GameteGuard™ improves membrane quality in frozen-thawed bull sperm

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INTRODUCTION

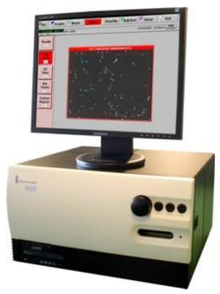
Artificial insemination with frozen-thawed bull semen is a common breeding practice used in the dairy and to a lesser extent in the beef industry. Frozen-thawed semen must be of high quality for optimal pregnancy. However, the handling, freezing and thawing of sperm cells can generate free radicals that often causes membrane damage which may then translate to reduced fertility. Here, we examine the effects of GameteGuard™, a novel semen extender additive, on post-thaw membrane permeability and acrosome quality in frozen-thawed bull sperm.

EXPERIMENTAL DESIGN

Semen collection, processing, and freezing

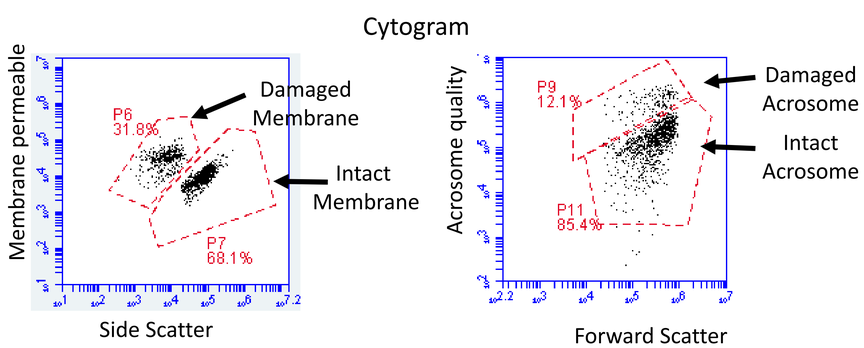
- Ejaculates were collected from 8 bulls using an artificial vagina.
- Concentrations were determined using a spectrophotometer.
- Sperm were initially diluted to either 40 or 20 x 10^6 cells/ml in
  1. TRIS egg-yolk citrate buffer (control)
  2. TRIS egg-yolk citrate buffer containing 10% (vol:vol) GameteGuard™.
- Cells were cooled to 4°C.
- After reaching 4°C, an equal part of TRIS egg-yolk citrate buffer containing 14% glycerol was added resulting in a final concentration of either 20 or 10 x 10^6 sperm cells/ml in 7% glycerol with or without 5% GameteGuard™.
- Sperm were loaded into 0.5cc straws and allowed to equilibrate for 4 h at 4°C.
- After equilibration, straws were frozen over liquid nitrogen vapor using a standard protocol and plunged into liquid nitrogen.

Sperm analysis



- Motility parameters were determined at immediately post-thaw (0) and 3 h post-thaw (at 37°C) using a computer assisted sperm analysis system.
- Membrane permeability and acrosome quality was determined at 0 and 3 h post-thaw (at 37°C) using flow cytometery.
  1. A triple stain protocol was used to determine membrane permeability and acrosome quality

Staining protocol



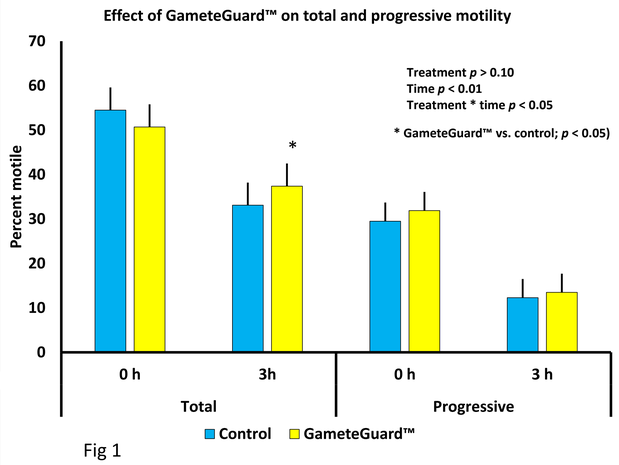


Fig 1

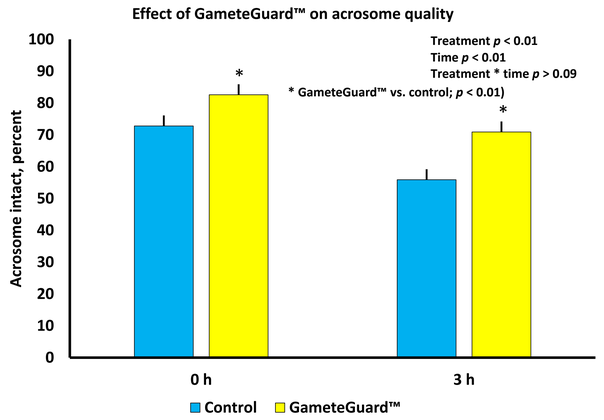


Fig 2

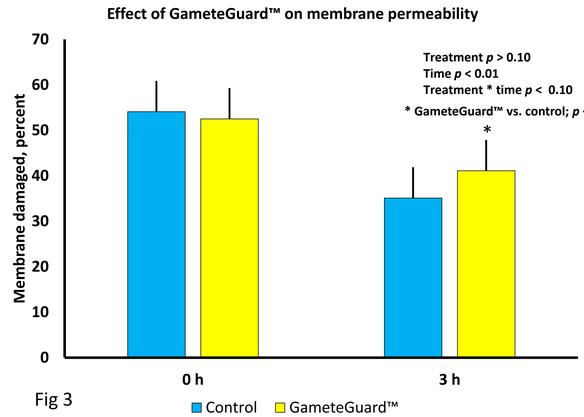


Fig 3

RESULTS

- **Effect of GameteGuard™ on sperm motility is shown in FIG 1.**
  1. Total motility at 3h for GameteGuard™ treated sperm was 12% higher ($p < 0.05$) than control sperm.
  2. As expected, for both treatments, total and progressive sperm motility was reduced at 3 h post-thaw ($p < 0.01$) when compared to 0 h analysis.
- **Effect of GameteGuard™ on acrosome quality is shown in FIG 2.**
  1. There was a 14% improvement immediately post-thaw (0 h) for GameteGuard™ treated sperm ($p < 0.01$)
  2. There was a 40% improvement at 3 h post-thaw in acrosome quality for GameteGuard™ treated sperm ($p < 0.01$).
  3. In fact, acrosome quality at 3 h post-thaw for GameteGuard™ treated sperm was similar to control sperm immediately post-thaw.
- **Effect of GameteGuard™ on membrane permeability is shown in FIG 3.**
  1. GameteGuard™ improved membrane quality by 17% at 3 h post-thaw when compared to control ($p < 0.05$).
  2. For both treatments membrane permeability decreased by 3 h ($p < 0.01$).

CONCLUSIONS

- **In this, as well as many previous studies, GameteGuard™ greatly improves post-thaw membrane and acrosome quality particularly in stressed cells. In addition, GameteGuard™ improves DNA quality, although such an analysis was not performed on this data set. The post-thaw sperm quality improvement imparted by GameteGuard™ seems to positively affect pregnancy rates in both heifers and lactating cows.**
- **Similar post-thaw results have been observed in other species including horses, roosters and humans.**
- **GameteGuard™ is a novel semen extender that prevents oxidative damage to sperm during collection, handling, freezing, and thawing thereby improving AI pregnancy rates in dairy and beef cows.**

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