Evaluating a novel apparatus during density gradient centrifugation for the elimination of bacteria, HIV-1 RNA and proviral DNA from human semen

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ESHRE Campus Belgium Genk: 15 December 2009
INTRODUCTION

- Seminal pathogens
  - Compromise ART outcome
  - Sero-conversion of ♀ partner or infection of offspring

OBJECTIVES

- To determine the prevalence of bacteria in the semen patients participating in the assisted reproductive program at the Unit

- To evaluate the effectiveness of density gradient centrifugation (DGC) & a novel apparatus to remove from semen:
  - bacteria & yeast spiked at different concentrations
  - in vivo derived HIV-1
METHODS

Prevalence of bacteria in semen
Surveyed 2007-2009
N = 929

Semen spiked
prevalent bacteria and yeast

Informed consent
HIV-1-seropositive patients
N = 50

*Semen viral validation
DNA (qualitative) & RNA (quantitative)

Semen processing
DGC (PureSperm®, Nidacon, Sweden) with novel polypropylene (FDA approved) ProInsert™ (Nidacon, Sweden)

Bacterial quantification
Culture on blood agar plates

DNA → Amplicor HIV-1 DNA Test, version 1.5, Roche Diagnostics

RNA → RT-PCR Cobas Ampliprep/Cobas Taqman HIV-1 Test, version 2, Roche Diagnostics; sensitivity: <40 copies/ml

## RESULTS

Presence of bacteria in sperm samples processed with and without the use of the ProInsert™

<table>
<thead>
<tr>
<th>Spiking Concentration</th>
<th>Enterobacter cloacae</th>
<th>Enterococcus faecalis</th>
<th>E. coli</th>
<th>CNS</th>
<th>Staphylococcus aureus</th>
<th>Candida albicans</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>In-house Strain</td>
<td>ATCC 29212</td>
<td>ATCC 25922</td>
<td>In-house Strain</td>
<td>ATCC 25923</td>
<td>ATCC 90028</td>
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<tr>
<td>Without Insert</td>
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<tr>
<td>1 x 10^3</td>
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</tbody>
</table>

**Staphylococcus aureus** colonies present in processed sperm (spiking concentration 1 x 10^5 CFU/ml)
**RESULTS (Cont)**

Presence of HIV-1 RNA and proviral DNA in semen samples (n=50) and purified sperm samples of HIV-1-seropositive patients (N=27)

<table>
<thead>
<tr>
<th>HIV-1 positive semen samples (Total 64%)</th>
<th>HIV-1 positive purified sperm samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA+</td>
<td>RNA+</td>
</tr>
<tr>
<td>20%</td>
<td>26%</td>
</tr>
</tbody>
</table>

Range: 138–801,440 copies/ml

Mean: 127,543 copies/ml
DISCUSSION & CONCLUSION

The novel ProInsert™ facilitated:
- Precise density gradient layering
- Access to the treated sperm pellet without re-contamination
- Bio-secure disposal

Incorporating the insert in semen decontamination procedures:
- Affordable way to improve risk reduction
- Streamlines semen processing by providing the option to omit an extra swim-up step
- Standard in the assisted reproductive program
The end

Thank you!

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