FERTILITY AND STERILITY®
Vol. 68, No. 5, November 1997

Printed on acid-free paper in U. S. A.
Published by Elsevier Science Inc.

Detection of cytomegalovirus in semen from a population of men seeking infertility evaluation

Rachel Levy, M.D.
Fatiha Najioullah, Ph.D.
Bertrand Keppi
Daniele Thouvenot, Ph.D.
Sylvie Bosshard, Ph.D.
Jacqueline Lornage, Ph.D.
Bruno Lina, Ph.D.
Jean-François Guerin, Ph.D.
Michele Aymard, Ph.D.

Laboratoire de Biologie de la Reproduction et du Développement, Hôpital Edouard Herriot; and the Laboratoire de Virologie, Faculté de Médecine, Lyon Grange Blanche, Domaine Rockefeller, Lyon, France

Objective: To determine the incidence of cytomegalovirus in the ejaculates of infertile men who were seropositive for IgG antibodies to cytomegalovirus.

Design: Prospective study.

Patient(s): We tested cytomegalovirus infection in the semen of men participating in an IVF-ET program.

Main Outcome Measure(s): IgG and IgM antibodies to cytomegalovirus were measured in sera. We used polymerase chain reaction (PCR) and cell culture to look for both cytomegalovirus DNA and infectious virus in the semen of 70 men with cytomegalovirus-specific antibodies detected in sera.

Result(s): Of the infertile couples, 13.5% exhibited "mismatching" serology (i.e., detection of IgG antibodies to cytomegalovirus in male serum only and not in female serum) and constituted a potential risk for cytomegalovirus transmission. Cytomegalovirus was identified in the semen of two patients who were positive for IgG antibodies to cytomegalovirus. Cytomegalovirus DNA also was detected in one positive sample after centrifugation through a three-layer Percoll gradient.

Conclusion(s): Human cytomegalovirus was present in the semen from a population of infertile men. Rapid detection can be achieved by molecular techniques such as PCR combined with a hybridization assay. Even though cytomegalovirus was infrequently detected in semen, these data must be considered in determining the risk of transmission and developmental anomalies in infected fetuses. (Fertil Steril® 1997;68:820–5. © 1997 by American Society for Reproductive Medicine.)

Key Words: Human cytomegalovirus (HCMV), polymerase chain reaction (PCR), semen

Human cytomegalovirus, a member of the herpesvirus group, is a common agent throughout the world. About 50%–80% of the adult population in central Europe or North America is infected with cytomegalovirus during early childhood (1). Human cytomegalovirus infection in healthy people occurs exogenously via close or intimate contact with a person excreting human cytomegalovirus in saliva, tears, breast milk, urine, and other excretions, and is usually asymptomatic. After the acute but asymptomatic phase of infection, these subjects harbor the virus in a latent form in various tissue cell types (monocytes/macrophages, epithelial cells, or fibroblasts) for the rest of their lives, with intermittent excretion. From time to time, these persons experience endogenous reinfection from viral reactivation (2).

Besides immunocompromised patients, cytomegalovirus can be responsible for diseases observed in nonimmunocompromised hosts and remains the leading cause of human congenital viral infection,

Received March 3, 1997; revised and accepted July 16, 1997.
Presented at the 10th World Congress on In Vitro Fertilization, Vancouver, British Columbia, Canada, May 24–28, 1997.
Reprint requests: Rachel Levy, M.D., Laboratoire de Biologie de la Reproduction et du Développement, Hôpital Edouard Herriot, Place d'Arsonval, 69437 Lyon Cedex 03, France (FAX: 04-78-77-72-64).

0015-0282/97/$17.00
PII S0015-0282(97)00340-3

affecting 0.4%–2.3% of live-born infants (3). Human cytomegalovirus can be transmitted from mother to child, either in utero or during the perinatal phase, even when the mother is known to have been infected months to years before conception (4).

Prenatal transmission often results in severe disturbance of development and in disease that may become manifest at birth (5) or may be observed later, such as hearing loss and mental retardation (6). It is frequently concluded that congenital cytomegalovirus infection is mainly due to reactivation of maternal cytomegalovirus infection during pregnancy because most women acquire cytomegalovirus before reaching childbearing age (5).

Newborns infected after a reactivation of maternal cytomegalovirus infection rarely have clinically apparent disease or severe sequelae, whereas congenital infection after a primary maternal cytomegalovirus infection during pregnancy may result in damage to the fetus with long-term sequelae (7). Mid- to upper-income groups of women have lower rates of cytomegalovirus immune response and therefore are more often susceptible to cytomegalovirus during pregnancy.

Some reports have postulated that cytomegalovirus infection of the female genital tract during pregnancy may cause spontaneous abortion, but it is difficult to identify the strict role of cytomegalovirus in this pathology (8, 9).

Considering the severity of prenatal and intrauterine cytomegalovirus infections and the recent demonstration of developmental anomalies in mouse fetuses by transfer of murine cytomegalovirus DNA–injected eggs to surrogate mothers (10), we were interested to know whether cytomegalovirus could be detected in the ejaculates of healthy men, thus allowing semen to constitute a reservoir for transmission.

The presence of cytomegalovirus in human semen has been reported, but most of these studies concerned selected populations, such as men in an immunosuppressed state (11–14). Two recent studies of patients who sought evaluations at an infertility clinic reported opposite results. In contrast to the results presented by Bantel-Schaal et al. (15), Shen et al. (16) reported the presence of cytomegalovirus in the semen of 32.7% of men in Taiwan as determined by polymerase chain reaction (PCR).

Amplification of DNA by PCR currently is used for the diagnosis of cytomegalovirus infection. Our purpose was to use both cell culture and PCR to detect cytomegalovirus in the semen of our patients. This report assessed shedding of human cytomegalovirus in semen. Human cytomegalovirus was detected in sperm samples obtained after centrifugation through a three-layer Percoll gradient, which is the suspension used for insemination during IVF or IUI.

MATERIALS AND METHODS

Clinical Specimens

This experimental protocol included 250 patients who enrolled in our IVF program and gave their informed consent for using spare semen for research or diagnostic purposes. Semen was collected by masturbation after 3 days of abstinence from patients attending the IVF program in our unit. These samples were examined using routine semen analysis techniques.

One milliliter of the semen sample was centrifuged, and the seminal fluid was separated from the cells. Another milliliter was submitted to centrifugation through a three-layer discontinuous Percoll gradient (90%–70%–50%), and each layer was subsequently separated, washed, and resuspended with 1 mL of B2 medium (B2 Menezo; bioMérieux SA, Marcy l'Etoile, France). IgG antibodies (Enzygnost Anti CMV IgG; Behring, Rueil-Malmaison, France) and IgM antibodies (Captia CMV; Centocor, Malvern, PA) to cytomegalovirus were measured in all serum samples by ELISA. We looked for both cytomegalovirus DNA and infectious virus in the semen of men who had IgG antibodies to cytomegalovirus detected in the sera.

Seventy collected semen samples were inoculated freshly onto culture and then stored at −20°C for PCR assay. We also tested 30 semen samples from men who were seronegative for IgG antibody to cytomegalovirus and from the patients with IgM antibodies. Semen samples obtained after centrifugation (seminal fluid or cellular pellet) or after centrifugation through the Percoll gradient were analyzed only using the PCR assay.

Tissue Cell Culture

Semen was inoculated immediately onto human embryonic fibroblast cell cultures (MRC5). Virus detection and identification were performed by using an immunoperoxidase (IP) assay with a monoclonal antibody directed against the IE1 antigen (17). During 21 days, detection of a cytopathic effect was performed daily in IP-negative samples.

DNA Extraction

A rapid DNA extraction procedure was used. In a 0.6-mL Eppendorf tube, 100 μL of semen was added to 50 μL of an extraction buffer (10 mmol/L Tris-HCl, pH 8.3; 50 mmol/L KCl, 2.5 mmol/L $MgCl_2$, 0.45% Tween 20, and proteinase K at 100 μg/mL [Boehringer Mannheim, Mannheim, Germany]). The

mixture was homogenized using a vortex mixer for 10 seconds and then incubated for 60 minutes at 56°C and for 10 minutes at 95°C. The mixture was centrifuged briefly, and the supernatant was collected and used directly for amplification without further purification.

DNA Amplification

For amplification of cytomegalovirus DNA by PCR, a couple of primers were used, with sequences as follows: HXLF 4 1, US 11755–11780, 5′ CGG ATC AAC ATA AGG ACT TTT CAC AC 3′; and HXLF 4 2, US 11895–11920, 5′ GAA TAC AGA CAC TTA GAG CTC GGG GT 3′ (EurogenTec, Belgium) (18).

We performed PCR for two concentrations of semen (undiluted and 1:10) to avoid false-negative PCR results due to Taq inhibitors; PCR was performed in a 100-μL reaction mixture containing 50 mmol/L KCl, 10 mmol/L Tris-HCl (pH 8.3), 1.5 mmol/L $MgCl_2$, 10 μL of extraction product, and 0.5 U of Taq DNA polymerase (Promega).

The PCR mix contained 200 μmol/L of adenine, cytosine, and guanine triphosphates and 40 pmol/L of each oligonucleotide primer. In this mix, deoxyuridine triphosphate replaced deoxythymidine triphosphate (400 μmol/L), and uracil DNA glycosylase (1 μL of a 1-U/μL stock solution; GIBCO BRL) was added to the mixture to reduce contamination (19).

Polymerase chain reaction consisted of 35 cycles in which the primers were denatured at 94°C (30 seconds), annealed at 58°C (30 seconds), and extended at 72°C (30 seconds), with a hot start (94°C, 4 minutes) and a hold temperature at 72°C for 10 minutes.

Each PCR test included three positive and two negative controls (strain AD 169 was used as the positive control at three dilutions). To avoid contamination by PCR product carryover and false-positive results, samples were prepared in a separate laboratory. We also used currently recommended precautions (e.g., aliquoted reagents and positive displacement pipettes).

Detection of Amplified DNA

Ten microliters of the PCR products was run on a 3% agarose gel in 1× Tris Borate EDTA (TBE) buffer (Sea Kem GTG agarose) containing 10 μg/mL of ethidium bromide at 200 V for 20 minutes. A DNA ladder was used in each gel (DNA Molecular Weight Marker V; Boehringer Mannheim). The gels then were photographed with a Polaroid camera (Instant Camera System, United Kingdom) (MP4+) under UV light. The expected size of the PCR products is 166 bp.

Table 1 Serologic Status of Couples Seeking Treatment for Infertility

	Men with anti-CMV IgG	Men without anti-CMV IgG	Total
Women with anti-CMV IgG	36 (44.0)	14 (17.2)	50 (62.3)
Women without anti-CMV IgG	11 (13.5)	20 (24.7)	31 (38.2)
Total	47 (58.0)	34 (41.9)	81 couples

CMV = cytomegalovirus. Values in parentheses are percentages.

Gen-Eti-K-DNA Enzyme Immunoassay

Detection of amplified cytomegalovirus DNA with the Gen-Eti-K-DNA enzyme immunoassay (Sorin Biomedica, Italy) was carried out according to the recommendations of the manufacturer (20). Briefly, streptavidin-coated microtiter plates were incubated with 100 ng of the 5′ biotinylated capture probe overnight at 4°C. After washing five times, the microtiter plate was ready for use or could be stored for 1 week. After a 10-minute denaturation at 96°C, 20 μL of each PCR product to be tested was incubated for 1 hour at 50°C in one well containing 100 μL of hybridization buffer. After five washings, the anti-dsDNA antibody diluted at 1:50 in a specific buffer was added and incubated for 60 minutes at room temperature. Then, after washing five times, bound anti-dsDNA antibody was detected by adding 100 μL of horseradish peroxidase–labeled rabbit anti-mouse Ig antibody.

After a last incubation of 60 minutes, 100 μL of chromogen-substrate mixture was added, and the colorimetric reaction was allowed to develop for 30 minutes. The reaction was stopped with 200 μL blocking solution (1 mol/L sulfuric acid), and the absorbance was measured rapidly at an optical density (OD) of 450 nm against a reference OD of 630 nm.

In this study, the Gen-Eti-K-DNA enzyme immunoassay was performed using the following coated probe: 5′ CAG GAC TGT GTA CCG TGT ATC CAG CGC TTA CTA TC 3′, 35-mer US probe (18).

RESULTS

Serology

Among the 250 sera (108 men and 142 women), 146 (58.4%) showed detectable IgG antibody to cytomegalovirus by ELISA. In one man's case, IgM and IgG antibodies to cytomegalovirus were detected. A total of 81 couples 25–40 years old were analyzed serologically (Table 1). Infertile couples exhibited "mismatching" serology (detection of IgG antibodies to cytomegalovirus in male serum only and not in

female serum) in 13.5% (11/81), and these constituted a potential risk for cytomegalovirus transmission in case of IVF or IUI.

Tissue Cell Culture and PCR

Cytomegalovirus was not detected in the 30 semen samples from IgG-seronegative patients or in the sample from the IgM-seropositive patient. Two (2.85%) of 70 semen samples from cytomegalovirus IgG-positive patients were positive for cytomegalovirus in cell culture and PCR (Fig. 1). It took 3 weeks to culture one of the cytomegalovirus isolates. Concordant results in cell culture and PCR were observed in both cases.

The presence of Taq polymerase inhibitors in seminal fluid was tested by adding increased amounts of cytomegalovirus DNA to the raw semen before submitting the samples to extraction and PCR. Polymerase inhibitors were detected in 20% of the semen samples. Even after adding cytomegalovirus DNA, these samples remained negative after PCR and subsequent hybridization (Fig. 1).

For one positive sample, cytomegalovirus DNA also was detected by PCR in the seminal fluid and the cell pellet after centrifugation and in the different spermatozoa suspensions obtained after centrifugation through a three-layer Percoll gradient (Fig. 2).

Electrophoresis Gel Analysis

To determine the sensitivity of the DNA detection assays, 10-fold dilutions of a 10^4 tissue culture infectious dose 50% (TCID50) suspension of the reference strain AD 169 were submitted to PCR amplification and subsequent hybridization. The detection limit of PCR combined with the enzyme immunoassay was a 10^{-5} to 10^{-6} dilution (0.1–0.01 TCID50). The hybridization step did not increase the number of positive specimens, just assessing the specificity of the method (2/2).

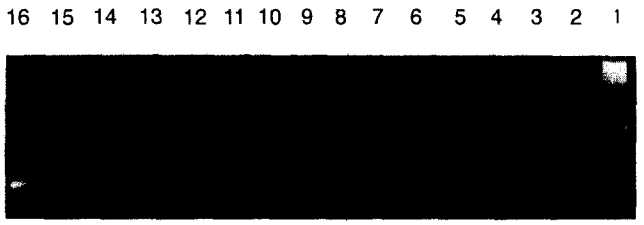

Figure 1 Ethidium bromide staining of 3% agarose gel showing samples infected with cytomegalovirus and the detection of Taq inhibitors in semen. *Lane 1,* molecular-weight marker (DNA V; Boehringer); *lanes 2–3,* negative controls; *lane 4,* raw semen no. 1 + positive control; *lane 5,* raw semen no. 1 after 1:10 dilution + positive control; *lane 6,* raw semen no. 2 + positive control; *lane 7,* raw semen no. 2 after 1:10 dilution + positive control; *lane 8,* positive control AD 169; *lanes 9–14,* negative samples; *lane 15,* positive control + 10^{-5} cytomegalovirus AD 169; *lane 16,* positive control + 10^{-2} cytomegalovirus AD 169.

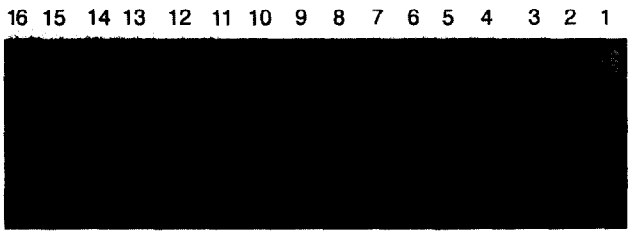

Figure 2 Ethidium bromide staining of a 3% agarose gel showing samples infected or not infected with cytomegalovirus and the fractions. *Lane 1,* molecular-weight marker (DNA V; Boehringer-Mannheim); *lanes 2–3,* negative controls; *lane 4,* negative sample; *lane 5,* cell pellet obtained from a positive semen sample after sperm washing and subjected to 35 cycles; *lane 6,* seminal fluid obtained from a positive semen sample after sperm washing and subjected to 35 cycles; *lane 7,* 90% fraction obtained from a positive semen sample after centrifugation through a three-layer discontinuous Percoll gradient; *lane 8,* 70%–50% fractions obtained from a positive semen sample after centrifugation through a three-layer discontinuous Percoll gradient; *lanes 9–12,* same as *lane 8* but from a negative semen sample; *lanes 13–14,* negative samples; *lane 15,* positive control + 10^{-5} cytomegalovirus AD 169; *lane 16,* positive control + 10^{-2} cytomegalovirus AD 169.

DISCUSSION

The difficulty in detecting asymptomatic latent infections associated with the time and cost of performing systematic cultures has led to nearly universal dependence on rapid, inexpensive cytomegalovirus antibody (IgM and IgG) assays to determine the status of patients attending an IVF program. However, our study clearly demonstrated that the only patient with IgG antibodies to cytomegalovirus did not have any virus in his semen, and both patients with cytomegalovirus detected in semen had no IgM in their sera.

IgG and IgM antibodies to cytomegalovirus can be considered as an easy tool for selecting patients who are at risk of cytomegalovirus infection. In the present study, IgG antibodies to cytomegalovirus were detected in 146 (58.4%) of the 250 serum samples. These results corroborate serologic data concerning the adult immunocompetent population in central Europe or North America (1).

Among these 250 patients, 13.5% of infertile couples exhibited "partner mismatching" or "discordant" serology, consisting of detection of IgG antibodies to cytomegalovirus in male serum only and not in the female serum. Because these women are seronegative for human cytomegalovirus, the risk of transmission might be higher in this population (Table 1). The semen of patients entering this last group should be analyzed to check for the absence of cytomegalovirus.

As shown in Table 2, many investigators at-

Table 2 Recent Studies Concerning Detection of Human Cytomegalovirus in Semen

Study	Year	Patients	Methods	IgG to CMV in sera (%)*	Results in semen
Bayle et al. (unpublished data)	1986	456 men seeking fertility evaluation	Cell culture	50	Detection in 3.1%
Leach et al., 11	1993	220 HIV-1-positive men	Cell culture	100	Detection in 45%
Bantel-Schaal et al., 15	1993	63 men seeking fertility evaluation	Dot-blot-positive cell culture	50	No virus detected
Shen et al., 16	1994	217 men seeking fertility evaluation	PCR DNA CMV	92	Detection in 32.7%
Leach et al.	1994	234 HIV-1-positive men	PCR DNA CMV	100	Detection in 40%
Detels et al., 12	1994	164 HIV-1-positive men (4 samples)	Cell culture	100	Detection in 33%
Yang et al., 22	1995	250 couples seeking fertility evaluation	PCR DNA CMV	99.6	Detection in 33.5%
Rasmussen et al., 13	1995	35 HIV-1-positive men	PCR DNA CMV	100	Detection in 31.5%
Krieger et al., 14	1995	56 HIV-1-positive men	Cell culture	100	Detection in 30%
Current study	1997	81 couples seeking fertility evaluation	PCR DNA CMV; cell culture	50	Detection in 2.85%

Note. CMV = cytomegalovirus; HIV = human immunodeficiency virus.
* Determined by ELISA.

tempted to detect human cytomegalovirus in semen. Among the different methods tested, DNA amplification with PCR seemed to be the most sensitive and specific (21). In this study, human cytomegalovirus was detected in only two semen samples with both cell culture and PCR. Our results agree with previous studies in western countries, where cytomegalovirus seroprevalence is low (15). These rates are largely lower than those reported in studies in Taiwan (16, 22) or those concerning human immunodeficiency virus-1 (HIV)-seropositive men (11–14).

Although tissue culture remains the method currently used for the diagnosis of cytomegalovirus infection, this technique is time-consuming and expensive and does not detect a low amount of virus. The dramatic gains in sensitivity and rapidity of viral detection made possible by the PCR technique offer new hope for routine application of this technique in the detection of human cytomegalovirus infection.

Gass et al. (23) revealed the role of Taq polymerase inhibitors. We tested this hypothesis by adding increased amounts of cytomegalovirus DNA to the raw semen before submitting the samples to extraction and PCR. Twenty percent of the semen samples remained negative after PCR and subsequent hybridization. We demonstrated the presence of Taq polymerase inhibitors and/or damaging DNA enzymes in the samples of semen; these results led us to test each semen sample at two concentrations: undiluted and 1:10.

Human cytomegalovirus DNA could be detected in one of the positive samples after sperm washing and after centrifugation through a three-layer Percoll gradient, which is performed routinely in our infertility center to prepare the sperm suspension that will be used for IVF or IUI. The persistence of virus after centrifugation through a three-layer Percoll gradient can be related to the persistence of leukocytes (24), which constitute a known reservoir for human cytomegalovirus. It could be of interest to use a six-layer Percoll gradient (100%–90%–80%–70%–60%–40%) if cytomegalovirus is detected in the raw semen. This technique is time-consuming but is efficient to remove or decrease dramatically leukocytes in semen samples (25).

We propose a strategy for infertile couples who need artificial insemination or IVF (Table 3). In all cases, serologic tests for detection of previous cytomegalovirus infection are recommended. If the serologic status is discordant (i.e., if the man is seropositive and the woman is seronegative for cytomegalovirus), the absence of viral shedding in semen by viral culture or preferably by DNA PCR followed by hybridization should be confirmed immediately

Table 3 How to Manage the ART Decision Considering Both Cytomegalovirus Serologic Status and PCR Results in Semen

Men's serologic status	Women's serologic status		PCR CMV in semen indication	ART decision
IgG	IgM	IgG		
0	0	+	No	Yes
0	0	0	No	Yes
0	+	0 or +	No	Delay
+	+	0 or +	No	Delay
+	0	0	Yes	Six-layer Percoll gradient
+	0	+	No	Yes

Note. CMV = cytomegalovirus; ART = assisted reproductive technology.

before each IUI or IVF, inasmuch as cytomegalovirus detection in the ejaculate is discontinuous (12). If human cytomegalovirus is detected in the raw semen and if the attempt cannot be delayed, centrifugation through a six-layer Percoll gradient should be performed to try to remove cytomegalovirus from the semen.

In conclusion, cytomegalovirus is present in the semen in a population of infertile men. Although cytomegalovirus was infrequently detected in semen and although cytomegalovirus infection does not seem to play an important role in infertility, these data must be considered in determining the risk of transmission and developmental anomalies in infected fetuses. In western developed countries, where the prevalence rate of cytomegalovirus is low, there is still risk of severe congenital cytomegalovirus infection and general screening for cytomegalovirus seropositivity followed by direct viral detection in the ejaculate in infertile couples should be performed.

Acknowledgments. The authors thank Sorin Biomedica for providing DNA enzyme immunoassay (DEIA) kits and Gaëlle Peronnet, Christine Prive, and Marc Carteron for their excellent technical assistance.

REFERENCES

1. Onorato IM, Morens DM, Martone WJ, Stansfield SK. Epidemiology of cytomegaloviral infections: recommendations for prevention and control. Rev Infect Dis 1985;7:479–97.
2. Sinzger C, Plachter B, Grefte A, The TH, Jahn G. Tissue macrophages are infected by human cytomegalovirus *in vivo*. J Infect Dis 1996;173:240–5.
3. Demmler GJ. Summary of a workshop on surveillance for congenital cytomegalovirus. Rev Infect Dis 1991;13:315–29.
4. Huang ES, Alford CA, Reynolds DW, Stagno S, Pass RF. Molecular epidemiology of cytomegalovirus infections in women and their infants. N Engl J Med 1980;303:958–62.
5. Stagno S, Whitley RJ. Herpesvirus infections of pregnancy. Part I: Cytomegalovirus and Epstein-Barr virus infections. N Engl J Med 1985;313:1270–4.
6. Pass RF, Stagno S, Myers GJ, Alford CA. Outcome of symptomatic congenital CMV infection: results of long term longitudinal follow-up. Pediatrics 1980;66:758–62.
7. Fowler KB, Stagno S, Pass RF. The outcome of congenital cytomegalovirus infection in relation to maternal antibody status. N Engl J Med 1992;326:663–7.
8. Putland RA, Ford J, Korban G, Evdokiou A, Tremaine M. Investigation of spontaneously aborted concepti for microbial DNA: investigation for cytomegalovirus DNA using polymerase chain reaction. Aust N Z J Obstet Gynaecol 1990;30:248–50.
9. Cook MS, Himebaugh KS, Frank TS. Absence of cytomegalovirus in gestational tissue in recurrent spontaneous abortion. Diagn Mol Pathol 1993;2:116–9.
10. Baskar JF, Furnari B, Huang ES. Demonstration of developmental anomalies in mouse fetuses by transfer of murine cytomegalovirus DNA-injected eggs to surrogate mothers. J Infect Dis 1993;167:1288–95.
11. Leach CT, Cherry JD, English PA, Hennessey K, Giorgi JV, Visscher BR, et al. The relationship between T-cell levels and CMV infection in asymptomatic HIV-1 antibody-positive homosexual men. J Acquir Immune Defic Syndr 1993;6:407–13.
12. Detels R, Leach CT, Hennessey K, Liu Z, Visscher B, Cherry JD, et al. Persistent cytomegalovirus infection of semen increases risk of AIDS. J Infect Dis 1994;169:766–8.
13. Rasmussen L, Morris S, Hamed K, Merigan TC. Human cytomegalovirus DNA is present in CD45+ cells in semen from human immunodeficiency virus-infected patients. J Infect Dis 1995;171:432–6.
14. Krieger JN, Coombs RW, Collier AC, Ross SO, Speck C, Corey L. Seminal shedding of human immunodeficiency virus type 1 and human cytomegalovirus: evidence for different immunologic controls. J Infect Dis 1995;171:1018–22.
15. Bantel-Schaal U, Neumann-Haefelin D, Schieferstein G. Cytomegalovirus is absent from semen of a population seeking fertility evaluation. J Infect Dis 1993;168:518–9.
16. Shen CY, Chang SF, Yang SL, Wu CW, Yang YS, Ho HN, et al. Cytomegalovirus is present in semen from a population of men seeking fertility evaluation. J Infect Dis 1994;169:222–3.
17. Swenson PD, Kaplan MH. Rapid detection of cytomegalovirus in cell culture by indirect immunoperoxidase staining with monoclonal antibody to an early nuclear antigen. J Clin Microbiol 1985;21:669–73.
18. Weston K, Barrell BG. Sequence of the short unique region, short repeats, and part of the long repeats of human cytomegalovirus. J Mol Biol 1986;192:177–208.
19. Longo MC, Berninger MS, Hartley JL. Use of uracil DNA glycosylase to control carry-over contamination in polymerase chain reactions. Gene 1990;93:125–8.
20. Levy R, Najioullah F, Thouvenot D, Bosshard S, Aymard M, Lina B. Evaluation and comparison of PCR and hybridization methods for rapid detection of cytomegalovirus in clinical samples. J Virol Methods. 1996;62:103–11.
21. Mazeron MC, Alain-Albertini S. Human cytomegalovirus infection: new methods for virological diagnosis. Pathol Biol 1993;41:487–94.
22. Yang YS, Ho HN, Chen HF, Chen SU, Shen CY, Chang SF, et al. Cytomegalovirus infection and viral shedding in the genital tract of infertile couples. J Med Virol 1995;45:179–82.
23. Gass P, Kiessling M, Schäfer P, Mester C, Schmitt HP, Kühn JE. Detection of human cytomegalovirus DNA in paraffin sections of human brain by polymerase chain reaction and the occurrence of false negative results. J Neurol Neurosurg Psychiatry 1993;56:211–4.
24. Fedder J. Nonsperm cells in human semen: with special reference to seminal leukocytes and their possible influence on fertility. Arch Androl 1996;36:41–65.
25. Forman R, Guillet-Rosso F, Fari A, Volante M, Frydman R, Testart J. Importance of semen preparation in avoidance of reduced in vitro fertilization results attributable to bacteria. Fertil Steril 1987;47:527–30.