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Pure Sperm; prevention of sperm pellet re-contamination by HIV-1 RNA, DNA and bacteria during density gradient centrifugation

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INTRODUCTION:
The presence of pathogens in semen can compromise the outcome of assisted reproductive procedures, and can lead to the infection of the female partner or offspring. Therefore, effective risk reduction protocols are critical when attempting to provide a service to patients with seminal pathogens. The present study evaluates sperm preparation by density gradient centrifugation (DGC), wherein a novel centrifuge tube insert is used for retrieval of the sperm pellet.

AIM:
To evaluate the effectiveness of DGC with a novel apparatus to remove a variety of bacteria and yeast from *in vitro* spiked semen samples and subsequently test the method for the removal of *in vivo* derived HIV-1 RNA and pro-viral DNA from semen.

MATERIALS AND METHODS:
(**i)** Bacteria and yeast commonly found in semen (*Enterococcus faecalis, Enterobacter cloacae, Escherichia coli,* coagulase-negative staphylococci, *Staphylococcus aureus* and *Candida albicans*) were added to pooled semen in triplicate at concentrations of 1 x 10^3, 10^4, 10^5 and 10^6 colony forming units/ml (CFU/ml). The spiked samples were processed using DGC (PureSperm®, Nidacon, Sweden) with and without the use of a novel polypropylene (FDA approved) tube insert (ProInsert™, Nidacon). Bacteria and yeast quantification in processed sperm samples were performed by culture on blood agar plates.
(ii) Semen samples (N=50) from HIV-1-1-seropositive men were processed using DGC with the insert. HIV-1 RNA quantification of raw semen and sperm pellets were performed using RT-PCR (Cobas Ampliprep/Cobas Taqman HIV-1 Test, version 2, Roche Diagnostics, Indianapolis, USA; sensitivity: <40 copies/ml). DNA evaluation was performed qualitatively (Amplicor HIV-1 DNA Test, version 1.5, Roche Diagnostics).

RESULTS:
DGC using the novel ProInsert™ removed significantly more (96%) bacteria from semen compared to processing without the insert ($P<0.004$). Of all semen samples from HIV-1 infected patients, 64% tested positive for HIV-1 RNA, DNA, or both (26%, 20%, and 18%, respectively). DGC using the insert was effective in removing HIV-1 RNA and proviral DNA from all semen samples.

CONCLUSION/DISCUSSION:
The high prevalence of seminal pathogens in South Africa warrants the need for effective semen processing procedures. In this study, the ProInsert™ facilitated density gradient layering and retrieval of treated sperm pellet without recontamination.