Effects of peri-AI nutritional management on embryo development and pregnancy success

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Introduction

Heifers need to calve by 24 months of age to achieve maximum life-time productivity (Patterson et al., 1992), and heifers that lose a pregnancy or conceive late in the breeding season are unlikely to have enough time to rebreed during a defined breeding season. However, heifers that calve early with their first calf have more time to resume normal estrous cycles by the start of the subsequent breeding season. Therefore, early calving heifers are more likely to breed back as two year olds and continue to calve early in the calving season. This is important to overall profitability since heifers that calved during the first 21 days of the calving season had increased ($P < 0.01$) longevity in the cow herd compared to heifers that calved in the second 21 day period, or later (Cushman et al., 2013). Furthermore, analysis of 3700 calves at the USDA- Meat Animal Research Center indicated that for each day of age after the beginning of the breeding season that a calf is born 2.4 pounds of weaning weight is lost (personnel communication R. Cushman).

Role of nutrition during the breeding season on fertility

Fertilization rates are reported to be between 89% and 100% when animals are detected in estrus and semen is present at the time ovulation occurs (Kidder et al., 1954; Bearden et al., 1956; Diskin and Sreenan, 1980; Maurer and Chenault, 1983; Gayerie de Abreu et al., 1984). While fertilization usually takes place, conception rates (number of animals that conceive divided by number of animals inseminated) are usually around 60% to 70% for natural service or artificial insemination. Although nature (poor oocyte quality, disease, chromosomal abnormalities, etc.) contributes much of this loss, management practices can also increase embryonic mortality, and nutritional stress can be detrimental to embryo survival and decrease pregnancy success.

In order to understand how stress may increase embryonic mortality, one must first understand the development of the embryo (Table 1). Just like the estrous cycle, embryo development begins on day 0, or the day of standing estrus. This is the day the female is receptive to the male and insemination occurs. Ovulation occurs on day 1 or about 30 hours after the first standing mount (day 0, Wiltbank et al., 2000). If viable sperm is present, fertilization occurs inside the oviduct shortly after ovulation. The first cell division occurs on day 2, and by day 3 the embryo has reached the 8-cell stage (Shea, 1981). Between days 5 and 6 the embryo migrates into the uterine horn and by day 7 to 8 it forms into a blastocyst (Flechon and Renard, 1978; Shea, 1981; Peters, 1996). At this stage two distinct parts of the embryo can be seen: 1) the inner cell mass, which will form into the fetus and 2) the trophoblast, which will form into the placenta. Between days 9 and 11 the embryo hatches from the zona pellucida, a protective shell that has surrounded the embryo to this point (Shea, 1981; Peters, 1996). Then, on days 15 to 17, the embryo produces a chemical signal to prevent corpora lutea destruction and allow the cow to remain pregnant (Peters, 1996). The embryo attaches to the uterus beginning on day 19, and around day 25, placentation, an intricate cellular interface between the cow and the calf, begins. By day 42 the embryo has fully attached to the uterus of the cow (Peters, 1996).

Table 1. Time course of early bovine embryo development

Event	Day
Estrus	0
Ovulation	1
Fertilization	1
First cell division	2
8-cell stage	3
Migration to uterus	5-6
Blastocyst	7-8
Hatching	9-11
Maternal recognition of pregnancy	15-17
Attachment to the uterus	19
Adhesion to uterus	21-22
Placentation	25
Definitive attachment of the embryo to the uterus	42
Birth	285

Data adapted from: (Flechon and Renard, 1978; Shea, 1981; Telford et al., 1990; Peters, 1996)

Stress from Change in Diet

Grazing skills and dietary habits are learned early in life (Provenza and Balph, 1988). This learning resulted in the development of motor skills necessary to harvest and ingest forages (Provenza and Balph, 1987), and allowed animals to increase their consumption of forage (Lyford, 1988). These skills learned between weaning and breeding have been reported to carry through to the next grazing season (Olson et al., 1992). Furthermore, the willingness to try novel food declines with age (Provenza and Balph, 1988). Young livestock ingest small amounts of novel food and gradually increase the amount ingested if no adverse effects occur (Chapple and Lynch, 1986; Burritt and Provenza, 1987). Therefore, when introduced to novel food/environment livestock may spend more time and energy foraging (Osuji, 1974), but ingest less food (Arnold and Maller, 1977; Hodgson and Jamieson, 1981; Curll and Davidson, 1983). Thus when heifers grazed forage from weaning to breeding rather than being placed in drylots they appeared to retain better grazing skills and had increased average daily gains into the subsequent summer (Olson et al., 1992; Perry et al., 2013). Similar to the losses in weight that occurred (Figure 1) when heifers that were developed in a feedlot from weaning until the next spring were moved from a feedlot to grass (Perry et al., 2013); a decrease in feed intake from 120% of maintenance to 40% of maintenance resulted in a loss of 56.3 lbs over 2 weeks (4.03 lbs/day, Mackey et al., 1999). However, heifers that were developed from weaning until the next spring on range with supplementation showed no weight loss the following spring (Perry et al., 2013). Furthermore, heifers that were kept in a drylot until AI (n = 214) had decreased (P = 0.04) pregnancy rates compared to heifers that had previous grazing experience (n = 207; 59.4% vs. 49.1%; Table 2). Therefore, post-insemination nutrition may have a tremendous influence on embryonic survival.

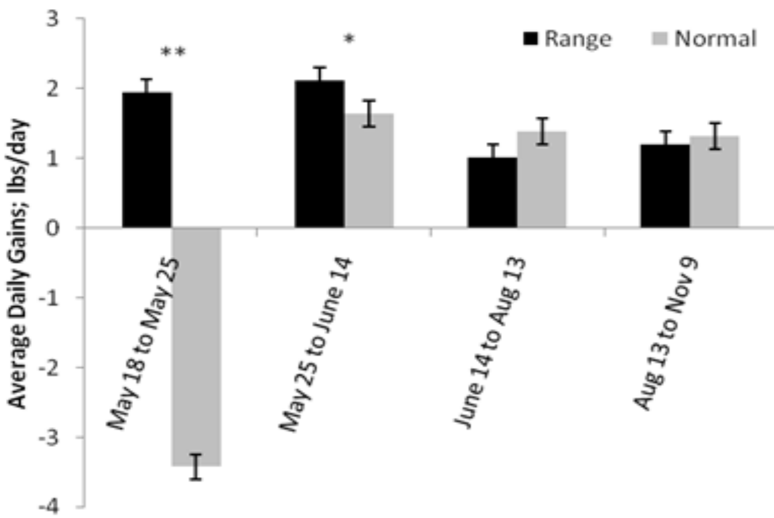


Figure 1. Average daily gain (lbs/day) of heifers weaned and developed on range (Range) compared to heifers weaned and developed in a drylot (Normal). All heifers were moved to the same pasture on May 18th (*P = 0.06; **P < 0.05)

Table 2. Reproductive performance of heifers that were weaned and developed on range (Range) compared to heifers weaned and developed in a drylot (Lot) (all heifers were moved to grass following AI on the first day of the breeding season).

	Range	Lot
Number of heifers	91	92
Puberty status, (%)[a]	89/91 (94)	90/92 (98)
Synchronized conception rate, (%)[b]	52/91 (57)[y]	41/92 (45)[z]
Final pregnancy rate, (%)[c]	82/91 (90)	81/92 (88)

[a] Percentage of heifers that had reached puberty before the start of the breeding season
[b] Percentage of heifers pregnant during the 10 d synchronization period to natural service
[c] Overall pregnancy rate (60 d breeding season)
[yz]Means within a row having different superscripts tended to differ (P = 0.108)

Since energy is used for all body functions, a hierarchy must exist designating which function is most important when nutrients are limiting. This is often referred to as nutrient partitioning. The priority for nutrients taken into the body is usually listed as follows: 1) basal metabolism, 2) activity, 3) growth, 4) basic energy reserves, 5) pregnancy, 6) lactation, 7) additional energy reserves, 8) estrous cycles and initiation of pregnancy, 9) excess reserves (Short et al., 1990). Therefore, a change in energy intake could have a significant impact on reproductive success as it is far down the list in order of importance. When nutrients are limited at or immediately after insemination, this lack of energy may perturb fertility through direct or indirect regulation of the uterine environment. Nutritionally mediated changes to the uterine environment can occur by changing components of uterine secretions or by influencing the circulating concentrations of progesterone that regulate uterine environment (see review by Foxcroft, 1997). More specifically, heifers fed 85% of maintenance requirements of energy and protein had reduced embryo development on day 3 and day 8 compared to heifers fed 100% maintenance (Hill et al., 1970) indicating decreased embryonic growth. Therefore, under nutrition can have an impact on embryo survival and the ability of heifers to conceive during a defined breeding season.

To test if increasing nutrient intake immediately after AI could impact pregnancy success, beef heifers at two locations (n = 140 and 161 at location 1 and 2, respectively) were developed in a drylot from weaning to breeding (Perry et al., 2015). At time of insemination heifers were randomly allotted to one of two treatments: 1) heifers were moved from drylot to graze spring forage (PASTURE), or 2) heifers were moved to graze spring forage and supplemented with DDGS (5 lbs/hd/day) for 42 days (PASTURE-SUPP). Pregnancy success was determined 42 days after AI. At both locations, PASTURE heifers were placed on the higher quality pasture that had more available forage. However, when moved to pasture immediately following AI, there was a treatment ($P < 0.01$) and a treatment by herd interaction ($P < 0.01$) on weight change, but no effect of herd ($P = 0.17$). Overall, PASTURE-SUPP heifers gained weight from AI to pregnancy determination while RANGE heifers lost weight (Table 3). Similarly at location 2, PASTURE-SUPP heifers gained weight and PASTURE heifers lost weight. However, at location 1, there was no difference ($P = 0.79$) between treatments. Furthermore, conception rates to AI were affected by treatment ($P = 0.02$; Figure 2), with PASTURE-SUPP heifers having increased pregnancy success compared to PASTURE heifers. However, there was no effect of herd ($P = 0.64$), treatment by herd ($P = 0.21$), BCS at AI ($P = 0.40$), or weight change from AI to pregnancy determination ($P = 0.47$) on AI conception rates. Breeding season pregnancy rates were not different ($P = 0.20$) between PASTURE and PASTURE-SUPP heifers (91% and 94%, respectively).

Table 3. Weight change from AI to pregnancy determination on day 42 after AI.

	Location 1		Location 2		Combined	
	Pasture	**Pasture-supp**	**Pasture**	**Pasture-supp**	**Pasture**	**Pasture-supp**
Weight at AI (lb)	940 ± 9.9	962 ± 9.7	865 ± 9.9y	919 ± 8.8z	902 ± 7.1y	939 ± 6.6z
Weight at pregnancy diagnosis (lb)	957 ± 8.8	977 ± 8.6	838 ± 8.8y	965 ± 7.7z	897 ± 6.2y	970 ± 5.7z
Weight change (lb)	17 ± 4.0	15 ± 4.0	-37 ± 4.0y	45 ± 3.1z	-5.5 ± 4.0y	32 ± 3.5z

[xyz] Means within a row and location having different superscripts are different ($P < 0.01$)

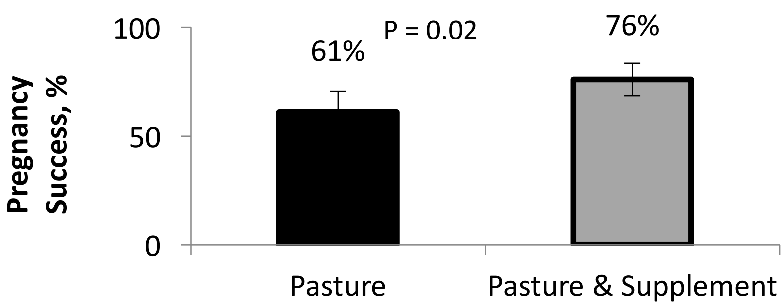


Figure 2. Artificial insemination conception rates for heifers developed in a drylot from weaning to AI, and moved to pasture following AI. Heifers were moved from drylot to graze spring forage (Pasture), or moved to graze spring forage and supplemented with DDGS (5 lbs/hd/day) for 42 days (Pasture and Supplement). Pregnancy success was determined 42 days after AI.

To further investigate if a short term (first week after AI) change in energy intake could impact embryo survival, we recently conducted a study in beef heifers to further elucidate the direct effects of an immediate change in nutrition at AI on early embryonic development. The objective of this study was to determine if post-AI nutrient restriction directly impacted early embryo quality and the number of live/

dead blastomeres. This study was conducted at two locations, University of Minnesota's North Central Research and Outreach Center (UMN) and South Dakota State University (SDSU). All heifers were on a common diet during development. Heifers were bred by timed-AI. On the day of AI, heifers were placed in one of two nutritional treatments. Half of the heifers continued on the pre-AI diet (approximately 120% NRC requirements), targeting an ADG of 1.5 lbs/hd/d (treatment designation = GAIN). The remaining heifers were fed at 50 to 80% NRC requirements (treatment designation = LOSE). Dietary treatments were fed until embryo collection was done using non-surgical embryo flush techniques six days after AI. Recovered embryos were microscopically evaluated and classified as per International Embryo Transfer Society standards for quality (scale 1 to 4; 1 = excellent/good, 4 = dead or degenerate) and stage (scale 1 to 9; 1 = unfertilized, 9 = expanded hatched blastocyst). Then embryos were transferred to the laboratory where number of dead blastomeres and total number of blastomeres was evaluated using epifluorescent staining. Nutrient restriction immediately following AI resulted (Table 4) in poorer quality embryos that were developmentally retarded as indicated by being at an earlier stage of development and having fewer total blastomeres (Table 4). In addition, embryos from nutrient restricted heifers had a decreased (P = 0.01) percentage of live blastomeres.

Table 4. Effect of post-AI nutrition on d 6 embryo development collected from heifers either fed at 120% of NRC requirements (control; CON) or below maintenance (restricted; RES) immediately following AI

TRT	n[a]	% Embryos Recovery	Embryo Stage[b]	Embryo Quality[c]	Accessory Sperm (n)	Dead Cells (n)	Total Cells (n)	% Live Cells
CON	44	67.7 (44/65)	4.4 ± 0.16	1.6 ± 0.26	19.9 ± 3.93	7.9 ± 1.04	66.9 ± 5.05	80.9 ± 4.19
RES	41	62.1 (41/66)	3.7 ± 0.16	2.0 ± 0.25	15.4 ± 3.99	9.5 ± 1.11	47.9 ± 5.41	69.7 ± 4.39
P-value	-	-	= 0.003	= 0.03	= 0.37	= 0.28	= 0.009	= 0.09

[a] Defined as embryo number; not heifer with the exception of recovery rate

[b] Stage of development (1-9; 1 = UFO; 9 = expanded hatched blastocyst; per IETS Standards)

[c] Quality of embryo (1-4; 1 = excellent; 4 = dead/degenerate; per IETS Standards)

These results indicate that the early embryo, oviduct, and uterus are sensitive to immediate changes in nutrition. It is proposed that the immediate retardation of embryonic development observed is likely responsible for reduced pregnancy rates due to an inability of the embryo to successfully signal maternal recognition of pregnancy at later stages of development. Currently, the mechanisms by which an abrupt change in nutritional inputs immediately following AI affects early embryonic development are not definitive and numerous physiological and endocrine processes may contribute.

To investigate the idea that the decrease in AI pregnancy success may be due to grazing behavior and not a change in diet, we conducted an experiment where heifers were moved from a grazing environment to a drylot following AI. Beef heifers at one location (n= 333) were developed on a forage diet from weaning to breeding (Perry et al., 2016). All heifers were brought into a feedlot and synchronized with a 7-d CO-Synch + CIDR protocol. At time of insemination heifers were randomly allotted to one of three treatments: 1) heifers were moved to graze spring forage (RANGE), 2) heifers were moved to graze spring forage plus supplemented with DDGS (5 lbs/hd/day) for 42 days (RANGE-SUPP), or 3) heifers were returned to the feed lot for 42 days (LOT). Pregnancy success was determined 42 days after AI. Body condition increased ($P < 0.01$) from the day synchronization began (day -7; 5.4 ± 0.05) to day 42 in both the heifers that were supplemented on pasture (RANGE-SUPP) and the heifers that were kept in the feed lot (LOT; 5.9 ± 0.04 and 5.8 ± 0.04, respectively; Table 5). Body condition did not change from day -7 to day 42 among the heifers that were on grass alone (Table 5). Pregnancy success did not differ among treatments [59% (65/111), 57% (63/111), and 56% (62/111) for heifers on grass alone (RANGE), heifers on grass plus supplemented (RANGE-SUPP), and heifers in the feed lot (LOT), respectively. Therefore, when heifers were developed on grass, there was no effect on pregnancy success whether they were returned to grass with or without supplementation or even kept in the feed lot.

Table 5. Reproductive performance of heifers that were weaned and developed on range and following AI were returned to range (Range), returned to range and supplemented (Range-SUPP), and moved to a drylot (LOT).

	Range	Range-SUPP	LOT
Number of heifers	112	112	112
Percent of heifers with a CL on d -7, (%)[a]	90/112 (80)	88/112 (79)	81/112 (72)
Body Condition Score on d -7	5.4 ± 0.05	5.4 ± 0.05	5.4 ± 0.05
Body Condition Score on d 42 after AI	5.4 ± 0.04[x]	5.9 ± 0.04[y]	5.8 ± 0.04[z]
Synchronized conception rate, (%)[b]	66/112 (59)	64/112 (57)	63/112 (56)
Final pregnancy rate, (%)[c]	99/112 (88)	100/112 (89)	96/112 (86)

[a] Percentage of heifers that had circulating concentration of progesterone > 1ng/mL on d -7 (day of CIDR insertion)
[b] Percentage of heifers pregnant during the 10 d synchronization period to natural service
[c] Overall pregnancy rate (28 d breeding season)
[xyz] Means within a row having different superscripts are different ($P < 0.01$)

To further investigate if method of heifer development could impact grazing behavior, we conducted an experiment to measure daily activity between drylot developed heifers that had been moved to grass before AI compared to heifers that were moved to grass on the day of AI (Perry et al., 2015). Sixty-nine drylot developed heifers were randomly allotted to one of two treatments 42 days before AI: 1) heifers remained in the drylot until AI, or 2) heifers were moved to graze spring forage for the 42 days prior to AI. Daily activity was measured by a pedometer. Prior to AI, heifers that were grazing spring forage took more ($P < 0.01$) steps per day compared to heifers in the drylot (Figure 3). However; following AI, heifers that had remained in the drylot until AI had increased activity compared to heifers that had previous experience grazing spring forage (Figure 4). Furthermore, when heifers were move to pasture ADG was decreased compared to heifers that had 42 days of prior grazing experience (Figure 5). When activity is increased energy requirements are also increased. Cows that were forced to walk 3.2 km per day had a greater than 30% increase in energy requirements compared to cows that were held in a drylot (Bellows et al., 1994). Hence, heifers switched from a drylot to pasture are not accustom to grazing, forced to eat a novel diet, and exert increased energy during the period following AI. These factors combined may be the reason some heifers developed in a drylot and move to forage after insemination have reduced conception rates.

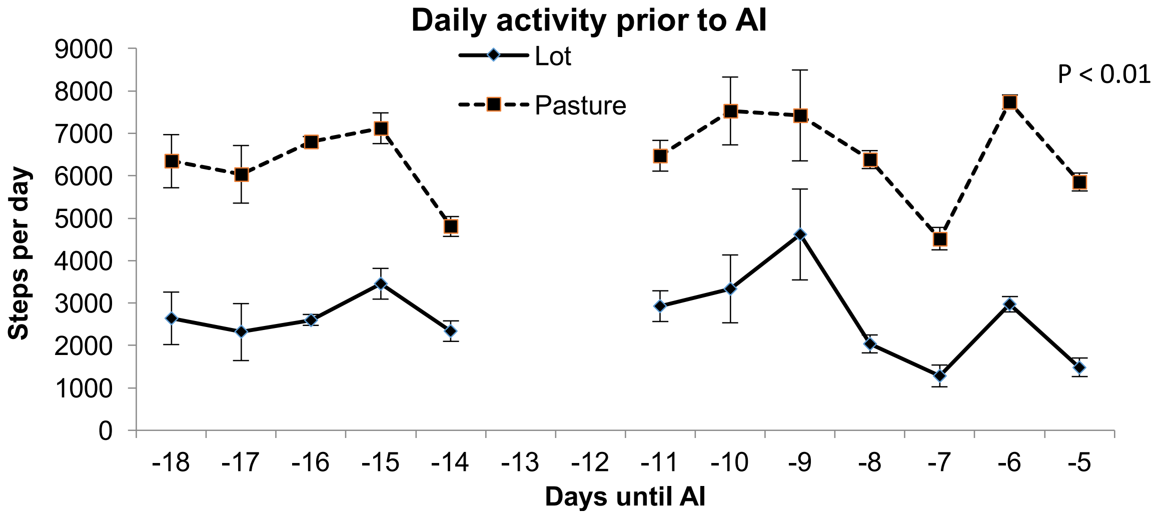
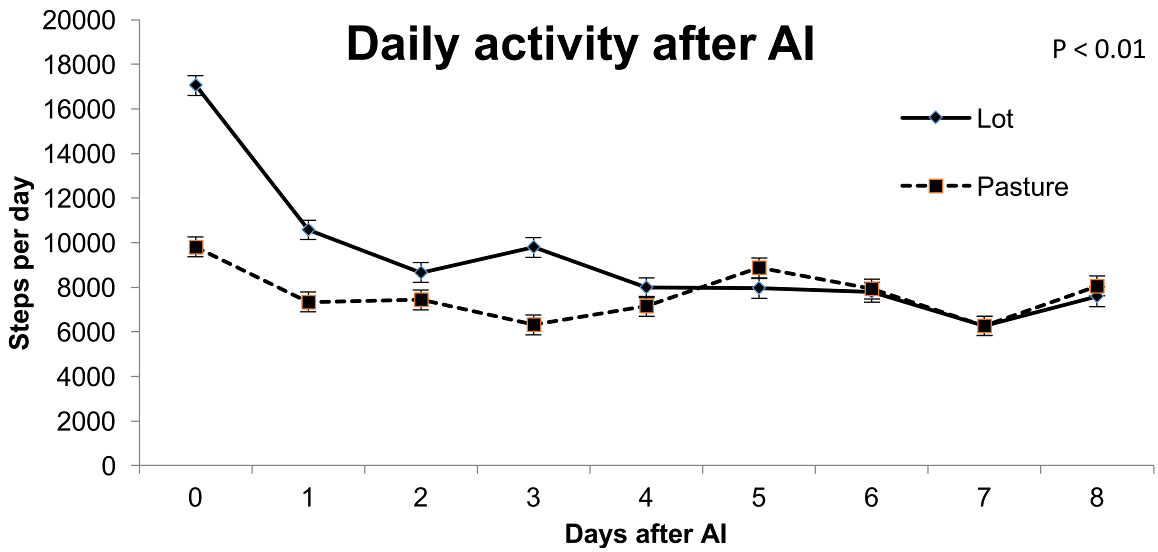


Figure 3. Daily activity for heifers that remained in the drylot until AI (LOT), and heifers moved to graze spring forage for the 42 days prior to AI (Pasture).



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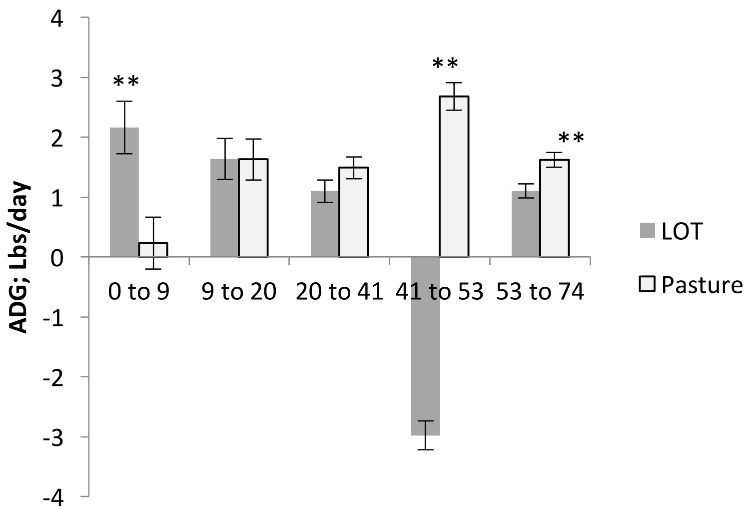

Figure 5. Average daily gain (means ± SE) for heifers moved from the drylot to forage on d –44, and heifers were moved from the drylot to forage on d 0. **P < 0.01 within day.

Shipping stress and embryonic mortality

Knowing that nutritional changes around the time of AI can have tremendous effects on embryo survival, a common question regards the best time to move heifers. With the knowledge of the critical time points in embryonic development, it is possible to understand how stress from shipping could also result in increased embryonic mortality in cows (Table 6). When animals are loaded on a trailer and hauled to a new location, they become stressed and release hormones related to stress. These hormones lead to a release of different hormones that change the uterine environment in which the embryo is developing. During blastocyst formation, hatching, maternal recognition of pregnancy, and attachment to the uterus, the embryo is vulnerable to these changes. The most critical time points are between days 5 and 42 after insemination. Before day 5, the embryo is in the oviduct and is not subject to changes in the uterine environment. Therefore, stress does not influence embryo survivability at this time. The greater the length of time after day 42, the less severe the influence of shipping stress on embryonic loss appears to be. When the embryo is completely attached to the uterus, the embryo is supported by the dam and appears to be less easily affected by environmental changes. On the other hand, in between these time points (5 – 42 days), the embryo is at greatest risk. Shipping during this time can cause detrimental changes to the uterine environment and may result in embryonic mortality. Administration of the prostaglandin inhibitor flunixin meglumine to cows and heifers 10 to 13 days after AI (when they were transported) reduced pregnancy losses about 9% (Merrill et al., 2007). However, administration of flunixin meglumine 10 to 15 d after breeding did not increase pregnancy establishment in cows. In another study, handling heifers to administer flunixin meglumine (compared to leaving them in the pasture) reduced pregnancy rates by 6% (Geary et al., 2010). Taken together, these studies provide evidence that some heifers are more susceptible to the stress of handling.

Table 6. Effect of time of transport after insemination on pregnancy rates

	Days after insemination that transportation occurred			
	1 to 4	8 to 12	29 to 33	45 to 60*
Synchronized pregnancy rate	74%	62%	65%	
% pregnancy loss compared to transportation on days 1 to 4		12%	9%	6%*
Breeding season pregnancy rate	95%	94%	94%	

*Loss in heifers compared to percentage pregnant prior to transportation (pregnancy determined by transrectal ultrasonography)

Data adapted from (Harrington et al., 1995), and T. W. Geary unpublished data

Conclusion

In summary, abrupt changes in diet around the time of AI (moving heifers naive to grazing to grass or other forms of nutrient restriction) can have negative impacts on pregnancy success. When heifers that had been developed in a drylot situation from weaning to breeding were turned out to graze forage without any supplementation they had increased activity (steps per day), lost weight, and had decreased conception rates compared to heifers that had prior grazing experience. In addition, this nutritional stress (decreased intake) does not have to last long. Restriction of intake for only 6 days immediately after AI resulted in decreased embryo quality and delayed embryo development. Thus consistency is important for improved AI conception.

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