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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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

Informed consent

HIV-1-seropositive patients

N = 50

Semen spiked

prevalent bacteria and yeast

*Semen viral validation

DNA (qualitative) & RNA (quantitative)

Semen processing

DGC (PureSperm®, Nidacon, Sweden) with novel polypropylene (FDA approved) ProInsert™ (Nidacon, Sweden)[1]

Bacterial quantification

Culture on blood agar plates

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

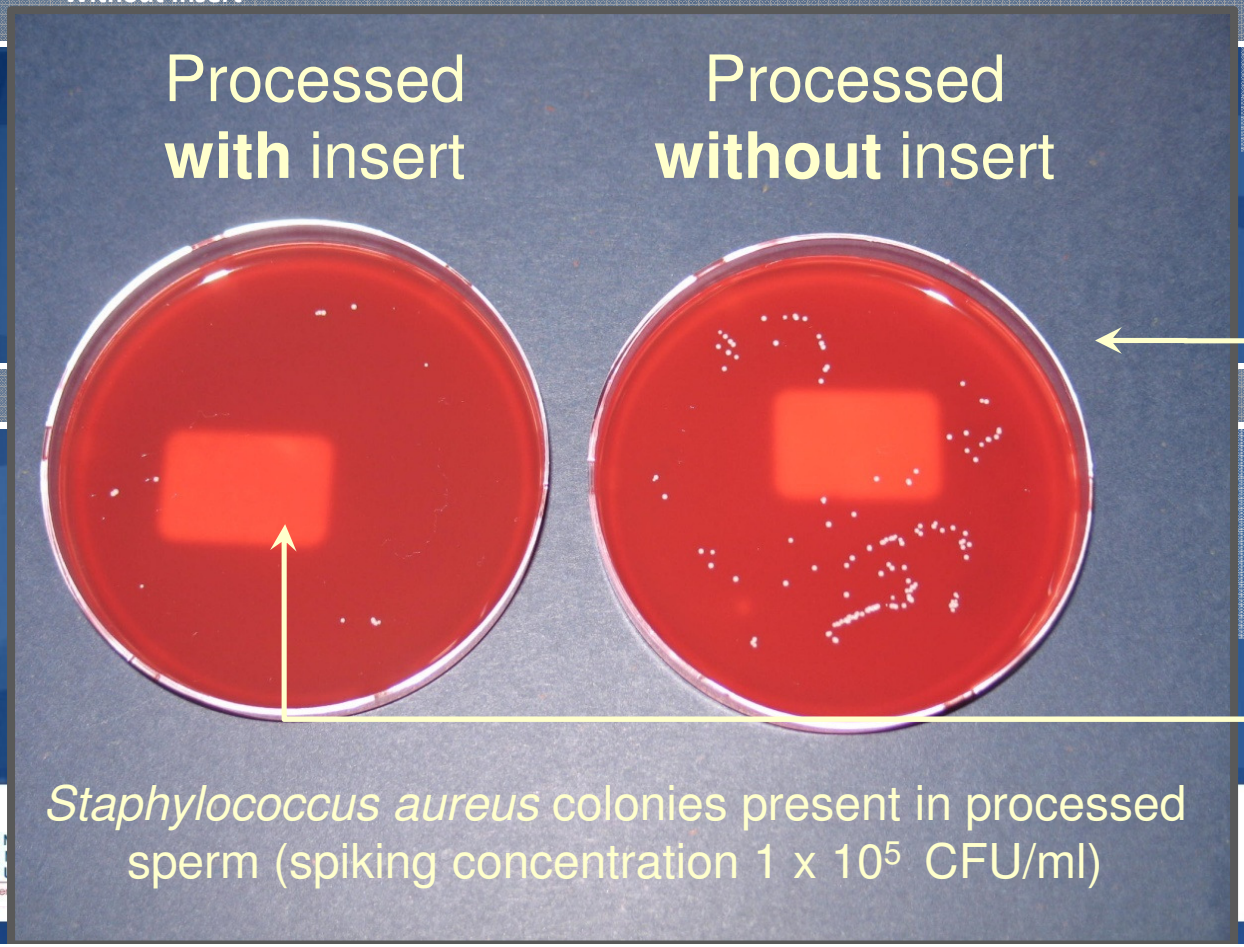
DNA → Amplicor HIV-1 DNA Test, version 1.5, Roche Diagnostics

1. Loskutoff *et al.* Fert Ster. 2005 84(4):1001-1010

RESULTS

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

Spiking Concentration	*Entero-bacter cloacae* In-house Strain	*Entero-coccus faecalis* ATCC 29212	*E coli* ATCC 25922	CNS In-house Strain	*Staphylo-coccus aureus* ATCC 25923	*Candida albicans* ATCC 90028
Without Insert						
1×10^3					0	0
					0	0
1×10^4					100	300
					100	400
1×10^5					2500	700
					5500	900
1×10^3					0	0
					0	0
1×10^4					0	0
					0	0
1×10^5					400	0
					900	0



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)

HIV-1 positive semen samples (Total 64%)			HIV-1 positive purified sperm samples
DNA+	RNA+	DNA+ & RNA+	
20%	26%	18%	0%
	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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