SPERM ONLY PLEASE:
Prevention of infections in an artificial insemination program

Carin Huyser
Reproductive and Endocrine Unit
Department Ob/Gyn
University of Pretoria
Steve Biko Academic Hospital
South Africa
carin.huyser@up.ac.za

ESHRE Campus workshop
Genk Belgium, 15 December 2009
I. Introduction
   ▪ Infection and contamination control
     ▪ Environment
     ▪ Staff
     ▪ Supplies
     ▪ Patients & specimens

II. Aim
   ▪ Origin & prevention

III. Prevalence (origin) of pathogens in ART
   ▪ Bacteria and STIs
   ▪ Viruses
   ▪ Interaction STI & HIV

IV. Risk reduction (prevention)
   ▪ Screening & counselling
   ▪ Semen washing
   ▪ Validation of specimens
   ▪ ART procedure: IUI versus IVF/ICSI

V. Discussion & Conclusion

VI. Acknowledgements
I. INTRODUCTION

‘Semen contains vitality & heredity, not germs’: seminal discourse in the AIDS era\(^1\).

Infection and contamination control in ART laboratory\(^2-4\):

- **Environment** (inside & outside of lab e.g. *Pseudomonas* spp. in water, fungal spores in air)
- **Staff** (individuals & techniques)
- **Facilities & supplies** (equipment, eg. cryostorage tanks; media)
- **Patients & specimens**

1. Khan *et al.* *J Health Popul Nutr* 2006; **24**:195-200;
2. Englert *et al.* *Hum Reprod Update* 2004; **10**:149-162;
I. INTRODUCTION

Infection and contamination control in ART laboratory¹:

- Patients & specimens (*semen*)
  - Prevalence of pathogens (*origin*)
  - Risk reduction (*prevent*)

I. INTRODUCTION

Infection and contamination control in ART laboratory:

Patients & specimens (*semen*)
- Prevalence of pathogens (*origin*)
- Risk reduction (*prevent*)

Fig 1: Prediction of reproductive potential

II. AIM

- The prevalence of pathogens (*origin*)\(^1\) and methodologies to prevent infections (*risk reduction*) from *male* → *female* in an ART program\(^2\):

*Male: HIV-seropositive and the female HIV-seronegative through:

- **Screening of patients**, thorough counselling, appropriate treatment and applying appropriate semen decontamination & ART (IUI) procedures

---

2. Englert *et al.* *Hum Reprod Update* 2006; 10:149-161
CONTENT

I. Introduction
   ▪ Infection and contamination control
     ▪ Environment
     ▪ Staff
     ▪ Supplies
     ▪ Patients & specimens

II. Aim
   ▪ Origin & prevention

III. Prevalence (origin) of pathogens in ART
   ▪ Bacteria and STIs
   ▪ Viruses
   ▪ Interaction STI & HIV

IV. Risk reduction (prevention)
   ▪ Screening & counselling
   ▪ Semen washing
   ▪ Validation of specimens
   ▪ ART procedure: IUI versus IVF/ICSI

V. Discussion & Conclusion
VI. Acknowledgements
Prevalence of micro-organisms in semen samples:

- Coagulase negative staphylococci
- *Ureaplasma* sp.
- α-Haemolytic streptococci
  - *Escherichia coli*
  - *Enterococcus faecalis*
  - *Enterococcus* sp.
- *Mycoplasma* sp.
- *Staphylococcus aureus*

Besides HIV, the most common STIs reported include\textsuperscript{1-2}:

chancroid,
human papiloma virus,
herpes simplex,
trichomoniasis and candidiasis,
while gonorrhoea,
syphilis and \textbf{chlamydia} also contribute to damages of Fallopian tubes.

\begin{itemize}
  \item \textbf{1.} Bambra \textit{Hum Reprod Update} 1999; \textbf{5}:1-20
  \item \textbf{2.} Ochsendorf \textit{Andrologia} 2008; \textbf{40}:72-75
\end{itemize}
III. PREVALENCE – Interaction STD & HIV

- Association of sexually transmitted diseases (STDs) and HIV
  
  **Epidemiologic synergy** –
  Transmission: *Susceptibility & Infectiousness*
  Duration of infectiousness:
  Rate of progression/recovery, recurrence of STDs

- STDs seems to have a stronger effect on *susceptibility* to HIV than on *infectiousness* of HIV; treatment of STDs in HIV+ patients should be targeted

- **Impact on:**
  - seminological parameters\(^1\),\(^2\),
  - leukocytospermia and/or seminal *viral load*\(^1\),\(^3\) and
  - shedding\(^4\) (e.g. cytomegalovirus\(^5\), *Herpes simplex*, *Candida* or *Trichomonas* infections\(^6\))

---

III. PREVALENCE – Viruses (origin)

Fig 2: Summary of viruses identified in the genital tract of the male

Adapted from Dejucq-Rainsford & Jégou. *Curr Pharm Des* 2004;10:557-75
### III. PREVALENCE – Viruses (blood-semen loads)

<table>
<thead>
<tr>
<th>Blood viral load</th>
<th>Semen</th>
<th>%</th>
<th>Sperm sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;50</td>
<td>7/41</td>
<td>17</td>
<td>0/41</td>
</tr>
<tr>
<td>&gt;50-&lt;1,000</td>
<td>7/20</td>
<td>35%</td>
<td>0/20</td>
</tr>
<tr>
<td>&gt;1,000-&lt;10,000</td>
<td>4/8</td>
<td>50%</td>
<td>0/8</td>
</tr>
<tr>
<td>&gt;10,000</td>
<td>15/16</td>
<td>94</td>
<td>6/16</td>
</tr>
<tr>
<td><strong>Total:</strong></td>
<td>33/85</td>
<td>39</td>
<td>6/85</td>
</tr>
</tbody>
</table>

- **Blood** viral load impact on **seminal** viral load\(^1\)
- **Seminal viral load** impact on efficiency of **sperm washing**\(^2\)
  
  Washed sperm samples (DGC + SU) contaminated with HIV-1 RNA, when seminal plasma $>1 \times 10^6$ copies/ml

---

1. Englert et al. *Hum Reprod Update* 2004; **10**:149-162;
CONTENT

I. Introduction
   ▪ Infection and contamination control
     ▪ Environment
     ▪ Staff
     ▪ Supplies
     ▪ Patients & specimens

II. Aim
   ▪ Origin & prevention

III. Prevalence (origin) of pathogens in ART
   ▪ Bacteria and STIs
   ▪ Viruses
   ▪ Interaction STI & HIV

IV. Risk reduction (prevention)
   ▪ Screening & counselling
   ▪ Semen washing
   ▪ Validation of specimens
   ▪ ART procedure: IUI versus IVF/ICSI

V. Discussion & Conclusion

VI. Acknowledgements
IV. RISK REDUCTION

Screening & counselling of male patient

**Decisions:**

- **Diagnostic**
  - **Infectious disease tests**
    - Swab – bacterial infections
    - Blood - viral load/CD4 count
    - Semen – spermiogram
    - bacteria culture
      - viral load - DNA/RNA

- **Treatment**
  - Antibiotics/HAART
  - Semen decontamination technique
    - Density gradient centrifugation (DGC)
    - Washing cycles/Swim-up/Inserts
  - Viral validation - DNA/RNA

- **Appropriate ART procedure** – IUI - IVF/ICSI
IV. RISK REDUCTION – semen washing

Fig 3.1: Schematic presentation of spermatozoa and non-sperm cells (not according to scale)
Fig 3.2: Schematic presentation of spermatozoa and non sperm cells, with viral particles (not according to scale)
IV. RISK REDUCTION – semen washing

Fig 3.3: Schematic presentation of spermatozoa without contaminating cells (not according to scale)
Fig 5: Schematic presentation of sperm processing using a tube insert: 1) Layering; 2) Prevention of recontamination using an elongated micro-pipette to bypass contaminated layers

IV. RISK REDUCTION - (DGC + tube insert 2 - video)

<table>
<thead>
<tr>
<th>Raw semen</th>
<th>Processed and washed sperm</th>
</tr>
</thead>
</table>

[Images of raw semen and processed and washed sperm]
CONTENT:

I. Introduction

- Infection and contamination control
  - Environment
  - Staff
  - Supplies
  - Patients & specimens

II. Aim

- Origin & prevention

III. Prevalence (origin) of pathogens in ART

- Bacteria and STIs
- Viruses
- Interaction STI & HIV

IV. Risk reduction (prevention)

- Screening & counselling
- Semen washing
- Validation of specimens
- ART procedure: IUI versus IVF/ICSI

V. Discussion & Conclusion

VI. Acknowledgements
IV. DISCUSSION – ART procedure

To test or not to test\(^1\text{-}^3\):

- **Type of test:**
  - Various tests with different thresholds of detection:
    - Nuclisense Kit RNA extraction; NASBA, bDNA, RT-PCR, nPCR
    - False-negative results due to inhibitors of PCR extraction or dilution below detection limit\(^1\)

---

IV. DISCUSSION – ART procedure

To test or not to test\(^1-3\): 

- **Type of test:**
  - Various tests with different thresholds of detection:
    - Nuclisense Kit RNA extraction; NASBA, bDNA, RT-PCR, nPCR
    - False-negative results due to inhibitors of PCR extraction or dilution below detection limit\(^1\)

- **What to test:**
  - Semen or seminal compartments
  - Purified sperm sample (post-processing)
  - DNA and/or RNA

- **When to test:**
  - Prior to procedure, same-day evaluation/freeze-thaw processing
  - Follow-up female partner & child

IV. DISCUSSION – ART procedure

Fig 6: Conventional insemination (A) versus ICSI (B) – hypothetical risk to introduction external particles. Adapted from: Kvist U. ESHRE Campus Symposium 1-3 Oct 2009. http: www.eshre.com; See also Semprini & Fiore *Curr Opin Obstet Gynecol* 2004; 16:257-262
IV. DISCUSSION – ART procedure

Fig 6: Conventional insemination (A) versus ICSI (B) – hypothetical risk to introduction external particles. Adapted from: Kvist U. ESHRE Campus Symposium 1-3 Oct 2009. [http://www.eshre.com](http://www.eshre.com); See also Semprini & Fiore *Curr Opin Obstet Gynecol* 2004; 16:257-262
The debate on the presence of HIV-1 in human gametes

Baccio Baccetti *, Arrigo Benedetto *, Giulia Collodel *, Antonino di Caro *, Anna Rosa Garbuglia *, Paola Piomboni *

* Institute of General Biology, University and Center for the Study of Germline Cells, C.N.R., Via F. Ferrata 4, 21010 Sannio, Italy
* Center of Virology, L. Spallanzani Hosp, IRCCS, Rome, Italy

Spermatozoa capture HIV-1 through heparan sulfate and efficiently transmit the virus to dendritic cells

Ana Ceballos, Federico Remes Lenicov, Juan Sabaté, Christian Rodriguez Rodriguez, Mercedes Cabrini, Carolina Janiec, Silvina Raiden, Mónica Donaldson, Rodolfo Agustín Pasqualini Jr, Clara Marín-Briggler, Mónica Vázquez-Levin, Francisco Capani, Sebastián Amigo Reza, Jorge Goffin

Analysis of human immunodeficiency virus in semen: indications of a genetically distinct virus reservoir

Randal A. Byrn *, Ann A. Kiessling *

* Vertex Pharmaceuticals, 150 Water St, Cambridge, MA 02139-4202, USA
* Department of Surgery, Division of Urology, Beth Israel Deaconess Medical Center, Harvard Institutes of Medicine, Room 333, 4 Brookline Circle, Boston, MA 02115, USA

HIV-1 viral DNA is present in ejaculated abnormal spermatozoa of seropositive subjects

B. Muiaiccia, S. Corallini, E. Vicini, F. Padula, L. Gandini, G. Liuzzi, A. Lenzi and M. Stefanini

* Department of Histology and Medical Embryology, Sapienza University of Rome, Rome, Italy; † Department of Medical Sapienza University of Rome, Rome, Italy; ‡ National Institute for Infectious Disease Spallanzani (INMI), Rome, Italy
IV. DISCUSSION — ART procedure & viral validation

The adherence of viral particles & presence of HIV in spermatozoa has been a matter of debate.


**Fig 6:** Spermatozoa (arrows) transmit HIV-1 when they attach to dendritic cells (DC - red)² Spermatozoa acted as HIV carrier; attachment increase at pH 6-7, modulating the function of mucosal DC
IV. Conclusion – Current treatment, ART & sperm washing

**HIV-discordant couple** → **Sexual health screening**

**Plasma:** HIV-1 RNA + CD4

**Semen:** HIV-1 (RNA\(^1\) & DNA\(^2\)), bacteria culture & spermiogram

---

*Fig 7: Flow chart for the ART management* (adapted from: Ombelet et al., ESHRE Monograph p64-72 2008.)

1. RNA – quantitative Cobas Ampliprep/Cobas Taqman HIV-1, v 2, LLD 40 copies/ml;
2. DNA qualitative - Amplicor HIV-1 DNA, v 1.5, Roche Diagnostics
IV. Conclusion – Current treatment, ART & sperm washing

**HIV-discordant couple**

**Sexual health screening**

**Plasma:** HIV-1 RNA + CD4

**Semen:** HIV-1 (RNA\(^1\) & DNA\(^2\)), bacteria culture & spermiogram

♂ **Male Factor**

Morphology >5% Normal
IMC >1.5 x10\(^6\)

*Sperm washing + RT-PCR\(^1\)*

IUI/IVF

♀ **Male Factor**

Morphology <5% Normal
IMC <1.5 x10\(^6\)

-Low motility/ immotile

*Sperm washing + RT-PCR\(^1\)*

ICSI

**Results**

- Normal semen analysis/
  Morphology >5% Normal
  IMC >1.5 x 10\(^6\)

*Sperm washing + RT-PCR\(^1\)*

IUI

---

**Fig 7: Flow chart for the ART management** (adapted from: Ombelet et al., ESHRE Monograph p64-72 2008.)

1. RNA – quantitative Cobas Ampliprep/Cobas Taqman HIV-1, v 2, LLD 40 copies/ml;
2. DNA qualitative - Amplicor HIV-1 DNA, v 1.5, Roche Diagnostics
IV. Conclusion – Current treatment, ART & sperm washing

HIV-discardant couple → Sexual health screening

**Plasma:** HIV-1 RNA + CD4

**Semen:** HIV-1 (RNA¹ & DNA²), bacteria culture & spermiogram

♂ Male Factor
Morphology >5% Normal
IMC >1.5 x 10⁶

Sperm washing + RT-PCR¹
IUI/IVF

♀ Male Factor
Morphology <5% Normal
IMC <1.5 x 10⁶
-Low motility/ immotile

Sperm washing + RT-PCR¹
ICS

Normal semen analysis/
Morphology >5% Normal
IMC >1.5 x 10⁶

Sperm washing + RT-PCR¹
IUI

Cryopreservation → Repeat analyses → HIV testing follow-up

Fig 7: Flow chart for the ART management (adapted from: Ombelet et al., ESHRE Monograph p64-72 2008.)

1. RNA – quantitative Cobas Ampliprep/Cobas Taqman HIV-1, v 2, LLD 40 copies/ml;
2. DNA qualitative - Amplicor HIV-1 DNA, v 1.5, Roche Diagnostics
Acknowledgements:

Staff: Reproductive & Endocrine Unit
Krushmee Singh, Jozef Fourie, Melissa Stander, Prashan Maharaj, Shaan Maharaj, Laura Grant, Grace Ramaotsoa, Tarina Mathe

Funding:
Medical Research Council (MRC).

Disclaimer: Views & opinions expressed do not necessarily reflect the views of the MRC

Graphics:
Creative Studios (Health Sciences, University of Pretoria, SA)