Prevalence of human herpes virus types 1–7 in the semen of men attending an infertility clinic and correlation with semen parameters

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Objective: To determine the prevalence of herpes viruses in the semen of an asymptomatic male cohort with and without infertility problems and its association with altered semen parameters.
Design: A prospective randomized study.
Setting: Medical school and IVF clinic.
Patient(s): One hundred seventy-two male patients undergoing routine semen analysis: 80 with normal semen parameters (control group) and 92 with abnormal semen parameters.
Intervention(s): Semen samples were collected by masturbation.
Main Outcome Measure(s): The DNA from the *Herpesviridae* family (herpes simplex virus 1 [HSV-1], herpes simplex virus 2 [HSV-2], Varicella zoster virus [VZV], Epstein-Barr virus [EBV], cytomegalovirus [CMV], human herpes virus type 6 [HHV-6], human herpes virus type 7 [HHV-7]) and routine semen parameters.
Result(s): Viral DNA was detected in 143/172 (83.1%) of the total samples for at least one herpes virus: HSV-1, 2.5%; VZV, 1.2%; EBV, 45%; CMV, 62.5%; HHV-6, 70%; HHV-7, 0% in the normal semen samples and HSV-1, 2.1%; VZV, 3.2%; EBV, 39.1%; CMV, 56.5%; HHV-6, 66.3%; HHV-7, 0% in the abnormal semen samples. No association was found between the presence of viral DNA and semen parameters. Interestingly, a statistical significance between leukocytospermia and the presence of EBV DNA was observed.
Conclusion(s): The DNA of herpes viruses is frequently detected in the semen of asymptomatic fertile and infertile male patients. Further studies are required to investigate the role of herpes viruses in male factor infertility. (Fertil Steril® 2009;91:2487–94. ©2009 by American Society for Reproductive Medicine.)

Key Words: Infection, herpes virus, PCR, leukocytospermia, infertility, semen

Infertility, defined as the inability to conceive after 1 year of unprotected intercourse, is a problem that affects almost 15%–20% of couples in European countries. Male and female factors coexist in about one-third of infertility cases; 60% of cases are due to a male factor, and studies on the causative factors of male factor infertility reveal that 30% of the patients suffer from idiopathic infertility (1).

A number of causes have been implicated in male factor infertility such as failure to produce or blockage of sperm, chronic diseases, and erectile dysfunction (2, 3). Moreover, treatments for certain illnesses or cancer, for example, chemotherapy and radiation therapy, exposure to harmful substances, and environmental or work hazards can lead to infertility problems, while injury to the testicles may also affect sperm production.

Human pathogens have been recognized as having a considerable possible effect on male factor infertility or low sperm counts in men. Bacterial infections may lead to male factor infertility with a prevalence of 6.6%–48% (1). Accumulating evidence indicates that viral infections contribute to male factor infertility, either directly through toxic effects on the cells of the male genital tract or indirectly, causing local infectious or immunological responses that in turn can negatively affect reproductive procedures (4–6). Semen is thought to facilitate the spread of viral diseases as these have been detected in both cell-free semen and cells (e.g., spermatozoa and white blood cells [WBCs]) (4, 6).

Common infections include mumps and certain types of sexually transmitted diseases (STDs). Human papilloma virus (HPV) has been shown to alter sperm motility, while specific genes seem to be affected (7). Human immunodeficiency virus (HIV) does not infect spermatozoa (8) and has shown no changes in semen pH, sperm concentration, total motility, vitality, and morphology (9). Hepatitis viruses (HBV, HCV) are harbored in semen, indicating that the male genital tract may act as a reservoir (10).

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Members of the herpes virus superfamily have been detected in various studies in semen, establishing STDs that cause chronic and/or lethal diseases and can be transmitted through insemination (11) or from mothers to their children during pregnancy and/or delivery (6, 12).

Viral DNA from members of the *Herpesviridae* family, such as herpes simplex virus (HSV) -1 and -2, cytomegalovirus (CMV), Epstein-Barr virus (EBV), and human herpes virus (HHV) -6, -7, -8, has been detected in the semen of asymptomatic infertile patients (4–6, 10). In particular, HSV-2 and CMV, which are sexually transmissible, have been extensively studied and can lead to fetal and neonatal abnormalities (2).

Despite the clinical significance of these observations, the role of herpes viruses in male factor infertility remains obscure. The prevalence of herpes viruses varies, as it has been reported until recently in the literature, particularly for HSV-1, which ranges between 3.1% and 49.5%, depending on the viral DNA detection method used (polymerase chain reaction [PCR], in situ hybridization, etc.) (10, 13–17). CMV has been found in the semen of both randomized male populations attending an infertility clinic (13, 18) and infertile males (16) but does not seem to play an important role in infertility (13, 18), while the role of other members of the superfamily that have only been detected at low frequencies remains unclear and controversial (10, 18).

The aim of our study was to use PCR to determine the prevalence of herpes viruses in the semen of a randomized asymptomatic male cohort attending an infertility clinic. Furthermore, the possibility of viral infections affecting semen parameters, and thus fertility, by comparing a control group to an "abnormal" semen group was also examined.

MATERIALS AND METHODS

Samples

Semen samples were collected by masturbation from 172 men who were attending the infertility clinic at the University Hospital of Crete because of couple fertility problems. Informed consent was obtained from each patient for the purposes of the current study. None of the men studied or their spouses had reported any clinically confirmed genital herpetic infection in their medical history. In all cases, a complete semen analysis was performed, including sperm count, motility, pH, viscosity, and morphology.

Semen Collection and Analysis

Semen samples were obtained by masturbation in sterile containers after sexual abstinence of 48–72 hours. Samples were analyzed within 1 hour of collection and processed for DNA extraction within 2 hours of collection. Sperm concentrations and motility were observed under a light microscope using a Makler chamber according to World Health Organization (WHO) guidelines. Additional parameters such as volume, progression, pH, white blood cells (WBCs), vitality, and mixed agglutination reaction (MAR) test were also evaluated. Sperm morphology was assessed on methanol-fixed smears of fresh ejaculate stained with eosin and thiazine under a light microscope and evaluated according to strict Krueger criteria. Normal samples were considered to be the semen specimens that fulfilled all the aforementioned criteria. An excessive number of WBCs alone did not render the sample abnormal. All semen specimens that had one or more altered semen parameters were characterized abnormal.

DNA Extraction from Semen Samples

All preparations for the PCR assays were performed in a "clean room" (no post-PCR DNA products) inside a laminar flow hood to minimize contamination. After collection, each fresh semen sample was centrifuged at 13,000 rpm for 30 minutes. The supernatant (seminal fluid) was transferred to a new eppendorf tube, while the pellet containing the spermatozoa remained in the original eppendorf tube. Proteins were lysed using proteinase K. DNA extraction was performed using the standard phenol-chloroform protocol. All DNA samples were subjected to spectrophotometry for the quantification of DNA at 260 nm, while the ratio 260/280 nm, indicating the purity of DNA, was also determined.

PCR Amplification Reactions

All specimens were examined for the presence of amplifiable DNA using a set of primers for the *β2-globin* gene. Both spermatozoa and the corresponding seminal fluids from all participants were examined for the presence of HSV-1, HSV-2, Varicella zoster virus (VZV), EBV, CMV, HHV-6, and HHV-7 DNA by PCR using the appropriate set of primers (Table 1). One hundred nanograms of extracted DNA of each sample was initially amplified in a reaction solution of 20 μL, containing 50 mM $MgCl_2$, 25μM of each primer, 10 mM of each dNTP, 10× PCR buffer, and 0.5 U Taq polymerase (Fermentas Life Sciences, St. Leon-Rot, Germany). The first-round PCR products from the EBV and HHV-6 reactions were used as templates for nested PCR, using an additional set of inner primers (Table 1).

The PCR conditions for *β2m* and herpes viruses are shown in Table 1. In all reactions, the primary PCR mixture was heated for 4 minutes at 94°C and subjected to amplification for 35 cycles according to the data shown in Table 1; the PCR reaction ended with an elongation step at 72°C for 10 minutes. Typing of HSV was carried out after the digestion of the initial PCR product with the restriction enzyme *Ava*II and determined depending on the digestion pattern produced.

The PCR products for each virus were cloned into the plasmid vector pCR2.1-TOPO (Invitrogen, Harilaou, Greece) and were amplified in parallel with the samples, serving as positive controls. To ensure that our PCR assay was sensitive enough to detect relatively low levels of viral DNA, as described elsewhere (19), serial dilution assay was employed (data not shown). The PCR products were examined by

TABLE 1

List of primers used for the detection of HSV-1, HSV-2, VZV, EBV, CMV, HHV-6, HHV-7, and HHV-8 and the amplification of *beta2 microglobulin*.

Virus	Gene	Sequence	PCR conditions	PCR product
HSV-1 and -2	*DNA polymerase UL30*	5′-CAG TAC GGC CCC GAG TTC GTG A-3′	94°C/50 s	478 bp
			64°C/40 s	
		5′-TTG TAG TGG GCG TGG TAG ATG-3′	72°C/50 s	
VZV	*ORF62 gene for IE62 transactivator*	5′-ATG TCC GTA CAA CAT CAA CT-3‘	94°C/40 s	267 bp
			60°C/30 s	
		5′-CGC AGA GAG AAC CTT TTA GC-3′	72°C/30 s	
		Outer primers	94°C/30 s	102 bp
EBV	*Nuclear antigen*	5′-AGC ACC CCC ACA TAT CTC TTC TT-3′	65°C/30 s	
		5′-CGA GTC ATC TAC GGG GAC ACG GA-3′	72°C/30 s	
		Inner primers	94°C/30 s	
		5′-GGA GAA GGT CTT CTC GGC CTC-3′	69°C/30 s	
		5′-TTC AGA GAG CGA GAC CCT GC-3′	72°C/20 s	
		5′-GTC ACC AAG GCC ACG ACG TT-3′	94°C/40 s	
CMV	*IE1*	5′-TCT GCC AGG ACA TCT TTC TC-3′	57°C/30 s	167 bp
			72°C/30 s	
		Outer primers	94°C/30 s	
		5′-GAC AAT CAC ATG CCT GGA TAA TG-3′	58°C/30 s	
		5′-TGT AAG CGT GTG GTA ATG GAC TAA-3′	72°C/20 s	
HHV-6	*Major capsid protein*	Inner primers	94°C/30 s	70 bp
		5′-GTT AAA TTG ATA GTA CTT ACG TG-3′	53°C/30 s	
		5′-ATC AAA ATA TAA AGA GCA CAG CA-3′	72°C/30 s	
HHV-7	*Major capsid protein*	5′-GGA AAT AGG ATC TTT TCA AAT TC-3′	94°C/30 s	122 bp
		5′-GTT ACT TTC AAA AAT GTT TGT CCC-3′	59°C/30 s	
			72°C/30 s	
HHV-8	*Major capsid protein*	Outer primers	94°C/30 s	173 bp
		5′-AGC CGA AAG GAT TCC ACC AT-3′	58°C/30 s	
		5′-TCC GTG TTG TCT ACG TCC AG-3′	72°C/20 s	
		Inner primers	94°C/30 s	
		5′-GTG CTC GAA TCC AAC GGA TT-3′	53°C/30 s	
		5′-ATG ACA CAT TGG TAT AT-3′	72°C/30 s	
	Housekeeping gene: *beta 2 microglobulin*	5′-TCC AAC ATC AAC ATC TTG GT-3′	94°C/30 s	102 bp
		5′-TCC CCC AAA TTC TAA GCA GA-3′	55°C/30 s	
			72°C/30 s	

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electrophoresis in a 2% or 3% agarose gel, depending on the size of the PCR product, and photographed on an ultraviolet light transilluminator. Finally, all viral PCR products were subjected to direct sequencing analysis, verifying the initial amplification results.

Statistical Analysis

The results were analyzed through standard statistical methods applied in case-control studies. The correlation of each of the studied characteristics with viral infection was evaluated by Pearson's χ^2 analysis or Fisher's exact test where indicated (expected frequencies <5). Numerical values were expressed as mean ± SEM, and differences between means were compared by the two-tailed, unpaired Student's *t*-test. In all cases, $P<.05$ was considered statistically significant. The analyses were performed using SPSS software, version 10 (SPSS Inc., Chicago).

RESULTS

Herpes Virus Prevalence in Total Semen Samples

Viral DNA, in general, was detected by PCR in 143 (83.1%) of 172 total semen samples for at least one member of the herpes virus family (Table 2). The results from the detection of viral DNA for all 172 samples available are as follows: CMV was detected in 98 specimens (56.9%), HHV-6 in 116 (66.8%), EBV in 70 (40.6%), VZV in four (2.3%), and HSV-1 in four (2.3%). The simultaneous presence of two DNA viruses was detected as follows: CMV and HHV-6 in 76 subjects (44.1%), EBV and HHV-6 in 55 (31.9%), and EBV and CMV in 48 (27.9%), whereas the combination of HSV with EBV or HSV with HHV-6 was found in four cases (2.3%) and the combination of VZV with HHV-6 was found in three cases (1.7%). Concurrent detection of the DNA of three viruses was as follows: EBV-CMV-HHV-6 was found in 39 cases (22.6%), VZV-EBV-CMV in two (1.1%), VZV-CMV-HHV-6 in two (1.1%), and VZV-EBV-HHV-6 in two (1.1%). The DNA of four viruses, VZV-EBV-CMV-HHV-6, was found in only two samples (1.1%) (Figure 1).

Herpes Viral Prevalence in Spermatozoa and Seminal Fluid of Total Semen Samples

All previous studies examined the prevalence of viral DNA in total semen samples. We found the possibility of detecting viral DNA in spermatozoa, in seminal fluid, or in both very intriguing, and, therefore, we separated these two biological materials by high-speed centrifugation. Interestingly, herpes virus DNA was detected at variable frequencies for each virus tested, when spermatozoa and seminal fluid were compared (Table 2). In spermatozoa, CMV was detected in 89 samples (51.7%), HHV-6 in 82 (47.6%), EBV in 49 (28.4%), HSV-1 in three (1.7%), and VZV in one (0.5%). With respect to seminal fluid, CMV was found in 66 cases (38.3%), HHV-6 in 65 (37.7%), EBV in 35 (20.3%), and VZV in four (2.3%). We also combined those samples, which showed herpes virus presence both in spermatozoa and seminal fluid, constituting male semen. The presence was as follows: CMV was detected in 50 semen samples (29%), HHV-6 in 39 (22.6%), EBV in 22 (12.7%), and VZV in one (0.5%). Interestingly, high concentrations of WBCs were found in 20 (40.8%) of 49 seminal fluid samples, which were positive for EBV; however, no statistical association could be established.

Herpes Viral Prevalence in Normal Semen Samples

Eighty of 172 semen samples were classified as normal (normozoospermia) according to WHO guidelines. Viral DNA of HHV-6 was detected in 56 cases of normal semen samples (70%), CMV in 50 (62.5%), EBV in 36 (45%), HSV-1 in two (2.5%), and VZV in one (1.2%; Table 3).

Herpes Viral Prevalence in Abnormal Semen Samples

Viral DNA was also detected in the group of abnormal semen samples from 92 male subjects (Table 3). In this group, the samples were sorted into the following subgroups: 85 oligozoospermic, 59 asthenozoospermic, 52 oligoasthenozoospermic (including those samples in which both sperm concentration and motility were altered), three teratozoospermic, and 14 with leukocytospermia. Fifty-two of 92 abnormal semen samples were CMV-positive (56.5%), and 61 samples were positive for HHV-6 (66.3%). EBV was found in 36 cases (39.1%), VZV

TABLE 2

Cumulative results of the detection of herpes viruses in spermatozoa and seminal fluid.

Virus	Spermatozoa	Seminal fluid	Semen (sperm and seminal fluid)	Total virus presence
HSV-1	3 (1.7)	1 (0.5)	—	4/172 (2.3)
HSV-2	—	—	—	—
VZV	1 (0.5)	4 (2.3)	1 (1)	4/172 (2.3)
EBV	49 (28.4)	35 (20.3)	22 (12.7)	70/172 (40.6)
CMV	89 (51.7)	66 (38.3)	50 (29)	98/172 (56.9)
HHV-6	82 (47.6)	65 (37.7)	39 (22.6)	116/172 (66.8)
HHV-7	—	—	—	—

Note: Data in parentheses are percents.

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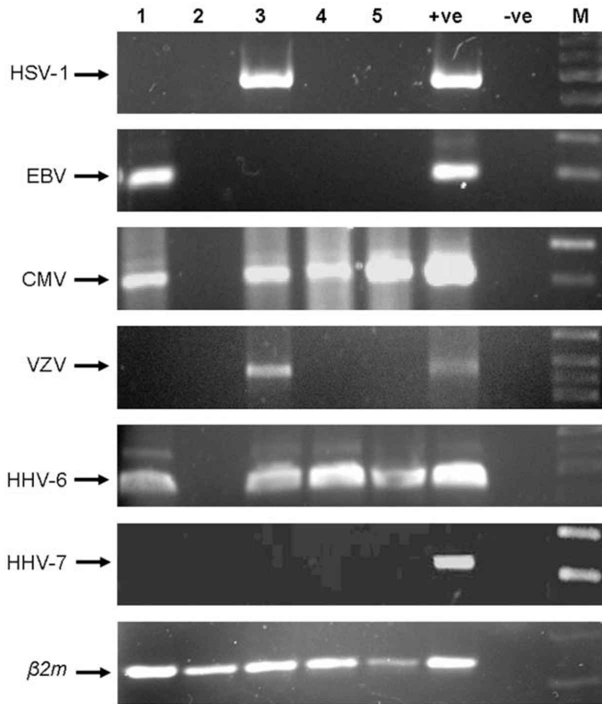
FIGURE 1

PCR detection of herpes viruses HSV-1, EBV, CMV, VZV, HHV-6, HHV-7, and the housekeeping gene *b2microglobulin*. The PCR products were visualized in 2% agarose gel. Lanes: 1–5, semen samples; ve: positive control; −ve: negative control; M: molecular weight marker.



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in three (3.2%), and HSV-1 in two (2.1%). Statistical analysis was carried out between the presence of each herpes virus and each abnormal subgroup, but no statistical correlation was observed.

Herpes Viruses and Leukocytospermia

Thirty-seven of the total 172 semen samples, 23 normal and 14 abnormal samples, were found presenting high concentrations of WBCs. PCR analysis resulted in the following data: HHV-6 was detected in 27 samples (72.9%), CMV and EBV in 24 (64.8%), and HSV-1 in two (5.4%). The statistical analysis revealed a significant association between the presence of EBV DNA in the tested specimens and leukocytospermia (P=.021).

Herpes Simplex Viruses and Teratospermia

A statistical significance was observed, indicating an association between VZV and teratospermia (P=.037). However, VZV-positive samples were extremely rare (Table 3), and this conclusion should be carefully considered.

TABLE 3

Detailed results of the detection of herpes viruses in normal and abnormal semen.

Virus	Normozoospermia (80/172)	Abnormal sperm (92/172)	Oligozoospermia (85/172)	Asthenozoospermia (59/172)	Oligoasthenozoospermia (52/172)	Teratozoospermia (3/172)	Leukocytospermia (37/172)
HSV1	2 (2.5)	2 (2.1)	1 (1.1)	1 (1.6)	—	—	2 (5.4)
VZV	1 (1.2)	3 (3.2)	3 (3.5)	3 (5)	2 (3.8)	1 (33.3)	—
EBV	36 (45)	36 (39.1)	30 (35.2)	20 (33.8)	16 (30.7)	2 (66.6)	24 (64.8)
CMV	50 (62.5)	52 (56.5)	44 (51.7)	31 (52.5)	27 (51.9)	3 (100)	24 (64.8)
HHV-6	56 (70)	61 (66.3)	54 (63.5)	38 (64.4)	32 (61.5)	1 (33.3)	27 (72.9)

Note: Data in parentheses are percents.

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Normal Semen versus Abnormal Semen

Statistical analysis failed to reveal any association between the influence of herpes virus presence on the semen parameters and the subsequent characterization of samples as normal or abnormal semen, and, thus, viral existence does not seem to dramatically influence qualitative or quantitative characteristics of the semen. Viral DNA was almost equally distributed in both normal and abnormal semen. On the other hand, EBV, CMV, and HHV-6 appeared to be more frequent in oligozoospermic semen than in asthenozoospermic semen but with no statistical significance.

Mean Sperm Count in Association with Herpes Viral DNA

The mean sperm count of EBV-positive semen samples showed a statistical significance (P=.034), indicating an association between the presence of EBV DNA and an increased mean sperm count. On the contrary, HSV-1, VZV, CMV, and HHV-6 infection did not show any influence on mean sperm count (Table 4).

Mean Motility in Association with Herpes Viral DNA

HSV-1, EBV, CMV, and HHV-6 infection did not show any influence on mean sperm motility. The statistical significance observed in the mean motility of semen samples infected by VZV (P=.018), however, does not allow us to make any conclusions because of the small number of samples (Table 5).

TABLE 5

Herpes virus presence and mean sperm motility.

	Specimens, n[a]	Motility[b]	P[c]
HSV-1 positive	4	53.8 ± 1.7	.8
HSV-1 negative	168	51.3 ± 6.3	
CMV positive	95	52.3 ± 2.2	.4
CMV negative	74	49.5 ± 2.4	
VZV positive	4	26.5 ± 18.5	.018
VZV negative	168	51.9 ± 1.6	
EBV positive	70	53.7 ± 2.7	.2
EBV negative	102	49.7 ± 2	
HHV-6 positive	110	52.1 ± 2	.5
HHV-6 negative	55	49.5 ± 3	
HHV-7 positive	0		
HHV-7 negative	172		

[a] Number of specimens/patients.
[b] Mean ± SEM.
[c] P-values are for comparisons between the positive and negative of each virus.

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DISCUSSION

The demand for assisted reproduction has increased over the last few years because of infertility problems among young couples (20). Male factor infertility in the majority of the cases remains asymptomatic and with unknown causes. We designed our study to investigate the prevalence of seven herpes viruses, types 1–7, in the semen of healthy fertile and infertile males, using a sensitive PCR assay. Moreover, we wished to study the relationship between virus presence and semen parameters.

TABLE 4

Herpes virus presence and mean sperm count.

Virus	Specimens, n[a]	Sperm count[b]	P[c]
HSV-1 positive	4	74 ± 38.2	.063
HSV-1 negative	168	34.3 ± 3.2	
CMV positive	95	37.8 ± 4.6	.3
CMV negative	74	30.2 ± 3.9	
VZV positive	4	35.8 ± 3.3	.3
VZV negative	168	13 ± 6.1	
EBV positive	70	43.5 ± 5.9	.034
EBV negative	102	29.6 ± 3.5	
HHV-6 positive	110	37.3 ± 4.2	.3
HHV-6 negative	55	30.3 ± 5.3	
HHV-7 positive	0		
HHV-7 negative	172		

[a] Number of specimens/patients.
[b] Mean ± SEM.
[c] P-values are for comparisons between the positive and negative of each virus.

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Our results indicate a high prevalence of all herpes viruses tested (83.1%) in semen regardless of their categorization as normal or abnormal semen samples. This overall detection frequency in our study appears to be very high compared with previous studies that reported prevalences of 56.6% [Kapranos et al. (15)] or 17.1%–18.7% [Bezold et al. (18)]. There are also a number of relevant studies that did not conclude on an overall percentage of viral detection. A possible explanation for this high percentage could be the examination of a very large range of herpes viruses compared with most of the previous studies. Apart from one study that screened for all herpes viruses (10), the rest included only a few members of the *Herpesviridae* family, for example, Kapranos et al. (15), who investigated only HSV, CMV, and EBV, or Bezold et al. (18), whose study was designed to examine CMV, HHV-6, and EBV. The number of samples that was included in our study (n = 172) was larger than the number tested by Kapranos et al. (15) (113 samples), Aynaud et al. (21) (111 samples), and Krause et al. (22) (130 samples), and this could also be a reason for the higher viral prevalence.

CMV and HHV-6 were present in 56.9% and 66.8% of the cases, respectively. CMV belongs in the beta herpes virus family and causes serious symptoms especially in immunocompromised patients (23, 24). It is one of the herpes viruses

that has been studied the most in relation to male semen (10, 22, 25, 26). In our study, CMV, along with HHV-6, was the most commonly detected in both subgroups of male semen, in normal and abnormal semen. Fifty out of 80 normal semen samples were found positive for CMV DNA (62.5%), whereas 52 out of 92 (56.5%) abnormal semen samples were positive. We did not observe any impact of the presence of CMV DNA on semen parameters, such as sperm count and motility. Our data are consistent with previous studies that do not show any association of CMV infection with a reduction in semen parameters (15, 26). This result strongly opposes those shown by Kapranos et al. (15), notwithstanding that CMV seems to be the most common herpes virus in a number of studies (15, 18, 21, 22).

HHV-6 also belongs to the beta herpes virus family and causes a childhood disease called roseola infantum as well as multiple sclerosis. It is associated with exanthema subitum (27, 28) and EBV- and CMV-negative cases of mononucleosis in young adults (28, 29). The DNA of this virus has been detected in semen only twice before (0.4% and 3.7%, respectively) (10, 18), and its unexpectedly high prevalence in our cohort is noteworthy. Fifty-six (70%) out of 80 of the normal samples and 61 (66.3%) out of 92 of the abnormal semen samples were found to be positive. Since no statistical significance was found despite the high prevalence of HHV-6 DNA, this could imply that HHV-6 presence does not affect semen parameters.

EBV is a member of the gamma herpes virus family and causes infectious mononucleosis and two cancer types including Burkitt's lymphoma (19). EBV has been previously been detected in semen (10, 15, 18), and it is considered to be a sexually transmitted pathogen. Normally, a low EBV detection rate would be expected because of the fact that the virus's primary host cells, the B-lymphocytes, represent only a small proportion of the WBCs in semen (3). However, we detected EBV DNA in 40.6% of the cases, at a relatively equal frequency among normal and abnormal groups. This frequency is relatively high compared with previous studies in which frequencies range from 0.4% to 16.8% (10, 15, 18).

Leukocytospermia or pyospermia is defined by the WHO as the presence of peroxidase-positive leukocytes in concentrations greater than 1×10^6/mL of semen (3). Physiologically, most leukocytes appear to originate from the epididymis (4, 31) and are thought to play an important role in immunity (4, 31) and phagocytic clearance of abnormal sperm (4), but the origin of the excess leukocytes remains undetermined. It may be a symptom of male accessory gland infection, but the incidence of leukocytospermia ranges between 10% and 20% among infertile men. Moreover, the role of leukocytospermia in the pathogenesis of male factor infertility remains highly controversial (21). The fact that we found a 20.3% presence of EBV DNA in seminal fluid and a statistical significance between leukocytospermia and EBV presence allows us to assume that EBV could be one of the reasons for the increased concentrations of WBCs in seminal fluid.

HSV-1 causes oral and occasionally genital cold sores, whereas HSV-2 is the most common cause of genital herpes. Generally, direct or indirect contact with herpetic lesions is infectious, but HSV-1 and HSV-2 have been detected in semen (10, 15, 18, 32) and in sperm (17), and HSV-2 was transmitted through donor insemination (12). In the current study, HSV-1 was only detected in two (2.5%) of 80 normal semen samples and in two (2.1%) of 92 abnormal samples. The frequency of HSV, which was observed only in the 2.3% of the normozoospermic males, is at variance with the findings of Kapranos et al. (15) but close to those demonstrated by Bezold et al. (18), with the difference that they were detected in infertile males. No association was found between HSV-1 and HSV-2 DNA and low sperm count and motility and infertility as in previous studies (32). Notably, it has been shown that antiviral treatment (acyclovir and valacyclovir) can help previously infertile men and women achieve pregnancy (30, 31).

HHV-3 or VZV causes chicken pox and herpes zoster. Chicken pox can affect male factor infertility in two ways (33). First, a high fever associated with the illness can temporarily decrease semen production. Typically, semen production returns to normal within 90 days after the fever ends. Second, infectious diseases such as chicken pox may cause inflammation of the testis (orchitis). This may result in testicular shrinkage (atrophy) and infertility (33). To date, VZV has been studied only once, and it was not detected at all (31). In the present study, VZV DNA was detected in one (1.2%) sample out of the 80 normal ones and in three (3.2%) out of 92 abnormal samples. The fact that a statistical significance was observed, indicating an association between VZV and teratospermia, suggests that further research on its influence on spermatozoa morphology is required. However VZV-positive samples were extremely rare, and such a conclusion should be carefully evaluated.

Lastly, HHV-7 was discovered in 1990 and is a member of the beta herpes virus family, closely related to both HHV-6 and CMV (20). No data are available concerning its involvement in male factor infertility. It has only been detected in infertile women, who were subsequently treated with valacyclovir (31). The detection rate of the virus in our study is consistent with the only available study by Bezold et al. (10). Since almost 100% of the population is thought to be infected by HHV-7 during infancy and since it remains in a latent state in the body thereafter, we can assume that it does not enter in the male genital tract and, thus, is not detected in semen (20).

Analysis of the cumulative results shows that there is a significant difference between the presence of herpes viruses in spermatozoa and in seminal fluid. Viruses seem to be hosted in spermatozoa rather than in the seminal fluid, which can support their preference in infecting human cells. Their presence in seminal fluid has never been examined before because in all previous studies, the fluid was discarded and only the cells tested. In fact, we found viral DNA to be present in seminal fluid of both normal and abnormal semen. Furthermore, we showed the concurrent presence of viruses in both spermatozoa and seminal fluid. The presence of viral DNA in the

seminal fluid may be the reason why the high percentages that some viruses have shown in abnormal semen have no direct influence on fertility, as only the spermatozoa are examined for the parameters that categorize them as normal or abnormal and, hence, infertile.

A statistical significance between EBV presence and mean sperm count was observed, suggesting that EBV may affect sperm count by increasing the numbers of spermatozoa produced. Nevertheless, this is an observational study, and further studies are needed to clarify this finding.

Interestingly, an observed statistical significance between VZV and mean sperm motility shows that VZV presence could affect sperm motility. The fact that VZV was mainly found in seminal fluid and not in spermatozoa could indicate that the actual factor that inhibits spermatozoa movement is localized in seminal fluid. Nevertheless, the small number of VZV-positive samples does not allow us to draw any safe conclusions.

In conclusion, the screening of normal and abnormal semen samples for the presence of viral infection caused by any of the seven herpes viruses, for the first time, has revealed relatively high percentages of viral infection in both groups. EBV provided an indication that it might be the reason why abnormally high concentrations of WBCs are present in semen. Nevertheless, further studies are needed to clarify the exact role that herpes viruses play in semen and infertility.

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