Spermatotoxic potential of insemination lubricant

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Introduction

In a typical 2400 sow breeding barn approximately 100 sows are artificially inseminated each week. If every sow receives two inseminations, 200 times a week sperm may contact the lubricant used to assist insertion of the AI catheter. If the lubricant is toxic to the sperm there is a chance of reduced reproductive potential. Although insemination lubricants may claim they are "non-spermicidal", preliminary results indicate spermatotoxic effects of a popular brand of lubricant across a range of exposure levels and contact times.

The specific aim of this study was to evaluate the effect of two different catheter lubricants on boar sperm motility, acrosome integrity and membrane viability using computer-assisted semen analysis (CASA) and flow cytometry. Samples extended in both a short-term and a long-term extender were evaluated across three different lubricant concentrations (1%, 2.5% and 5% v/v) at multiple durations of exposure (0, 30, 60 min).

Materials and methods

Insemination lubricants

Kuster Research and Consulting, Inc. (KRC) performed replicate testing on extended semen samples using two different insemination lubricants: Clarity, supplied by Aurora Pharmaceutical (Northfield, MN) and Product B – a frequently used commercial catheter lubricant purchased from an industry vendor.

Semen extenders

Semen doses from two different extender classifications: short-term, (BTS: IMV USA, Maple Grove, MN) and long-term, proprietary non-BSA , (NUTRIXcell⁺, IMV USA, Maple Grove, MN) were analyzed across three lubricant concentrations (1%, 2.5% and 5% v/v) and three exposure times (0, 30 and 60 minutes). A third extender representing the category of extenders containing bovine serum albumin (BSA) will be added for the meeting presentation.

Boar semen doses

- Pooled semen doses were obtained from commercial boar studs during routine collection.
- Ejaculates were partially diluted, pooled and fully extended in either short-term (n = 4) or NutriXcell⁺ (n = 6) according to the stud targets of approximately 35-50 million sperm per ml.
- Insemination doses were cooled and maintained at 17°C during same day transportation or overnight shipping to KRC.
- Only pooled semen doses that met standard production criteria (75% total motility; 75% normal morphology) were included in this study.

Semen incubation with insemination lubricants

Treatment groups: Extended semen samples were mixed 1:1 with lubricant diluted in extender to a total volume of 1ml and incubated at room temperature (RT, 20-22°C) for 0, 30 or 60 minutes for CASA analysis, and 60 minutes prior to flow cytometry. Control samples: Semen doses were mixed 1:1 with the same extender to match the sperm concentration in the treatment groups. Undiluted control samples were also incubated at RT.

CASA motility analysis

A CASA system (IVOS, Hamilton Thorne Biosciences, Beverly, MA) was used to analyze gross and progressive motility in control and treatment groups following exposure to lubricant (1%, 2.5% or 5% v/v) at 0, 30 or 60min.

Flow cytometric analysis

Sperm plasma membrane viability and acrosome integrity were assessed at a single time point (60 min), using the fluorescent dyes propidium iodide (PI) and fluorescein-labelled *Arachis hypogaea* (peanut) agglutinin (FITC-PNA) to determine the percentage of viable sperm with intact acrosomes (VIA) in both control and treatment groups. The VIA assays were performed on a Guava EasyCyte Plus flow cytometer (Millipore Corp., Hayward, CA).

Results

Statistics

Further replicates are planned with statistics presented at the meeting pending attainment of full sample size.

Effect of insemination lubricant on boar sperm motility

Incubation of extended semen with Clarity gel induced only mild sperm agglutination in some samples, while sperm agglutination was routinely noted at higher levels when exposed to Product B. Motility remained comparable to the Controls for samples exposed to Clarity in the long-term extender (Table 1 and Figure 2), with relatively small differences at the longer time points in the short-term extender (Table 1 and Figure 1). In contrast, sperm motility reductions were apparent after exposing semen to Product B for all lubricant concentrations in both short-term and long-term extenders (Figure 1 and Figure 2). For Product B, sperm motility was reduced to ≤ 60% for all time points at the 2.5% and 5% levels in the short term extender (Figure 1), as well as for all time points at the 5% level in BSA-free long term extender (Figure 2).

Table 1: Total sperm motility (average % ± SD) after 30 minutes exposure to lubricant.

Treatment/extender type	Control	Lubricant concentration 1% (v/v)	2.5% (v/v)	5% (v/v)
Clarity – short term	78 ± 6%	78 ± 3%	76 ± 3%	72 ± 6%
Product B – short term	78 ± 6%	65 ± 3%	47 ± 7%	23 ± 14%
Clarity – long term	85 ± 4%	83 ± 5%	81 ± 5%	81 ± 6%
Product B – long term	85 ± 4%	67 ± 6%	65 ± 6%	33 ± 14%

Effect of insemination lubricant on sperm viability and acrosome integrity

There was no apparent effect of Clarity lubricant on sperm VIA results (percentage of sperm with viable plasma membranes and intact acrosomes) in either short-term or long-term extenders as determined by flow cytometry (Figures 3 and 4). However, a downward trend in VIA was observed as the exposure concentration increased for Product B. The average control sperm VIA was 76.4 ± 3.6% in short-term extender and 59.1% ± 8.5% following exposure to Product B (5% v/v) for 60 minutes (Figure 3). A lower magnitude trend was visible in VIA results for sperm exposed to Product B in the long-term extender.

Conclusions

Although insemination lubricants may claim non-spermicidal status, the current data demonstrates that spermatotoxicity varies by lubricant, semen extender, lubricant concentration and duration of exposure. These effects can be observed immediately after exposure, and tend to become more severe over time. In the current study, motility suppression was a more sensitive indicator than sperm membrane viability status for the spermatotoxic lubricant. Exposure to Product B for specific concentration*time combinations reduced motility to the extent that litter size could be compromised.[1,2] In contrast, exposure to the highest concentration of Clarity gel (5% v/v) for the longest duration (60 min) had virtually no effect on sperm viability or acrosome status, with a relatively small change in motility for only the short-term extender. The BSA-free long-term extender appeared partially protective against the membrane toxicity effects of Product B when compared to the short-term extender. Based on both the sperm motility and viability results obtained in this study, Product B expresses spermicidal effects, while Clarity lubricant does not.

References

1. Hirai M et al. Objectively Measured Sperm Motility and Sperm Head Morphometry in Boars (*Sus scrofa*): Relation to Fertility and Seminal Plasma Growth Factors. *J Androl*. 2001;22:104-110.

2. Broekhuijse MLWJ et al. Application of computer-assisted semen analysis to explain variations in pig fertility. *JAS*. 2015;90:779-789.

Figure 1: Total sperm motility in short-term extender following exposure to lubricant over increasing time.

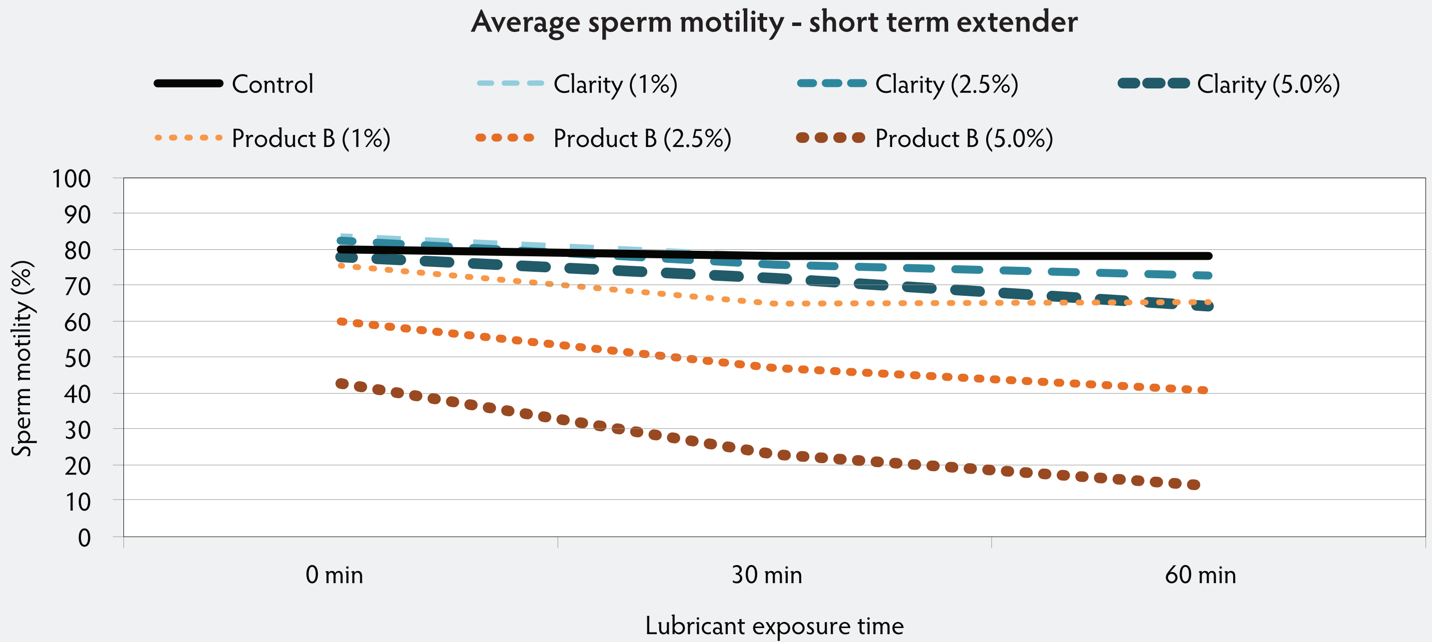

Figure 2: Total sperm motility in long-term (non-BSA) extender following exposure to lubricant over increasing time.

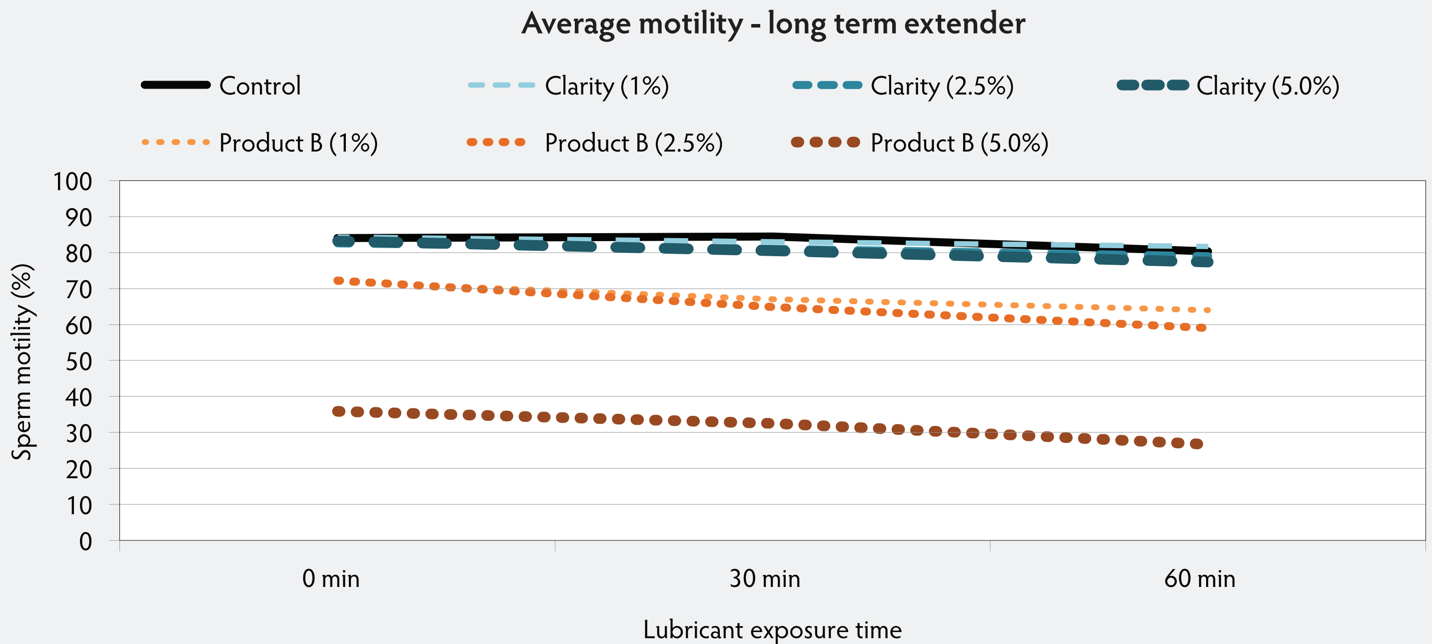

Figure 3: Sperm VIA (viable with intact acrosomes) in short-term extender.

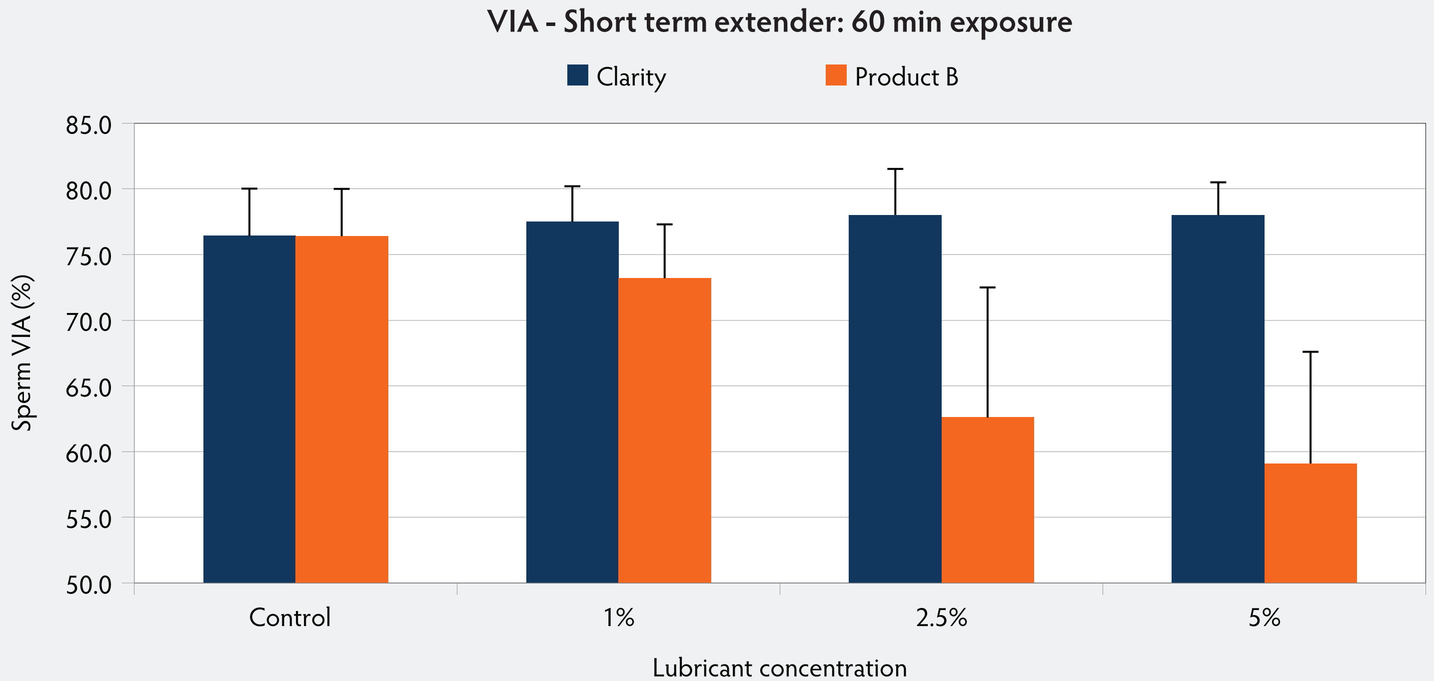

Figure 4: Sperm VIA (viable with intact acrosomes) in long-term extender after 60 minute lubricant exposure.