Embryo Quality Characteristics from Superovulated Cows Receiving a Blend of Bioactive Peptides and Oligosaccharides to Support Immune Function (Grade One™)

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Treatment of embryo donor cows with NHG1 (Nutrition Horizons Grade One™) during the super-stimulation protocol did not alter the absolute number of embryos collected but it increased the quality of the embryos by increasing the percentage of grade 1 embryos relative to the number of transferable embryos harvested.

Summary

We determined whether supplementation of a blend of bioactive peptides and oligosaccharides to support immune function (Nutrition Horizons Grade One™; Brookville, OH; US Patent no. 6,962,718) would alter quality, stage, and fertilization rate of embryos recovered after superovulation. Cows were superovulated using follicle stimulating hormone as NIH-FSH-P1 and were stratified by breed before random assignment to treatment: 1) donors received six Grade One™ boluses (13 g/capsule) containing a blend of bioactive peptides and oligosaccharides (NHG1; n = 35); or 2) donors received 6 placebo capsules (13 g/capsule; Control; n = 37). Superovulation of donors was initiated by insertion of a controlled internal drug release device (CIDR) on d 0, eight injections of follicle stimulating hormone (FSH) administered at 12 h intervals initiated on d 4, plus two injections of prostaglandin $F_{2\alpha}$ (PGF) 12 h apart on d 7. At 0 and 12 h after detected estrus cows received an artificial insemination (AI). Boluses were inserted into the esophagus utilizing a balling gun. Cows received two boluses at CIDR insertion (d 0), at the first (d 4), and third (d 5) injection of FSH. Embryos were collected 7 d after first detected estrus and were recovered by nonsurgical embryo collection procedure. The embryos were evaluated and classified by stage and quality. Total ova (14.1 ± 1.8) and transferable embryos (5.2 ± 1.1) per flush for NHG1 and Control did not differ. Mean number of grade 1 (2.5 ± 0.8) and 2 (2.7 ± 0.5) were similar between treatments and no differences existed between treatments for degenerate (1.9 ± 0.4) or unfertilized ova (7.0 ± 1.4). However, the percentage of grade 1 embryos collected compared to recovered transferable embryos was greater (P = 0.06) for NHG1 (39.4%) than Control (23.4%). In addition, the percentage of grade 2 embryos collected compared to recovered transferable embryos was greater (P < 0.05) for Control (76.6%) than NHG1 (59.9%). We conclude that the number of transferable embryos collected per flush did not differ between treatments; however, the quality of transferable embryos was improved after embryo donor cows received NHG1 prior to embryo collection.

Introduction

Embryo transfer has been developed to increase the number of offspring from a superior genetic donor. The technique enhances the opportunity to create multiple offspring from a genetically superior cow rather than a single offspring per year. The results are accomplished by using recipients (regular genetic cow/heifers with good maternal ability) that serve as the surrogate for the embryo collected from the donor cow. Donors are selected based on an outstanding characteristic that results in more value such as

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greater fertility, milk production, heavier calves, disease resistance, etc. Recipients usually are selected for their maternal ability such as milk production and ability to nurture their young, along with good temperament. Physiologically, donors are super-stimulated with natural hormones that act to recruit more than one regular follicle, increasing the likelihood that multiple follicles become dominant, ovulate, and may be fertilized at the same time. Donors are flushed 7 d after insemination to remove the fertilized embryos. Embryos are then either frozen or transferred fresh to recipients that were previously synchronized to be at the same stage of the estrous cycle of the donor. The development of the non-surgical embryo transfer technique, pushed the embryo transfer (ET) industry to significantly increase the number cattle derived from ET. Conception rates of more desirable quality embryos (excellent or good) are greater than embryos qualified as fair or poor quality (IETS, Savoy, IL); therefore, conception rates would increase if a greater percentage of grade 1 (excellent) embryos are harvested and transferred after collection.

Research in the field of ET is very expensive and many of the basic procedures were established some years ago. Consequently, little academic research currently is being conducted that is likely to immediately benefit the commercial ET industry. Because ET success rates in well-managed cattle herds are generally quite high, most recent improvements involve rather small, albeit important, increments. In a 1992 review article, Hasler (1992) stated "There have been no significant improvements in techniques for the superovulation of cattle in the last 15 years". This statement remains largely true today (Hasler, 2010). In fact, data provided by both the American and Canadian Embryo Transfer Associations indicate that mean embryo yields per donor range from 5 to 7 and basically have not changed for many years. These embryo means are a composite average of individual means of both beef (6.6) and dairy cattle (5.7) flushed by the certified members of the AETA.

A product, Nutrition Horizons Grade One™ (NHG1), was originally developed to provide formulations of immune factors in combination with minerals, antioxidants, amino acids, and other nutraceuticals administered orally to treat animals exhibiting disease symptoms but also to lower general morbidity. Due to its ability to improve immune function, NHG1 may possibly improve uterine health and embryo quality.

Materials and Methods

The experimental was designed as a completely randomized design. Seventy-two cows underwent a super-stimulation protocol with follicle stimulating hormone (FSH) prior to embryo collection. Cows were superovulated using FSH as NIH-FSH-P1 and were stratified by breed before random assignment to treatment: 1) donors received 6 Grade One™ boluses (13 g/capsule) containing a blend of bioactive peptides and oligosaccharides (NHG1; n = 35); or 2) donors received 6 placebo capsules (13 g/capsule; Control; n = 37). Superovulation of donors was initiated by insertion of a CIDR on d 0, eight injections of FSH administered at 12 h intervals initiated on d 4, plus two injections of PGF 12 h apart on d 7. At 0 and 12 h after detected estrus cows received an AI. Boluses were inserted into the esophagus utilizing a balling gun. Cows received two boluses at CIDR insertion (d 0), at the first (d 4), and third (d 5) injection of FSH (Figure 1). Embryos were collected 7 d after first detected estrus and were recovered by nonsurgical embryo collection procedure. Embryos were evaluated under a stereomicroscope and classified by stage and quality according to standards set forth by the International Embryo Transfer Society (developmental stage codes were: 3 = early morula; 4 = morula; 5 = early blastocyst; 6 = blastocyst; and 7 = expanded blastocyst). Quality codes were: 1 = symmetrical and spherical embryo mass with individual blastomeres that were uniform in size, color, and density with at least 85% of the cellular material intact (excellent or good); 2 = moderate irregularities in overall shape of embryonic mass or in size, color and density of individual cells

with at least 50% of the cellular material intact (fair); 3 = major irregularities in shape of the embryonic mass or size, color and density of individual cells with at least 25% of the cellular material intact (poor); 4 = dead or degenerating; and 5 = unfertilized.

Results

Table 1 represents the nutrients analysis of the placebo and NHG1 boluses. Overall mean total ova (14.1 ± 1.8) and transferable embryos (5.2 ± 1.1) per flush for NHG1 and Control did not differ ($P > 0.05$; Table 2). Mean number of grade 1 (2.5 ± 0.8) and 2 (2.7 ± 0.5) were similar ($P > 0.05$) between treatments and no differences ($P > 0.05$) existed between treatments for degenerate (1.9 ± 0.4) or unfertilized ova (7.0 ± 1.4; Figure 2). However, the percentage of grade 1 embryos collected compared to recovered transferable embryos tended ($P = 0.06$) to be greater for NHG1 (39.4%) than Control (23.4%; Figure 2). In addition, the percentage of grade 2 embryos collected compared to recovered transferable embryos was greater ($P < 0.05$) for Control (76.6%) than NHG1 (59.9%; Figure 3). We conclude that the number of transferable embryos collected per flush did not differ between treatments; however, the quality of transferable embryos was improved after embryo donor cows received NHG1 prior to embryo collection.

Literature Cited

Hasler. 1992. J. Dairy Sci. 75:2857.
Hasler. 2010. Proc. Applied Reproductive Strategies in Beef Cattle, Nashville, TN pp. 265.

Acknowledgements

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Table 1. Nutrients analysis for placebo (Control) and NHG1 boluses used to treat donor cows prior to super-stimulation.

	Treatment[1]	
Components	NHG1	Control
	--------- DM --------	
Crude fat, %	40.2	4.1
Calcium, %	0.32	0.13
Phosphorous, %	0.94	0.97
Potassium, %	1.93	1.01
Sodium, %	0.335	0.035
Sulfur, %	0.24	0.15
Iron, ppm	145	271
Zinc, ppm	1,050	122
Manganese, ppm	19	152

[1]Donors received six Grade One™ boluses (13 g/capsule) containing a blend of bioactive peptides and oligosaccharides (NHG1) or donors received 6 placebo capsules (Control).

Table 2. Effect of nutrient supplementation on embryo recovery characteristics in superovulated cows.

	Treatments[1]			
Item	NHG1	Control	SEM	*P*-value
Number of donors	35	37		
Total embryos/ova, no.[2]	15.0	12.4	1.80	0.30
Transferable embryos, no.	5.2	4.5	1.09	0.64
Grade 1 embryos, no.	2.8	1.7	0.80	0.34
Grade 2 embryos, no.	2.5	2.8	0.47	0.58

[1]Donors received six Grade One™ boluses (13 g/capsule) containing a blend of bioactive peptides and oligosaccharides (NHG1) or donors received 6 placebo capsules (Control). SEM = standard error mean.

[2]Total unfertilized, degenerate, and transferable structures recovered.

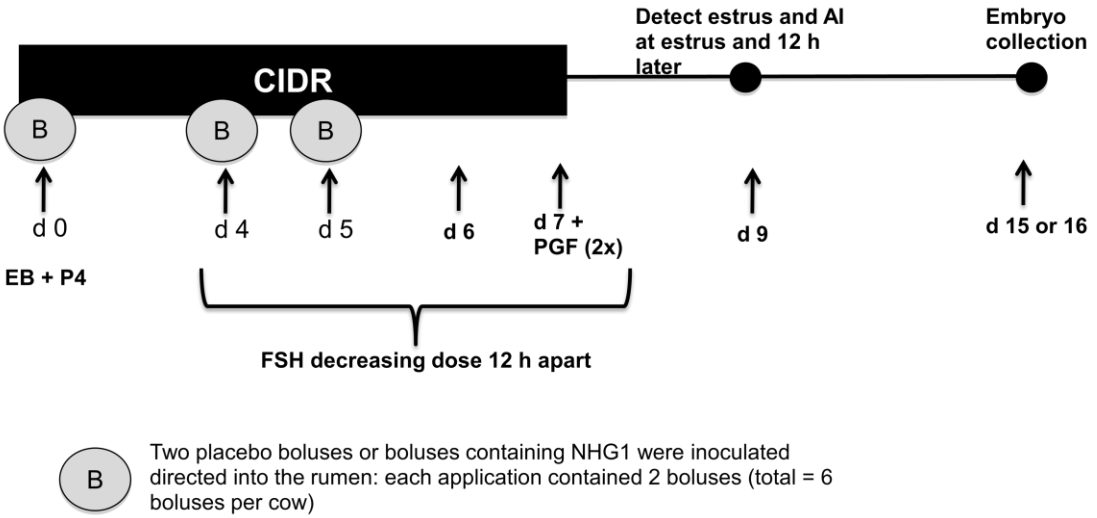

Figure 1. Schematic of experimental design for cows treated with or without NHG1 on d 0, 4 and 5.

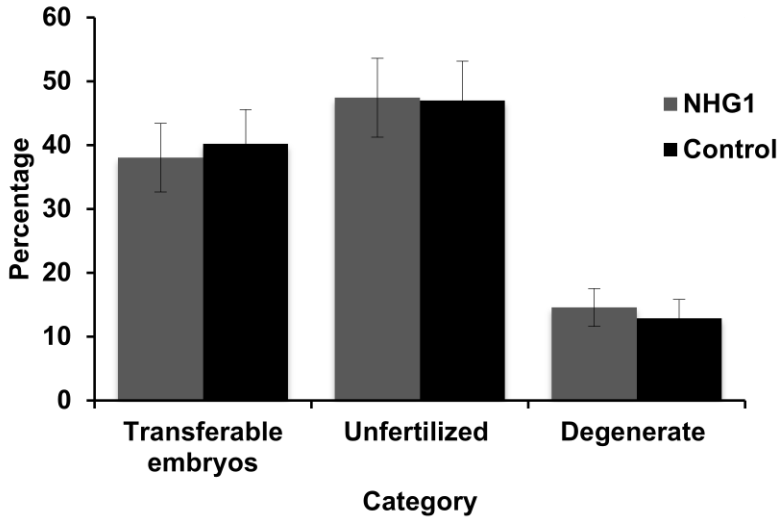

Figure 2. Effect of nutritional supplement on embryo classification

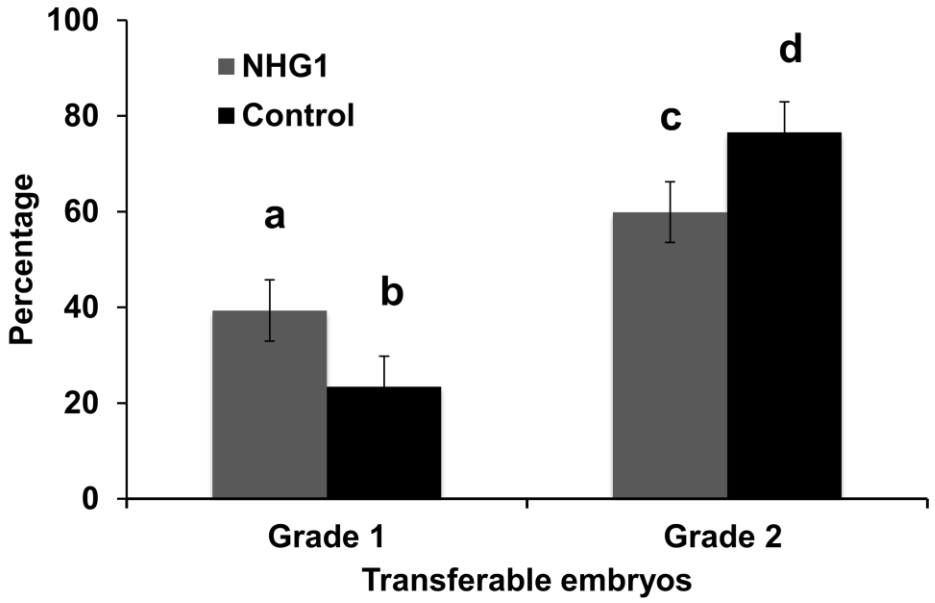

Figure 3. Effect of supplement on embryo grade. [ab]Percentage tended to differ ($P = 0.06$) [cd]Percentage differs ($P < 0.05$).