

# Guidelines for Handling Infectious Patients in the IVF Lab

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# Learning Objectives

- Viruses & exposure risk
- Virus+ patients in ART program
- Virus+ patients in IVF lab
- Semen freezing & storage
- Washing sperm for IUI
- PCR detection of virus
- New developments

# Viruses & Exposure Risk

# Sexually Transmitted Viruses

- HIV types 1 & 2
- Hepatitis C
- Hepatitis B
- Hepatitis A
- HTLV I & II
- HPV, Herpes, CMV, etc.

# HIV

- A retrovirus (RNA to DNA)
- Becomes part of the cell's genes
- Can lead to AIDS
- No vaccine!
- Must reduce risk of exposure to prevent disease
- Virus detectable in blood, semen & follicular fluid

# Hepatitis C

- Viral disease spread through bodily fluids
- Can lead to liver damage & disease
- No vaccine!
- Must reduce risk of exposure to prevent disease
- Virus detectable in blood, semen & follicular fluid

# Risks in ART Programs

- Protection of staff
- Protection of other patient's gametes
- Protection of patient's own gametes
- Main risk to staff is through needle stick & splash injuries

# What is the Risk of HIV Transmission by Needle Stick?

- Infection with HIV in the workplace represents a small but real hazard to healthcare workers
- Needle stick or other percutaneous exposure is concern with bloodborne pathogens, including HIV
- If source patient is a member of the general population, the chance that the exposed worker will contract HIV is 1 in 10 million
- If the source patient is known to be HIV-positive, the risk of infection of exposed employee is approximately 0.3%
- Prophylactic treatment with HAART reduces this risk
- Compare to case in which the patient has hepatitis B: the risk of infection for non-vaccinated workers is 30%



# **Virus+ Positive Patients in ART Program**

# Overview:

## *Treating HIV-discordant Couples*

***HIV-discordant couples (male positive, female negative) have been treated by three methods:***

- Unprotected intercourse at the time of ovulation
  - Not allowed under CDC regulations & not recommended by the ASRM
- Sperm washing for IUI in HIV-discordant couples
  - Widely used outside the U.S. & gaining acceptance here
- IVF with ICSI in HIV-discordant couples
  - Considered to be the safest method by U.S. practitioners
  - Used in cases of IUI failure or other indications outside the U.S.

# Infectious Disease In ART

- A subset of ART clinics in USA offer some ART to HIV+ couples
- Most deny treatment to these patients due to institutional policies, lack of lab infrastructure, fear of transmission of HIV.
- This denial of care may no longer be legal according to the ADA

# Viral Screening

- FDA mandates viral infectious screening for third party reproduction
- Screening not mandated but is standard-of-care for sexually intimate couples seeking ART

# Virus+ Patients in Reproduction

- Virus+ women & men seek to have families
- 75% of Hep C+ or HIV+ people are in their reproductive years
- Increasingly these couples are looking to ART for risk reduction in conceiving a pregnancy
- Serostatus of couple becomes a guide to appropriate treatments

# Notification of Other Patients?

- Is it necessary to notify other patients that HIV-positive specimens are processed in your laboratory?
  - Not necessary
  - Appropriate procedures & sanitation are in place to control for cross-contamination & reduce hospital-acquired infection
- Analogy
  - When a patient is scheduling a surgery, they do not need to be notified that surgeries are performed on HIV-positive patients using the same space & instruments

# Requirements for Treatment

- Multidisciplinary approach (psychological, medical, OB)
- Informed consent
- Patients agree to harm reduction during treatment (*e.g. condom use, serial diagnostic testing of mother and offspring for 1<sup>st</sup> year*)

# Work-up for ART: *The HIV-negative Woman*

- Normal PAP & no evidence of inflammation or lesions
- Vaginal culture & treatment of
- Screening for STIs
- Normal menstrual cycles & documentation of ovulation
- FSH testing for ovarian reserve,
- Tubal patency assessed by hysterosalpingography
- Age <40 years





# Informed Consent and Counseling

1. Options explained & alternatives encouraged:  
Use of donor semen, adoption or remaining childless
2. Risks explained (in addition to standard risks of the procedure)
  - Risk of the woman becoming infected
  - Risk of resulting child being infected
  - Risk of adverse reactions to HAART if used by the woman for prophylaxis & during pregnancy
  - Risk of cancelled cycle if PCR is used & HIV is detected in inseminate (3-8%)
  - IUI has low pregnancy per cycle (10-15%) vs. IVF/ICSI (30-40%)
  - Time & finances required per cycle of treatment

# Informed Consent and Counseling (Cont'd)

3. Consult with psychologist
  - Experienced in counseling couples for assisted reproduction and for HIV
4. Consult with obstetrician
  - Experienced treating woman with HIV
  - Prenatal counseling
5. Couple signs safe-sex agreement
  - Including agreement to use condoms for every coital act and abstain from any high risk behavior

# Ovarian Stimulation

- Due to the risk involved, conception per insemination should be optimized<sup>1</sup>
- In many European centers using for HIV-discordant IUI, ovarian stimulation and/or ovarian monitoring & ovulation induction are used for all cycles<sup>2-4</sup>

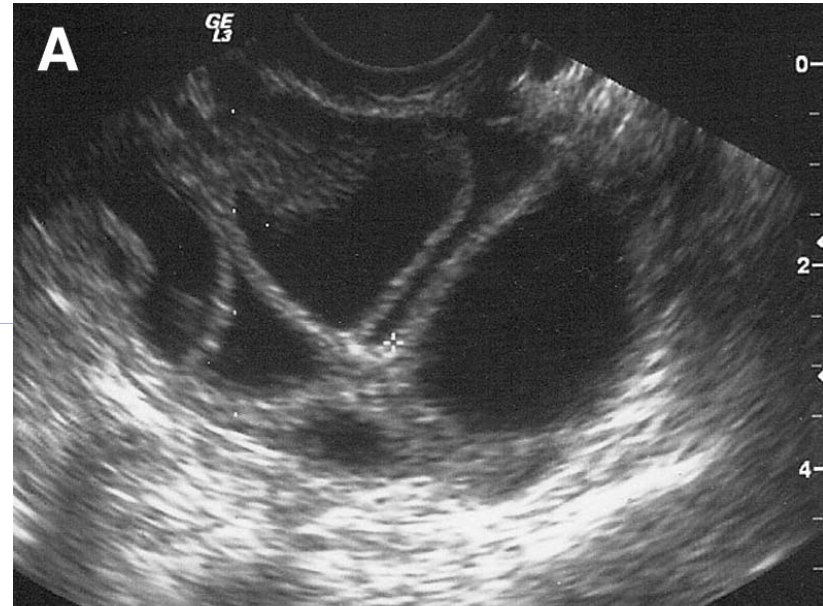


Photo used with permission from Oktay et al.  
*Fertil Steril.* 2010 Jul;94(2):753.e15-9.

1. Semprini et al., 1992 2. Marina et al., 1998 3. Gilling-Smith et al 2006 4. Savasi et al., 2008

# Work-up for ART: *The HIV-positive Man*

- History & physical examination
- Consult with infectious disease specialist
- Semen analysis
- Semen culture & treatment of infections
- Screening for STIs



**HIV +**

# Consult with Man's Infectious Disease Specialist

- Disease is under control & stable for 1 year
- Man is on HAART if appropriate
  - Trade off: Improved health & CD4 count is associated with improved semen quality while HAART causes reduced semen quality
  - Decision based on appropriate treatment of the man's infection rather than on reproductive concerns
- High CD4 count (*e.g.*,  $CD4 >200$  cells/mL)
- Low peripheral viral load

# **Virus+ Positive Patients in IVF Lab**

# Is HIV Associated with the Sperm Cell? *Controversial*

- Some authors report no association of HIV with washed, motile sperm using PCR for HIV DNA/RNA<sup>1-2</sup>
- Although there are no HIV receptors on the sperm surface, HIV attaches avidly to sperm via sperm surface mannose or heparin sulfate receptors<sup>3-7</sup>
- HIV may infect morphologically abnormal sperm in semen<sup>8</sup>
- Highly motile sperm selected from semen of HIV-infected men usually have undetectable HIV nucleic acid levels<sup>9</sup>
- **Leukocytes in semen are main mode of HIV infection**<sup>9-10</sup>

1. Quayle et al 1997, 1998 2. Kim et al., 1999 3. Baccetti et al., 1991, 1994 4. Barboza et al., 2004  
5. Bandivdekar et al., 2003 6. Fanibunda et al., 2008 7. Ceballos et al., 2009 8. Muciaccia , 2007  
9. Anderson et al., 2010 10. Nicopoulllos et al , 2010



# How is Semen Quality Affected by HIV Infection?

- Multiple studies compared HIV-positive men with a control group of HIV-negative men<sup>1-7</sup>
- Semen quality generally poorer than in uninfected men
  - Low proportion of progressively motile sperm
  - High leukocyte concentration in semen
- In most studies
  - Asymptomatic men had more normal semen than men with more significant disease
  - Men with higher CD4 counts had better semen quality
- Sperm motility often positively correlated with peripheral CD4 count

1. Krieger et al., 1991 2. Crittenden et al., 1992 3. Politch et al., 1994 4. Dondero et al., 1996  
5. Muller et al., 1998 6. Dulioust et al., 2002 7. Bujan et al., 2007c



# Semen Analysis

- If semen analysis is performed at a clinic, it should also be available for known HIV-positive patients
- No special expertise or equipment is required other than that used under Universal Precautions

# IVF with ICSI in HIV-Discordant Couples

- Outside the U.S., ICSI is generally used only in patients with an indication other than HIV-status<sup>1-3</sup>
- Some advise against ICSI for HIV-infected men because it could introduce HIV virus into the oocyte<sup>2,4</sup>
- Concern about state criminalization of insemination<sup>5-6</sup>
- Largest series of sperm washing for HIV-positive men in the U.S. using ICSI exclusively<sup>7</sup>
  - No evidence that ICSI is safer than IUI
  - Minimize risks/costs of ART by use of IUI for some couples
- Risk/benefit analysis must consider the higher success rate of IVF/ICSI compared with IUI: each attempt is associated with some risk<sup>8</sup>
- TESE from an HIV-positive man, followed by ICSI, was recently reported<sup>9</sup>

1. Ohl et al., 2003 2. Gilling-Smith et al., 2006 3. Savasi et al., 2007 4. Bujan et al., 2006, 2008

5. Pena et al., 2003 6. Sauer, 2008 7. Sauer et al., 2009 8. Vitorino et al., 2011 9. Garrido et al., 2009

# Virus and the Oocyte

- Virus may be in follicular fluid & may attach to granulosa cells surrounding the oocyte
- Oocyte is protected by zona pellucida
- Wash oocyte thoroughly in sterile media to dilute viral titer
- Presence of viral RNA does not connote infectivity!

# Separation in Space or Time

- ART procedures involving semen from HIV-positive patients should be separated in “space or time” from those for other patients to reduce risk of cross-contamination<sup>1-3</sup>
  - Disposable contact materials
  - Separate instruments/equipment
  - A physically separate area
- Scheduling HIV-positive patients at a different time allows
  - Undivided attention of personnel
  - Time to sanitize completely before handling specimens from other patients
- Process samples within a biosafety cabinet



*Image from CDC.gov*

1. Ohl et al., 2003 2. Gilling-Smith et al., 2006 3. Savasi et al., 2008

# Virus in the IVF Lab

## *Universal Precautions*

- Scrubs, hat, shoe covers
- Gloves
- Mask
- Faceshield or goggles
- Avoid sharps
- Extra decontamination
- Extra hand washing

# Virus in ART

- Sanitization and sterilization carried out routinely
- Red bag all blood products, dishes etc.
- Use ethanol/methanol
- Use viracidal wipes
- Air exchanges in lab every 10 minutes
- Use high security embryo straws
- No mouth pipetting
- Cover tubes so no aerosol-borne viruses escape

# **Semen Cryopreservation & Storage**

# ASRM Guidelines

- Refers to HIV, HBV, HBC
- Counseling, education, informed consent
- Refers to FDA screening regulations for semen & egg donors
- Recommend:<sup>1</sup>
  - Separation in time or space
  - Separate frozen storage
  - Special sperm washing ± viral check prior to freezing

1. ASRM Practice Committee, Fertil Sterility 2012



# Virus & Cryopreservation

- HFEA recommends separate tanks for storage
- A practical option: CBS straws
- Shown to provide highly effective seal against migration of microorganisms into or out of straws
- Short term evaluation is positive
- Long term safety & efficacy remains to be evaluated

# Virus & Cryopreservation

- Heat-sealed straws are used by most European IVF centers treating HIV & Hep B and Hep C positive patients with no reported cases of cross-contamination
- Ideally: CBS straws, vapor phase storage in a separate tank

# Washing Sperm for IUI

# Sperm Washing for HIV in USA: *Recent Past*

- Sperm washing appears to significantly decrease the risk of passing HIV infection from an HIV-positive man to an HIV-negative woman.
- However, it is still controversial. In 1990, CDC issued a recommendation against sperm washing
- (<http://www.cdc.gov/mmwr/preview/mmwrhtml/00001604.htm>), citing a case in which a previously HIV-negative woman was found to be HIV-positive after she was inseminated with washed sperm from her HIV-positive husband. That recommendation has never been revised.

# Sperm Washing for HIV in USA: *Current Status*

- New guidelines from ASRM in 2012<sup>1</sup>
- Cannot eliminate risk, but can reduce risk
- In our practice, the male partner should be on anti-retroviral therapy if appropriate, have good CD4 counts, & undetectable viral load

1. ASRM Practice Committee, Fertil Sterility 2012

# Sperm Washing for IUI in HIV-discordant Couples

- IUI after specialized sperm washing for HIV-discordant patients first described in 1992<sup>1</sup>
- Sperm are first separated from leukocytes & seminal plasma by gradient separation **followed by a swim-up**
- Sperm washing reduces HIV from the sperm suspension to undetectable levels in most specimens<sup>2</sup>

1. Semprini et al., 1992 2. Kim et al., 1999

# Sperm Washing for IUI

- CREAThE (Centres for REproductive Assistance Techniques to HIV couples in Europe)
- >4500 inseminations of HIV-negative women with sperm separated from the semen of their HIV-positive partners
- None of the women or the hundreds of children produced have been infected<sup>1-3</sup>



1. Gilling-Smith et al., 2006 2. Anderson et al., 2010 3. Vitorino et al., 2011

# PCR Detection of Virus

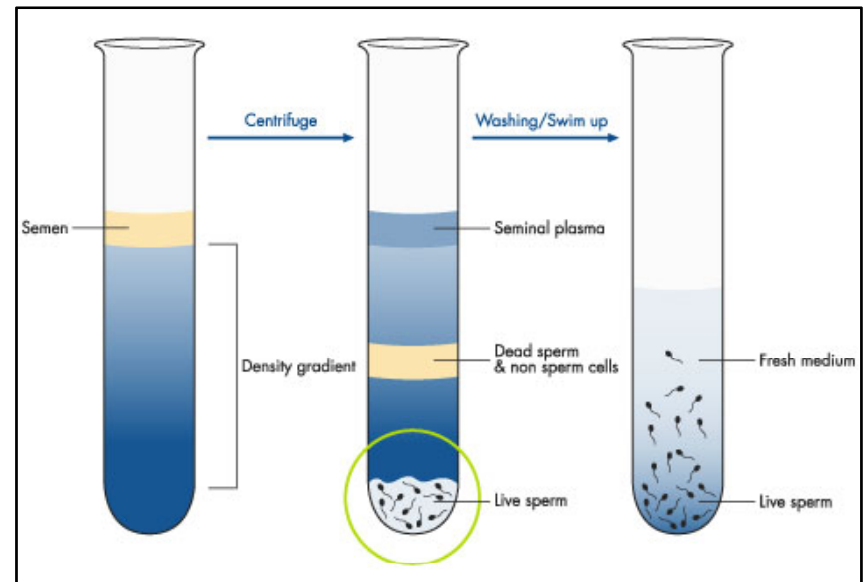


# Sperm Washing for IUI + PCR

- Gradient followed by swim-up
- PCR testing of prepared sperm specimen
  - The final sperm suspension can be stored while testing for HIV is performed by sensitive RT-PCR
  - Specimens found negative for HIV are then used for IUI
  - Insemination is not performed if HIV is detected (3-8%)
  - Processed sperm are held during PCR at room temperature, at 4°C, or are cryopreserved and stored until the day of IUI

# Sperm Washing for IUI + PCR

- Gradient followed by swim-up
- PCR testing of prepared sperm specimen
- The final sperm suspension can be stored while testing for HIV is performed by RT-PCR
- Insemination is not performed if HIV is detected (3-8%)
- Holding prepared sperm at room temperature for 8 hours while PCR is performed



# Sperm Washing for IUI + PCR

- About 3-8% of washed specimens contain detectable HIV virus after washing & cannot be used<sup>1-4</sup>
- As this is within the range of false positive results for this test, these may represent samples that test positive but contain no HIV virus
- It remains controversial if this test is required, particularly for men with undetectable viral loads
- PCR requires up to 20 hours during which the processed sperm lose some viability
- Cryopreservation of the washed sperm is used by some groups so PCR can be done before the day of insemination<sup>5</sup>
- Strict quality control is required

1. Persico et al., 2006   2. Gilling-Smith et al., 2006   3. Nicopoulos et al., 2011  
4. Vitorino et al., 2011   5. Bujan et al., 2007a

# Quality Control of PCR

- Quality control of PCR not consistently applied by all programs for HIV-discordant couples
- PCR testing of 11 centers that offered IUI and IVF to HIV-discordant couples<sup>1</sup>
- Different methods (for detection of 500 RNA copies/million sperm)
  - Sensitivity 82%
  - Specificity 95%
- Similar results were obtained for HIV DNA detection
- As more centers offer this alternative, routine QC must be applied to PCR methods

# New Developments

# Addition of Microbicide to Sperm

- Treatment the sperm with trypsin before washing to reduce the infectivity of HIV RNA<sup>1</sup>
- Addition of a microbicide, PPCM, to washed sperm to reduce HIV infectivity<sup>2</sup>
  - PPCM treatment did not decrease motility significantly over 2 hour incubation
  - Some decrease may become significant during the time required for PCR
- These proposed treatments are promising

1. Loskutoff et al., 2005 2. Anderson et al., 2011

# The Double Tube Method

- Contamination by leukocytes during sperm processing<sup>1</sup>
- Double tube method to recover more sperm with less contamination by leukocytes<sup>2</sup>
- Modified double tube with tube insert will not yet commercially available<sup>3</sup>

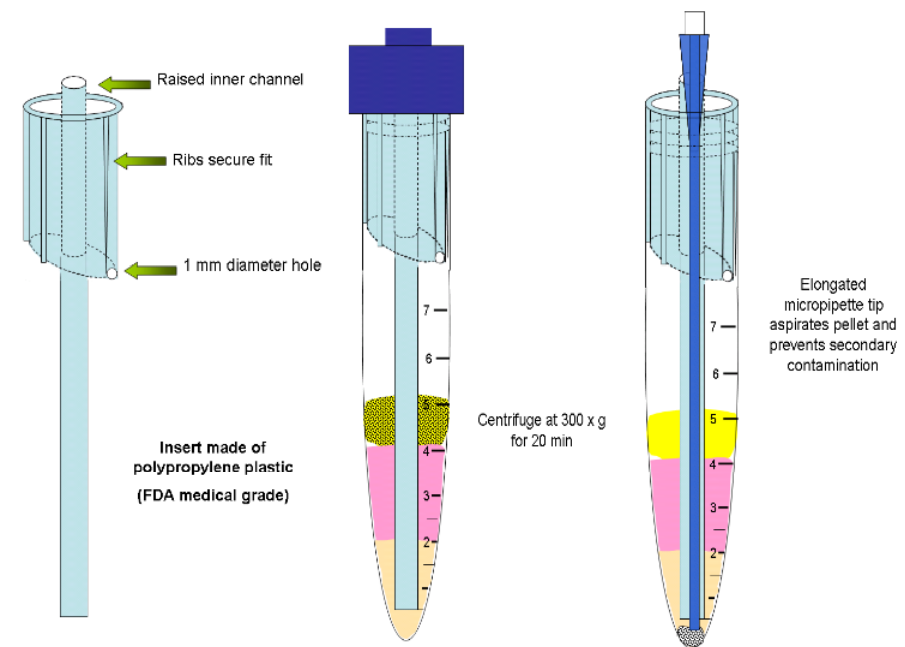


Figure courtesy of Dr. Loskutoff. Used by permission.

1. Kuji et al., 2008
2. Politch et al., 2004
3. Loskutoff et al., 2005

# CDC Recognized Change in HIV Status

- 2007: CDC Working Group on Infertility
- Published a paper on infertility prevention, detection & management<sup>1</sup>
- ART may assist patients with chronic diseases, including HIV-discordant couples, to maintain the ability to reproduce

1. Macaluso et al., 2010



# Conclusions

- Strongly recommend update to 1990 CDC recommendation
- Recommend that ART programs treat patients with chronic infectious diseases

# Conclusions

- Recommend IUI with density gradient + swim up for HIV-positive men on antiretroviral therapy if applicable, with good CD4 counts & undetectable viral loads. Do PCR prior to IUI?
- Recommend IUI with density gradient + swim up for Hep C positive men
- More cautious treatment: ICSI for Hep C, HIV positive men regardless of male factor infertility

# ***THANK YOU!***

