Improving goat sperm post-thaw quality using GameteGuard™ extender

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INTRODUCTION

The use of artificial insemination (AI) in the goat dairy and meat industries is increasing. However, quality of post-thaw goat sperm is highly variable. Current methodology used for cryopreservation of goat sperm has been adopted from bull industry standards, which may account for this variability. Therefore, the need to develop a buck-specific cryopreservation protocol to enhance post-thaw sperm quality is warranted. In this study, GameteGuard™ was used as an extender additive to improve post-thaw quality of goat sperm. GameteGuard™, a proprietary blend of plant derived antioxidants, has previously provided significant improvement in post-thaw bull sperm motility, membrane protection, and acrosome integrity, which translated to increased pregnancy per AI [1].

EXPERIMENTAL DESIGN

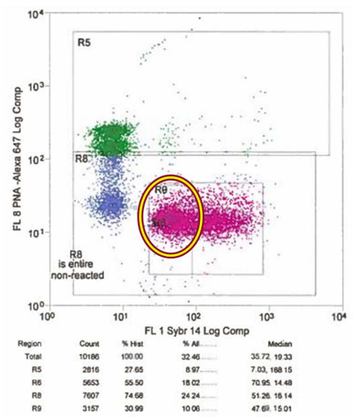
Semen collection, processing, and freezing

- ❑ Ejaculates were collected from 9 bucks using an artificial vagina.
- ❑ Ejaculates were immediately diluted with 10 mL of 37°C Tris buffer solution.
- ❑ Semen samples were placed in a pre-warmed 37°C water bath and allowed to cool to room temperature during transport to the lab (approximately 30 min).
- ❑ Upon arrival at the lab, samples were centrifuged at 500 × g for 10 min. All seminal plasma was aspirated and the sperm pellet was resuspended in 1 mL of Tris buffer.
- ❑ Sperm were extended to 50 × 10^6 cells/mL in either:
  - Tris egg-yolk citrate buffer with 5% glycerol (vol:vol; **control**), or
  - Tris egg-yolk citrate buffer containing 5% (vol:vol) **GameteGuard™** and 5% glycerol (vol:vol).
- ❑ Extended ejaculates were cooled to 4°C, packaged in 0.5 cc straws, allowed to equilibrate for 2 hr then frozen over liquid nitrogen vapor and plunged into liquid nitrogen.

Sperm analysis

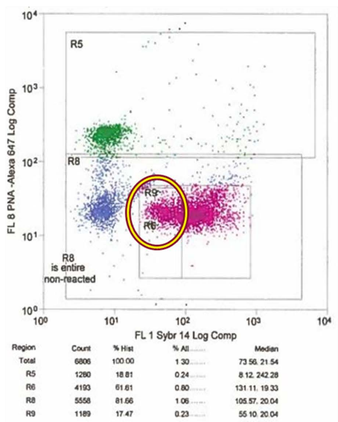
- ❑ Motility parameters were determined immediately post-thaw (0 hr) and after 3 hr incubation at 37°C using a computer assisted sperm analysis system.
- ❑ Both membrane permeability and acrosome integrity were determined at 0 and 3 hr post-thaw using flow cytometry.
  A triple stain protocol was used to determine membrane permeability and acrosome integrity
  - SYBR®-14
  - PNA –Alex Fluor® 647
  - Propidium Iodide
- ❑ Sperm parameters were analyzed using 1-tailed Two Sample Student's *t*-test.

EYC Control



Region	Count	% Hist	% All	Median
Total	10186	100.00	32.46	35.72. 19.33
R5	2816	27.65	8.97	7.03. 188.15
R6	5653	55.50	18.02	70.95. 14.48
R8	7607	74.68	24.24	51.26. 16.14
R9	3157	30.99	10.06	47.69. 15.01

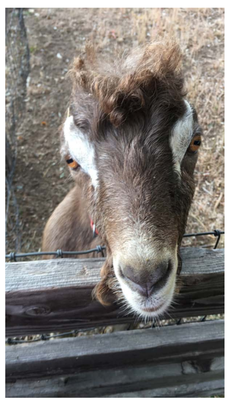
GameteGuard™ Treatment



Region	Count	% Hist	% All	Median
Total	6806	100.00	1.30	73.56. 21.54
R5	1280	18.81	0.24	8.12. 242.28
R6	4193	61.61	0.80	131.11. 19.33
R8	5558	81.66	1.06	105.57. 20.04
R9	1189	17.47	0.23	55.10. 20.04

FIG 1

Flow Cytometric dot plots comparing acrosome integrity (Y-axis, PNA-Alexa® 647) and membrane quality (X-axis, SYBR® 14) of post-thaw sperm in control or GameteGuard™ extenders. The populations are identified as follows: dead cells with reacted acrosomes (green population), dead cell with intact acrosomes (blue population), and live cells with intact acrosomes (magenta population). The circles illustrate the decrease in SYBR-14 mean fluorescence. GameteGuard™ treated sperm has a smaller population in this region (17.47%) compared to control (30.99%).



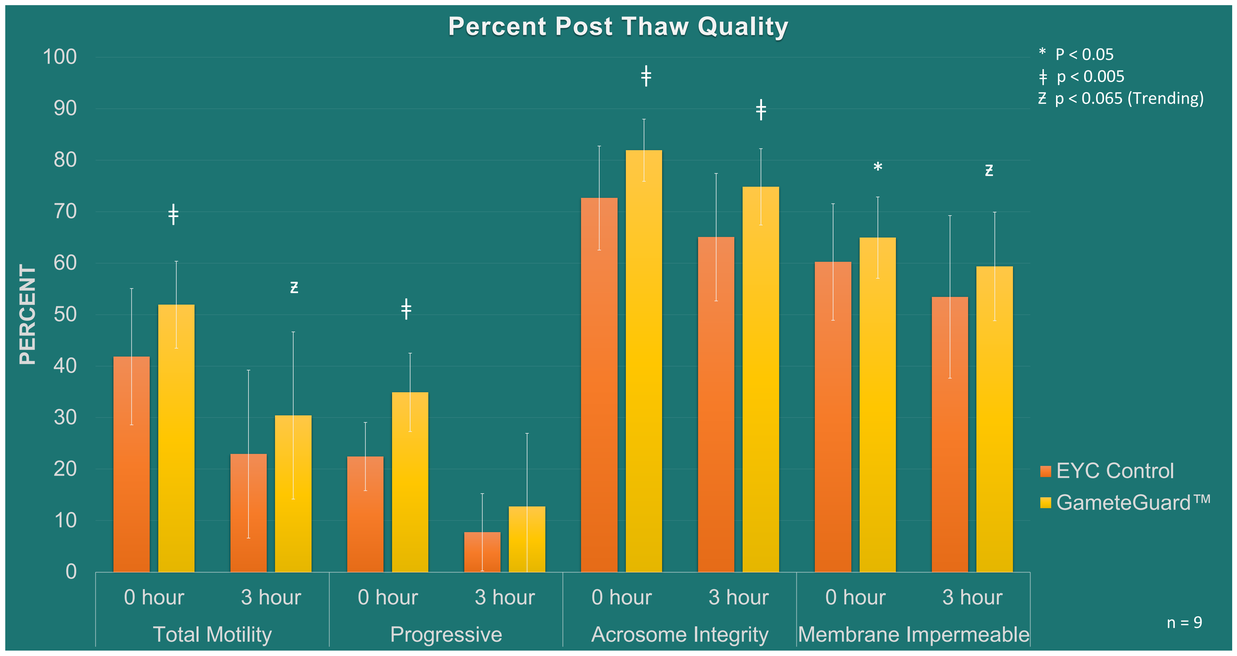


FIG 2

	0 hour		
	EYC Control	GameteGuard™	% Improvement
% Total Motility	41.9	51.9	19.4 *
% Progressive	22.5	34.9	35.7 *
Path Velocity (VAP)	71.7	79.8	10.2 *
% Acrosome Integrity	72.7	81.9	11.3 *
% Membrane Impermeable	60.2	65.0	7.3 *

TABLE 1: Percent Improvement at 0 hr. Data represent an average of 9 samples from 9 bucks analyzed in duplicate. *p<0.05

	3 hour		
	EYC Control	GameteGuard™	% Improvement
% Total Motility	22.9	30.4	24.7 ƶ
% Progressive	7.8	12.8	39.1
Path Velocity (VAP)	36.8	44.9	17.9 *
% Acrosome Integrity	65.1	74.8	13.1 *
% Membrane Impermeable	53.5	59.4	10.0 ƶ

TABLE 2: Percent Improvement at 3 hr. Data represent an average of 9 samples from 9 bucks analyzed in duplicate. *p<0.05 ƶ p<0.065 (trending)

CONCLUSIONS

- **Flow cytometry data demonstrate that GameteGuard™ increases post-thaw membrane and acrosome quality in buck sperm (Fig 1).**

The increased percentage of membrane intact cells (from 24.52% to 44.14%) and brighter mean fluorescence in SYBR-Green 14 channel indicate cells were healthier in GameteGuard™ compared to control. Lower fluorescence is an indicator of cells having increased membrane permeability allowing internalization propidium iodide [2]. The decrease in dead, acrosome reacted sperm cells from 27.65% to 18.81% (control vs. GameteGuard™) also supports this conclusion.

- **Motility parameters confirm improvement in post-thaw sperm health by GameteGuard™ (Fig 2, TABLE 1, and TABLE 2).**

The increase in VAP in GameteGuard treated sperm is an indicator of improved sperm mobility, and deceased DNA damage resulting from cryopreservation [3].

Together, these data indicate that GameteGuard™ -EYC can be used as an alternative cryopreservation extender to improve post-thaw quality of goat sperm. It is hypothesized that improved acrosome integrity coupled with improved motility parameters will result in increased pregnancy/artificial insemination.

ACKNOWLEDGEMENTS

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References

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[3]Gillan, L., Kroetsch, T., Maxwell, W. C., & Evans, G. (2008). Assessment of in vitro sperm characteristics in relation to fertility in dairy bulls. Animal reproduction science, 103(3), 201-214.