30) led to the concept of a "quarantine period" for semen specimens in sperm banks, to allow the men to undergo repeat infectious disease testing before the sperm are cleared for use. This is in contrast to the recommendation by the British Andrology Society that only CMV seronegative men be allowed to donate sperm to sperm banks (36), a position that sparked a lively debate pointing out that approximately half of sperm donors would be excluded by this criterion (37). Men testing positive for CMV IgG have been considered to be at low risk of shedding CMV into semen specimens (38), a position called into question by the more recent PCR-based detection methods (32–34).

In 1997 Mansat et al. (25) analyzed 178 donor semen specimens from 97 sperm donors to two sperm banks in France for CMV by PCR and by virus culture. Cytomegalovirus was detected in five samples from two donors by cell culture and in 10 samples from five donors by PCR. Their consequent recommendation was that all donor sperm be tested for CMV by PCR and not used if it tested positive.

In 2000 a French consortium, CECOS (Center for the Study and Preservation of Human Ova and Sperm), attempted to clarify issues around CMV in donor semen with the following objectives: [1] determine the frequency of sperm donors carrying CMV, [2] assess the intermittence of the excretion, [3] determine what semen fraction is infectious, [4] specify the predictive value of serologic data concerning the risk of CMV transmission, [5] assess the performance of the virologic tests used, and [6] evaluate the relevance of screening standards in use by sperm banks and if necessary propose more suitable recommendations. They tested 635 semen samples from 231 men, collected at 16 sites in France. Disparities between culture results, PCR tests, and serology led these authors to suggest that the safest way to limit CMV infection from sperm bank specimens was to quarantine the specimens for 6 months and retest the sperm donors before specimen release, despite the information that one CMV DNA PCR$^+$ donor remained seronegative at 6 months (30).

In contrast, the most recent report (33) of herpesviruses in semen specimens argues against using a quarantine period for safety because of intermittent virus in semen: "…implementation of quarantine for a donor shown to shed a herpesvirus is not a tenable solution… each sperm sample rather than the donor should be characterized for the presence of virus."

Current American Society for Reproductive Medicine guidelines, used by many sperm banks and fertility clinics around the world, are that if the CMV-seropositive donor is "…without active infection…," their sperm are recommended for use only by CMV-seropositive women (31). This recommendation is called into question by the lack of a clear standard for "active infection" because serology may not be an accurate predictor of semen CMV (30, 33) and because preconception immunity only partially protects against new CMV infection (16–18).

Gay men may parent through egg donation and surrogacy in many US states. According to US Food and Drug Administration (FDA) guidelines (39), sperm to be used in their assisted reproduction procedures with a surrogate needs to have been collected within 7 days of a tissue donor infectious disease screening panel of blood (antibody tests for HIV, hepatitis B and C, CMV, human T-cell leukemia virus, West Nile virus, and syphilis) and urine (chlamydia and gonorrhea). Follow-up tests for the presence of virus are available to further evaluate the clinical status of men testing antibody positive for HIV, hepatitis B and C, human T-cell leukemia virus, and West Nile virus, thus allowing an assessment of the potential risk of transmission to the surrogate and planned offspring. However, similar tests are not available for CMV, which was rarely recovered from the blood of HIV-infected men whose semen specimens were CMV-infected (24). For this reason, to provide more complete information about semen pathogen burden to the gestational surrogate and the IVF clinic staff, we designed a bracket-nested PCR assay for CMV DNA that offers a sensitive, rapid, and specific means of identifying a few copies of World Health Organization DNA control spiked into semen (40, 41). These assays are based on the semen HIV assay in use in this laboratory for two decades, which has improved sensitivity and reproducibility over earlier assays for semen pathogens (41, 42).

Herein we report the results of examining 10 semen specimens by electron microscopy and PCR-testing a total of 163 semen specimens from 58 homosexual men with known HIV sero-status in an effort to discover clinical parameters that reliably predict CMV in semen.

MATERIALS AND METHODS

Study Subjects

Ten HIV-infected homosexual men in a study of HIV in semen (43) and 58 (52 HIV-infected) undergoing evaluation to satisfy FDA requirements for sperm tissue donors