Screening of semen donors for infectious diseases

Corinne A. Liesnard

Laboratory of Virology, AIDS Reference Laboratory, Erasme Hospital, Université Libre de Bruxelles, Brussels, 1070, Belgium

The aim of this work was to prepare European guidelines concerning the screening for infectious diseases in the setting of gamete donation. Existing guidelines have been reviewed and commented. In particular, the problem of cytomegalovirus (CMV) was a matter of debate following the decision by the French Government to reject CMV seropositive donors. Other remarks and suggestions concerning various pathogens and perspectives of new molecular techniques for the detection of pathogens are also included.

Key words: cytomegalovirus/European guidelines/infectious diseases/screening/semen donors

Introduction

Several infectious agents can be present in semen and be transmitted by artificial insemination. The transmitted organisms may produce local or disseminated disease in the inseminated woman and may adversely affect the outcome of the resulting pregnancy or reduce the woman's fertility. Selection of semen donors must include a screening for sexually transmitted diseases (STD). Moreover, as there is no definitive way to ensure that pathogens will not be transmitted by artificial insemination, the use of fresh semen should be prohibited. Semen must be kept frozen, quarantined for 6 months and released only when the donor has been retested and found to be negative for STDs. This procedure allows the elimination of semen from donors who seroconverted during quarantine, e.g. for human immunodeficiency virus (HIV) or hepatitis C virus (HCV) antibodies, and were potentially infectious 6 months previously, when anti-HIV or HCV antibodies were not detectable. Guidelines for the screening of infectious diseases have been edited by the British Andrology Society (1993), and by the American Fertility Society (1993). These guidelines are summarized in Table I and involve initial screening tests (medical history, physical examination and laboratory tests) and continuing surveillance every 6 months. Table II shows the incidence and/or prevalence of infectious diseases that are covered by the guidelines, but also of other new viral infections that we should probably take into account in the near future. The incidence and prevalence is that in Western countries, as epidemiology in other part of the world may be very different. Prevalences in semen refer to semen donor series when available, or to chronic carriers series; methods of detection are indicated.

Medical history

Guideline

The donor candidate should be interviewed by a health care professional with regard to behaviour that could have exposed him to STDs. In particular, a history of homosexuality, bisexuality, multiple sex partners, prostitution, and i.v. drug use are reasons for exclusion. A recent stay in a geographical region with a high incidence of HIV should lead to other questions concerning sexual contacts with natives of this country, particularly with prostitutes or i.v. drug users, accidents, surgery, blood transfusions, and the use of skin piercing instruments (tattoos, razors, etc). A history of genital herpes or warts is also a cause of exclusion.
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| Table I. Guidelines for screening for infections in semen donors—exclusion criteria |
|---------------------------------------------|----------------------------------------------|
| British Andrology Society | American Fertility Society | Remarks or suggestions |
| **Initial screening** | | |
| **History** | | |
| HIV risk: | | |
| Homosexuality, i.v. drug users, haemophilia (factor VIII before heat treatment) high prevalence areas, etc. | HIV risk: | Recent exposure to known or suspected HIV blood (health care workers) |
| Past history of STD: | | |
| If bacterial and correctly treated → no exclusion | | |
| if viral (HSV, warts) → exclusion | | |
| **Physical examination** | | |
| Urethral discharge | | |
| genital warts or ulcers | | |
| **Laboratory tests** | | |
| 1. Urethral culture | | |
| Neisseria gonorrhoeae | | |
| Chlamydia trachomatis (or urines) | | |
| (not warranted: Escherichia coli, Proteus spp., Trichomonas vaginalis, Gardnerella vaginalis, Streptococcus faecalis, etc) | | |
| 2. Serology | | |
| Hbs Ag | | |
| HIV, HCV, syphilis antibodies | | |
| CMV IgG antibodies: | | |
| if positive → used for seropositive women only | | |
| if negative → used for seronegative women in priority | | |
| semen is frozen and quarantined for ≥180 days | | |
| Surveillance every 6 months | | |
| **Physical examination** | | |
| Urethral cultures | | |
| Serology: Hbs Ag HIV, HCV antibodies | | |
| CMV follow-up has been forgotten in BAS guidelines | | |
| CMV IgM should be recommended together with CMV IgG antibody detection | | |
| **CMV follow-up has been forgotten in BAS guidelines** | | |
| **HSV, HPV?** | | |

**Comment**

It should be kept in mind that many genital herpes and papillomavirus infections are asymptomatic and that transmission of human papillomavirus (HPV) has not been described in the setting of artificial insemination with donor (AID). Thus, medical history is insufficient to exclude latent, asymptomatic herpes simplex virus (HSV) or HPV infections. Other methods to detect these infections could be proposed (see ‘laboratory tests’).

With reference to the risk of HIV, as health care workers and medical students are frequently semen donors, another precaution is to exclude people who have recently been exposed to known or suspected HIV-positive blood.

Human T-cell lymphotropic virus (HTLV) I and II viruses have a low prevalence in Western countries (Table I). They are principally endemic in Japan, Central Africa, the Caribbean and Melanesia for HTLV I (Hinuma et al., 1982; Fleming
<table>
<thead>
<tr>
<th>General population</th>
<th>Sexual transmission or presence in semen</th>
<th>Transmission by therapeutic insemination</th>
<th>Prevalence in semen</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV: incidence 8/1 × 10^5/year (Belgium) (SIDA, 1995)</td>
<td>yes</td>
<td>yes</td>
<td>PCR: 77% in recent seroconversion (Tindall et al., 1992)</td>
</tr>
<tr>
<td>seroprevalence: 0.08% (Belgium) (SIDA, 1995)</td>
<td></td>
<td></td>
<td>Culture: 10–16% (sperm), 55% (semen cells), (Van Voorhuis et al., 1991; Vernaza et al., 1996)</td>
</tr>
<tr>
<td>HPV hybridization: 10% women (USA) (Bauer et al., 1991)</td>
<td>yes</td>
<td>NA</td>
<td>RT-PCR: up to 87% (Hamed et al., 1993)</td>
</tr>
<tr>
<td>PCR: 46% women (USA) (Bauer et al., 1991)</td>
<td></td>
<td></td>
<td>Hybridization: 0% (Finland) (Nieminen et al., 1991)</td>
</tr>
<tr>
<td>HCV: seroprevalence: 0.3–0.5% (blood donors, France) (Roudot-Thorval et al., 1994)</td>
<td>yes (low)</td>
<td>no</td>
<td>PCR: 0% HCV chronic carriers (USA) up to 24% (China) (Fried et al., 1992; Liou et al., 1992)</td>
</tr>
<tr>
<td>1% (general population France)</td>
<td></td>
<td></td>
<td>(three cases)</td>
</tr>
<tr>
<td>HBV: incidence: 4.2/1 × 10^5/year (USA)</td>
<td>yes</td>
<td>yes</td>
<td>Hybridization: 20–77% chronic carriers (USA) (Jenison et al., 1987; Davison et al., 1987)</td>
</tr>
<tr>
<td>seroprevalence: &lt;0.5% (Western countries) (Hollinger, 1996)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HSV: seroprevalence: 15–20% (HSV2; US, UK) (Johnson et al., 1989; Cowan et al., 1994)</td>
<td>yes</td>
<td>yes</td>
<td>Culture: 0% men with recurrences (USA) (Douglas et al., 1988)</td>
</tr>
<tr>
<td>7.6% (blood donors, UK) (Cowan et al., 1994)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HTLV I and II: seroprevalence: 0.0004–0.011% (blood donors, Western countries) (Salker et al., 1990; Goubeau et al., 1992)</td>
<td>yes</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>CJD-TSSE: incidence 1/1 or 2 × 10^6/year worldwide) (Collee and Bradley 1997)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>CMV: seroprevalence: 50–70% (women in Sweden, USA) (Chandler et al., 1985; Demmler, 1991)</td>
<td>yes</td>
<td>NA</td>
<td>seroprevalence: 15% (UK), 45% (Belgium) semen donors (Prior et al., 1994; Liesnard et al., 1997)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Culture: 3% (USA), 0.4% (Belgium) semen donors (Lang and Kammer, 1975; Tjiam et al., 1987)</td>
</tr>
<tr>
<td>HGV: seroprevalence: 2% (blood donors, Germany) (Stark et al., 1996)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>HHV8: seroprevalence: 25% (US) (Lennette et al., 1996)</td>
<td>NA (suspected)</td>
<td>NA</td>
<td>PCR: 0% semen donors (US) (Howard et al., 1997)</td>
</tr>
<tr>
<td>0–4% (blood donors - Western countries) (Gao et al., 1996; Kedes et al., 1996)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gonorrhoea: incidence: 182/1 × 10^5/year (US)</td>
<td>yes</td>
<td>yes</td>
<td>Culture: 0% (Belgium, Netherlands) (Tjiam et al., 1987)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0% (Australia), semen donors (Haddad and Steigrad, 1991)</td>
</tr>
<tr>
<td>Chlamydia: incidence: 182/1 × 10^5/year (US)</td>
<td>yes</td>
<td>yes</td>
<td>Culture: 0% (Australia) (Haddad and Steigrad, 1991)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6.3% (Belgium, Netherlands) semen donors (Tjiam et al., 1987)</td>
</tr>
<tr>
<td>Syphilis: incidence: 6.3/1 × 10^5/year (USA)</td>
<td>yes</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

NA = not available; PCR = polymerase chain reaction; RT-PCR = reverse transcriptase-polymerase chain reaction; HIV = human immunodeficiency virus; HCV = hepatitis C virus; HBV = hepatitis B virus; HGV = hepatitis G virus; HSV = herpes simplex virus; HPV = human papilloma virus; CJD/T SSE = Creutzfeldt-Jacob disease/transmissible spongiform encephalopathies; HTLV = human T cell lymphotropic virus; CMV = cytomegalovirus; HHV8 = human herpes virus type 8.
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et al., 1986; Wiktor et al., 1990; Yanagihara et al., 1990; Murphy et al., 1991) and Central America and the southern USA for HTLV II (Hjelle et al., 1990; Heneine et al., 1991; Levine et al., 1993). HTLV II is more frequently isolated from i.v. drug users (Lee et al., 1990; Zella et al., 1990; Khabbaz et al., 1992). Both viruses are sexually transmitted (Kajiyama et al., 1986; Murphy et al., 1989; Hjelle et al., 1992b; Vitek et al., 1995), although parenteral transmission (Donegan et al., 1989; Feigal et al., 1991; Sullivan et al., 1991) and perinatal transmission through breast feeding (particularly for HTLV I) are also very efficient (Kusuhara et al., 1987; Heneine et al., 1992). Tropical spastic paraparesis (TSP) and T-cell associated leukaemia and lymphoma are associated with HTLV I. T-cell associated leukaemia and lymphoma develop in 2-4% of infected patients and have a latent period of few decades (Kondo et al., 1985). TSP develops in <1% of infected persons, affects women more frequently than men and has a shorter latent period of a few years (Kaplan et al., 1990; Osame et al., 1990). HTLV II has not been clearly associated with any disease. Rare cases of hairy-cell leukaemia, neurological illnesses and other rare malignancies have been reported in HTLV II infected persons (Rosenblatt et al., 1986; Hjelle et al., 1991, 1992a). It is now mandatory in some countries (USA, France) to test all blood and organ donors for HTLV I-II antibodies. Guidelines for therapeutic insemination should be adapted to local regulations and epidemiology. Although the low HTLV I-II prevalence in the general population in Western countries does not justify a systematic screening of semen donors, it seems wise at least to test semen donors coming from endemic areas for the presence of HTLV I-II antibodies and to exclude seropositive donors.

The recent description of a Creutzfeld–Jacob disease (CJD)-like illnesses in young people and farmers in the UK and the hypothesis that bovine spongiform encephalopathy (BSE) could be related to these cases has raised the interest in transmissible spongiform subacute encephalopathies (TSSE) and has prompted questions regarding the safety of biological products, blood, organ and tissues donations (Will et al., 1996). CJD is a degenerative disease of the central nervous system. It is transmissible and characterized by a clinically silent stage which may evolve over decades, although recent cases in UK have a shorter incubation period. A double causality, transmissible and genetic, is generally admitted, as outbreaks of CJD among people treated with human growth hormone and familial forms of CJD have been described (Billette de Villemeur et al., 1991; Collinge et al., 1991; Gibbs et al., 1993). To date, only corneal transplants and dura mater grafts have been reported to cause CJD in recipients and CJD has also been associated with neurosurgical instruments or electrodes contaminated and inadequately sterilized (Dormont, 1995). No data are available concerning sexual transmission in humans. Infectivity in testis and seminal vesicles from sheep with scrapie is undetectable (Kimberlin, 1994). No laboratory tests are available to detect infected asymptomatic individuals. With regard to blood donors, the medical history of semen donors should include questions concerning past treatment with human growth hormone, TSSE cases in the family and dura mater grafts, although the probability of contamination by therapeutic insemination is very low.

Physical examination

Comment

This is a very simple way of discovering the health status of the donor. The examiner should look for urethral discharge, for lesions in relation to STD, not only on the genitals but also on the skin and into the mouth, and for signs of possible HIV infection, such as adenopathies, marks of i.v. drug use and evidence of recent weight loss.

Laboratory tests

Urethral culture

Guideline

For both the British Andrology Society and the American Fertility Society, a urethral culture for Neisseria gonorrhoeae and Chlamydia trachomatis is mandatory. These organisms are major causes of pelvic inflammatory disease in women and both pathogens have been transmitted by semen of asymptomatic donors (Mascola and Guinan, 1986; Nagel et al., 1986).
Comment

Current culture systems are not satisfactory. Sensitivity is low in asymptomatic donors with low amount of organisms. Sensitivity is even lower in semen which is toxic for the cellular culture detection system for Chlamydia. Other tests, such as enzyme immunoassays, immunofluorescence and hybridization should not be recommended as they show problems of sensitivity and specificity in a low-prevalence population (Taylor-Robinson, 1992; Ehret et al., 1993; Talbot and Romanowski, 1994). For the detection of C. trachomatis, adaptation of these tests for urine specimens offers new promise of non-invasive screening tests. However, their sensitivity has to be carefully evaluated in low-risk populations (Ehret et al., 1993; Talbot and Romanowski, 1994).

As previously mentioned, the guidelines propose excluding potential donors on the basis of a history of genital warts or herpes. However, many of these infections are atypical and unrecognized by the infected person. Episodes of asymptomatic shedding are also common (Strand et al., 1986; Barton et al., 1987). Transmission of HSV by fresh semen used for therapeutic insemination has been described (Moore et al., 1989). HSV generally remains infectious when cryopreserved, so there is a potential risk of transmitting HSV infection using semen from donors with a negative medical history. Two possibilities could be proposed to diminish this risk: firstly, donors can be serologically screened for HSV antibodies. HSV1 is more prevalent in the general population than HSV2. Thus a majority of donors would probably have HSV antibodies without having genital herpes. HSV2 antibodies can now be measured specifically. However, a significant proportion of genital herpes is caused by HSV1. This percentage varies from one country to another and could be as high as 50% and carries an unknown risk of transmission (Corey et al., 1983; Kinghorn 1993), although genital HSV1 infection seems to recur less frequently than genital HSV2 infections (Lafferty et al., 1993). Thus some donors with asymptomatic genital herpes would not be detected by HSV2 antibody screening.

With regard to cytomegalovirus (CMV), one can propose screening donors and receivers for HSV1 and HSV2 antibodies. Seronegative donors would be reserved by priority for seronegative recipients. HSV2 seropositive donors would be reserved for women with HSV2 antibodies, and HSV1 seropositive donors without HSV2 antibodies would be reserved by priority to women possessing HSV1 antibodies but no HSV2 antibodies. Two main problems persist using this strategy; firstly, to date the only reliable serological test for discriminating between HSV1 and HSV2 antibodies is a Western blot assay which is time consuming and not routinely available (Koutsy et al., 1992). Secondly, protection against reinfection with the same type of virus is incomplete and reinoculation with HSV1 is well-known. Thus, reinfection with HSV2 might be possible in HSV2 seropositive receiver. The rate of transmission in these conditions is unknown. The second possibility of reducing the risk of HSV transmission by therapeutic insemination is to detect the virus in semen or in urethral samples. Detection in semen by culture could be hampered by the toxicity of semen for cells of the culture. Moreover, isolation of HSV from semen has been rarely described even in men with patent HSV recurrences (Douglas et al., 1988). Thus a urethral culture would probably be more efficient in detecting an actual HSV shedding than a semen culture. Urethral culture could be more cost-effective than serological screening as, in many HSV infected patients, there is a decrease in recurrence after the first genital episode of HSV due to progressive development of immunity and it is estimated that individuals with genital HSV2 infections shed virus on 2–5 days per year (Mertz, 1990).

Some types of HPV have been associated with the development of cervical and penile neoplasia (high-risk HPV). HPV can be sexually transmitted and has been recovered from semen, including in men free of genital symptoms (Inoue et al., 1992; Kyo et al., 1994; Lai et al., 1996). Although no transmission of HPV by therapeutic insemination has been described (most probably because data are lacking), the risk of transmitting high-risk HPV deserves attention. No routine serology for HPV exists and the medical history of genital warts is insufficient to exclude asymptomatic carriers. HPV is not cultivable and, for a long time, detection
has relied on hybridization (Caussy et al., 1988). Molecular amplification techniques have recently been introduced to detect HPV. For example, polymerase chain reaction (PCR) allows detection of high-risk HPV (van den Brule et al., 1990; Vandevelde and Van Beers, 1993; Polyak and Semé, 1996). The frequency of detection by PCR is generally higher than with hybridization (Gjöen et al., 1991; Hallam et al., 1991). None of these methods can give an indication of the amount of infectious virus present in the specimen. Further studies are needed to analyse the significance of a positive result obtained by PCR. In the near future, detection of HPV in semen, urine or urethral samples may be a better way of preventing the risk of high-risk HPV transmission than using medical history of the donor.

**Serology**

**Guideline**

Testing for HIV, HCV, syphilis and CMV antibodies and for hepatitis B surface (HBs) antigen is mandatory. Donors with a positive test for one of these markers, except for CMV, are excluded. A 6 month interval is recommended for surveillance and control of the serology. Semen is frozen and must not be used until the donation is validated after obtaining the results of the control serology. All these precautions are taken to prevent the use of infected semen sampled in seronegative donors incubating one of these infections.

**Comment**

**HIV:** Concerning HIV testing, exclusion of a donor should be based on the result of the screening test, not on the confirmation test. Over the last few years, the sensitivity of enzyme-linked immunosorbent assays (ELISA) for HIV antibodies have been greatly improved. Confirmation tests, e.g. Western blot, remain insensitive during the first days or weeks of a seroconversion (Phair and Wolinsky, 1992; Barbé et al., 1994). Thus a positive ELISA test, with a negative Western blot could correspond with an HIV seroconversion. Even with quarantine precautions, it is wiser not to conserve a potentially positive semen sample among other semen samples waiting for release, as contamination of cryopreservation tanks by viruses has been demonstrated (Tedder et al., 1995).

**Hepatitis B virus (HBV):** Screening for HBs antigen is, of course, obvious. Another precaution could consist in proposing vaccination against HBV to potential receiver. Even if HBV vaccination takes months to be complete, sufficient protection can be achieved after one or two injections without delaying the insemination.

**CMV:** In the USA and Europe, the annual incidence of congenital CMV infection is 0.4–2.5 % of all live births (Peckham, 1991). Of these newborns, 10% will be symptomatic at birth and ~20–30 % of them will die. Among the survivors, the majority will present important sequelae such as bilateral deafness, paralysis, mental retardation and microcephaly. Among the 90% of asymptomatic newborns, a further 5–20% will develop later sequelae such as deafness and mental retardation (Stagno, 1990). It is clear from various studies, that symptomatic CMV disease in the newborn is related to the occurrence of a primary CMV infection in the mother during pregnancy (Stagno et al., 1982; Yow et al., 1988). When primary infection occurs during pregnancy, transmission of the virus takes place in 30–40% of the cases. Among infected fetuses, 10% will be symptomatic at birth and a further 5–20% will develop sequelae later in life. Stagno et al. (1986) have shown that a primary infection during the first part of pregnancy is associated with significant handicap and newborn deaths. In CMV seropositive women, the infection of the fetus is a rare event, occurring in Western countries in no more than 1% of these pregnancies. Among these infected fetuses, the large majority are asymptomatic at birth and <10% may develop minor sequelae later in life. Fowler et al. (1992) compared the influence of primary and recurrent infection of the mother on the development of CMV disease and sequelae in 189 babies born with a congenital CMV infection. They have shown that death and sequelae, e.g. bilateral hearing loss, mental impairment and severe retinitis, were seen exclusively in the group with maternal primary infection. In this group, 25% of the newborns were affected. In the group of infected neonates born from mothers with recurrences, 8% of minor sequelae were observed. For example, deafness was never bilateral, nor was retinitis associated with visual impairment.
Different studies have demonstrated the presence of the virus in semen. Excretion of the virus in semen is high and persists for months after a primary infection but reinfection and reactivation of the virus in a CMV seropositive man can induce excretion of the virus in the semen at any moment of his life (Lang and Kummer, 1975; Collier et al., 1989). Let us say that a CMV seropositive donor is a potential virus excretor. We also know that CMV is transmitted by sexual contact but we do not know the frequency and the efficiency of this mode of transmission (Demmler et al., 1986). Finally, the virus remains infectious when frozen, so there is a potential risk of transmitting the virus by insemination.

In the case of a primary infection in a previously seronegative women, virus transmission to the fetus is very high. With artificial insemination, we are in the worse conditions, or shall I say, in the best conditions, to provoke a primary infection in the first part of pregnancy. This situation is associated with a more severe disease of the fetus which takes place in up to 30% of the infected fetuses (Stagno et al., 1986). In these conditions, it is clear that the only thing to do is to reserve CMV seronegative donors for CMV seronegative women.

What about seropositive recipients? We know from past studies that CMV antibodies do not prevent viral transmission but confer protection from fetal CMV disease and sequelae in the great majority of the cases. In European countries, except perhaps in Scandinavia, the exclusion of CMV seropositive donors would eliminate the majority of potential donors. In natural infections no more than a small percentage of seropositive women transmit the infection to their fetuses and 90–95% of them will be perfectly well at birth. Until now, there has been no reason to think that CMV introduced by insemination into a seropositive women will succeed more frequently in infecting and causing damage to the fetus than would the natural infection.

These are the reasons why I think that the exclusion of CMV seropositive donors is not efficient and probably not justified. However, as some countries have chosen to use seropositive semen, it is highly desirable to evaluate the risk of transmitting CMV by insemination. We should organize a programme of detection of CMV congenital infection and disease in the setting of AID.

Concerning the way of performing screening test for CMV antibodies, it will be more informative to test donors and recipients for CMV immunoglobulin (Ig)G and IgM antibodies. The CMV IgG antibody test will determine the immune status toward CMV. The CMV IgM antibody test could detect or exclude a current CMV infection. Current CMV infection is a cause of donor exclusion. For the recipient, starting a pregnancy whilst infected with the virus carries a risk of congenital infection, the detection of CMV IgM antibodies should postpone any insemination.

Other laboratory tests

Other screening tests for new pathogens will be probably be recommended in future guidelines. Two candidates are hepatitis G virus (HGV) and human herpes virus type 8 (HHV8). HGV is a recently discovered RNA virus belonging to the flavivirus family. Although HGV has been associated with hepatitis, the clinical significance of acute and chronic HGV infection is presently not well known. The virus is transmitted parenterally. Sexual transmission is suspected (Jarvis et al., 1996; Schmidt et al., 1996; Wang et al., 1996) and vertical transmission has been demonstrated, but the consequences of vertical HGV transmission are unknown (Feucht et al., 1996; Moaven et al., 1996). The only available method to detect HGV is nucleic acid amplification. Serological testing is also available but a correlation with the presence of the virus has not been established (Tacke et al., 1997). Further studies are needed to assess whether testing for HGV is useful in the setting of therapeutic insemination and which method is the most appropriate.

Genomic sequences of HHV8, a new human herpes virus have been recently detected in Kaposi’s sarcoma (KS) lesions. HHV8 DNA has been also detected in the peripheral blood of 50% of HTV infected patients with KS, but only rarely in blood donors (Blakbourn et al., 1997). Seroepidemiological studies confirmed these findings, although seroprevalence can depend on the antigens used in the assay (Gao et al., 1996; Kedes et al., 1996; Lennette et al., 1996; Simpson et al., 1996). Data are consistent with sexual transmission
of the virus. Conflicting results have been obtained concerning the detection of HHV8 in semen. Detection of HHV8 in semen of healthy men varies from 0 to 90% (Monini et al., 1996; Howard et al., 1997). More studies are needed to define the potential oncogenic role of HHV8 in KS and to describe the epidemiology and diseases associated with HHV8 infection.

**Place of new molecular techniques**

New molecular techniques, particularly PCR, allow a rapid diagnosis of fastidious microorganisms. PCR also plays an important role in the identification of emerging pathogens such as HGV, HHV8, new hantaviruses, etc. Commercial assays based on these methods are now becoming available (HCV, HIV, C. trachomatis, etc) and are sometimes proposed in place of older techniques, e.g. serology or culture. What can be the place of such assays in the setting of therapeutic insemination?

I have already mentioned the problem of detection of *C. trachomatis* for which current assays are not specific or sensitive enough. The problem in therapeutic insemination is that screening for *Chlamydia* concerns a low prevalence population and must be highly specific, but also that the purpose of the screening is to reduce the risk of transmission to almost zero and thus the screening test used must be highly sensitive. Commercial PCR and ligase chain reaction (LCR) assays are now available. The PCR test developed by Roche Diagnostics (AmpliCor) is approved for cervical and male urethral specimens and has been evaluated in a low prevalence population with a sensitivity of 90% and a specificity approaching 100% (Bianchi *et al.*, 1994; Skulnick *et al.*, 1994). More conflicting results were obtained with male urine, some studies showing a lowered sensitivity of the assay on urine compared with urethral specimens (Jaschek *et al.*, 1993; Stary *et al.*, 1996). The LCR test developed by Abbott Laboratories has been evaluated as giving good performances in urine from low prevalence male groups (Chernesky *et al.*, 1994). Thus both tests correspond to the definition of a good assay for *C. trachomatis* screening in semen donors. Moreover, if good performances on urine are confirmed, this will suppress the inconvenience imposed on donors by repetitive urethral sampling. A recent PCR study for *Chlamydia* detection in semen of semen donors has shown a prevalence of 16% among 30 donors (van den Brule *et al.*, 1993). Such a high prevalence in semen was not confirmed in a larger study of 205 men from an infertility clinic in Germany (Wolff *et al.*, 1994).

These techniques could improve other diagnoses, e.g. HCV infection. The transmission risk of HCV by semen is not well defined (Fried *et al.*, 1992; Liou *et al.*, 1992). Sexual transmission seems to be low but has not been extensively investigated (Kao *et al.*, 1992; Honda *et al.*, 1993). Transmission of HCV from mother to child is described and concerns ~10% of the newborns of HCV seropositive women (Lam *et al.*, 1993; Ohto *et al.*, 1994). The current sensitivity of the HCV antibody screening test is satisfactory. However, at this moment HCV serology does not enable a distinction between chronic HCV carriage and past infection, although a positive HCV antibody test is strongly associated with the presence of the infection (Lavanchy *et al.*, 1994). Moreover, at seroconversion, the ‘window’ period without detectable HCV antibodies could be several weeks or even months (Vallari *et al.*, 1992). In these conditions, it should be investigated if the detection of the virus in the plasma or in the semen would be more sensitive and accurate for the screening of semen donors.

The problem of a ‘window’ period at seroconversion is essentially the same for HIV. Generally the ‘window’ period is 2–6 weeks. One HIV-infected patient with persistent lack of detectable HIV antibody lasting for months has been described, but this kind of case is an exception (Centers for Disease Control, 1996). The best way to exclude the infection remains the detection of HIV antibodies and the quarantine of semen for 6 months until the obtention of the control serology. Sensitivity performances of PCR for the detection of HIV DNA in peripheral blood lymphocytes could be impaired by the high heterogeneity observed between HIV strains sequences (Gürtler *et al.*, 1994). Should PCR be used for HIV screening of semen donors, each fertility center will be confronted to the necessity of developing multiple pairs of specific HIV primers according to the local HIV epidemiology, with the substantial risk
of missing the diagnosis in cases of genetically distant strains (Vandamme et al., 1995). Recently, it has been shown in a high-risk population that detection of HIV RNA in plasma by RT-PCR could help in the diagnosis of primary HIV infection in the 'preseroconversion' period (Phair et al., 1997).

In conclusion, molecular techniques are very promising for the diagnosis of STD in the setting of AIDS but should be evaluated carefully before introducing them for this purpose. One major drawback of these techniques are their high cost.

References


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C.A. Liesnard


