MICROBIAL PATHOGEN REMOVAL FROM PORCINE SEMEN WITH ACOUSTOPHORESIS

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ABSTRACT

Microbial pathogens in semen used for artificial insemination (AI) do not only transmit diseases, but also reduce semen quality. A continuous pathogen removal separation technique overcomes the limits of current processing and separation techniques. We have applied acoustophoresis to separate bacteria and viruses from porcine semen while maintaining spermatozoa viability and achieved high spermatozoa recovery.

KEYWORDS: Artificial insemination, Acoustophoresis, Bacteria, Viruses, Separation, Spermatozoa

INTRODUCTION

In the veterinary industry (VI), semen samples are collected and processed into doses before the semen is used for insemination. To prevent the spread of diseases, it is legally obligated to add antibiotics to semen samples and to screen for viruses. However, antibiotic resistant bacteria have been identified in semen [1] and virus screening techniques are time consuming as well as labor-intensive. Bacteria (~1-5 µm) and viruses (10-400 nm) present in semen samples are smaller than spermatozoa (head: ~5 µm, total length (including tail): ~50 µm). When removing pathogens from semen, not only disease spread is prevented, but also semen quality is improved [2]. We applied the size-based separation technique acoustophoresis to remove microbial pathogens from semen.

EXPERIMENTAL

Acoustic separation was performed with the AcouWash (AcouSort AB, Lund, Sweden), a benchtop research instrument for automated particle separation. The microfluidic glass chip has a typical acoustophoretic channel structure. The main separation channel has a meander shape (2.5 cm x 420 µm x 150 µm) to increase the separation efficiency (figure 1). The standing wave field for acoustic separation was created using a 2 MHz piezoceramic transducer glued underneath the separation channel.

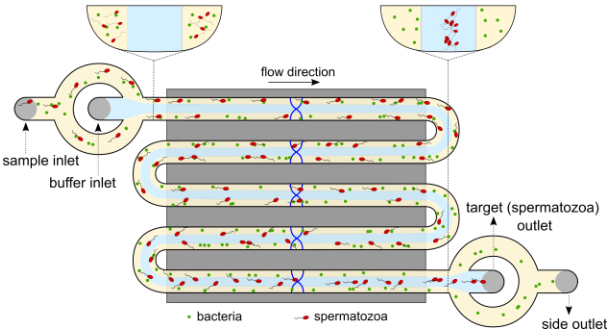

Figure 1: Schematic representation of the acoustic separation principle: The sample (spermatozoa and bacteria) is injected through the sample inlet, bifurcated around the center buffer inlet, and enters the separation channel laminated close to the side walls. An acoustic standing wave forces larger particles (spermatozoa) to move towards the pressure node in the center of the channel, such that these are collected at the target outlet. The smaller particles (bacteria/beads) remain along the side walls of the channel and can be collected at the side outlet.

RESULTS AND DISCUSSION

Acoustophoretic separation of bacteria from semen was modelled by spiking porcine semen with Escherichia coli (E. coli) bacteria (2 x 10^6 spermatozoa/ml; 10 x 10^6 bacteria/ml). The effect of the density of the central buffer solution (figure 2A) and the effect of the sample flow rate (figure 2B) on the separation quality were investigated. Considering both the spermatozoa recovery and bacteria present in the target outlet, a Ficoll concentration of 1% and a sample flow rate of 30 µL/min have been chosen to achieve the best separation efficiency (figure 2C).

It is important that spermatozoa retain their quality to assure successful fertilization. Therefore, it was investigated whether the spermatozoa viability decreases after acoustophoretic separation (figure 3). No statistical difference (Wilcoxon Signed Ranks Test, P-value: 0.05) in spermatozoa viability before and after processing was observed.

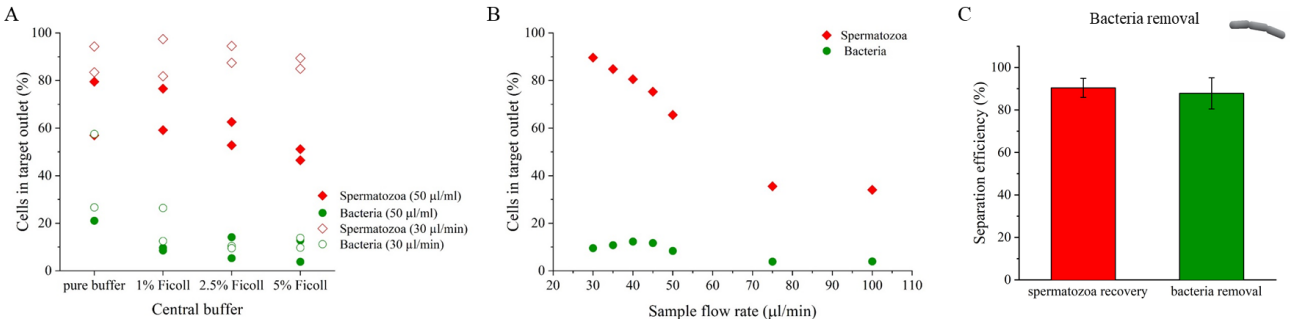

Figure 2: A) Effect of medium buffer with increasing Ficoll concentration on the separation quality in the target outlet. An increase in Ficoll concentration decreases the number of bacteria and spermatozoa in the target outlet. B) Effect of sample flow rate on the percentages of cells present in the target outlet. The percentage of spermatozoa decreases with increasing sample flow rate, whereas the percentage of bacteria is for all flow rates below 20%. The central buffer solution contained 1% Ficoll. C) Separation efficiency at a sample flow rate of 30 µl/min and with a central buffer solution with 1% Ficoll. (N=9)

Virus contaminated semen was modelled by spiking porcine semen with fluorescent labelled cowpea chlorotic model virus (CCMV) (diameter: 28 nm, concentration: 120 ng/ml). After processing the semen sample with acoustophoresis, the virus concentrations at both outlets were determined with a fluorescence microplate reader (figure 4). The model virus exited the chip at the waste outlet and were separated from the spermatozoa.

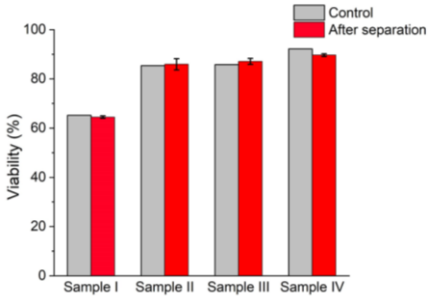

Figure 3: The effect of acoustophoresis on the spermatozoa viability. No effect of spermatozoa viability between the control and processed sample was observed. (Control: N=1; After separation: N=3)

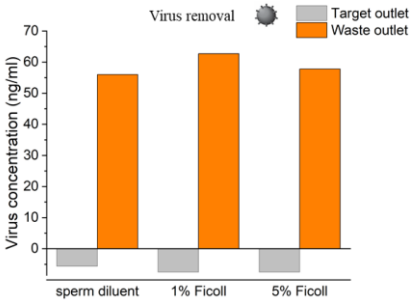

Figure 4: Virus concentration in both outlets after separating with varying buffer solution (sample flow rate: 30 µl/min). CCMVs were detected in the waste outlet and not in the target outlet.

CONCLUSION

Both microbial pathogens, bacteria and viruses, were removed from semen samples using acoustophoresis. A bacteria removal of 88±7% and a spermatozoa recovery of 90±4% were achieved. Viruses were mainly detected in the waste outlet. Pathogen removal from semen increases the biosecurity of AI in the veterinary industry.

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