



# GameteGuard™ treatment improves post-thaw sperm quality and pregnancy per insemination in dairy cows

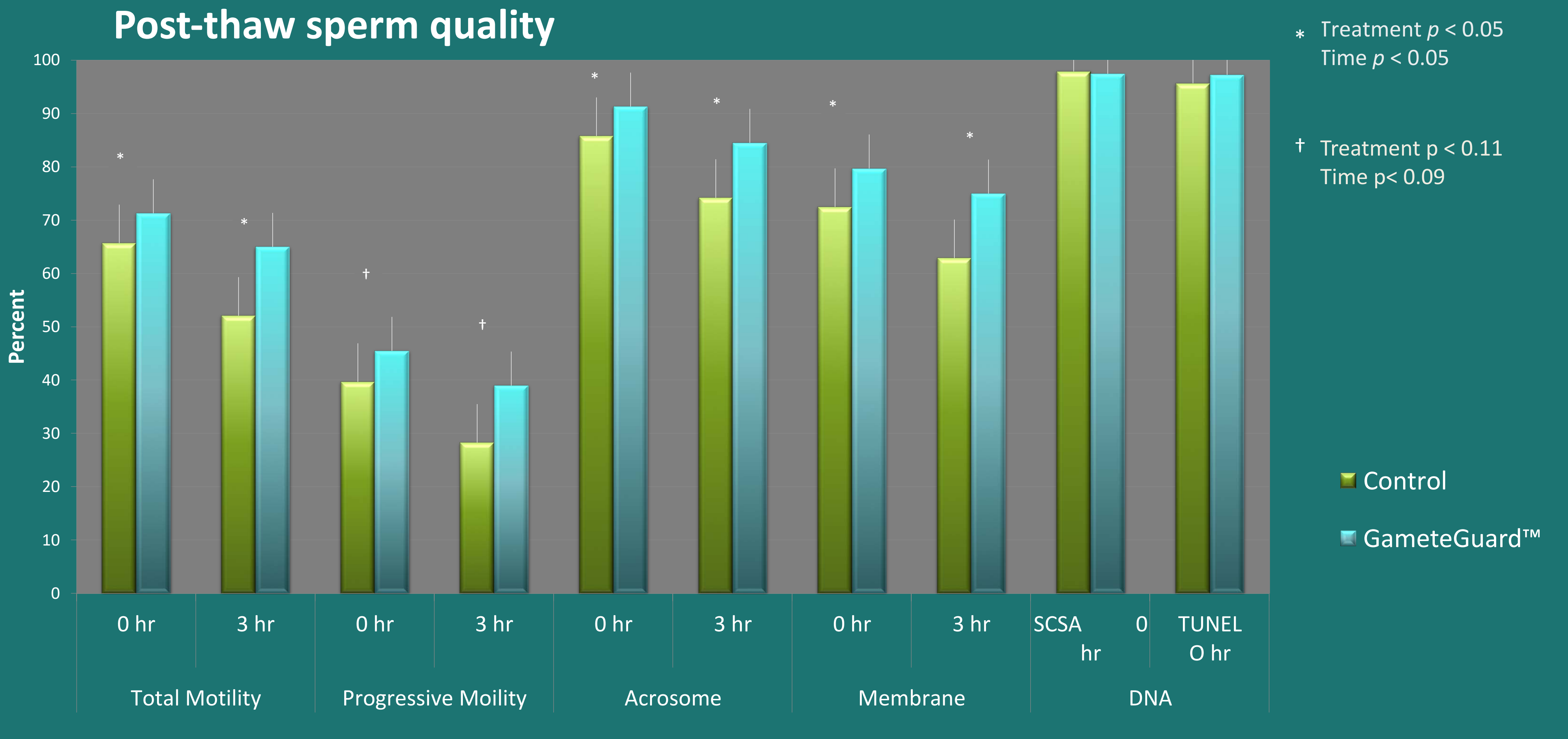
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## INTRODUCTION

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 sperm must be of high quality for optimal pregnancy. However, the handling, freezing and thawing of sperm cells can generate free radicals that cause membrane damage which may then translate to reduced fertility. Here, we report the effects of GameteGuard™, a novel semen extender additive, on post-thaw motility, membrane permeability, acrosome quality as well as the resulting increased pregnancy rates in frozen-thawed bull.



## EXPERIMENTAL DESIGN

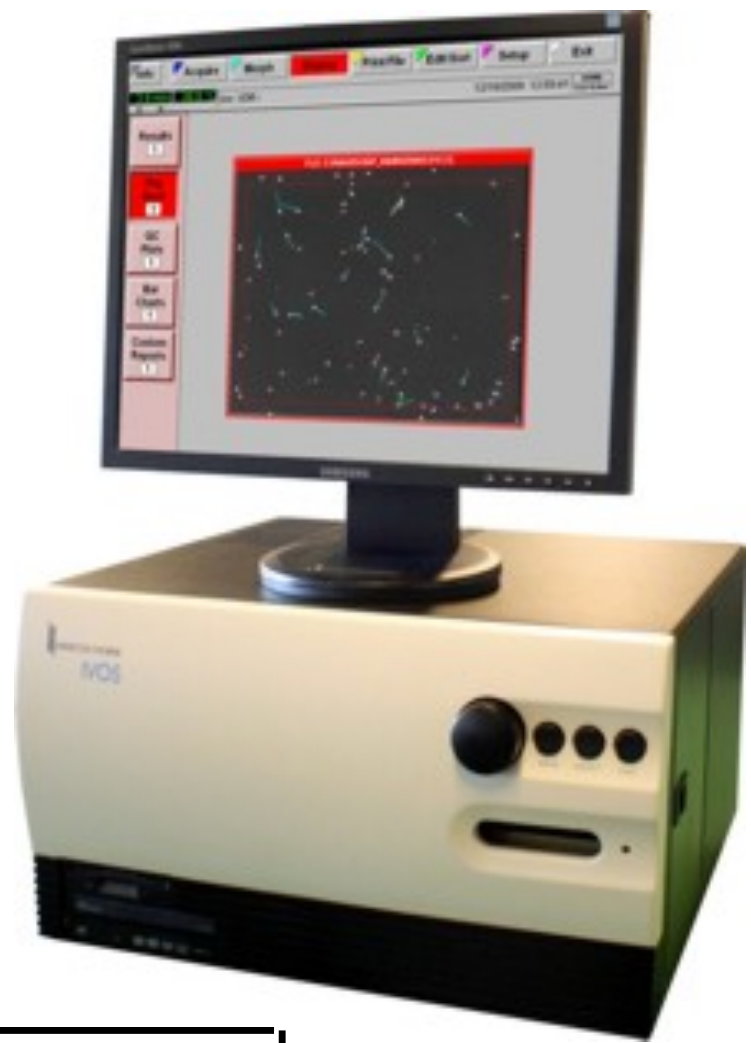
### Semen collection, processing, and freezing

- Ejaculates were collected from 2 ABS production bulls using an artificial vagina.
- Concentrations were determined using a spectrophotometer.
- Sperm were initially diluted 60 x 10<sup>6</sup> cells/ml in either TRIS egg-yolk citrate buffer (control), or 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 30 x 10<sup>6</sup> sperm cells/ml in 7% glycerol with or without 5% GameteGuard™.
- Sperm were allowed to equilibrate for 4 h at 4°C. Sperm were loaded into 0.5cc straws during equilibration.
- After equilibration, straws were frozen over liquid nitrogen vapor using a standard protocol and plunged into liquid nitrogen.



### Sperm analysis

- Motility parameters were determined immediately post-thaw (0) and 3 h post-thaw using a computer assisted sperm analysis system
- Membrane permeability and acrosome quality was determined at 0 and 3 h post-thaw using flow cytometry.  
A triple stain protocol was used to determine membrane permeability and acrosome quality (see below)
- Sperm parameters were analyzed by 1-way Analysis of Variance for effects of treatment.



### Staining protocol

0 min	15 min	20 min	25 min
PNA – Alexa fluor 674	SYBR-Green 14	PI	Analyze

### Artificial insemination

- Cows were cleared for breeding by the farm veterinarian then observed for visual signs of estrus such as disturbed tail chalk.
- Cows of any lactation, but only first service post-parturition were eligible for use in this study.
- Cows visually determined to be in estrus were inseminated within 12 hours with either bull 1 or 2, treated or control sperm, where treatment was blind to all farm personnel.  
All cows were bred by one inseminator over a 7 month period (May- Dec 2014).
- Bull and treatment combinations were randomized and dictated by calendar day. For example, May 6 all eligible cows were bred with Bull 1 Treatment 1 and on May 7 all cows were bred with Bull 2 Treatment 1.
- Cows were determined to be pregnant using transrectal ultrasonography between 35-45 days post-insemination.
- Cows were removed from the study if they were found to have earlier post-partum problems or current reproductive issues (e.g. ovarian cysts).
- A total of 208 cows were included in the study. Data were analyzed using Chi-Square.



FIG 1

	# inseminated	# pregnant	% pregnant	% improvement
Control	108	42	38	31%*
GameteGuard™	100	50	50	

TABLE 1: Overall pregnancy per treatment

\*p<0.05

	# pregnant/# insemination		% improvement
	Control	GameteGuard™	
45-83 days post calving	22/61	31/63	36.1%*
First lactation	30/59	28/48	13.7%

TABLE 2: Pregnancy by cow characteristics

\*p<0.11

	# pregnant/# inseminated		% improvement
	Control	GameteGuard™	
Bull 1	24/55	22/45	11.9%
Bull 2	18/53	28/55	50.1%*

TABLE 3: Pregnancy by bull

\*p<0.07

## RESULTS

### Effect of GameteGuard™ on post-thaw sperm quality is shown in FIG 1.

- GameteGuard™ treated sperm had improved immediate post-thaw total motility by 9%, acrosome quality by 3%, and decreased membrane permeability by 10% ( $p < 0.05$ ).
- The differences in post-thaw sperm quality were pronounced after 3 hr at 37°C. In fact, 3 hr GameteGuard™ treated sperm was similar to control sperm immediately post-thaw.

### Effect of GameteGuard™ on pregnancy per insemination is shown in Tables 1, 2 and 3.

- GameteGuard™ improved pregnancy rates by 31% across two bulls (Table 1;  $p < 0.05$ ). Both bulls utilized in the study showed improvement with GameteGuard™ treatment.
- Pregnancy rate per AI was improved by 36.1% for GameteGuard™ bred animals bred within 83 days of calving (Table 2;  $p < 0.11$ ).
- First lactation cows were 13.7% more likely to become pregnant if inseminated with GameteGuard™ treated sperm (Table 2).
- Ejaculates from both ABS production bulls benefited from the inclusion of GameteGuard™ in the extender (Table 3;  $p < 0.07$  for bull 2).
- Calves born from GameteGuard™ treated sperm (previous studies) are phenotypically normal.

## CONCLUSIONS

GameteGuard™ is a novel semen extender that prevents oxidative damage to sperm during handling, freezing, and thawing thereby improving AI pregnancy rates in dairy and beef cows. In this, as well as many previous studies, GameteGuard™ greatly improves post-thaw membrane and acrosome quality particularly in stressed cells. In addition, in most studies GameteGuard™ improves DNA quality, although not in this data set. The post-thaw sperm quality improvement imparted by GameteGuard™ positively affected pregnancy rates lactating cows. Importantly, by achieving more pregnancies per AI in the first service, the dairy farmer experiences significant financial benefits. This study was extended and now includes >400 cows. The full data set will be published later this summer.

Additional breeding trials have been conducted in both heifers and lactating cows and similar results were achieved. From previous breeding trials, calves conceived with GameteGuard™ treated sperm are phenotypically and reproductively normal. Similar post-thaw sperm quality data has been observed in other species including horses, roosters and humans. In addition GameteGuard™ cooled sperm in horses and boars shows promise.

## ACKNOWLEDGEMENTS

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