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Potential Mechanical and Antiviral Methods to Insure PRRSV Free Semen

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Introduction

PRRSV can be shed in boar semen causing transmission of the virus, so there is risk of wide dissemination of the virus through AI. "On-farm" approaches to reduce the risk of PRRSV shedding in semen were evaluated. This included a density gradient to purify semen from PRRSV and antivirals that when added to semen have the potential to reduce or eliminate the viral load. HIV, hepatitis virus and *Brucella abortus* have all been successfully removed from semen by density gradients.[1] PRRSV has been eliminated using a 15 ml density gradient, but a quicker method is needed to obtain more sperm after the gradient. Antivirals (cysteine protease inhibitors) were evaluated since they potentially block viral proteins needed for replication.

Materials and Methods

For gradient evaluations (NidaCon, Intl., Gothenburg, Sweden), 29 boars were inoculated IN with $10^{4.6}$ $TCID_{50}$ of PRRSV MN 30-100 (2-layer gradient). Semen was collected 3X/wk for 2 wks or, 8 boars were inoculated IN with SD-23983 PRRSV isolate (10^6 FFU/ ml) (1-layer gradient). Semen was collected 2-3X/wk for 4 wks. PRRSV positive semen from the 2-layer (n=34) or 1-layer gradients (n=45) was centrifuged to concentrate sperm and layered on the gradient in a 50 ml tube using a novel insert to prevent contamination of purified sperm (Diversified Plastics, Mpls, MN). Samples were centrifuged through the gradient and tested before and after the gradients by PCR (Tetracore Inc., Rockville, MD). Any PCR positive samples after the 1-layer gradient treatment were also evaluated by a swine bioassay to determine infectivity.[2]

The cysteine protease inhibitors, chymostatin, E-64, antipain, cystatin C and α-2-macroglobulin were evaluated for antiviral activity using a modified FFN assay.[3] Endpoints were determined by 90% virus inhibition when compared to the positive control.

Results

No viral RNA was detected in 26 of 34 (76%) or 32 of 45 (71%) sperm samples after the 2 or 1 layer gradients, respectively. Swine bioassay piglets were all PCR positive by 3 days post inoculation (dpi) and seropositive by 13 dpi even if PCR Ct levels indicated very low levels of viral RNA.

The protease inhibitor chymostatin was shown to inhibit PRRSV replication *in vitro* by 90% at a 62.5μm concentration. The other antivirals did not demonstrate viral inhibition up to 2000 μm concentrations.

Discussion

The 1 and 2-layer gradients demonstrate some risk reduction from PRRSV-contaminated semen to protect sow and gilt units receiving the semen. Although further refinements will be needed for 100% protection, these processes may also prevent transmission of other pathogens and eliminate non-viable sperm, thus optimizing the semen for insemination. It is important to note that even low levels of PRRSV RNA detectable by PCR can be infectious as determined by the swine bioassay.

The addition of antivirals in semen would provide added protection against PRRSV. Since chymostatin demonstrated reduction in PRRSV growth *in vitro*, next, pigs will be inoculated (swine bioassay) with various doses of chymostatin and PRRSV positive semen to evaluate their efficacy *in vivo* along with evaluations of sperm quality after adding the antiviral.

References

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