GameteGuard® in Maturation Media Improves Bovine Embryo Quality and Quantity

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Introduction

The number of **in vitro produced** (IVP) bovine embryos generated in the US grew 11% from 2017 to 2018, demonstrating a demand and acceptance of technology to improve herd genetics rapidly and efficiently (1). Unfortunately, embryo production on a per oocyte basis remains low, limiting the number of high-quality commercial embryos available for transfer or freezing. **Reactive oxygen species** (ROS) negatively affect embryo production rates in IVP systems and are especially problematic in oocyte maturation media. This is due to high numbers of mitochondria in the bovine oocyte producing ROS and is further exasperated by the in vitro environment.

Antioxidants can be added to IVP media to decrease ROS yet data from previous studies focus on the addition of only one antioxidant such as cysteine (2), cysteamine, catalase (3), or transferrin and selenium (4).

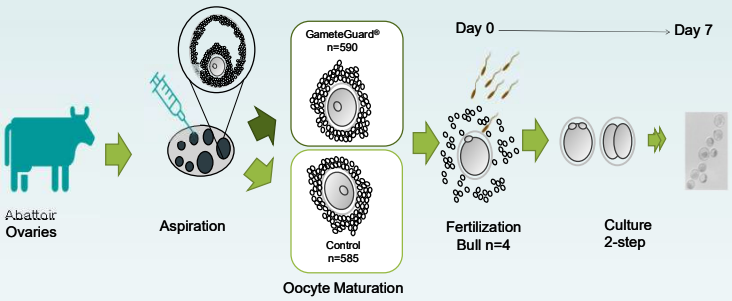
GameteGuard® is a blend of all natural, organic, plant-derived extracts providing **antioxidants** and membrane stabilizers, in a uniquely well-balanced formulation. As a family of antioxidant mixtures, GameteGuard® is known to mitigate the effects of environmental and cellular stress in sperm. Formulations of GameteGuard® have been utilized to protect bull sperm from the effects of oxidants during cryopreservation (5,6), improving pregnancy rates 10 to 25% in several split-ejaculate trials. Other formulations are being developed for applications in swine for both cool-stored and frozen sperm (7,8), and caprine (9) and ovine sperm processing. Semen frozen in the presence of GameteGuard® has resulted in physiologically and reproductively normal offspring. The benefits of using GameteGuard® when sperm may be exposed to increased levels of ROS led to the exploration of GameteGuard® in the in vitro production system of embryos, specifically **in vitro maturation media**.

KEYWORDS: **Oocyte Maturation** **Antioxidants** **Bovine**

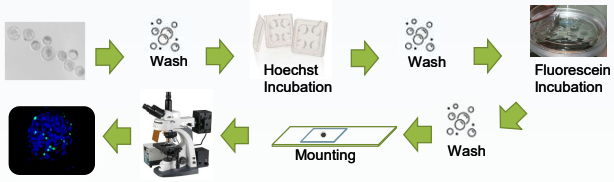
Objectives

1. Evaluate oocyte competence after in vitro maturation in the presence or absence of GameteGuard®
2. Evaluate the effects of supplementing maturation medium with GameteGuard® on embryo quality

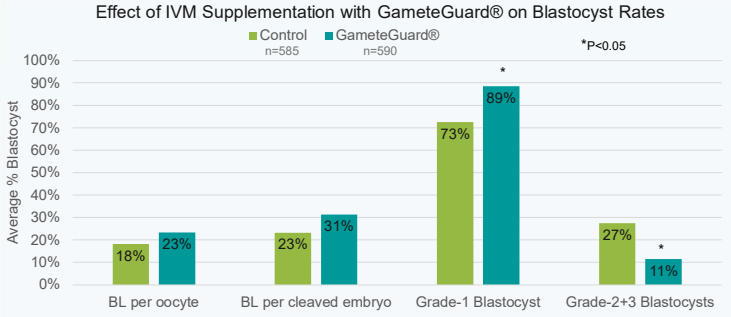
Methodology



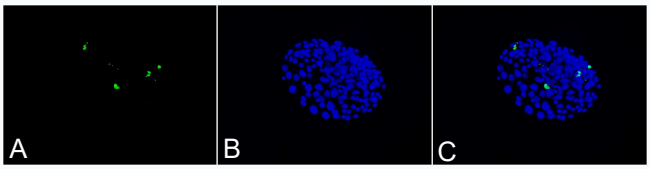
Four replicates of in vitro embryo production (Colorado State University protocol and media) were performed. Half of the oocytes in each replicate were matured in the presence (n=585) or absense (n=590) of GameteGuard®. Blastocysts were evaluated on day 7 according to IETS embryo grading standards. A subset of grade-1 blastocyst and expanded blastocysts (n=91) were fixed and stained post-grading for apoptosis using an In Situ Cell Death Detection Kit (Fluorescein; Millipore Sigma, Burlington, MA) and Hoechst 33342 (Invitrogen, Waltham, MA). The percentage of apoptotic cells to total cell count was calculated. Because multiple bulls were used, a linear mixed model was required for data analysis in R program. Treatment was considered the fixed effect and bulls as a random effect to determine the impact of the treatment on stage and quality of blastocysts.

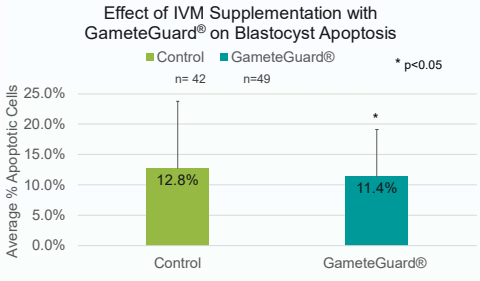


Results



Improvement of 22% with GameteGuard® supplement to maturation medium compared to control maturation media. Total blastocysts per oocyte, blastocysts per cleaved embryo count, and blastocysts by grade were assessed. A significant increase in blastocysts rates and number of grade-1 embryos was observed when maturation medium was supplemented with GameteGuard®.





Fluorescent images of a grade-1 expanded blastocyst that has been evaluated for apoptosis (above). (A-apoptotic cells, B-blastomeres, C-merged)

Oocytes matured with GameteGuard® produced grade-1 blastocysts with 11% fewer apoptotic cells than oocytes matured in control medium (left).

Conclusion

GameteGuard® supplementation of the maturation medium significantly increased the production of high-quality IVP bovine embryos thereby providing potential marketable gain for bovine embryo producers.

- **GameteGuard® increased the number of grade-1 embryos** per total blastocysts by 22% (P<0.01; n=79/106 control versus n=120/137 GameteGuard® grade-1 blastocysts) even though cleavage rates were not different (P>0.5).
- **GameteGuard® promoted development of better-quality embryos overall.** Percent of grade 2 and 3 embryos per total blastocysts decreased, (P<0.01; 27%, n=27/106 control and 11%, n=14/137 GameteGuard® grade 2 and 3 blastocysts).
- **GameteGuard® lowered percentage of apoptotic cells** per blastocyst (P<0.02; avg 11.4%, n=38 control and 12.8%, n=42 GameteGuard®).

With sales of bovine embryos averaging $50-250, the production of more freezable embryos per donor could lead to significant financial gain for bovine embryo producers in a system where each cycle of IVF for a donor animal is of a fixed cost independent of the number of oocytes collected and embryos produced.

Future research will be conducted to determine the ideal concentration of GameteGuard® in other media systems. Improvement in the production of high-quality embryos through GameteGuard® oocyte maturation has applications beyond the bovine industry making it a valuable, translational tool for assisted reproductive technologies.

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References

1. Demetrio, D. (2019). Statistics Committee Report - AETA 2019 (2018 Data). Colorado Springs.
2. Ali, A., et al. (2003). Antioxidant requirements for bovine oocytes varies during in vitro maturation, fertilization and development. *Theriogenology*, 59(3-4), 939-949.
3. Rocha-Frigoni, et al. (2016). Improving the cytoplasmic maturation of bovine oocytes matured in vitro with intracellular and/or extracellular antioxidants is not associated with increased rates of embryo development. *Theriogenology*, 86, 1897-1905.
4. Dallemagne, M., et al. (2018). Oxidative stress differentially impacts male and female bovine embryos depending on the culture medium and the stress condition. *Theriogenology*, 117, 49–56.
5. Graham, P.E., GameteGuard: A Novel Method to counteract fertility decline in dairy cattle. 2014: Proceedings: 25th Technical Conference on Artificial Insemination and Reproduction.
6. Herickhoff, L., GameteGuard treatment improves post-thaw sperm quality and pregnancy per insemination in dairy cows. 2015, *Journal of Dairy Science*.
7. Shepherd, M., et al. (2019). GameteGuard® improves boar post-thaw sperm health. *Theriogenology*, 137, 129.
8. Gonzalez-Castro, R., et al. (2019). Effect of Extender, Cryoprotectants and GameteGuard® on the Post-Thaw Motility in Boar Sperm. *Theriogenology*, 137, 129-130.
9. Shepherd, M., et al. (2017). Improving goat sperm post-thaw quality using GameteGuard extender. *Journal of Dairy Science®*, Volume 100, Supplement 2, 124, M332.