Nutrition and Reproductive Efficiency: Transition Period Management, Energy Status, and Amino Acid Supplementation Alter Reproduction in Lactating Dairy Cows

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Introduction

A number of reviews have highlighted the importance of nutrition in regulating bovine reproductive efficiency (Cardoso et al., 2013; Grummer et al., 2010; Santos et al., 2010; Wiltbank et al., 2006). The effects of nutrition in the embryo donor cow have been particularly emphasized (Santos et al., 2008; Sartori et al., 2010; Sartori et al., 2013; Velazquez, 2011; Wu et al., 2013). This review will specifically focus on some of our results related to how changes in nutrition can alter reproductive efficiency of dairy cattle.

Inadequate or excessive energy, protein, or specific amino acids can have effects at multiple stages of the reproductive process. First, effects during the early postpartum period have been postulated to alter the oocyte and subsequent embryo development after fertilization of this perturbed oocyte (Britt, 1992). Second, changes in circulating hormones and metabolites such as insulin, glucose, urea, or amino acids during the final stages of oocyte development, before ovulation, can profoundly impact fertilization or embryo development (Adamiak et al., 2005; Adamiak et al., 2006; Bender et al., 2014). A third obvious target of nutrition on the embryo is during the first week of development when changes in oviductal and uterine environment could alter development of the embryo to the blastocyst stage (Steeves and Gardner, 1999a; b; Steeves et al., 1999). Finally, changes in circulating energy metabolites such as glucose and propionate, and building blocks for cells such as amino acids, could alter the uterine lumen composition and subsequently influence hatching and embryo elongation. The elongating embryo secretes the protein interferon-tau that is essential to rescue the corpus luteum and can alter the concentrations of many substances in the uterine lumen (Groebner et al., 2011; Hugentobler et al., 2010). Alternatively, select nutrients in the uterine lumen can also alter interferon-tau expression (Kim et al., 2011). Thus, deficiencies or excesses of energy, protein, or specific amino acids could have targeted impact on a specific stage of oocyte/embryo development or may have multiple, potentially additive effects, on reproductive processes. Due to space limitations, many specific nutritional effects will not be approached in this particular review article, including effects of fatty acid supplementation, as well as vitamin and mineral

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supplementation or deficiencies; however, some of these aspects have been recently reviewed (Leroy et al., 2013; 2014; Santos et al., 2008; Velazquez, 2011).

Transition Period Nutritional Management: Effects of Dry Period Length

One extreme way alter energy balance during the transition period is to eliminate the dry period. Our studies, in collaboration with Ric Grummer's laboratory, have shown a positive effect of dry period elimination on energy balance, return to estrous cyclicity, and fertility (Grummer et al., 2010). When the dry period was eliminated, negative energy balance during the early postpartum period was effectively eliminated (Rastani et al., 2005). Time to first ovulation was reduced when comparing cows that had a 56 day dry period (31.9 + 4.4 d) with cows with a 28 d dry period (23.8 + 3.4 d) and cows with no dry period (13.2 + 1.2 d) (Gumen et al., 2005). Of particular interest to this review, the pregnancies per artificial insemination (**P/AI**) were increased in cows with no dry period (55%) compared with cows with a 56 day dry period (20%) (Gumen et al., 2005). Thus, changes in dry period management can reduce/eliminate negative energy balance during the early postpartum period and increase P/AI (Grummer et al., 2010; Gumen et al., 2005; Watters et al., 2009).

Transition Period Nutritional Management: Effects of Changes in Body Condition

The relationships between energy intake, energy output, and form of dietary energy (fiber vs. non-fiber carbohydrate, **NFC**) have been shown to produce profound effects on metabolic status of the cow and, in some cases, reproductive performance of both dairy and beef cattle. Part of this effect is due to a delayed return to cyclicity. Negative energy balance decreases dominant follicle growth and estradiol (**E2**) production probably related to the decrease in luteinizing hormone (**LH**) pulses as well as the decrease in circulating insulin and IGF-1 (Butler, 2003; 2005; Canfield and Butler, 1990). The magnitude of body condition score (**BCS**) loss after calving can increase in the percentage of cows that are not cycling at the end of the voluntary waiting period (Gumen et al., 2003; Lopez et al., 2005; Santos et al., 2004; Santos et al., 2009). An increase in percentage of anovular cows will lower reproductive efficiency in programs using detection of estrus or synchronized ovulation and timed artificial insemination (**TAI**) (Gumen et al., 2003; Santos et al., 2009). Cows with lower BCS near the time of AI have decreased fertility (Moreira et al., 2000; Souza et al., 2008) and this may be related to increased anovulation as BCS decreases (Santos et al., 2009).

In a recent retrospective study (Carvalho et al., 2014), we evaluated the effect of BCS near TAI on reproductive performance of lactating dairy cows treated with Double-Ovsynch protocol (Herlihy et al., 2012; Souza et al., 2008) to induce cyclicity and synchronize ovulation. Cows with low BCS (≤ 2.5) compared to cows with BCS ≥ 2.75 had greater incidence of anovulation (12.3% [21/171] vs. 4.9% [22/451]; $P = 0.0006$) and decreased P/AI (40.4% [105/260] vs. 49.2% [415/843]; $P = 0.03$). Thus, BCS near AI has a small but significant effect on fertility even when cows are induced into cyclicity using a GnRH-based protocol, such as Double-Ovsynch.

Potentially even more important to fertility than the absolute BCS at the time of AI is the amount of BCS loss between parturition and first AI (López-Gatius et al., 2003; Santos et al., 2009). Consistent with this idea, in experiment 2 of our study (Carvalho et al., 2014), we observed a much more dramatic effect on P/AI when we evaluated cows for BCS change between calving and 21 d after calving. The P/AI differed ($P < 0.001$) dramatically among BCS change categories and was greater for cows that gained BCS (83.5%; 353/423), intermediate for cows that maintained BCS (38.2%; 258/675), and least for cows that lost BCS (25.1%; 198/789). Thus, these results are consistent with the idea first introduced by Britt (1992), who postulated that energy status during the early post-partum period could alter follicular/oocyte quality resulting in negative effects on subsequent fertility in lactating dairy cows.

In experiment 3 (Carvalho et al., 2014), we decided to directly test this hypothesis by evaluating the effect of early postpartum body weight loss on embryo quality from superstimulated cows (Carvalho et al., 2014). The body weight of lactating dairy cows (n = 71) was measured weekly from first to ninth week postpartum and then all cows had superovulation induced using a modified Double-Ovsynch protocol. Cows were divided into quartiles by percentage of body weight change (Q1 = least change; Q4 = most change) from calving until third week postpartum. There was no effect of quartile on number of ovulations, total embryos collected, or percentage of oocytes that were fertilized; however, the percentage of fertilized oocytes that were transferable embryos was greater for cows in Q1, Q2 and Q3 than Q4 (83.8%, 75.2%, 82.6%, and 53.2%, respectively). In addition, percentage of degenerated embryos was least for cows in Q1, Q2, and Q3 and greatest for Q4 (9.6%, 14.5%, 12.6%, and 35.2% respectively). Thus the effect of changes in BCS during the early post-partum period on subsequent fertility at first AI could be partially explained by the reduction in embryo quality and increase in degenerate embryos by d 7 after AI in cows that lost more body weight from first to third week postpartum. This result is obviously consistent with the hypothesis introduced by Britt (1992). Thus, BCS and particularly BCS change can have dramatic effects on fertility and early embryo development in dairy cattle.

Effects of High Energy Diets on Fertility

Another somewhat opposite idea related to dietary energy intake and energy balance is an observed reduction in embryo quality when cows were fed excessive energy in the diet near the time of AI. Increases in feed intake or increased dietary NFC have been found to alter insulin (Adamiak et al., 2005; Adamiak et al., 2006) and progesterone (**P4**) concentrations (Sangsritavong et al., 2002; Vasconcelos et al., 2003), and superestimulatory success (Yaakub et al., 1999). Superestimulated beef heifers that were fed a high energy diet ad libitum (excessive energy) compared to 81% of ad libitum intake had reduced number of CL, reduced number of recovered structures, and dramatically reduced yield of transferrable embryos (Yaakub et al., 1999). Thus, excessive energy consumption can alter embryo development, although the mechanism(s) for these effects and whether the effects are on the oocyte or directly on the early embryo are not yet fully described.

We tested this idea by comparing the reproductive records of 49 free-stall Holstein-dairy herds in WI (herds using Dairy Comp 305 [n = 44] and PCDart [n = 5] software for management) with the composition of total mixed ration (**TMR**) diets. The nutritional information included all ingredients and nutrient composition of all mixes used. Size of herds enrolled in the data collection varied from 143 to 2,717 lactating cows (average 719.6 ± 77.2), were milked 2 (n = 6) or 3 (n = 43) times per day, with average production per cow of 39.0 ± 1.3 Kg/day, and average dry matter intake (**DMI**) of 25.1 ± 0.5 Kg/day. There was substantial variation in diet composition. For example, crude protein (**CP**) varied from 16.0 to 18.7%, rumen-degrable protein (**RDP**) from 9.1 to 12.3%, neutral detergent fiber (**NDF**) from 24.9 to 35.1%, NFC from 31.7 to 46.6%, starch from 20.1 to 30.8%, and fat from 3.1 to 6.7%. Milk production level was not associated with P/AI at first AI or other reproductive measures ($P > 0.10$). However, greater DMI tended to be associated with lower first service P/AI ($r = -0.25$, $P = 0.10$). Dietary content of CP, RDP, and fat was not associated with P/AI ($P > 0.10$). Percentage of dietary NDF was positively associated with first service P/AI ($r = 0.36$, $P = 0.01$). Most interestingly, greater energy content in the diet measured as NFC, NFC-intake, or starch were found to be detrimental to first service P/AI (NFC: $r = -0.54$, $P < 0.01$; NFC intake: $r = -0.42$, $P < 0.01$; starch: $r = -0.37$, $P = 0.03$), and all P/AI combined (NFC: $r = -0.51$, $P < 0.01$; NFC intake: $r = -0.44$, $P < 0.01$; starch: $r = -0.21$, $P = 0.20$). In conclusion from this study, diets containing more fiber and less rapidly digestible carbohydrates were associated with improved reproductive performance in high-producing dairy herds.

An important idea that needs to still be adequately tested is that excessive energy could lead to overstimulation of the follicle and oocyte leading to subsequent reductions in embryo development (Garnsworthy et al., 2008a; b; Rooke et al., 2009; Webb and Campbell, 2007). Some evidence for negative effects of overfeeding on embryo development is provided by a study using super-stimulated ewes in which overfeeding (2.2 times maintenance) dramatically reduced embryo quality compared to underfed (0.5 times maintenance) ewes (Lozano et al., 2003). This last study, as well as others in lactating cows (Sangsritavong et al., 2002; Vasconcelos et al., 2003), also observed that animals with greater feed intake had reduced circulating P4 concentrations. Previous studies have shown that increased circulating P4 concentrations during super-stimulatory treatments increased embryo quality and number of transferrable embryos (Nasser et al., 2011; Rivera et al., 2011). Lower circulating P4 may lead to increased LH pulses possibly leading to premature resumption of meiosis and ovulation of an oocyte of reduced fertility, as has been observed in persistent follicle models (Revah and Butler, 1996; Roberson et al., 1989). In addition to the effect of P4 during preovulatory follicle development, increasing circulating P4 concentrations after breeding, during early embryo development, can increase embryo development, particularly increasing length of the preimplantation embryo (Lonergan and Forde, 2014; Lonergan et al., 2013; Maillo et al., 2014; O'Hara et al., 2014a; O'Hara et al., 2014b; Wiltbank et al., 2014).

Excessive concentrations of insulin may decrease oocyte quality and subsequent embryo development. Adamiak et al. (2005) conducted an elaborate experiment

collecting oocytes via ultrasound-guided trans-vaginal follicular aspiration in beef x dairy crossbred heifers exposed to either maintenance or two times maintenance feeding levels over a period of three successive estrous cycles. The study found that the effect of feeding level on oocyte quality is dependent on body condition of the heifers; thus, the two times maintenance had a positive impact on oocytes recovered from heifers in a low BCS but had a negative impact on oocytes recovered from heifers of a moderately high BCS. In addition, many of the moderately fat heifers were hyperinsulinemic, which also had a negative impact on oocyte quality. In a similar study, heifers exposed to a high starch diet had a corresponding increase in circulating insulin concentrations and a subsequent decrease in blastocyst production rate (Adamiak et al., 2006). Thus, excessive energy intake may reduce embryo quality through elevations in LH pulses or through excessive insulin or other metabolic signal associated with consumption of a high carbohydrate diet or excess energy.

In later lactation Holstein dairy cows, energy intake generally exceeds energy output and therefore cows are in positive energy balance and circulating insulin is elevated. Acute restriction of feed intake reduced circulating insulin and increased circulating P4 in late lactation dairy cows (Ferraretto et al., 2014). We used this model to test specific hypotheses related to feed intake (ad-libitum intake vs. 25% feed restricted) and LH (± additional LH) in super-stimulated Holstein cows in late lactation using a 2 X 2 Latin square design (Bender et al., 2014). As expected, feed restriction had a substantial effect on circulating insulin concentrations without changing plasma glucose concentrations (Bender et al., 2014). Large changes were not observed in numbers of large follicles on the final day of super-stimulation, in the percentage of these follicles that ovulated, or in the number of CL on the day of flushing. Probably the most consistent and biologically-interesting result from this study was an interaction that was found between feed restriction and amount of LH during the superovulation protocol on the percentage of oocytes that were fertilized, and on the percentage of total structures that were graded as 1 and 2 embryos (best quality embryos) compared with degenerate embryos. It appears that combining ad libitum feeding and high LH reduced percentage of oocytes that were fertilized and subsequent embryo quality of fertilized oocytes. This is consistent with the idea that high LH combined with high insulin can reduce embryo quality. Conversely, feed-restricted cows with low LH in the super-stimulation protocol also had reduced fertilization of oocytes, reduced percentage of grades 1 and 2 embryos (of total structures), and increased degenerate embryos. However, increasing LH in feed-restricted cows increased embryo quality. Thus, there was an interaction between these two treatments on embryo quality that is consistent with the idea that optimizing super-stimulatory success requires consideration of both the hormonal and metabolic state of the treated cow with conditions that produce both high LH and high insulin (excess energy consumption) apparently being negative for fertilization and embryo quality (Bender et al., 2014).

In conclusion, it seems clear that negative energy balance during the first 3 weeks after calving can have a negative impact on fertility at the first AI, even though the AI occurred more than 5 weeks after the original negative energy balance. The harmful effect of negative energy balance during the transition period is manifest in

reduced embryo development during the first week after AI, suggesting a lingering effect of the transition problems on oocyte competence. In late lactation Holstein cows, feed restriction had a positive effect on embryo quality when supplemented with LH, but was negative in cows without additional LH. In dry Holstein cows on a maintenance diet, elevations in insulin reduced fertilization, suggesting a negative effect of insulin on oocyte quality, but did not alter subsequent embryo development or quality. Thus, breed, BCS, and current metabolic status of the cow need to be considered when deciding the optimal nutritional and hormonal programs to use during embryo production.

Effects of supplementation of specific amino acids on fertility

Some amino acids are limiting for optimal milk production as evidenced by an increase in milk and protein yields, and percentage of protein in milk after supplementation with specific, rumen-protected amino acids (Cho et al., 2007; Patton, 2010; Socha et al., 2005). Generally the first three rate-limiting amino acids for milk production are considered to be methionine (**Met**), lysine (**Lys**), and histidine (**His**) in most diets fed to lactating cows. In addition, many amino acids can have positive effects on physiological processes that are independent of their effects on synthesis of proteins. This has been termed “functional effects” of amino acids and methionine and arginine effects are the best studied “functional amino acids” that have been linked to reproduction (Bazer et al., 2011; Penagaricano et al., 2013).

Most amino acids are more concentrated in the oviduct and uterus than in the blood (Hugentobler et al., 2007). In addition to the mechanisms that concentrate amino acids in the uterus in non-pregnant ruminants, there are additional mechanisms that result in further increases in concentrations of amino acids in the uterine lumen in pregnant ruminants near the time of embryo elongation (Day 14 to 18 of development). Of particular interest for dairy cattle, the three amino acids that are considered rate-limiting for milk production, Met, His, and Lys, are the amino acids with the greatest increase in concentrations in the uterine lumen during embryo elongation (>10-fold increase on average (Gao et al., 2009c; Groebner et al., 2011)). Arginine is another amino acid that has been studied extensively in relation to reproduction (Wu et al., 2013) and it is also highly concentrated in the pregnant uterus.

The increase in specific amino acids in the uterus near the time of embryo elongation appears to be due to an induction of specific amino acid transporters in the uterine endometrial cells (Gao et al., 2009a; b; Groebner et al., 2011). The induction of these amino acid transporters is most likely induced by the protein interferon-tau that is secreted by the elongating conceptus (tissues that will generate the embryo and placenta). For example, interferon-tau treatment dramatically increased one specific amino acid transporter, SLC15A3, in both glandular epithelial (36-fold) and stromal epithelial (177-fold) uterine cells (Groebner et al., 2011). Thus, there is likely a positive feedback system occurring during this critical time of embryo elongation with uterine amino acids being essential for rapid embryo growth and embryonic interferon-tau production; whereas, interferon-tau stimulates active amino acid transport through the uterine epithelial cells to increase amino acid supply to the elongating embryo.

Disturbances in the temporal relationship between uterine blood flow, induction of uterine amino acid transport, uterine amino acid concentrations, embryonic growth, embryonic interferon-tau production, and rescue/regression of the corpus luteum may reduce fertility and increase pregnancy losses.

Numerous studies have evaluated the effects of rumen-protected amino acids, particularly methionine, on milk production. For example, a recent meta-analysis (Vyas and Erdman, 2009) evaluated the results from 35 experiments on production effects of postruminal supplementation with methionine. At low methionine intakes (25 g per cow per day) there were dramatic increases in milk protein (16 g of milk protein per gram of metabolizable methionine intake); whereas, the production response was more muted at high methionine intake (70 g per cow per day; increase of 4 g of milk protein per g of metabolizable methionine intake). Unfortunately, we have been unable to find studies in the scientific literature which were specifically designed and adequately powered to evaluate the effects of specific amino acids on reproductive efficiency of lactating dairy cows. The largest study (Polan et al., 1991) combined results from 259 cows at 6 Universities evaluating rumen-protected methionine and lysine supplementation. They detected no significant effect on days to first service, services per conception, or calving interval, although no details were provided on reproductive measures in each specific treatment group. It is obvious that large studies are needed to validly evaluate the effects of supplementing amino acids on measures of reproductive efficiency in lactating dairy cows.

One particularly interesting study (Coelho et al., 1989) used serum from lactating dairy cows in the media to grow head-fold stage rat embryos (Day 9.5 after breeding). Complete development of these embryos requires serum and development is normal in rat serum. When embryos were grown in serum from dairy cows embryonic development was abnormal when measured as total embryo protein, somite pairs, or percentage of the embryos that are abnormal (no neural tube closure, abnormal shape, no development of eyes and branchial arches). Supplementation of bovine serum with amino acids and vitamins produced normal development. Amino acid supplementation alone but not vitamin supplementation produced normal development. Supplementation of methionine alone was sufficient to produce normal development of the rat embryos in cow serum. In a separate experiment, use of serum from cows that were supplemented with rumen-protected methionine (110 g/d) also produced normal embryo development. Thus, bovine serum has such low methionine concentrations that normal development of rat embryos is retarded.

The requirements for complete development of bovine embryos have not yet been determined. Current culture conditions allow production of bovine embryos to the blastocyst stage (Day 7-8) and even allow hatching of a percentage of embryos (Day 9); however, conditions have not been developed that allow elongation of embryos in vitro, and definitely do not allow culture of bovine embryos to the head-fold stage that was analyzed in the rat embryo experiments. The methionine requirements for in vitro produced preimplantation bovine embryos (Day 7-8) was recently determined in studies from University of Florida (Bonilla et al., 2010). There was a surprisingly low methionine

requirement (7 µM) for development of embryos to the blastocyst stage by Day 7, however development to the advanced blastocyst stage by Day 7 appeared to be optimized at about 21 µM (Bonilla et al., 2010). Thus, the results of this study indicated that development of morphologically normal bovine embryos did not require elevated methionine concentrations (> 21 µM), at least during the first week after fertilization.

A recent study (Ikeda et al., 2012) evaluated whether methionine metabolism was required for normal development of bovine embryos. The researchers added ethionine or additional methionine to cultures of bovine embryos. Ethionine blocks metabolism of methionine into the one-carbon pathway (termed antimetabolite of methionine). Ethionine did not block development to the morula stage but blocked development to the blastocyst stage (Control = 38.5%; Ethionine = 1.5%). Development to the blastocyst stage in the presence of ethionine was partially restored by adding S-adenosylmethionine (**SAM**) which would restore the methylation pathway but not restore protein synthesis. Thus, methionine has an essential role in the development of the bovine embryo from morula to blastocyst, whichsuperstimulated is probably partially mediated by hypomethylation in the absence of sufficient methionine.

We recently evaluated the effect of supplementation with rumen-protected Met on early embryo development in superstimulated cows (Souza et al., 2012a; Souza et al., 2012b). We used superstimulated cows so that we would have sufficient statistical power by evaluating numerous embryos in order to validly test the in vivo effects of methionine supplementation on early embryo development in lactating dairy cows. In this experiment, cows were blocked by parity and calving date and randomly assigned to two treatments differing in level of dietary Met supplementation: 1) Met; diet composed of (% dry matter) corn silage (39.7), alfalfa silage (21.8), high-moisture corn (17.2), roasted soybeans (8.6), grass hay (4.6), canola meal (4.0), mineral-vitamin mix (2.7) and a blood meal-based product (ProVAAl Ultra; Perdue Agribusiness) with the rumen protected Met Smartamine (Adisseo), formulated to deliver 2,875 g of metabolizable protein (**MP**) with 6.8 Lys as % of MP and 2.43 Met as % of MP; 2) Control; cows fed the same basal diet but replacing ProVAAl Ultra by ProVAAl Advantage, which contains no added rumen protected Met, formulated to deliver 2,875 gr MP with 6.8 Lys as % of MP and 1.89 Met a % of MP. There was an increase in both kg of milk protein produced and percentage of protein in the milk (Souza et al., 2012b). Thus, from a protein production standpoint, Met appeared to be rate-limiting. We measured plasma Met concentrations in this study and found a large effect of feeding rumen-protected Met on circulating Met concentrations (Control = 16.8 µM vs. Met-supplemented = 22.9 µM).

Our primary interest was the effect of supplemental Met on embryo quality (Souza et al., 2012a). We evaluated a total of 570 embryos in this experiment and found no differences in fertilization or embryo quality. Thus, Met supplementation did not alter early embryo development, at least from a gross morphological standpoint.

Even though Met supplementation during the later stages of follicle development and early embryo development may not have produced morphological changes in the

early embryo, it is well known that Met during this time can have dramatic effects on the epigenome of the embryo (Sinclair et al., 2007). This means that the genes can be changed in such a way that they are not expressed in the same way due to addition of groups, generally methyl groups to the DNA of the cells. For example, a previous study in sheep restricted methyl donors by restricting Met, vitamin B12, and folate before and for the first 6 days after breeding (Sinclair et al., 2007). They then transferred normally-appearing embryos into control sheep and then evaluated the lambs after parturition. The embryos that were produced in low methionine produced lambs that had substantial differences in blood pressure and immune function. To test this idea in cattle, we evaluated whether the embryos that were recovered from cows that had been supplemented or not supplemented with Met had differences in gene expression (Penagaricano et al., 2013).

The objective of this part of the study was to evaluate the effect of maternal Met supplementation on the transcriptome of bovine preimplantation embryos (Penagaricano et al., 2013). Only high quality embryos from individual cows were pooled and then analyzed by a powerful technique that allows evaluation of all genes that are expressed in these embryos, called RNA sequencing (**RNAseq**). Remarkably, the small difference that we produced in circulating methionine produced a substantial difference in expression of genes in the embryo. A total of 10,662 genes were significantly expressed in the bovine embryos. A total of 276 genes were expressed differently, statistically, in embryos from cows supplemented or not supplemented with methionine. Most of these genes were turned off in embryos from cows that were supplemented with methionine. This would be expected since methionine supplementation leads to methylation of the DNA and this can inhibit expression of some specific genes until cells differentiate to the appropriate stage when gene expression should commence (Burdge et al., 2007; Wolff et al., 1998). Thus Met supplementation seemed to change gene expression in a way that may lead to improved pregnancy outcomes and improved physiology of the offspring. Many of the genes are involved in immune function and later stages of embryo development that may be critical for pregnancy progression and normal immune function after birth. Further studies are needed to determine if these changes in gene expression lead to changes in embryo development, reduced pregnancy loss, and altered physiology of the offspring.

Thus, supplementation of rate-limiting amino acids can have substantial effects on milk protein content and yield; however, effects on reproduction have not yet been adequately evaluated. The dramatic induction of the rate-limiting amino acids, Met, His, and Lys, in the uterine fluid of pregnant cows near the time of embryo elongation suggests that elevated amounts of these amino acids may be critical for this important stage of embryo development. Supplementation of cows with methionine during the final stages of follicular development and early embryo development, until Day 7 after breeding, did not lead to gross morphological changes in the embryos but did result in dramatic differences in gene expression in the embryo. Further studies are needed to evaluate whether supplementation with these essential amino acids to lactating cows

would have a beneficial impact on embryo survival and if these changes in the early embryo translate into changes in pregnancy outcomes or physiology of the resulting calf.

Conclusions

Fertility can be affected in a positive or negative way by deficiencies or excesses of energy/carbohydrates and protein/amino acids. Some of these effects may be occurring during the final stages of oocyte development within the preovulatory follicle but are only manifest by the blastocyst stage. For example, the effects discussed above using feed restriction and LH supplementation during follicle development can alter subsequent embryo development (Bender et al., 2014). In addition, some of the effects on embryo function may not be manifest in gross morphological appearance of the embryos but result in dramatic differences in gene expression as observed in the study that evaluated embryonic gene expression using RNASeq in embryos produced in dams that were supplemented or not supplemented with methionine (Penagaricano et al., 2013). There is still a great deal more fundamental biology that needs to be done to fully understand how embryo development can be most practically manipulated using nutritional strategies.

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SESSION NOTES