Influence of Pre- and Post-Insemination Nutrition on Pregnancy Success in Beef Cattle

G.A. Bridges and S.G. Kruse
North Central Research and Outreach Center, University of Minnesota, Grand Rapids

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Take Home Message

Without question, nutrition mediates reproductive function. It is well established that insufficient nutrition in cattle compromises general reproductive efficiency. Specifically in cattle, undernourishment can alter the secretion and circulating amount of various metabolic hormones including insulin, IGF and IGFBP, GH, and leptin. Alterations in these hormones have direct effects on the ovarian follicles and the oocyte to compromise fertility. In addition, nutrient restriction following breeding appears to alter oviductal and uterine support for embryo growth and pregnancy maintenance. Therefore, to maximize fertility, nutritional inputs to reproducing beef cows must be managed to allow for the animal to be in a positive energy balance. Caution is warranted however as over-nutrition may also compromise various reproductive parameters.

Introduction

It is well established that reproductive efficiency is the main factor influencing production efficiency of a cow/calf operation (Short et al., 1990), with the failure of cows conceiving contributing to the largest loss of potential calves (Wiltbank et al., 1961). Nutrition and subsequent metabolic status of the reproducing female is one of the major factors that contribute to reproductive competence. Nutritional cues can influence a variety of reproductive processes. Inadequate nutrition can result in an older age at puberty in replacement beef heifers (Ferrell, 1982; Yelich et al., 1995) and an extended period of postpartum anestrus in lactating beef cows (Randel, 1990). The function of the hypothalamic-pituitary-ovarian axis is also sensitive to nutritional insults as severe nutritional deprivation results in diminished gonadotropin secretion and reversion to a state of nutritionally-induced anestrus (Spitzer et al., 1978; Rhodes et al., 1995; Diskin et al., 2003; Mackey et al., 1999). Ovarian follicle growth and steroid production is also impacted by nutritional inputs (Mackey et al., 1999; Kruse et al., 2012b). Furthermore, specific metabolic substrates and hormones such as growth hormone (GH), nonesterified fatty acids (NEFA), insulin, insulin-like growth factor-I & -II (IGF), and leptin can have direct effects on various reproductive processes (Leroy et al., 2008). Given the complex interactions between nutrition and reproductive function, it is not surprising that failing to provide reproducing beef cows with adequate nutritional inputs can result in a suppression of general reproductive performance.

The aforementioned list of reproductive processes influenced by nutrition has been extensively investigated. However, the direct effects of nutrition and metabolic signals on oocyte maturation and competence and uterine functionality have not been fully elucidated. The ovulation of a competent oocyte is requisite for conception and pregnancy establishment. Moreover, proper uterine function must occur for embryo elongation, placentation, and ultimately conceptus (embryo proper + placental tissues) survival. Less is known about nutritional mediation of oocyte development and uterine function, but it is becoming evident that both inadequate nutritional inputs and excessive nutritional inputs (obesity) may influence these parameters. As such, a recent focus of our laboratory has been to directly investigate the role of nutrition on the oocyte and uterus in beef cattle. This review will discuss potential nutritional mediated alterations that can occur prior to insemination on

the follicular microenvironment and oocyte and the potential influence of post-conception nutrition on uterine secretions and receptivity to the developing conceptus.

Impact of Pre-Insemination Nutrition on Follicular Development and the Oocyte

In response to nutritional deficiency, in an effort to maintain homeostasis, various aspects of metabolic signaling are changed. Subsequent results are alterations to other biological processes and systems. The system that is most rapidly and drastically affected is reproduction. The effects of nutrition on reproduction are either mediated 1) directly via alterations in GnRH secretion at the level of the hypothalamus or on release of gonadotrophs from the anterior pituitary or 2) indirectly through changes in metabolic hormones such as insulin, growth hormone (GH), insulin-like growth factor (IGF) - I or II and its binding proteins, and leptin. These metabolic hormones signal nutritional status and can alter follicular growth, oocyte quality, and subsequent embryo survival. Furthermore, these metabolic hormones can affect the sensitivity of the developing follicle to the gonadotrophs and thus potentially impact follicular steroid production and that of the subsequent CL.

Insulin

Insulin is a metabolic hormone produced in the pancreas and is key in the regulation of fat metabolism. It induces cellular uptake of glucose from blood and stores it as glycogen. In periods of nutritional restriction, circulating concentrations of insulin are reduced (Webb et al., 2004). Several investigators have shown the importance of insulin as a signaling molecule mediating the effects of abrupt changes in metabolic status on follicular development in cattle. Armstrong et al. (2002) associated diet-induced increases in circulating concentrations of insulin with increased estradiol production from granulosa cells in culture, demonstrating direct action of insulin on follicular functionality. Furthermore, Armstrong (2001) demonstrated that the concentration of circulating insulin increased during the preovulatory period, corresponding with increases in follicular production of estradiol and estradiol has been reported to increase expression of mRNA encoding insulin and its secretion (Morimoto et al., 2001). As it directly relates to nutrition, nutrient restricted heifers infused with insulin had increased dominant follicle diameter and ovulation rate (Simpson et al., 1994; Harrison and Randel, 1986) compared to nutrient restricted heifers not receiving insulin. However, when hyperinsulinemia was induced in fat heifers, it resulted in impaired oocyte quality (Adamiak, et al., 2005). *In vitro* studies have further highlighted the importance of physiological insulin concentrations for developmentally competent oocytes (Glister et al., 2001). Addition of low levels of insulin to bovine granulosa cells showed a dose dependent increase in cell number and secretion of growth factors necessary for oocyte development. In contrast, addition of insulin to oocytes in culture at concentrations exceeding physiological levels resulted in decreased cleavage and blastocysts rates (Arias-Alvarez et al., 2011).

Insulin-like growth factor and growth hormone

Similar to insulin, many nutrition-induced impacts on the ovary are mediated by IGF-1 and GH. Insulin-like growth factor-I is a small peptide and one of the two ligands of the IGF system, which also includes receptors, high-affinity IGF binding proteins (IGFBPs) and IGFBP proteases (Velazquez et al., 2009). IGF-I is the primary mediator of the effects of GH and regulates cellular proliferation, differentiation, and survival (Benito et al., 1996). Furthermore, IGF-I has been shown to induce mitosis and prevent apoptosis in bovine embryos (Velazquez et al., 2011). Growth hormone acts directly and indirectly through IGF-I and together GH and IGF-I promote cellular growth and influence regulation of metabolism. Production of GH occurs in the anterior pituitary, is then released in the blood stream and acts upon the liver to produce IGF-I. In undernourished cattle, GH is increased whereas IGF-1 is decreased, resulting in low plasma concentrations of IGF-I which are associated with poor fertility (Webb et al., 2004). Although, GH has been demonstrated to affect follicular growth (Gong et al., 1991; Lucy et al., 1999), its direct action on follicles is questionable as

mRNA encoding its receptor has not been found in bovine follicles (Lucy et al., 1999) nor have *in vitro* experiments supported its role in proliferation or steroidgenesis of bovine granulosa cells (Jimenz-Krassel et al., 2002). More likely, GH acts via other metabolic hormones to influence follicular development.

Unlike GH, IGF-I directly mediates events within the follicle in response to nutritional changes in cattle. However, by itself, changes in IGF-I amounts does not fully explain how nutrition imparts IGF-induced regulation of reproductive function. Actions of IGF-I are further mediated by IGF binding proteins. IGF binding proteins serve to transport and increase the half-life of IGF-I with nearly 98% of IGF-1 is bound to binding proteins. A decreased plane of nutrition leads to a decline in IGFBP in blood (Webb et al., 1999) limiting the availability of IGF to such structures as the ovarian follicle and the oocyte. IGF-I and its binding proteins act to mediate several reproductive processes. As an example, nutritional insults can lead to alterations in expression of mRNA encoding mechanisms of the ovarian IGF system to influence the sensitivity of follicles to gonadotrophins (Webb et al., 2004). In addition, IGF-I accelerates preantral follicle growth *in vitro* (Saha et al., 2000), and several *in vitro* experiments have demonstrated that adding IGF-I to oocytes in culture increases blastocyst development rate. Wasielak et al. (2007) demonstrated that addition of IGF-I to maturation media (100 ng/ml) inhibited apoptosis in bovine oocytes. More recently, investigators established that supplementing IGF-I to oocytes recovered via ovum pick-up (OPU) resulted in a greater number of oocytes reaching the blastocysts stage and increased the number of cells within the blastocyst (Sakagami et al., 2012). Also, injecting cows with IGF-I directly into the ovarian stroma during folliculogenesis was shown to be beneficial to embryo production. Cows treated with IGF produced more embryos that reached the blastocyst stage, with these embryos having increased inner cell mass proliferation (Velazquez et al., 2012). Thus, even a short *in vivo* exposure of oocytes to a supraphysiological IGF-I microenvironment can increase ICM cell proliferation *in vitro*.

Leptin

Leptin is an adipose-derived hormone key in modulating feed intake, with high plasma concentrations known to suppress appetite (Foster and Nagatani, 1999). Concentrations of plasma leptin have been associated with nutritional changes in cattle. Current research has proposed that leptin may serve as an important link between metabolic status and reproductive function. One study conducted by Amstalden et al. (2000) showed that fasting heifers for only 48 h lead to decreased leptin gene expression and circulating leptin. This decline in leptin was associated with a decline in circulating concentrations of insulin and IGF-I and LH pulse frequency, suggesting that leptin plays an inhibitory role in the interaction between gonadotropins and insulin. *In vitro* studies further support this hypothesis, demonstrating the ability of leptin to negate actions of insulin on steriodogensis, specifically inhibiting estradiol production by granulosa cells and androstenedione by theca cells (Armstrong et al., 2003).

Direct relationships between pre-insemination nutrition and fertility in cattle

Our laboratory recently performed a study in lactating postpartum beef cows with the objective to determine the influence of pre-breeding, postpartum nutrition on oocyte viability, early embryonic development, and pregnancy establishment (Kruse et al., 2012a,b). Cows were fed to calve at a common body condition score (BCS, where 1 = emaciated; 9 = obese) of 5.0. Upon calving, body condition score was manipulated resulting in donor and recipient groups that were either BCS 6 or BCS 4 at embryo recovery and transfer, respectively. Cows in each BCS (4 vs 6) were subjected to ovarian superstimulation, AI, and embryo flushing Embryos were recovered 7 to 7.5 d post-estrus and insemination and evaluated microscopically to determine quality and stage. Compact morulae, blastocysts, and expanded blastocysts of quality #1 or #2 were cryopreserved for embryo transfer (ET). Embryos generated from donor cows (E4 or E6) were then transferred into recipients (BCS4 vs BCS6) 7 d post estrus resulting in the following treatments: BCS6:E6, BCS6:E4, BCS4:E6, and

BCS4:E4. At induced ovulation, ovulatory follicle size and preovulatory estradiol and progesterone concentrations were assessed. At ET, corpus lutem (CL) diameter and blood flow, as well as progesterone concentrations were determined. Seven days after ET (day 14 of estrous cycle), a final blood sample was collected for analysis of progesterone concentration. Cows remained on their respective diets until pregnancy diagnosis conducted 23 days following embryo transfer.

Cows with a BCS of 6 tended ($P = 0.09$) to yielded more embryos that were of great enough quality to be frozen for subsequent transfer and had a greater ($P < 0.05$) percentage of freezable embryos (Table 1). Hence, in nutritionally suppressed cows, the number of embryos collected after ovarian superstimulation was suppressed. Recipient cows with a BCS of 4 were induced to ovulate a follicle of lesser ($P < 0.01$) diameter, which resulted in a CL of decreased ($P < 0.01$) diameter (Table 2). In addition, progesterone concentrations were reduced ($P < 0.05$; Table 2) in the nutritionally restricted cows at ET and day 14 of the estrous cycle (7 days after ET). Given BCS4 treated recipient cows had smaller follicles and CL and produced less progesterone we speculate that the decrease in nutritional inputs resulted in a reduction of pulsatile LH release and/or altered sensitivity to LH at the level of the ovary. LH both drives estradiol production (Schallenberger et al., 1984) and growth of the dominant follicle. In periods of nutritional restriction circulating concentrations of insulin and IGF-I, known decrease and subsequently alter LH pulse frequency (Amstalden et al., 2000). Despite considerable difference in embryo recovery and quality and alterations to follicular and endocrine function known to negatively impact pregnancy establishment (Bridges et al., 2010) in cows with a BCS of 4, neither BCS of the recipient nor BCS of the embryo donor impacted pregnancy success (Table 3). It is unclear why pregnancy rates were not affected within the BCS4 treatment. Limited numbers of animals used in the experiment and the use of estrous synchronization to stimulate estrous cycle activity may have contributed to this inability to detect differences in pregnancy success.

Table 1. Effect of BCS on embryo donor.

TRT	n	DPP @ Flush	BCS @ Flush	BCS Change[a]	Total Bodies (n)[b]	UFO (n)	Freezable Embryos (n)[c]	Percent Freezable (%)
BCS4	10	78.3 ± 5	4.0 ± 0.1	-1.1 ± 0.1	10.8 ± 1.8	3.1 ± 1.1	7.0 ± 1.4	65.8 ± 0.1
BCS6	10	82.9 ± 3	6.2 ± 0.1	+1.1 ± 0.1	14.4 ± 2.6	1.3 ± 0.3	12.1 ± 2.4	82.6 ± 0.1
P-value	.	NS	< 0.05	< 0. 05	0.27	0.13	0.09	0.05

[a] Difference in BCS between calving and embryo flush.
[b] Total number of structures recovered at embryo flush.
[c] Quality grade 1 and 2 embryos that were frozen.

Table 2. Effect of BCS of embryo recipient on reproductive performance

	BCS6[a]	BCS4[a]	*P*-Value
BCS @ Calving	5.1 ± 0.03	5.1 ± .03	NS
BCS @ ET	6.1 ± 0.05	4.1 ± 0.04	< 0.001
DPP @ ET	79.5 ± 1.8	75.3 ± 2.1	NS
Ov. Follicle Diam., mm	14.0 ± 0.3	13.2 ± 0.3	< 0.01
CL Diam., mm	23.7 ± 0.5	21.4 ± 0.5	< 0.01
E2 Conc. @ PGF, pg/mL	2.2 ± 0.1	3.2 ± 0.3	< 0.01
E2 Conc. @ GnRH, pg/mL	7.7 ± 0.5	7.2 ± 0.6	NS
P4 Conc.@ ET, ng/mL	3.9 ± 0.1	3.4 ± 0.2	< 0.05
P4 Conc. @ d 14, ng/mL	8.2 ± 0.4	6.7 ± 0.5	< 0.01

[a] BCS of embryo recipient at time of embryo transfer.

Table 3. Effect of BCS of donor and embryo recipient on pregnancy success.

	Recipient BCS[a]		Donor BCS[b]		P-Value		
	BCS6	BCS4	EB6	EB4	Receipt	Donor	R*D
BCS @ Calving	5.2 ± 0.04	5.2 ± 0.04	5.2 ± 0.04	5.2 ± 0.03	NS	NS	NS
BCS @ ET	6.1 ± 0.05	4.1 ± 0.04	.	.	< 0.001	NS	NS
Pregnancy rate, n (%)	24/44 (54.5%)	20/41 (48.8%)	22/43 (51.2%)	22/42 (52.4%)	NS	NS	NS

[a] BCS of embryo recipient at time of embryo transfer.
[b] BCS of embryo donor at time of embryo recovery.

Impact of Post-Insemination Nutrition on Uterine Function and Conceptus Survival

Potential nutrition influences on uterine function

Unequivocally, proper uterine function is required for the establishment and maintenance of pregnancy. In addition, unlike many other species, in cattle implantation of the conceptus and placentation do not occur immediately following fertilization, but rather the conceptus spends a prolonged period (> 20 d) within the uterus without adhering to the uterine endometrium. During this period the embryo and eventual conceptus is dependent upon oviductal and uterine secretions, termed histotroph, to supply the required nutrients for continued growth. Uterine histotroph is comprised of hormones, growth factors, enzymes, mitogens, cytokines, vitamins, transport proteins, ions, lipids, glucose, fructose, and amino acids that are delivered into the uterine lumen via active and passive transport (Satterfield et al., 2010). While considerable research has been conducted investigating the endocrine mechanisms responsible for histotroph secretion (reviewed by Bazer et al., 2011) and identifying the functions of specific histotroph components, limited research is available on the impact of maternal nutrition on histotroph composition and/or secretion. Conceivably, under-nutrition or over-nutrition may alter amounts histotroph secreted or ratios of individual components, thus potentially influencing embryonic growth. Obvious candidates for this regulation include IGF-1, IGF-II, their respective binding proteins, glucose, lipids, and amino acids.

Reducing and/or altering the composition of uterine histotroph has been demonstrated to result in reduced embryonic growth in both the ewe (Satterfield et al., 2010) and the cow (Forde et al., 2011). Forde et al. (2011) altered histotroph secretion by reducing post-ovulatory concentrations of progesterone. Such a manipulation resulted in alterations in numerous genes responsible for production and secretion of various components of the uterine histotroph and ultimately resulted in reduced embryonic growth that would likely have resulted in eventual embryonic mortality. While this study did not address direct nutritionally-mediated changes in uterine histotroph, we have demonstrated (Kruse et al., 2012b) that cows that are in lesser body condition pre-insemination have smaller CL that producer lesser concentrations of progesterone. Furthermore, Hill et al. (1970) observed an immediate reduction in CL size and circulating concentration of progesterone in heifers exposed to nutrient restriction at insemination. However, both Spitzer et al. (1978) and Rhodes et al. (1995) reported no difference in circulating concentrations of progesterone in heifers immediately following nutrient restriction. Hence, it is unclear if nutrient restriction imparted immediately following AI can impact subsequent progesterone concentrations. The potential exists however, that negative energy balance may indirectly impact uterine function through alterations in luteal progesterone production. Lesser amounts of progesterone early in gestation may delay conceptus growth (Kerbler et al., 1997; Green et al., 2005) through decrease uterine histotroph secretion and result in smaller conceptuses that fail to signal maternal recognition of pregnancy. Alternatively, depressed nutrition may directly alter uterine secretions, which ultimately impact conceptus growth.

During early pregnancy oviductal and uterine accumulation of such nutritional factors as IGF-I, glucose and amino acids (Gao et al., 2008; Block et al., 2011) may be critical for proper embryonic growth. Similar to other organs of the body, the oviduct and uterus produce IGF-I and IGF-II. Both of these nutritional signaling molecules can stimulate cell proliferation and cellular differentiation in the uterine endometrium and developing conceptus (Wathes et al., 1998; Sosa et al., 2009). It is unclear if an abrupt period of nutrient restriction immediately following insemination can impact the oviductal and uterine secretions of IGF-I and IGF-II or their respective binding proteins. However, it is known that changes in nutrient hormonal signals can occur rapidly following a nutritional insult. Kiyma et al. (2004) demonstrated that in nutrient restricted ewes circulating concentrations of insulin and IGF-I decreased by 24 and 48 hours, respectively. Changes in IGF binding proteins were evident within 72 hours. The importance of IGF-1 for early embryonic development is critical and has been well documented (reviewed by Block et al., 2011). The study of Kiyma et al. (2004) demonstrates that changes in regulatory hormones are extremely responsive to perceived changes in homeostasis

such as nutrient restriction. As such, the brevity of these signals suggests that nutrient restriction could alter embryo development within days of insemination and thus ultimately influence pregnancy establishment.

Glucose and fructose are the main energy substrates of the developing conceptus with their concentrations increasing considerably in the uterine lumen of pregnant ruminants during early gestation (Bazer et al., 2011). Although circulating concentrations of glucose are closely monitored in ruminants, it is unclear if undernourishment can impact glucose and/or fructose secretion and sequestering in the uterine lumen. If so, alterations in glucose concentrations may impede embryonic development in nutritionally suppressed livestock. Recently, the importance of amino acids, specifically arginine and arginine derivatives, for facilitating embryonic growth has been demonstrated. Glucose and arginine, in coordination with other uterine secreted proteins such as secreted phosphoprotein 1 (SPP1), are involved with the mechanistic target of rapamycin (MTOR) cell signaling pathway (reviewed by Bazer et al., 2012). The MTOR pathway is a "nutrient sensing system" stimulated by IGF-II, glucose, and select amino acids (Kim et al., 2008; Bazer et al., 2012) that is critical from proper embryonic growth and development (Kim et al., 2010). Future research is warranted to determine if general undernourishment or acute nutritional deficiencies can impact the MTOR cell signaling pathway and thereby impede proper embryonic development.

Direct relationships between post-insemination nutrition and fertility in cattle

It is well established that general under-nutrition during the prepartum and postpartum period negatively impacts pregnancy success and reproductive efficiency in beef cattle (Diskin et al., 2003 review). Recently however, greater attention has been given to potential cattle management strategies that result in a period, albeit it brief, of nutritional insult immediately following insemination and potential impacts this abbreviated period of negative energy balance has on fertility. Many spring-born heifers are developed from weaning to breeding in a dry-lot scenario and fed a diet consisting of a combination of forage and concentrate needed to gain approximately 1.5 lb. per day, ultimately targeting a final weight of 65% of estimated mature body weight at the time of breeding. Often estrous is synchronized and AI is conducted while in the dry-lot to better facilitate protocol implementation. Immediately following AI, heifers are often moved to pastures to expose them to clean-up bulls, take advantage of lush spring forage, and reduce the incidence of embryonic loss associated with handling and moving animals at later stages of early gestation (day 5 through implantation; Harrington et al., 1995). Such an immediate change in nutrition, due to shift in diet delivery method and/or quality and quantity of nutrients, may negatively impact metabolism, body weight gains, and ultimately reproductive efficiency in these beef heifers.

Recently, investigators at Purdue University and the University of Wyoming jointly examined the role of post-insemination nutrition on AI pregnancy rates in beef heifers (Arias et al., 2012). At two locations (Purdue; n = 53, Wyoming; n = 99) heifers were fed at 125% of NRC maintenance requirements (approximate ADG of 1.5 lbs/d) from weaning until estrous synchronization and AI. Immediately following estrous synchronization and AI, feed delivery to heifers was tightly controlled as heifers were specifically fed diets formulated to: 1) maintain pre-breeding plane of nutrition (125% of maintenance requirements; GAIN), 2) 100% of maintenance requirements (Maintain), or 3) 80% of maintenance requirements (LOSE). Heifers remained on these diets for 21 days following AI. Pregnancy diagnosis was conducted at 30 days post-AI. Although limited numbers prevented detection of statistical differences between treatments within location, when locations were combined (Table 4) contrast analyses revealed that heifers that were fed to continue their pre-breeding plane of nutrition (GAIN treatment) for 21 days post-AI had greater (P = 0.04) AI pregnancy rates compared to both groups of heifers that had a decrease in dietary plane of nutrition (Maintain and LOSE heifers). In addition, it appears that severity of decreased energy intake did not matter, as pregnancy rates were similar between heifers fed the Maintain and LOSE treatments. These results indicate that failing to maintain a pre-breeding plane of nutrition that results in heifer gain following

insemination reduces the probability of AI pregnancy success. The results are in agreement with results reported by Perry et al. (2009). In a series of studies, these investigators demonstrated that developing heifers in a dry-lot scenario and then immediately moving heifers to pasture following AI can result in reduced pregnancy rates to AI, if heifers lose weight once placed on pasture. Moreover, if heifers transitioned to pasture immediately following AI are supplemented with a concentrated feedstuff such as distillers grains to prevent post-AI weight loss, pregnancy rates are not negatively impacted.

Interestingly, Perry et al. (2009) reported that heifers transitioned from a feedlot to pasture can lose greater than 3 lbs per day in body weight in the first week after entry to the pasture. Hence, with such a dramatic nutritional insult, concomitant with the likely alterations in metabolic signaling occur in response to this insult, it is not surprising the reproductive performance is negativity impacted.

Table 4. Effect of post-AI nutrition on AI pregnancy rates in yearling heifers.

	Ave. Daily Gain, lbs.		AI Pregnancy Rate[1,2], % (n)		
	Wyoming	Purdue	Wyoming[3]	Purdue[4]	Combined[5]
Gain (NE_m 125% NRC)	1.44	2.09	67.6 (23/34)	94.7 (18/19)	77.4 (41/53)
Maintain (NE_m 100% NRC)	0.12	0.15	46.9 (15/32)	75.0 (12/16)	56.3 (27/48)
Lose (NE_m 80% NRC)	-0.83	-0.75	51.5 (17/33)	77.8 (14/18)	60.8 (31/51)

[1] Location; P = 0.002
[2] Location x Treatment; P = 0.73
[3] Wyoming AI Pregnancy Rate; Contrast of Gain vs. Others; P = 0.09
[4] Purdue AI Pregnancy Rate; Contrast of Gain vs. Others; P = 0.13
[5] Combined AI Pregnancy Rate; Contrast of Gain vs. Others; P = 0.04

Two classic studies have also evaluated the impact of nutrient restriction in heifers on fertilization rates (Hill et al., 1970; Spitzer et al., 1978). Although these studies slightly differ in design from the proposed study and only fertilization rate was evaluated, they do provide insight into nutrient regulation of fertility in heifers. Using embryo collection at 3, 8, 13, and 18 days post insemination, Hill and colleagues (1970) reported that nutritionally suppressing heifers (85% maintenance) for one estrous cycle prior to insemination reduced pregnancy rates compared to heifers fed to maintenance (6/15 vs. 11/14, respectively). These authors contributed reduced fertilization rates in nutrient restricted heifers as the main reason for decreased pregnancy success. Conversely, Spitzer et al. (1978) concluded that breeding heifers at their second estrous cycle following nutrient restriction (33% maintenance) did not impact fertilization rates when evaluated via surgical ova recovery conducted at 48 to 96 h post-AI. Moreover, these authors concluded that reduced pregnancy rates in nutrient restricted heifers are not due to fertilization failure, but subsequent embryonic death.

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 the 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 day 6 embryos collected from heifers that were fed restricted, sub-maintenance diets would have poor embryo quality (assessment of quality grade) with fewer total blastomeres and greater proportion of dead blastomeres than heifer fed diets that allow weight gain post-insemination. All heifers were on a common diet during development. Estrus

was synchronized and timed-AI was conducted. 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 125% NRC requirements), targeting an ADG of 1.5 lbs/hd/d (treatment designation = GAIN). The remaining heifers were fed at 80% NRC requirements (treatment designation = LOSE). Dietary treatments were fed until embryo collection using non-surgical embryo flush techniques six days after AI. Recovered embryos were microscopically evaluated, classified by developmental stage (morula, blastocyst, expanded blastocyst) and graded on a 1 to 5 scale (1 = excellent, 2 = good, 3 = fair, 4 = poor, and 5 = degenerate) to evaluate embryo quality. Then embryos were transferred to the laboratory where accessory sperm, number of dead blastomeres, and total number of blastomeres, was evaluated using epifluorescent staining. Nutrient restriction immediately following AI resulted in embryos being of lesser quality (Table 5; embryo quality score) and developmentally retarded as indicated by being at an early stage of development and having fewer total blastomeres (Table 5). In addition, embryos from nutrient restricted heifers tended to have a reduce percentage of live blastomeres.

Table 5. Effect of post-AI nutrition on day 6 embryo development.

TRT	n[a]	% Embryos Recovered	Embryo Stage[b]	Embryo Quality[c]	Accessory Sperm (n)	Dead Cells (n)	Total Cells (n)	% Live Cells
GAIN	29	70.7 ± 7.2 (29/41)	4.5 ± 0.2	2.0 ± 0.2	20.6 ± 4.6	6.8 ± 1.3	65.1 ± 6.1	82.5 ± 4.6
LOSE	27	65.9 ± 7.2 (27/41)	3.8 ± 0.2	2.9 ± 0.2	14.2 ± 3.2	9.6 ± 1.2	48.3 ± 4.5	70.7 ± 5.2
P-value	.	NS	0.04	0.01	0.56	0.25	0.04	0.10

[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 (1-5;1 = excellent; 5 = degenerate; per IETS Standards)

These results suggest 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 and the manifestation of negative energy balance affects early embryonic development are not definitive and numerous physiological and endocrine processes may contribute. Further evaluation of circulating progesterone concentrations, IGF-1, and IGF-binding proteins in this study are currently being conducted. Given the importance of nutritional hormones (e.g. IGF-1, glucose, and insulin) on early embryonic development (Block et al., 2011), diet induced alterations in these factors could influence early embryo development and ability to establish pregnancy. Furthermore, Perry et al. (2009) reported that pregnant heifers had greater glucose than open heifers on d 11 post-AI (P= 0.04; 77.4 ± 1.24 and 73.8 ± 1.24 mg/dL) but not on d 0. Lastly, the contribution of oviductal and uterine histotroph to embryo development is critical. It is unclear if an immediate change in nutritional status can impede histotroph secretion or if nutritional status can dictate composition of the histotroph. Further studies are warranted to investigate this potential phenomenon.

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