Laboratory safety during assisted reproduction in patients with blood-borne viruses

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For couples where one or both partners are infected with human immunodeficiency virus or hepatitis C, the doors to receiving fertility care are opening as a result of better antiviral medication, better long-term prognosis and consequent changes in attitude. In line with this, fertility centres electing to treat couples with blood-borne viral (BBV) infection need to re-examine their policies and procedures to ensure the safety of their staff and both non-infected and infected patients during assisted reproduction treatments. At a time when the European Tissue Directive aims to introduce quality standards for assisted reproduction throughout Europe, we highlight the risks involved when treating patients with known BBV infections and argue that safety cannot be met with any certainty unless samples from such patients are handled within a separate high security laboratory or laboratory area, technically adapted to ensure minimal cross-contamination risk to uninfected gametes and embryos.

Key words: assisted reproduction/hepatitis/HIV/reproduction/safety

Introduction

There has been a substantial increase in the demand for assisted reproduction technology in patients infected with blood-borne viral (BBV) infections such as human immunodeficiency virus (HIV), hepatitis B (HBV) or hepatitis C virus (HCV) in recent years. Although the ethics of offering assisted reproduction to couples in whom one or both partners are infected with HIV has been, and continues to be, extensively debated (Smith et al., 1990; Minkoff and Santoro, 2000; Englert et al., 2001a; Gilling-Smith et al., 2001; Ryan, 2001; Ethics Committee of the ASRM, 2002; Sauer, 2003; Sharma et al., 2003), the combination of better prognosis and reduced vertical transmission risk in developed countries has led to an increased demand for assisted reproduction in these patients who either wish to have risk-reduction procedures such as sperm washing, or who have fertility factors and need IVF or ICSI. In 2002, following the Human Fertilisation and Embryology Authority (HFEA) recommendation that patients considering cryopreservation of gametes or embryos be screened for HIV, HBV and HCV prior to offering assisted reproduction, a UK audit of demand for assisted reproduction in HIV-infected patients found that 16% of men and 4% of women attending HIV specialist clinics had enquired about fertility treatment. The audit also found that 30% of fertility centres indicated intent to treat HIV positive males and 26% positive females (Frodsham and Gilling-Smith, 2003). Many European countries now subscribe to the view that these patients should be given the opportunity to have children safely (Marina et al., 1998a,b; Tur et al., 1999; Semprini, 2000; Englert et al., 2001a; Weigel et al., 2001; Gilling-Smith and Almeida, 2003; Marina et al., 2003; Ohl et al., 2003) with a priority being placed on preventing the uninfected partner or future child from acquiring HIV through horizontal or vertical transmission. In contrast to HIV, few question the ethics of providing fertility care to those infected with HBV or HCV, provided the risk of infection to health care workers and other patients is addressed. A vaccine which is 90% immunogenic against HBV is available to protect health care workers, uninfected partners and the newborn and in the assisted reproduction setting the main concern is transmission to uninfected gametes and embryos during laboratory procedures. There is no effective vaccine against HCV. Sexual transmission risk is low, unless the patient is co-infected with HIV (MacDonald et al., 1996).

The treatment of patients with BBV in assisted reproduction clinics raises questions over the safety of clinical and laboratory procedures. To date, published guidelines on
the subject are limited. In the UK, for example, the HFEA’s
_Code of Practice_ states that licensed centres must follow
good laboratory practice, be fully aware of microbiological
hazards and comply with the Association of Clinical Embry-
ologist’s and Control of Substances Hazardous to Health
(COSH) regulations. The European Union Tissues and
Cells Directive (European Parliament and the Council of the
European Union, 2004), which has to be implemented by all
member states by 2006, states that centres involved in the
handling of human tissues and cells (including gametes and
embryos) must adopt a ‘quality system approach working to
Community Standards and specifications’. The latter have
still to be defined. General statements such as these do not
provide guidance on the specific measures which should be
taken when handling known high risk samples. This is of
concern as the number of centres offering treatment to
infected patients rises to meet increased demand. In this
paper we argue the case for handling and storing high risk
samples in a separate laboratory or laboratory area to ensure
the safety of both infected and non-infected patients, as well
as staff, in centres electing to treat patients with BBV.

Screening before assisted reproduction treatment
It is important that assisted reproduction centres have in place
an effective means of identifying patients with BBV before
they embark on treatment. In the past, systematic screening
for HIV, HBV and HCV was only performed by a few centres
(Edelstein _et al._, 1990; Abusheikha _et al._, 1999) and treatment
denied to the majority of patients infected with HIV, as rec-
commended by various authorities at that time (Ethics Commit-
tee of the American Fertility Society, 1994; FIGO Commit-
tee of the Study of Ethical Aspects of Human Reproduction:
Schenker, 1997). In the absence of systematic screening
(Balet _et al._, 1998), patients who were chronic carriers of HIV
or other viruses were almost certainly treated in many centres,
with neither patient nor staff being aware of the consequences
of the virus on patient health, vertical transmission and cross-
contamination risk during assisted reproduction and cryopres-
servation. The European Society of Human Reproduction
and Embryology (Van den Eede, 1995) and the HFEA have both
recommended the screening of all patients for HIV, HBV and
HCV before offering assisted reproduction, and this is
increasingly becoming common practice in fertility clinics
(Edelstein _et al._, 1990; Balasch _et al._, 1992; Balet _et al._, 1998;
Abusheikha _et al._, 1999; Hart _et al._, 2001). This approach
confers many benefits. From an ethical and medico-legal
standpoint, doctors treating infected patients are involved in
the potential infection of any child born as a result of treat-
ment or in the contamination of an uninfected individual, e.g.
the negative partner in HIV discordant couples or uninfected
patients in the clinic. By identifying patients with BBV infec-
tion, measures can be taken to inform them of their condition,
prognosis and treatment, refer them to an appropriate phys-
ician and counsel them about their reproductive options
(Englert _et al._, 2001b). Reproductive care can be targeted to
minimize transmission risk to the uninfected partner or future
child, i.e. semen processing for infected men in cases of HIV
or HCV (see below), early vaccination at birth in cases of
HBV-infected women and antiretroviral drugs, Caesarean sec-
tion and bottle-feeding for HIV-infected women (British HIV
Association, 2001; Ministry of Health, French Republic,
2002). Finally, and as discussed further below, screening
enables patients presenting a risk to health care staff and other
patients to be identified pre-treatment and the risk of trans-
mition in the operating theatre and laboratory to be mini-
mized. If universal screening is not acceptable to a centre, an
opt-out policy is a workable alternative, provided the centre
handles the gametes and embryos of those who opt out as if
the patient were a chronic viral carrier.

Assisted reproduction in patients with BBV
Infected patients attending fertility centres fall into two
groups. The first are those who have no fertility issues and
require techniques such as sperm washing to reduce viral
transmission risk. The second are infertile couples requiring
traditional assisted reproduction methods to conceive. In some
couples, risk reduction methods may need to be combined
with assisted reproduction procedures such as IVF or ICSI.

Sperm washing to reduce risk
The risk of infection through sexual contact in a stable
couple (Vernazza _et al._, 1999) is between 0.1 and 0.5% (De
Vincenzi, 1994; Gray _et al._, 2001). The risk appears to be
effectively reduced by sperm washing, during which live
sperm, which do not carry HIV, are separated from HIV-con-
taminated seminal plasma and non-germinal cells by centrifu-
gation before being used in assisted reproduction (Semprini,
1992). The treatment is simple and provides a significant risk
reduction over timed, unprotected intercourse, which has
been reported to carry a risk as high as 4% of infecting the
female partner (Mandelbrot _et al._, 1997). The efficacy of the
wash should be verified with a post-wash HIV assay before
the sperm are used in treatment (various commercial tests are
currently available which have been found to be effective in
detecting different HIV strains) (Chew _et al._, 1999; Swiss
Cohort Study, 2000). The treatment has a good safety record,
with no reported cases of seroconversion in either female
partner or child born in >3000 cycles of sperm washing
(combined with either intrauterine insemination, IVF or
ICSI) where the above protocol has been followed (Marina
_et al._, 1998a,b; Tur _et al._, 1999; Semprini, 2000; Weigel _et al._,
2001; Sauer and Chang, 2002; Gilling-Smith and Almeida,
2003). Although the presence of detectable HCV in seminal
fluid from men chronically infected with HCV is debatable
(Fried _et al._, 1992; Semprini _et al._, 1998), sperm washing,
with HCV testing of the processed sample, has also been
shown to be effective in removing HCV RNA from seminal
fluid (Pasquier _et al._, 2000; Bourlet _et al._, 2003). Sperm
washing is not necessary in serodiscordant male HBV-
infected patients to prevent sexual transmission risk, unless
the female partner has failed to be effectively vaccinated
against HBV (Practice Committee of the American Society
for Reproductive Medicine, 2004).
Assisted reproduction in infected patients

One of the principle concerns in managing infected samples during assisted reproduction is that of cross-contamination risk to uninfected samples. Oocyte retrieval is an invasive procedure and blood contamination of follicular fluid samples difficult to avoid. It has been argued that patients with an undetectable HIV viral load present a low risk. In a series of seven cycles of IVF in HIV positive women, HIV was not detected in any of the follicular fluid samples of the four women with undetectable viral load but was detected in the follicular fluid of the only patient in the series with a detectable serum viral load (Bertrand, 2004). By contrast, in the series by Frodsham et al. (2004) significant levels of HIV were detectable in follicular fluid in seven out of nine HIV positive patients undergoing vaginal oocyte collection for IVF, irrespective of serum viral load or antiretroviral treatment. In another study on HCV positive women, HCV RNA was detected in 89% of follicular fluids and in 25% of the culture media at day 1 from 22 IVF trials of HCV positive but HIV negative women (Devaux et al., 2003). Although the numbers in these series are small, it is clear that samples from HCV and HIV positive women, irrespective in the latter case of viral load or whether they are on antiretroviral treatment, present a cross-contamination risk within the laboratory setting. Although we agree that all follicular fluid samples should be handled as if infected and universal precautions adopted at all times, the risk to uninfected samples is inevitably higher when samples known to be infected are handled within the same laboratory area and hardware, such as flow cabinets and incubators, shared.

Viral transmission risk during assisted reproduction

The main risk to health care workers is through needle stick or splash injuries and has been documented for HIV, HBV and HCV. HBV is a well-recognized occupational risk for all health care workers involved in handling blood products, body fluids and clinical waste although exposure risk is reduced significantly by the availability of a vaccine. Vaccination of all health care personnel with immune status verified by regular 5-yearly anti-HBV antibody checks is now well-accepted practice throughout Europe (Bonanni and Bonaccorsi, 2001). Unfortunately there are no effective vaccines for HIV or HCV. It is well accepted that cross-contamination risk within the laboratory setting can be minimized by using universal precautions when handling all samples, whether known to be infected or not, i.e. handling with latex gloves, using eye protection and, where possible, avoiding sharps. However, in the context of BBV, universal precautions have their limitations. When a health worker is injured accidentally with a patient/sample known to be infected with HIV, a period of antiretroviral treatment is recommended. Likewise, a health worker who fails to develop immunity to HBV will need anti-HBV immunoglobulin if accidentally exposed to the virus.

Although the risk of health care workers becoming infected when handling infected samples appears to be very low (Weiss et al., 1988), nosocomial contamination between patients has been described both for HIV (Blank et al., 1994) and during assisted reproduction for HCV and HBV (Quint et al., 1994; Lesourd et al., 2000). Cross-contamination in tanks storing biological material has also been clearly demonstrated (Tedder et al., 1995; Clarke, 1999). Cross-contamination between infected and uninfected patients and samples can potentially occur during oocyte retrieval and embryo transfer as well as during subsequent laboratory procedures such as insemination, injection, incubation and cryopreservation. We are aware that the Centers for Disease Control and Prevention (1998) recommend universal precautions, i.e. handling all specimens as if they were hazardous, and that this must be applied in all laboratories and in all patients (Duerr and Jamiesson, 2003). Clearly, it is imperative that sanitization and sterilization are carried out routinely in all assisted reproduction operating theatres and laboratories, irrespective of the viral status of the patient. However, an IVF laboratory is a complex structure where everything is planned to promote adequate conditions for cell survival and culture, conditions also favourable for viruses and bacteria. Therefore, when treating known infectious cases (or unscreened samples), we recommend that, over and above universal precautions, working surfaces and equipment used are cleaned with additional disinfecting agents, e.g. Virkon, to further minimize potential cross-contamination risk. The downside to this is that the chemicals used are potentially embryotoxic and incubators containing oocytes and embryos have to remain shut whilst cleaning takes place, and remain so for ≥ 30 min to allow residual traces to evaporate. These additional measures are time-consuming, which makes them impractical to adopt in screen negative cases. In practice, the most time-effective approach is to place known infected cases last on the list, as opposed to first, to allow sufficient time for this decontamination to take place and minimize delay to the operating list.

Clinical adaptations during operative procedures

In the operating theatre, samples should always be handled as if contaminated according to published guidelines (COSHH in the UK). All instruments used in the operating theatre and laboratory should be disposable where possible, and discarded after use in waste clinical disposal bags for incineration. Any non-disposable equipment should be sterilized using non-embryotoxic products such as Virkon and ethanol as recommended by the manufacturers. Measures used during ultrasound scanning are a matter of debate. Our practice is to cover vaginal ultrasound probes with a protective sheath and to ensure the probe is wiped with germicidal impregnated tissue before and after each patient is scanned (Milki and Fisch, 1998).

Laboratory adaptations

Our two centres have, independently of each other, elected to treat patients with known BBV in a separate ‘high risk’ laboratory, with specialized and dedicated equipment for assisted reproduction and safety procedures for staff entry and exit (Englert et al., 2002; Gilling-Smith and Almeida,
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2003). The laboratory designs include facilities for cleaning and disinfection, a separate storage area for general laboratory consumables (plastic-ware) and refrigerated facilities for holding culture media. A major reason behind choosing to segregate low and high risk cases is that assisted reproduction clinics have previously been subject to human errors, e.g. incorrect embryo being replaced, and, even in the presence of universal precautions, zero risk does not exist. For this reason we believe that it is safer, and more ethically acceptable, to handle patients with the same level of risk together, i.e. detected viral carriers in one laboratory and negatively screened patients in another, rather than mix patients with clearly different risk levels. This strategy assures non-infected patients and reduces the risk of cross-contamination of uninfected samples as previously reported (Quint et al., 1995; Tedder et al., 1995; Clarke, 1999; Lesourd et al., 2000). This approach of separating the treatment of infected and non-infected patients in space and time is, in France, imposed by law on assisted reproduction clinics which want to treat HIV- or HCV-infected patients (Decree about Assisted Reproductive Treatment of Patients with Viral Risks: Journal Officiel de la République Française, May 15, 2001 cited by Ohl et al., 2003). Some clinics in France have elected to separate infected cases in time, rather than space, as described in this paper. Set days/weeks are allocated for treating infected cases, when the service is closed for the treatment of non-infected cases. This may be a preferable solution when space is an issue, but it does not address the issues surrounding cryopreservation of infected samples.

In our approach, entry to the high risk laboratory is restricted to laboratory staff, who wear a clean set of theatre clothes, shoe covers, hat, eye protection, latex gloves, mouth masks and disposable plastic apron or gown when carrying out all embryological and andrological procedures. Hands are cleaned with a mild disinfectant (i.e. Hibiscrub) and hot water at the time of entry to and exit from the laboratory. A class II vertical laminar flow cabinet with 100% recirculation of filtered air is used (Englert et al., 2002). Mechanical pipetting devices are used at all times (mouth pipetting, previously acceptable in the assisted reproduction setting, should no longer be used). Micromanipulation procedures are carried out with a flat screen image projector or with safety goggles. Culture of oocytes and embryos takes place in mini-styled incubators with separate case-specific compartments. We believe this strategy lowers any risks of cross-contamination within a humidified environment. When preparing semen samples, covered centrifuge cups are used to prevent aerosol release during centrifugation. Detailed protocols and strict safety guidelines are available and health workers in our units receive regular training and updates on procedures used in infected cases, as we are sensitive to the fact that many staff working in fertility centres are not used to working with infected samples.

**Cryopreservation**

Cryopreservation of gametes and embryos in liquid nitrogen presents a risk of cross-contamination of HIV, HBV and HCV to other samples and patients (Tedder et al., 1995; Clarke, 1999). Straws may leak or shatter during freezing or blow open during thawing (Russel et al., 1997). One option, such as that currently recommended by the HFEA in the UK, is to freeze samples from known infected samples in a separate tank for each infection and infection combination (Tedder et al., 1995). The cost and space required for a separate tank and freezing machine for each infection/infection combination has, in practice, led to very few centres in the UK being able to offer cryopreservation to patients with known BBV infection. A more practical, cost-effective and safer approach is to use heat-sealed straws made from shatterproof ionomeric resin [e.g. Cryo Bio System (CBS) High Security straws; IMV Technologies, L’Aigle, France] for cryopreservation of all embryos and gametes. These have been shown to provide a highly efficient seal against migration of microorganisms either into or out of the straws (Clarke, 1999). Their long-term safety and efficacy for the cryopreservation of embryos generated through assisted reproduction technology remains to be evaluated. Microbiological evaluation of their short-term safety under cryopreservation conditions would suggest that their seal against migration of microorganisms is maintained. To assess their safety under conditions replicating those found in assisted reproduction laboratories, HIV-1-filled high-security ionomeric resin straws, thermosoldered at both ends, were tested at 37°C (Benifla et al., 2000) and under cryopreservation conditions in liquid nitrogen for up to 7 days (Letur-Könirsch et al., 2003) and compared with conventional polyvinyl chloride and polyethylene terephthalate glycol straws sealed ultrasonically. Leakage of HIV was demonstrated in all three types of straws at 37°C, confirming their lack of safety at this temperature (which is not applicable to storage conditions during assisted reproduction treatment). However, under cryopreservation conditions similar to those used during gamete and embryo storage, only the heat-sealed ionomeric resin straws were found to be safe against leakage of HIV-1 into the surrounding medium. This suggests that both straw and sealing system are safe for HIV-1 storage. There are as yet no published data on the safety of these straws for HBV or HCV. Heat-sealed straws are used by most European fertility centres treating HIV- and hepatitis-infected patients, with no reported cases of cross-contamination. A separate tank within the infectious zone of the laboratory, to house straws containing gametes or embryos frozen from patients known to be infected, would provide added security in the event of straw damage or breakage. Ideally heat-sealed straws should be housed in separate tanks for each infection if space and cost permits. It has been proposed that vapour phase storage in the case of known infected samples would offer more security against the risk of cross-contamination compared to liquid phase storage, without affecting embryo and sperm survival (Tomlinson and Sakkas, 2000). As with heat-sealed straws, vapour phase storage needs further evaluation to assess short- and long-term safety and efficacy in the assisted reproduction setting. A move towards vapour phase storage may well be recommended by the committee advising the European Directive.
Current practice and the way forward

In the USA, fertility units are obliged to provide reproductive treatment to patients infected with HIV, unless skill levels and facilities are inadequate (Ethics Committee of the American Society for Reproductive Medicine, 2002). In Europe the number of centres prepared to offer reproductive assistance to HIV- and hepatitis-infected patients is far higher and increasing along with the increase in demand, but few have committed themselves to ensuring that their clinical and laboratory practice meets the necessary safety standards to protect other patients and staff from nosocomial infection and cross-contamination. A recent UK audit would suggest that specialized facilities are lacking with only 4% (3/74) of units providing a separate laboratory for handling samples from known infected patients and 8% (6/74) providing separate storage tanks for freezing infected gametes and embryos (Frodsham and Gilling-Smith, 2003). In the USA, the need for a separate laboratory is appreciated but the costs involved appear to be used as an argument against treating HIV-infected women (Sauer, 2003). The cost of building a separate laboratory, and investing in the specialized equipment described in this paper, will vary according to the size of the build and local prices. However, when compared to the total cost of running an assisted reproduction centre, including equipment loan, staffing and laboratory material, a one-shot investment of between €200 000 and €250 000 (as our units have made) is relatively modest and more than justified if, as a consequence, the safety of all patients, infected and uninfected, and health workers is ensured. Many of the adaptations suggested may well become compulsory as part of the European Directive on Tissue and Cell banking to be enforced by April 2006.

A central ethical premise to good medical practice is non-maleficence (Sauer, 2003; Sharma et al., 2003). In that vein, assisted reproduction centres need to be mindful of the risks to other patients and staff when treating patients with known infection. The measures we have adopted in our centre, over and above universal precautions, when treating patients with BBV serve the purpose of both minimizing the risk of cross-infection to other samples and patients and the inevitable costly and complex medical litigation that would follow such infection. We accept that our approach must continue to be monitored prospectively to assess its safety, efficacy and cost-benefit.

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