



Influence of post-insemination nutrition on embryonic development in beef heifers



S.G. Kruse ^{a,1}, G.A. Bridges ^{a,**,2}, B.J. Funnell ^{a,3}, S.L. Bird ^a, S.L. Lake ^b, R.P. Arias ^b,
O.L. Amundson ^c, E.L. Larimore ^c, D.H. Keisler ^d, G.A. Perry ^{c,*}

^a North Central Research and Outreach Center, University of Minnesota, Grand Rapids, MN 55744, United States

^b Department of Animal Science, University of Wyoming, Laramie, WY 82071, United States

^c Department of Animal Science, South Dakota State University, Brookings, SD 57007, United States

^d Division of Animal Sciences, University of Missouri, Columbia, MO 65211, United States

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ABSTRACT

Previous studies have demonstrated that a decrease in nutrition immediately following AI reduces pregnancy success in beef heifers. The objective of this experiment was to determine if nutrient restriction following AI impacted early embryonic development among non-super ovulated heifers. Beef heifers in eight replications (Rep; Rep 1; n = 14, Rep 2; n = 15, Rep 3; n = 15, Rep 4; n = 14, Rep 5; n = 15, Rep 6; n = 15, Rep 7; n = 25, Rep 8; n = 25) across two locations (UMN, SDSU) were developed in a dry-lot and fed 125% NRC requirements from weaning to timed-AI (d 0). Heifers were timed-AI to a single sire in all replications. Immediately following AI, heifers were assigned, based on age, weight, and estrous response to one of two post-AI nutritional treatments. Half the heifers in each replication continued on the pre-insemination diet, serving as the control treatment (CON) and the remaining heifers were restricted to a sub-maintenance diet (RES). At UMN, heifers in the RES treatment were fed the same diet, but intake was limited to 80% NE_m, while at SDSU, DMI remained the same, but diet composition was altered with the addition of straw to reduce NE_m to 50% of requirements. On d 6, single embryos were collected nonsurgically and recovered embryos (CON; n = 46, RES; n = 42) were evaluated to determine quality (grade 1–9) and stage (1–4). Embryos were then stained and evaluated to determine the number of dead cells and total blastomeres. In Reps 1 through 6, concentrations of IGF-1 were assessed on d 0 and 6 and progesterone concentrations on d 4 and 6. Data were analyzed using the Mixed procedures of SAS. There were no treatment by Rep or treatment by location interactions for any embryo parameter evaluated, thus all data were pooled. Embryo stage and quality were improved ($P < 0.01$) in the CON (4.4 ± 0.16 , 2.2 ± 0.19 , respectively) compared to RES treatment (3.7 ± 0.16 , 2.9 ± 0.19 , respectively). Embryos in the CON treatment had greater total blastomeres (66.9 ± 5.05 ; $P < 0.01$) and tended to have a greater percentage of live cells ($P < 0.10$; $80.9 \pm 4.19\%$) compared to RES (47.9 ± 5.41 ; $69.7 \pm 4.39\%$, respectively). Progesterone and IGF-1 concentrations did not differ between treatments. In summary, nutrient restriction for 6 days immediately following AI resulted in poorer quality embryos that were delayed in stage of development, suggesting that immediate changes in nutritional status after insemination can alter early embryonic development.

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* Corresponding author. Department of Animal Sciences, Box 2170, ASC 214, South Dakota State University, Brookings, SD 57007, United States.

** Corresponding author. Elanco Animal Health, United States.

E-mail addresses: gabridges@elanco.com (G.A. Bridges), George.Perry@sdstate.edu (G.A. Perry).

¹ Current address: Boviteq, Monona, WI 53716, United States.

² Current address: Elanco Animal Health, Greenfield, IN 46140, United States.

³ Current address: Purdue University College of Veterinary Medicine, West Lafayette, IN 47907, United States.

1. Introduction

Numerous factors, beyond selection of an estrous synchronization protocol, influence the probability of pregnancy success to AI in beef heifers. Any management practice that detrimentally affects the ability of a female to conceive early in the breeding season ultimately reduces reproductive and production efficiency [9]. In the U.S., many spring-born heifers are developed in a dry-lot, where

estrous synchronization and AI are performed. Heifers are often moved to pastures immediately following AI to be exposed to clean-up bulls, graze forage, and reduce the likelihood embryonic loss associated with handling [17,27]. However, this immediate change in nutrition following AI has been demonstrated to negatively impact metabolism, BW gains, and reproductive efficiency in beef heifers [2,31,32].

A series of studies [31,32] demonstrated that moving beef heifers from a dry-lot environment to pastures with adequate forage quantity and quality for heifers to gain weight resulted in significant loss in body weight and if this change in environment occurred simultaneously with AI, resulted in reduced AI pregnancy rates. Furthermore, a recent study [2] compared pregnancy rates of beef heifers fed to gain, maintain, or lose weight immediately following AI for 21 d. If heifers failed to gain weight following AI (maintain or lose), AI pregnancy rates were reduced, and breeding season pregnancy rates tended to be reduced. Together, these studies provide evidence that nutrient intake and BW changes following AI influence pregnancy success. Therefore, the objective of this study was to determine if post-AI nutrient restriction directly impacts early embryo quality and the number of live/dead blastomeres. It was hypothesized that embryos collected from heifers undergoing nutrient restriction would have reduced embryo quality with fewer total blastomeres and a greater proportion of dead blastomeres than embryos from heifers fed diets that allowed weight gain post-insemination.

2. Materials and methods

Spring born virgin Angus heifers 12 to 14 months of age at the University of Minnesota North Central Research and Outreach Center (UMN; Replication (Rep) 1; n = 14, Rep 2; n = 15, Rep 3; n = 15, Rep 4; n = 14, Rep 5; n = 15, Rep 6; n = 15) and South Dakota State University (SDSU; Rep 7; n = 25, Rep 8; n = 25) were used. All animals were handled in accordance with the corresponding university's Institute of Animal Care and Use Committee guidelines.

2.1. Dietary treatments

Heifers were developed from weaning to breeding in a dry-lot scenario to gain approximately 0.7 kg/day prior to AI, targeting body weights of 60–65% estimated mature body weight at breeding. At UMN, 30 d prior to the initiation of the study, all heifers were fed an 11:1:1 diet of haylage, dried distillers grains, and corn (respectively) to gain approximately 0.7 kg/day at 125% of NRC requirements. Heifers at SDSU were fed a corn silage, ground alfalfa, ground grass hay ration, targeting gains of 0.7 kg/day. Immediately after insemination (d 0), heifers were allotted to one of two dietary treatments based on weight, age, and estrous response prior to timed-AI. At UMN (Reps 1–6), half of the heifers continued on the previously mentioned diet (targeting gains of 0.7 kg/day) of haylage, dried distillers grains, and whole corn at 125% of NRC requirements of maintenance (CON), whereas the other half was fed the same diet, however, intake was limited to 80% of NCR requirements of maintenance (RES). At SDSU (Reps 7–8), heifers in the CON group were fed the same diet following AI as fed before AI, targeting gains of 0.7 kg/day. The remaining half of the heifers were fed a similar amount of feed by adding oat straw to the diet, but the composition of the diet was altered to restrict energy supply to only 50% of maintenance requirements (RES). Body weights of heifers were determined and recorded on d 0 and 6. Average daily gain (ADG) between insemination (d 0) and embryo flush (d 6) was calculated. Diets were fed until d 6 at which time embryos were recovered nonsurgically by a trained veterinarian. Level of nutrient restriction was calculated to mimic weight

loss based on previous work [31,32] that demonstrated moving beef heifers from a dry-lot environment to pastures with adequate forage quantity and quality for heifers to gain weight resulted in significant loss in body weight and if this change in environment occurred simultaneously with AI, resulted in reduced AI pregnancy rates.

2.2. Ovulation synchronization

Ovulation was synchronized with the 5 d CO-Synch + CIDR protocol, which consisted of the insertion of a CIDR (Pfizer Animal Health, New York, NY) and administration of 100 µg of GnRH im (Factrel; Pfizer Animal Health) on d -8, CIDR removal and administration of 25 mg of PGF_{2α} im (PG; Lutalyse; dinoprost tromethamine; Pfizer Animal Health) on d -3 with a second administration of PG 6 h after the initial dose. Heifers were timed-AI with semen from a single bull at 72 h after CIDR insert removal concurrent with GnRH administration (d 0). Standing heat was detected using tail paint (Tell Tail; FIL, Mount Maunganui, New Zealand) and visual observation of mounting behavior. At AI, tail paint scores were assessed (1 = tail paint completely gone; 2 = tail paint partially gone, obvious signs of receiving some rubs from mountings; 3 = tail paint undisturbed; [43]). A total of 87 heifers (63%) were detected in estrus prior to fixed-time AI.

2.3. Embryo recovery and evaluation

Six days after insemination, single embryos were collected using nonsurgical embryo flush techniques. Briefly, the side of ovulation was confirmed by the presence of a CL using transrectal ultrasonography (7.5-MHz linear array transducer, Aloka 500V, Aloka Wallingford, CT), a catheter was placed in the uterine horn ipsilateral to the CL, and the horn was filled with flush media, (Vigro Flush Media; Bioniche Life Sciences, Belleville, Ont) the media was recovered, and filtered. Seven heifers were removed from the study at time of embryo flush either due to 1) inability to pass a catheter or 2) inadequate corpus luteum formation, resulting in a total of 131 heifers flushed in this experiment. Embryos were transported in flush media (Vigro Flush Media; Bioniche Life Sciences, Belleville, Ont) in 0.25 cc straws at 37 °C to the laboratory for evaluation. Within 3 h of recovery, embryos were evaluated microscopically by a technician that was blind to treatment to determine quality (per International Embryo Transfer Society standards; scale 1–4; 1 = excellent/good, 4 = dead or degenerate) and stage (scale 1–9; 1 = unfertilized, 9 = expanded hatched blastocyst). Any unfertilized oocytes (n = 3) recovered were removed from analysis as the failure of sperm to fertilize was assumed to be independent of dietary treatment. All embryos were then stained and evaluated via epifluorescent microscopy to determine the number of accessory sperm, dead blastomeres, and total blastomeres. All chemicals used in the embryo staining procedure were obtained from Sigma Aldrich (St. Louis, MO), unless otherwise specified. Initially, embryos were washed through three 50 µL drops of polyvinylpyrrolidone (PVP) solution (1 µg/L in PBS) to enhance stain absorption followed by a 10 min incubation period in 5 µg Hoechst 33342 mL⁻¹ (bisbenzimidazole trihydrochloride) in darkness. Embryos were then placed in PVP on a glass slide and visualized at 200 magnification with an inverted microscope equipped with epifluorescence filter (excitation 365 nm and emission 420 nm). Number of accessory sperm cells were counted three times and recorded. Embryos were then removed from the glass slide and washed through three 50 µL drops of (PVP) solution followed by a 5 min incubation period in propidium iodide (20 µg/mL) in darkness. Again, embryos were placed on a glass slide in a drop of PVP solution and visualized at 200-magnification using an

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