

EFFECTS OF PREPARTUM PLANE OF NUTRITION DURING MID- OR LATE
GESTATION ON BEEF COW BW, BCS, BLOOD HORMONE CONCENTRATIONS, AND
PREIMPLANTATION EMBRYO

BY

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THESIS

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ABSTRACT

The objectives were to evaluate the potential effect of prepartum plane of nutrition during mid- or late gestation on cow BW, BCS, blood hormone concentrations, and preimplantation embryos. In Exp. 1, multiparous Angus and Angus x Simmental crossbred cows ($n = 33$; $BW = 664 \pm 78$ kg) were fed diets formulated to provide 3 planes of nutrition: 100% NRC energy and protein requirement (**REQ**), 70% NRC requirement (**70%REQ**) or 130% NRC requirement (**130%REQ**) during late gestation (91 ± 4 to 8 ± 4 d prepartum). After treatment period, cows were fed a common diet formulated to meet NRC requirements. Cows fed 130%REQ tended ($P = 0.06$) to have greater BW at breeding when compared with cows fed REQ, cows fed 70%REQ were intermediate. Cows that were fed 130%REQ during late gestation tended ($P = 0.09$) to have greater average progesterone concentrations than cows that were fed REQ, cows fed 70%REQ were intermediate. There was a tendency ($P = 0.07$) for cows fed 70%REQ and REQ to have a greater number of total embryos recovered when compared to cows that were fed 130%REQ diet during late gestation. Nutritional plane during late gestation did not affect ($P \geq 0.53$) cow cyclicity, embryo quality, embryo development, or the total number of embryos that were frozen. In Exp. 2, multiparous Angus and Angus x Simmental crossbred cows ($n = 35$; $BW = 601 \pm 72$ kg) were fed the same diets as Exp. 1 but were fed during mid-gestation (195 to 112 ± 4 d prepartum). Cows fed REQ and 130%REQ had greater ($P = 0.02$) BW at breeding when compared with cows fed 70%REQ. Also, cows fed 130%REQ tended ($P = 0.06$) to have greater BCS at breeding than cows fed 70%REQ during mid-gestation and cows fed REQ were intermediate. Nutritional plane fed during mid-gestation did not affect ($P \geq 0.23$) blood hormone concentrations (progesterone, estradiol, and IGF-1). Cows fed 70%REQ and 130%REQ during mid-gestation had a greater ($P = 0.03$) count of total embryos recovered when compared with cows fed REQ. Nutritional plane during mid-gestation did not affect ($P \geq 0.27$) cow cyclicity,

embryo quality, embryo development, or the total number of embryos that were frozen. In conclusion, prepartum plane of nutrition tends to affect cow BW and BCS. Plane of nutrition effects on embryo production differed depending on the stage of gestation at which treatments were applied.

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CHAPTER 1

LITERATURE REVIEW

Introduction

The cow-calf sector of the beef industry has a constant challenge to provide cattle with the proper nutritional profile to optimize reproductive performance. Reproduction and nutrition are the two most important factors when considering profitability in this sector of cattle production (Hess et al., 2005). Feed costs account for 63% of the total annual cow cost, and, thus, it is evident that nutritional management plays a major role in the financial viability of beef enterprises (Miller et al., 2001). Female infertility and other reproductive diseases account for an estimated yearly cost between \$441 and \$502 million for beef producers (Bellows et al., 2002). With the increase in today's cattle prices in relation to 2002, the current cost associated to reproductive diseases and infertility are likely greater than the values reported by Bellows et al. (2002). Cattle need to be supplied with adequate nutrition to ensure reproductive success.

Season, forage type, and forage availability play a major role in determining the nutritional profile of beef cattle grazing pasture. Cattle grazing forages, depending on forage quality and cows' stage of production, are often supplied with deficient or excess levels of protein and energy needed to meet their requirements. As forages mature, there is an increased concentration of lignin in plant cell walls (Kamstra et al., 1958). Lignin is the major factor that limits the digestibility of cell-wall constituents (Jung and Allen, 1995). Jung and Allen (1995) also reported that lignin has a negative impact on cows' forage intake. Indigestible portions of forages slow the rate of passage and cause decreased intake due to gut fill. Voluntary intake is critical for animal performance and poor quality, mature forages can limit intake due to physical

fill and a decreased rate of passage, thus, resulting in cattle receiving inadequate nutrients to meet their requirements (Jung and Allen, 1995).

Energy balance refers to whether cattle have excess or deficient nutrient consumption to meet their requirements for the particular stage of production they are in. Cattle consuming excess nutrients in relation to their requirement are in a positive energy balance and typically gain BW and BCS, and cattle that consume deficient amounts of nutrients compared with their requirements are in a negative energy balance and will lose BW and BCS. Energy balance during gestation can be estimated through change in BW and BCS. However, during the last 60 days of gestation, BW change can be confounded by fetal growth and development (Selk et al., 1988). Therefore, additional weight gain during this period cannot be fully attributed to the energy balance of the cow. Body condition score is an estimate of body energy reserves assigned to cows based on fat cover over the ribs, spine, pin bones, tailhead, and in the brisket of the animal. Body condition score for beef cattle is typically represented with a 1 to 9 scale (Wagner et al., 1988). Body condition score prior to parturition and at the beginning of the breeding season have proven to be key indicators in estimating the reproductive performance and subsequent pregnancy rates of beef cows (Selk et al., 1988).

Cow requirements fluctuate throughout their production cycle. For instance, a 635 kg cow, pregnant, and 8 months postpartum requires 0.35 Mcal/kg of ME and 6.20% CP on a DM basis; whereas during peak lactation, the same cow will require 0.45 Mcal/kg of ME and 10.31% CP on a DM basis (NRC, 1996). It is important for producers to match nutrition with stage of production to ensure that cattle maintain adequate BCS. When pastures or forages do not meet the requirements of cows, supplementation needs to be considered to prevent cows from entering a negative energy balance. During lactation, when nutritional requirements are increased, cattle

can often enter a negative energy balance and lose BW and BCS. Consequently, cattle can enter the breeding season with poor BCS, and producers need to consider supplementing protein or energy to allow for proper reproductive function. Also, cattle grazing poor quality forages during gestation may not meet their nutritional requirements. Pregnant cattle may metabolize energy reserves to meet the additional energy required for fetal development (Wood et al., 2013) and this would render them in a negative energy balance. When cattle mobilize energy reserves and BCS decreases, it is important to realize that targeting maintenance nutrition may not allow cows to regain adipose tissue and BW or return cows to proper condition, to ensure reproductive efficiency. Abundant feed intake is important to allow compensatory gain in animals that have undergone nutrient restriction (NRC, 1996).

The energy balance of the cows and energy profile of their diet are crucial to reproductive success (Jones and Lamb, 2008). As discussed previously, maintaining cows on poor quality forage can result in deficient levels of energy and protein in the diet. Another management strategy is to dry lot cows during the winter months. In this case, producers use stored feeds to maintain cows as they are no longer grazing forages. The quality of stored feeds and diet during this dry lot period could dictate whether cows enter a positive or negative energy balance. The prepartum and postpartum energy balance of beef females are two of the most important factors associated with the interval from parturition to estrus (Hess et al., 2005). Reducing postpartum interval (**PPI**; the interval from calving to pregnancy) in beef cows is crucial to maximize production potential of the herd. Cows that calve earlier in the calving season are more likely to become pregnant as they have a greater opportunity to return to estrus during a set breeding season (Selk et al., 1988).

The beef industry has adopted many technologies to increase profitability and efficiency within their enterprises. From a reproductive standpoint, estrus synchronization through hormone administration as well as embryo transfer are 2 of the most important. Synchronization of estrus cycles using progesterone (P₄) releasing devices in beef cattle is commonly used for re-establishing ovarian function and cyclicity within a herd, and is well accepted throughout the world (Mapletoft et al., 2003). Williams (2005) discussed the underlying cause of anovulation in beef cattle is heavily influenced by the recovery of the hypothalamic-pituitary axis. Circulating concentrations of estradiol, nutritional status, calving season, as well as genetics play a very big role in a cow's ability to return to estrus (Williams, 2005). Controlled internal drug releases can help re-establish estrus in cows experiencing anovulation. The P₄ releasing devices maintain plasma concentrations of P₄ while the device is inserted in the cow, which is typically for 5 to 10 days (Baruselli et al., 2004). Controlled internal drug releases (**CIDR**) can be used to effectively synchronize estrus cycles and follicular development (Mapletoft et al., 2003). Pregnancy rates in cows treated with CIDR and Crestar (release progesterone and progestogen in cows, respectively) were superior to cows treated with Ovsynch protocol (GnRH protocol) when cows were bred via fixed-time AI (Baruselli et al., 2004). This technology has allowed producers the comfort of implementing timed AI protocols and increasing management to shorten calving intervals.

Just as importantly, embryo transfer opportunities became a viable option for the North American commercial beef industry during the early 1970s (Hasler, 2003). Embryo transfer has proven to be a useful technology to increase the number of offspring from elite genetic lines of cattle. However, nutrition can impact embryo quality (Nolan et al., 1998). Robinson et al. (2006) reported that increasing plane of nutrition can have negative effects on superovulated animals

involved in embryo production. Rhoads et al. (2006) showed that high plasma urea nitrogen (**PUN**) in the donor cow decreased embryo viability. To truly maximize the efficiency of embryo transfer, producers need to provide adequate, and not excess, nutrition to cows subject to superovulation and embryo transfer.

Prepartum Nutrition

Prepartum nutrition is important in ensuring cows remain in a positive energy balance and return to estrus efficiently (Hess et al., 2005). Selk et al. (1988) showed that cows grazing native range grass pastures and supplemented (0.3kg of 41% CP cottonseed meal) to lose BW for 5 months during gestation, had reduced ($P < 0.05$) pregnancy rates (29.5% lower) when compared with cows that were fed (1.4kg of 41% CP cottonseed meal) to maintain BW. This result suggests that cows that lose weight and energy reserves during gestation may suffer from reduced reproductive performance and pregnancy rates when compared with cows that maintain BW and BCS. When cows graze low quality forage, they may not meet their nutritional requirements and consequently lose BW (NRC, 1996). If forages contain inadequate amounts of protein to meet requirements, then protein supplementation could allow increased utilization of the forages and prevent weight loss. Bodine et al. (2000) showed that when cows were fed low quality forages (6% CP) and corn, adding degradable intake protein increased the OM digestibility of the hay. Feeding a 42% CP supplement or dried distillers grains and solubles (**DDGS**) during gestation can increase cow BW gains and BCS (Stalker et al., 2006; Winterholler et al., 2012).

Protein and Energy

Feeding diets deficient in CP from 150 d prepartum to 40 d postpartum can have a negative impact on reproductive performance (Sasser et al., 1988). To determine the impacts that protein content during prepartum plays in postpartum reproduction, these authors fed isocaloric diets to 40 Hereford, first-calf, beef heifers with varying amounts of CP (adequate, 0.96 kg CP/d and deficient, 0.32 kg CP/d). Cows that were fed the deficient diets had an increased ($P < 0.05$) PPI, decreased ($P < 0.05$) conception rates at first estrus and poorer ($P < 0.05$) overall pregnancy rates.

Protein supplementation during gestation can impact cow's BW and BCS (Stalker et al., 2006; Bohnert et al., 2013). Stalker et al. (2006) supplemented Angus, Gelbvieh, Hereford, and Simmental composite cows grazing native upland range pastures with 0.45 kg/(cow·d) of protein supplement (50% sunflower meal, 47.9% cottonseed meal, 2.1% urea for 42% CP and 73.3% TDN on DM basis) from 3 months prior to parturition until calving. They found cows supplemented with CP had increased BCS prior to calving ($P < 0.001$) and at the beginning of the breeding season ($P = 0.01$) when compared with cows that had not been supplemented with protein. There is evidence to suggest that when cows calve at a moderate (BCS ≥ 5 ; in a 1 to 9 scale) BCS as opposed to thin (BCS < 5) BCS, there can be a reduction in the interval from parturition to first estrus (Lents et al., 2008). However, Stalker et al. (2006) showed that even though cows receiving protein supplementation during gestation had greater ($P \leq 0.01$) BCS, there was no difference ($P = 0.26$) in cow's PPI. Also, there were no advantages ($P = 0.46$) of supplementing protein on overall pregnancy rates (93% and 90% for cattle fed supplement and those that were not supplemented, respectively). It is important to consider that even though supplemented cows had statistically greater BCS, biologically there was not a difference as both treatment groups were in a moderate BCS (5.1 vs 4.9 for supplemented cattle and non-

supplemented cattle, respectively; (Stalker et al., 2006). Winterholler et al. (2012) performed a similar study supplementing DDGS to 120 spring-calving beef cows that produced similar results to Stalker et al. (2006). As amount of DDGS supplementation increased (0.77, 1.54, and 2.31 kg/d), there was also an increase ($P < 0.01$) in BW (442, 462, and 492 kg, respectively) and BCS (4.65, 4.92, and 5.31, respectively); however, there was no effect ($P \geq 0.68$) of supplementation on AI conception rates (43.7, 58.8, and 70.6%, respectively) or overall pregnancy rates (76.3, 87.8, and 89.7%, respectively). However, there are numerical increases in the pregnancy data as the DDGS supplementation is increased. Cows that were supplemented greater amounts of DDGS were in a more positive energy balance and were potentially able to conceive more effectively than cattle that were supplemented less DDGS.

A study was performed to evaluate the effect of energy (1.22 kg/d of 20% CP soybean hull-based supplement; 7.0 Mcal ME/d) vs. protein (2.44 kg/d of 40% CP soybean meal-based supplement; 3.6 Mcal ME/d) in the diet from approximately 4 months prior to calving until parturition on the reproductive performance of Hereford and Hereford x Angus cows (Marston et al., 1995). The authors found that feeding supplemental energy, as opposed to protein, prepartum resulted in increased pregnancy rates (90% vs 80%; $P < 0.05$). Also, cows supplemented with energy prepartum had greater ($P < 0.04$) BW gain during gestation and lost less (0.2 units; $P < 0.001$) BCS prior to calving than cows that were fed protein.

Another study was performed to determine the impact of energy in the diet during gestation and its effect on postpartum reproduction (Corah et al., 1974). The authors fed 2 amounts of prepartum energy (11.4 vs 17.6 Mcal DE/d) from 100 d prior to parturition until calving. They observed that cows that were fed the greater amount of energy during gestation had increased BW gains ($P < 0.05$) and less change in BCS ($P < 0.05$) when compared with

those cows receiving a diet with less energy. Despite these changes in BW and composition, the varying amounts of energy did not affect PPI or reproductive performance. All cows, regardless of prepartum energy received, were in ideal BCS (≥ 5 mm fat cover) at the start of the breeding season and this may contribute to the lack of reproductive performance response observed.

Radunz et al. (2010) investigated different dietary energy sources and their impact on reproductive performance of 144 mature Angus crossbred cows. Cows were fed 1 of 3 dietary treatments at 167 ± 9 d of gestation until 1 wk prior to expected calving date. The treatments were as follows: ad libitum grass hay (8.2% CP, 68.1% NDF, and 41.1% ADF); limit fed grass hay and corn (11.4% CP, 24.2% NDF, and 13.0% ADF); and limit fed grass hay and DDGS (20.5% CP, 40.1% NDF, and 24.4% ADF). They reported no impact of gestational energy source on BCS ($P = 0.28$), or overall reproductive performance and pregnancy rates ($P \geq 0.66$). Yet there was a trend for cattle fed ad libitum hay to have increased plasma NEFA concentration at 3 h post feeding when compared with cows fed diets supplemented with corn and DDGS. This suggests that cattle fed ad libitum hay were mobilizing energy reserves. However, the severity of nutrient restriction in cattle fed ad libitum hay would appear minimal; as evident by the lack of difference in BCS among nutritional treatments.

Energy Balance

The amount of energy reserves present, represented by BCS, in cattle at parturition is 1 of the most important factors contributing to the timely resumption of estrus in beef cattle (Morrison et al., 1999). To better understand the impact of prepartum supplementation and BCS on postpartum reproduction, Bohnert et al. (2013) looked at the impacts of supplementation of DDGS during the last trimester of gestation in cows with two different body condition scores.

They used 120 Hereford x Angus mature cows in a 2 x 2 factorial design. The authors investigated the impact of DDGS supplementation on cows grazing flood meadow pasture and offered low-quality meadow hay (approximately 6% CP; DM basis) when cows were in an average BCS of 4.4 (**LBCS**) or 5.7 (**HBCS**). There were no BCS by supplementation interactions ($P > 0.05$) in this study. Similar to Stalker et al. (2006), Bohnert et al. (2013) found that supplementing DDGS during the last trimester of gestation resulted in elevated BW and BCS at calving and tended ($P = 0.08$) to increase BCS at weaning when compared with non-supplemented cows. Despite the increase in BCS, they found that supplementing DDGS during late gestation did not improve ($P = 0.94$) overall pregnancy rates. However, BCS of females during the last trimester of gestation did have an impact on cow reproductive performance. Not only did cows in the HBCS group have increased (62 kg greater; $P < 0.001$) BW in relation to LBCS cows; but just as importantly, HBCS cows experienced increased (92 vs 79%; $P = 0.05$) conception rates when compared with LBCS cows during a 60 d breeding season.

Body condition score at calving has been shown to affect the reproductive performance of beef cows, and managing beef cows to calve with moderate energy reserves (BCS = 5) can increase reproductive efficiency (Lents et al., 2008; Bohnert et al., 2013). Lents et al. (2008) offered varying amounts of a 42% CP supplement (0.7 vs 1.4 kg/d) to 45 multiparous Hereford x Angus cows at 115 d prepartum to manage cows to calve at a thin (4.3) or moderate (5.0) BCS. They found that when cows calved at a moderate BCS, they had decreased ($P < 0.01$) PPI and increased ($P < 0.05$) overall pregnancy rates at the end of the breeding season in relation to cows with a lower BCS. However, BCS at calving did not influence ($P = 0.72$) AI conception rates at first estrus. There is also evidence that contradicts the results of this study. A second study (Ciccioli et al., 2003) investigated the same concept with a very similar experimental design.

They designed a 2 yr study utilizing 82 primiparous cows (yr 1, n = 34; yr 2, n = 48). Breed composition of the cows, as well as BCS at calving, were similar to the Lents et al. (2008) study. They found the BCS of cows at calving (4.4 vs 5.1) did not impact ($P > 0.20$) resumption to estrus, ovarian function, or overall reproductive performance and conception rates.

Morrison et al. (1999) designed a study to investigate the impact of changes in energy reserves during late gestation on reproduction when cows calve at a moderate BCS. Spring-calving beef cows (n = 250) were randomly assigned and then divergently fed 3 months prior to the initiation of the experiment to achieve a BCS of ≤ 4 , 5 to 6, and ≥ 7 at the onset of the study (90 d prepartum). Cattle were then managed during late gestation (90 days before average expected calving date) to calve at a moderate BCS (5 to 6). This was accomplished by providing ad libitum hay or corn silage for thin and moderate BCS groups, while the over conditioned cows were limit fed hay to decrease body energy reserves. They found that the change in BCS during the latter part of gestation, regardless whether increasing or decreasing, did not impact ($P > 0.20$) reproductive performance of cows that calved in a moderate BCS. This study suggests that targeting a moderate BCS at calving and supplying a nutritional profile during late gestation that will move cattle toward moderate BCS at calving is a key factor to reproductive efficiency in beef cattle. Sorting cattle on BCS during gestation and providing greater planes of nutrition for thin cows and decreased planes of nutrition for over-conditioned cows could prove a valuable tool for beef producers to maximize reproductive performance and minimize feed costs within their herd.

Freetly et al. (2000) designed an experiment to further investigate the impact that BW change during gestation plays on reproductive performance in beef cows. There were 3 different treatments and they were as follows: **H-H-H**, where cows maintained BCS of 5.5 from the

second trimester of pregnancy until breeding; **L-H-H**, where cows lost BCS during the second trimester of pregnancy and regained it during the last trimester and had equal BCS to H-H-H at parturition; and **L-L-H**, where cows lost BCS during the second trimester and gained BCS after the 28th d of lactation so they were equal to other treatment groups at time of breeding. There were 48 cows per treatment. Total DMI for H-H-H and L-H-H were not different ($P = 0.23$); however, they were both greater ($P < 0.001$) than for L-L-H. Also, there were no differences ($P = 0.71$) in overall pregnancy rate (93, 92, and 88% for H-H-H, L-H-H, and L-L-H, respectively) across treatments. This study helps to illustrate that feeding cows an increased plane of nutrition during late gestation, and early lactation, to regain body energy reserves after a period of BCS loss can negate the poor reproductive performance associated with poor BCS at calving.

Cows need to have adequate body energy reserves to perform reproductive function. Spitzer et al. (1995) managed cows to calve at a BCS of 4 ($n = 73$), 5 ($n = 107$), and 6 ($n = 60$) from 90 d prior to calving until parturition. The authors reported that a greater ($P < 0.05$) percentage of cows that calved at a BCS of 5 (90%) or 6 (98%) had an estrus response by the end of the breeding season when compared with those that calved at a BCS of 4 (74%). Also, cows that calved in a BCS of 5 (80%) or 6 (96%) had greater ($P < 0.05$) overall pregnancy rates when compared with cows that calved at a BCS of 4 (56%). There was also a trend ($P < 0.10$) for cows that calved with a BCS of 6 to have increased pregnancy rates when compared with cows that calved at a BCS of 5. DeRouen et al. (1994) fed cows 74, 105, or 136% of NRC requirement for TDN from 90 d prepartum until calving and looked at its impact on reproductive performance of 476 spring-calving, primiparous cows. Cows that calved in a BCS of 6 (87%) or 7 (90.7%) had greater ($P < 0.05$) pregnancy rates in relation to cows that calved with a BCS of 4 (64.9%) or 5 (71.4%). The authors also reported that cows with a BCS ≥ 5 had 10 to 18 d shorter ($P < 0.05$)

PPI than cows calving with a BCS of 4. However, prepartum change in BCS and BW did not influence ($P > 0.05$) overall pregnancy rate or days to pregnancy. This would suggest that BCS at calving is a better predictor for reproductive performance of beef cows than BCS change during the prepartum period. This could prove valuable for production practices of beef producers, as supplying added nutrition for thin cows and restricting over-conditioned cows to target a moderate BCS could reduce feed costs and optimize reproductive performance.

Fat Supplementation

Dietary fat supplementation has been used extensively by the dairy industry to increase the energy density of diets. Within the beef industry, dietary fat has been used by producers to increase energy reserves and maintain BW in cows fed low quality forage diets. There may be some direct positive and negative effects that supplemental fats have on reproduction outside their addition of energy to diets. For instance, cholesterol serves as a precursor for P_4 synthesis (Funston, 2004) and it has been shown that increasing dietary fat supplementation can increase circulating cholesterol in cattle (Lammoglia et al., 1996). When cattle were fed 6.55% dietary fat (primarily derived from rice bran) in isocaloric and isonitrogenous diets, they had greater serum cholesterol concentrations ($P < 0.04$) when compared with cattle fed 5.20 and 3.74% dietary fat supplement (Lammoglia et al., 1996). This is important to keep in mind, as P_4 plays a major role in embryo implantation and survival (Grummer and Carroll, 1988). Also, linoleic acid (present in plants and fish meal) and eicosapentaenoic acid (present in fish meal) have been shown to decrease cyclooxygenase activity (Staples et al., 1998). Cyclooxygenase is a regulatory enzyme that converts linoleic acid to arachidonic acid, which is a precursor for $PGF_{2\alpha}$ (Funston, 2004). Suppression of $PGF_{2\alpha}$ could result in increased CL lifespan and embryonic survival (Funston, 2004).

Banta et al. (2011) investigated the impact of different sources of fat supplementation during gestation on reproductive performance of 127 multiparous Angus x Hereford cows. The supplements that these authors used were as follows: 1) 1.23 kg/d soybean hull based supplement (Control); 2) 0.68 kg/d linoleic sunflower seed and 0.23 kg/d control supplement (Linoleic); 3) 0.64 kg/d of mid-oleic sunflower seed and 0.23 kg/d of control supplement (Oleic). Supplement levels were formulated to provide similar CP, energy, and RDP. Treatment periods were concluded at calving and cows were then allowed to graze Bermuda grass or tall grass prairie pasture. These authors reported a difference ($P < 0.001$) in BW change (11, 3, -3 kg for control, linoleic, and oleic, respectively) during the first 62 d of the supplementation period across all supplemental treatments. This change in BW could be due to poor forage digestibility observed in a digestibility study using similar supplements and inclusion levels in Angus and Angus x Hereford cannulated steers (Banta et al., 2011). Supplement type did not have an impact on prebreeding ($P = 0.51$) and final ($P = 0.73$) BCS, PPI ($P = 0.95$), first-service conception rate ($P = 0.22$), or overall pregnancy rates ($P = 0.18$). Although lipid supplementation can prove a useful strategy to increase the energy density of a diet, it can decrease forage digestibility. This study suggests that feeding linoleic and oleic fats did not benefit the reproductive performance when compared with a soybean hull based control.

Bellows et al. (2001) performed two studies to evaluate the impact of increasing amounts of fat in the diet from d 215 of gestation until parturition on subsequent reproductive performance of pregnant beef heifers ($n = 149$). In exp. 1, they fed cattle isocaloric and isonitrogenous diets with different amounts of fat in the diet and they are as follows: control, 2.4% fat; safflower seed, 4.7% fat; raw soybeans, 3.8% fat; and sunflower seed, 5.1% fat. Dietary fat level did not affect ($P > 0.10$) the percentage of heifers in estrus at the beginning of

the breeding season; however, it did impact ($P < 0.05$) overall pregnancy rates. Heifers that were supplemented with fat during the last 65.5 ± 4.6 d of gestation had a 15.2% increase in overall pregnancy rates when compared with cattle fed the control diet (2.4% fat). In exp. 2, the authors fed cattle isocaloric and isonitrogenous diets with either low (2.2% fat) or high (sunflower seed, 6.5% fat) fat content from d 215 of gestation until calving. In exp. 2, nutritional treatment during gestation did not impact ($P = 0.13$) pregnancy rates (90.2% and 80.0% for cattle fed 2.2% and 6.5% fat, respectively). The authors hypothesized that the lack of reproductive response from increased fat in the diet in exp. 2 was due to the presence of higher quality forage in exp. 2. This suggests that feeding supplemental fat during gestation has the greatest impact on postpartum reproductive performance when cattle are grazing poor quality forage.

There has been minimal research on prepartum lipid supplementation and its impact on embryo recovery. Bader et al. (2005) utilized 40 mature beef cows with suckling calves in a study intended to investigate the effects of different supplements on postpartum estrus behavior, response to superovulation, and embryo recovery parameters. At 40 d prior to parturition until calving, cows were supplemented with either: whole soybeans (19.8% ether extract, 41.8% CP); or a soybean meal and soybean hull supplements (2.51% ether extract, 36.81% CP). At approximately 50 d postpartum, they synchronized estrus cycles using a GnRH synchronization protocol and superovulation was initiated with FSH injection. Cattle were bred using 4 total straws of semen and flushed 7 to 8 days post insemination. These authors reported that there was no difference ($P > 0.10$) in BCS between the supplemental treatments. Also, offering the higher fat supplement did not improve ($P > 0.10$) total number embryos recovered, number of transferrable embryos, number of degenerate embryos, or embryo quality. Graham et al. (2001) reported a 23% increase ($P \leq 0.05$) in first-service conception rates when cows were

supplemented with whole soybeans (85.7%) when compared with cattle receiving corn gluten and soybean meal supplement (62.8%). The Graham et al. (2001) study would suggest that cattle receiving the whole soybean meal supplementation should have increased reproductive performance in embryo production. However, Bader et al., (2005) reported that the increased lipid supplementation (19.8 vs 2.51% ether extract) from a whole soybean diet did not improve ($P > 0.10$) embryo recovery and quality.

Postpartum Nutrition

Postpartum nutrition is an important factor in reducing the postpartum interval and increasing reproductive efficiency in beef cows (Hess et al., 2005). When cattle enter a negative energy balance during gestation, they tend to mobilize energy reserves and consequently suffer a reduction in BCS. It has been shown that when cows calve at a poor BCS (4 or less on a 9 point scale), increasing postpartum dietary energy can decrease the PPI (Richards et al., 1986; Lalman et al., 1997) and increase conception rates (Richards et al., 1986).

Protein and Energy

Supplementing increased amounts of energy post calving has had conflicting results on reproductive performance in beef cattle. Studies supplementing increased energy during the postpartum period have shown to increase (Lalman et al., 1997; Ciccioli et al., 2003) reproductive efficiency while others (Lents et al., 2008) have not affected reproductive efficiency in beef cows. Ciccioli et al. (2003) designed an experiment to investigate the impact of supplementing additional energy levels during the postpartum period when cows calved at a BCS that was considered poor (BCS = 4) or moderate (BCS = 5) and its effect on reproductive performance. Ciccioli et al. (2003) designed a 2 year study using 82 Angus x Hereford cows (Yr

1, n = 34; Yr 2, n = 48) and fed 2 postpartum diets for 71 ± 3 d. Ciccioli et al. (2003) targeted moderate or increased gains in cows. Diets contained ad libitum prairie hay and 2 kg/d of 38% CP supplement or free access to a 1.61 Mcal NE_m /kg DM diet containing: rolled corn, ground alfalfa, cottonseed hulls, cane molasses, and salt for the moderate and increased gains, respectively. They reported that postpartum nutrition did not interact with BCS at calving to improve reproductive performance; however, increased energy supplementation postpartum did decrease ($P < 0.01$) cow PPI and increase ($P < 0.03$) conception rates at first estrus. The authors also observed that cattle fed the greater amount of energy had elevated BCS at the end of the treatment period. These authors concluded that additional supplemental energy put cows in a positive energy balance, which allowed them to return to estrus and conceive sooner when compared to cows that were supplemented with additional protein only.

Lents et al. (2008) used 48 Angus x Hereford cows that calved in a thin (< 5) or moderate (≥ 5) BCS to investigate the impact of postpartum protein supplementation on reproduction. They supplemented varying amounts of 42% CP (1.2 vs 2.5 kg/d) for 60 d postpartum to cows grazing native grass pasture. They reported that postpartum supplement amount did not impact BCS ($P = 0.24$), PPI ($P = 0.76$), or AI pregnancy rates ($P = 0.38$). This would suggest that feeding cows 1.2 vs 2.5kg/d of 42% CP supplement postpartum is not a sufficient difference to generate a reproductive response in beef cows. Postpartum nutrition did not impact ($P = 0.24$) BCS in the Lents et al. (2008) study and this would suggest that there were not enough differences in postpartum performance to induce a reproductive response.

Postpartum supplementation has been shown to minimize the negative impacts that poor BCS at calving has on reproductive performance in beef cattle (Richards et al., 1986). Richards et al. (1986) designed a 3 yr study to investigate the impact postpartum nutrition had on the

reproductive performance of 355 Angus and crossbred beef cows when they calved at a poor (≤ 4) or good (≥ 5) BCS. Cattle were stratified by age, BCS at calving, breed, and calving date. They had 4 postpartum nutritional treatments that are as follows: 1) a high energy, corn silage based diet (25.8 Mcal ME) that targeted a gain of 0.45 kg/d; 2) a maintenance energy diet (20.7 Mcal ME) that targeted cattle to maintain the same weight from parturition to breeding; 3) a low energy diet (14.7 Mcal ME) that targeted a loss of 0.45 kg/d; and 4) low flush diet where cattle were fed the low energy diet until 14 d prior to onset of the breeding season, and from then on were offered a 36 Mcal ME diet ad libitum. These authors reported that when cattle had calved in a good BCS (≥ 5), postpartum nutrition did not affect cow PPI or overall pregnancy rates. However, when cows calved at a poor BCS (≤ 4), cows that maintained or gained weight during the postpartum period had an increased cumulative percentage of females exhibiting estrus by d 20 ($P < 0.06$), 40 ($P < 0.01$), and 60 ($P < 0.01$) of the breeding season. Also, when cows calved at a poor BCS, providing nutritional supplementation to target maintenance or increased weight gain during the postpartum period did increase the cumulative percent pregnant by 40 and 60 d into the breeding season ($P < 0.05$). This suggests that when cows calve in a poor BCS, producers need to offer additional nutrition in the form of supplementation during postpartum period to ensure cows return to estrus and conceive.

A similar study (Spitzer et al., 1995) was designed to investigate the impact of different amounts of postpartum weight gain fed to cows with BCS ranging from 4 to 6 (on a 1 to 9 scale) on reproductive performance of 240 primiparous beef cows. Cows were randomly allotted and managed to calve at a BCS of 4, 5, or 6 by grazing manipulation and supplemental feed offered from 90 d prior to parturition to calving. At parturition, cows were blocked by breed, BCS, calving date, and then randomly allotted to one of two postpartum nutritional treatments. After

calving, cows were fed to target gains of either 0.45 or 0.90 kg/d from parturition to the beginning of the breeding season. Average weight gains during the postpartum treatment period were 0.44 and 0.85 kg/d and, thus, representative of the targeted gains these authors wished to achieve. A greater percentage of cows that were fed for increased ($P < 0.05$) postpartum BW gain were showing estrus by d 20, 40, and 60 of the breeding season. Also, cows that were fed for increased BW gain during the postpartum period had increased ($P < 0.05$) pregnancy rates by d 20, 40, and 60 of the breeding season.

Energy Balance

Assigning BCS to cattle within a herd is one of the most practical management tools for estimating the amount of energy reserves present in beef cattle. To better understand the relationship of BCS at breeding and reproductive performance of beef cows, Renquist et al. (2006) designed a 7 year study starting with 260 multiparous crossbred beef cows and 45, 54, 27, 68, 54, and 45 replacement heifers entering the study for years 2 through 7, respectively. Body weight and BCS were monitored at parturition, breeding, time of weaning, and between weaning and calving. The authors reported a quadratic relationship between BCS at breeding and pregnancy rate; whereas, optimal BCS at breeding for increased pregnancy rates were reported between 4.5 and 5.5 (on a 1 to 9 scale). They observed a dramatic decline in pregnancy rates when BCS was greater than 6.5 or less than 3 at time of breeding. Also, change in BCS from parturition to breeding did not impact ($P < 0.19$) overall pregnancy rates in cattle utilized in this study. Renquist et al. (2006) also reported that BCS at breeding had an impact on length of calving interval. Cows with a BCS of 3.5 at breeding had a longer calving interval than cows that calved with BCS greater than 4.5 ($P < 0.04$). When cattle were at a BCS of 6.5 at breeding, they had a shorter ($P = 0.04$) calving interval when compared with cows in a BCS 3.5 or 2.5;

however, cows with a BCS of 6.5 at breeding still had a reduction in pregnancy rates in relation to cattle in BCS between 4.5 and 5.5.

Rae et al. (1993) also investigated the impact of BCS at breeding on pregnancy rates in beef cattle raised in Florida. They used 8 commercial operations for a 2 yr study with 3,734 total records for analysis. Cows in this study were exposed to bulls for a 120 to 150 d breeding season. The authors reported a relationship between BCS at time of pregnancy examination (60 to 100 d following conclusion of the breeding season) and overall pregnancy rates. Rae et al. (1993) reported that average pregnancy rates were 31, 60, and 89% for cows in BCS of 3 or less, 4, and 5 or greater (using a 1 to 9 scale), respectively ($P < 0.05$). The authors also described peak pregnancy rates when cows were in BCS of 5 or greater, as these cattle experienced pregnancy rates of 85% or better (average 89%), and cows with BCS of 4 or less had decreased pregnancy rates (59% average; $P < 0.05$). This suggests that managing cattle to maintain or move toward a BCS of 5 at breeding is important to ensure cattle can perform reproductive function to maintain a defined calving window.

Fat Supplementation

Beef producers have utilized supplemental fats to increase dietary energy when cows have increased requirements and forages do not meet their needs. However, there are limitations and considerations that come with supplementing dietary lipids. Dietary fat has been shown to decrease forage digestibility (Pavan et al., 2007) and should not exceed 20% of dietary ME for ruminant diets (Palmquist, 1994). Yet fat supplementation is still a useful tool to increase energy density in beef diets.

There has been interest in the impact of different supplemental fat sources during the postpartum period on reproductive performance of beef cows. Bottger et al. (2002) investigated the impact of high linoleate and high oleate safflower diets on the reproductive performance of 36 Angus x Gelbvieh primiparous cows. Cows had ad libitum access to a 7.8% CP native grass hay and were supplemented for 90 d with one of the following nutritional treatments: 1) a corn and soybean meal supplement (9.67% ADF, 22.39% NDF, 18.51% CP, and 3.8% total fatty acid); 2) a high linoleate safflower supplement (31.20% ADF, 42.82% NDF, 20.14% CP, and 31.36% total fatty acid); or 3) a high oleate safflower supplement (34.73% ADF, 45.06% NDF, 17.78% CP, and 29.32% total fatty acid). The authors noted that supplemental treatment did not impact forage intake at d 30, 60, or 90 of the treatment period. Cows fed the high linoleate supplement had increased ($P \leq 0.04$) BCS at the conclusion of the treatment period when compared with high oleate and corn and soybean meal supplements. Yet, supplemental treatment did not affect PPI ($P = 0.80$), number cycling within the 90 d treatment period ($P = 0.50$), or overall pregnancy rate ($P = 0.96$). These data are representative to a similar study (Lake et al., 2005) that also investigated the impact of high linoleate and high oleate supplementation during the postpartum period on reproductive performance of 72 Angus x Gelbvieh cows. Lake et al. (2005) reported that postpartum fat supplementation did not impact first-service conception rate ($P = 0.56$) or overall pregnancy rate ($P = 0.55$).

Another study (Shike et al., 2013) investigated the impact of supplementing fats varying in fatty-acid composition on reproduction in beef cattle. The authors fed 4 different nutritional treatments to 480 Angus x Simmental cows from 36 ± 0.6 d prepartum to 72 ± 0.6 d postpartum. All cows grazed endophyte infected tall fescue, red clover, and white clover pastures and were supplemented with the following treatments: raw soybeans (41.05% CP, 20.36% ether extract on

DM basis); corn, soybean meal, and OmegaFlax (18.89% CP, 18.76% ether extract on DM basis); corn, soybean meal, and Energy Booster 100 (hydrolyzed animal fat; 18.89% CP, 21.52% ether extract on DM basis); corn and soybean meal (control; 18.34% CP, 5.69% ether extract on DM basis). Cows were synchronized using a CoSynch + CIDR or 7-11 estrus synchronization protocol and AI on d 76 ± 0.06 d postpartum. After AI, cows were exposed to bulls for a 45 d breeding season. Supplementing fat did not improve reproductive performance of cows when compared with the control supplement. There were no difference in the number of cows cycling prior to the onset of the breeding season ($P \geq 0.22$), first service AI conception rates ($P \geq 0.31$), or overall pregnancy rates ($P = 0.81$). However, within the fat supplement treatments there were differences in overall pregnancy rates. Cows that were supplemented with raw soybeans had lower pregnancy rates ($P = 0.01$) in relation to cows fed flaxseed supplement and tended ($P = 0.06$) to have lower pregnancy rates than cows fed hydrolyzed animal fat. Shike et al. (2013) attributed the poorer reproductive performance associated with feeding raw soybeans to the potential increased phytoestrogen content of the supplement, which can negatively impact reproduction.

The impact that supplemental fats during the postpartum period have on reproductive performance are inconsistent. They have been shown to influence (Sklan et al., 1991; Shike et al., 2013) and not influence (Bottger et al., 2002) pregnancy rates. When beef cows were fed calcium soaps of fatty acids (CSFA) for 105 d, beginning at 61 ± 36 d prepartum, the percentage of cows pregnant at 50 d into the breeding season was increased (62.5 vs 35.5 %; $P < 0.02$) when compared with cattle not fed additional fat supplementation (Espinoza et al., 1995). Feeding fats can be utilized to increase energy density of the diet, and perhaps prove more beneficial when cattle are grazing low quality forages as the utilization and digestibility of high quality forages

can be decreased when feeding supplemental fats (Pavan et al., 2007). However, its direct influence on reproduction appears to be inconsistent at this point.

Embryo Recovery

Nolan et al. (1998) designed a study to investigate the impact of short term nutrient change on follicle growth and embryo production of 61 beef heifers. Cattle were fed 2 different planes of nutrition ($28.6 \text{ Mcal}\cdot\text{kg}^{-1}\cdot\text{head}^{-1}\cdot\text{d}^{-1}$; and $9.6 \text{ Mcal}\cdot\text{kg}^{-1}\cdot\text{head}^{-1}\cdot\text{d}^{-1}$). Cattle were synchronized 10 d after the beginning of nutritional treatments and superovulation began on the fifth day after the initiation of the synchronization protocol. Twenty eight cows (14 per treatment) were used for embryo recovery and ovaries were recovered from a different 16 heifers (8 per treatment) that received 8 injections of porcine FSH (pFSH). This study showed that cattle receiving the $28.6 \text{ Mcal}\cdot\text{kg}^{-1}\cdot\text{head}^{-1}\cdot\text{d}^{-1}$ increased ($P < 0.0001$) weight gain during the trial when compared with cattle fed $9.6 \text{ Mcal}\cdot\text{kg}^{-1}\cdot\text{head}^{-1}\cdot\text{d}^{-1}$. During the last 3 days of synchronization, while the CIDR was inserted, heifers on $9.6 \text{ Mcal}\cdot\text{kg}^{-1}\cdot\text{head}^{-1}\cdot\text{d}^{-1}$ had greater ($P < 0.05$) concentrations of P_4 (4.72 and 3.75 ± 0.3 , for Low and High respectively) in relation to those fed $28.6 \text{ Mcal}\cdot\text{kg}^{-1}\cdot\text{head}^{-1}\cdot\text{d}^{-1}$. Along with this, cattle fed the Low diet also had a numerical increase in number of corpus luteum (CL) and total transferrable embryos recovered (52% vs 44%) when compared with cattle fed the High plane of nutrition. In embryos that were cultured for 24 hr after collection, there were a greater (78 vs 48; $P < 0.01$) number of embryos that developed into the blastocyst stage when recovered from heifers that were fed $9.6 \text{ Mcal}\cdot\text{kg}^{-1}\cdot\text{head}^{-1}\cdot\text{d}^{-1}$ in relation to embryos recovered from heifers fed $28.6 \text{ Mcal}\cdot\text{kg}^{-1}\cdot\text{head}^{-1}\cdot\text{d}^{-1}$. However, there were no differences in follicle size or number across nutritional treatments; nor was there any statistical difference in estradiol concentration in follicular fluid. Despite the lack of statistical significance, cattle fed $9.6 \text{ Mcal}\cdot\text{kg}^{-1}\cdot\text{head}^{-1}\cdot\text{d}^{-1}$ had numerically greater concentrations of P_4 in follicular fluid

when compared with cattle fed 28.6 Mcal·kg⁻¹·head⁻¹·d⁻¹ (90.5 ± 12.5 vs 66.9 ± 13.8 ng/mL for cattle fed 9.6 Mcal·kg⁻¹·head⁻¹·d⁻¹ and 28.6 Mcal·kg⁻¹·head⁻¹·d⁻¹, respectively). This could be attributed to increased blood flow to the liver and P₄ clearance from hepatic tissue caused by the increased plane of nutrition that the High diet provided (Nolan et al., 1998).

Another study (Yaakub et al., 1999) was designed to investigate the effect of type and quantity of concentrate fed to cows on embryo quality in 76 Charolais influenced crossbred heifers. There were 4 dietary treatments fed for 116 d prior to flush: limit fed barley at 3 kg/d and ad libitum silage (12.1% CP); ad libitum fed barley and 1 kg DM/d silage; citrus/beet pulp at 3 kg/d and ad libitum silage; and ad libitum citrus/beet pulp and 1 kg DM/d silage. The authors found that heifers that were limit fed concentrates had a greater ($P < 0.06$) response to FSH than heifers fed ad libitum concentrate feeds. Also, the number of freezable and transferrable embryos was increased ($P < 0.05$) when cows were fed 3 kg/d concentrates in relation to ad libitum. However, dietary treatment did not impact ($P > 0.05$) fertilization rate. Decreased embryo quality, reported in cattle that were fed for ad libitum intake could be due to increased P₄ clearance from hepatic tissue that is caused by an increased blood flow associated with greater nutrient intake (Nolan et al., 1998).

Metabolic and physiological relationship between nutrition and fertility

Postpartum resumption of cyclicity

Reestablishing estrus after parturition is an important factor when considering reproductive efficiency (Hess et al., 2005). There are several key hormones that initiate this process. In a review, Hess et al. (2005) described the physiological factors that reestablish estrus after parturition. Transportation of GnRH from neurosecretory neurons in the hypothalamus to

the anterior pituitary gland stimulates FSH and LH secretion and production (Senger, 2012). Follicle stimulating hormone is very important in early follicular growth (Senger, 2012) and LH plays a role in the maturation of the dominant follicle (Hess et al., 2005). Ovarian follicles produce estradiol and when its concentration reaches a threshold, there is a surge of GnRH released (Senger, 2012). This surge of GnRH is followed by a high-amplitude release of LH. The LH release results in ovulation (Senger, 2012). The recently formed CL produces P₄. Progesterone is crucial for embryo implantation and survival (Grummer and Carroll, 1988) and maternal recognition of the pregnancy must occur to continue CL lifespan and P₄ production must continue to suppress GnRH release (Hess et al., 2005). High levels of prostaglandin F_{2α} (PGF_{2α}) signals for CL regression at the conclusion of the estrus cycle (before maternal recognition of pregnancy occurs) and also has a role in uterine involution (Short et al., 1990). However, if pregnancy is maintained, the CL is responsible for primary production of P₄ until approximately d 90 of gestation. This is when the placenta becomes the major source for P₄ production.

Glucose and GnRH

Nutrition impacts reproduction through various changes in metabolic hormones due to cattle being in a positive or a negative energy balance. Glucose is one of the most important metabolic substrates involved with reproductive success in beef cattle (Hess et al., 2005). Glucose is the primary fuel source utilized by the central nervous system which plays a major role in the release of GnRH (Keisler and Lucy, 1996; Hess et al., 2005). Gonatropin-releasing hormone is crucial for the synthesis and release of FSH and LH (Senger, 2012). Propionate is the major gluconeogenic VFA that can be utilized to synthesize glucose in ruminant animals (Hawkins et al., 2000). However, when cows graze poor quality forage, the major VFA produced

is Acetate. When cattle enter a negative energy balance due to inadequate nutrition and lose weight, body fat is mobilized and used in oxidative metabolism in conjunction with catabolism of proteins from tissues to supply AA for glucose production (Hawkins et al., 2000). However, excess glucose does not necessarily provoke increased reproductive efficiency; as Keisler and Lucy (1996) described the potential for a glucose threshold that is permissive as opposed to causative. In this case, glucose would need to be at or surpass the threshold to ensure proper reproductive function.

Insulin and IGF-1

Insulin and IGF-1 are considered to be factors that signal nutritional status to the reproductive axis (Zulu et al., 2002; Hess et al., 2005). Growth hormone regulates the expressions and secretion of IGF-1 from the liver. When cattle enter a negative energy balance, IGF-1 concentrations are decreased. Lalman et al. (2000) reported that when 36 Angus and Angus sired crossbred cows were underfed (accomplished with low quality hay) during the last trimester of pregnancy and then transitioned onto diets containing 4 different amounts of ME (1.8, 2.1, 2.4, and 2.7 Mcal ME/kg) postpartum, there was an effect on IGF-1 concentrations. The authors reported that increasing ME in the diet resulted in a linear increase ($P = 0.001$) in the concentration of insulin and IGF-1. This suggests that when cattle that calve in a thin (BCS = 4.0 ± 0.1) BCS and are fed increasing levels of ME during the early postpartum period, there is an increase in IGF-1 and insulin concentration. This is important because plasma concentrations of IGF-1 are positively associated with circulating concentrations of insulin, glucose, and cow body condition (Zulu et al., 2002). Insulin-like growth factor-1 also plays a role in follicular dynamics in reproduction. Steroidogenesis of follicular cells by FSH and LH are supported by IGF-1 (Zulu et al., 2002). Also, IGF-1 can increase the sensitivity of follicular cells to FSH and LH, which

would promote follicular growth and maturation (Zulu et al., 2002). This suggests that not only does IGF-1 serve as an indicator of nutritional status of the animal, but also assists in follicular dynamics involved in reproduction.

NEFA

When cattle graze poor quality forages, or are fed a diet that is unable to meet the requirements for their stage of production, they enter a negative energy balance. When cattle enter a negative energy balance, stored energy reserves (fat) are mobilized to provide additional energy in order to meet their requirements. When fats are mobilized, lipolysis of stored fat results in the production of NEFA (Adewuyi et al., 2005). Elevated NEFA concentrations are typically seen during peak lactation when cow intake does not meet the nutritional requirements for lactation. Increased NEFA concentrations have been associated with negative energy balance in cattle (Canfield and Butler, 1990). Also, cows that are in a negative energy balance have been shown to have reduced concentrations of LH (Canfield and Butler, 1990). When cattle are undernourished and NEFA concentrations are increased, reproductive function could be compromised due to the potential inverse relationship between NEFA and LH.

Urea Nitrogen

Wiley et al. (1991) investigated the impact of feeding diets either at (5kg of TDN, 1kg of CP/animal/d) or below (2.5kg of TDN, 0.5kg of CP·animal⁻¹·d⁻¹) maintenance requirement of beef cows for 75 d prior to parturition and its impact on reproduction, blood urea nitrogen (BUN), and LH. Although this study did not illustrate a response of the diet on PPI and overall pregnancy rates, there were differences in blood hormone concentrations. Restricted fed cows during late gestation had increased ($P < 0.05$) levels of BUN (22.58 vs 20.81 mg/dl) at 51 d

postpartum. Research has shown that elevated amounts of circulating nitrogen in plasma (> 19 mg/dL) resulted in decreased pregnancy rates in dairy cattle (Butler et al., 1996). The lack of reproductive response due to elevated BUN may be contributed to the fact that both treatment groups were greater than the threshold reported by Butler et al (1996). Also, cattle were receiving diets that are in excess of NRC for CP and TDN requirements during the postpartum period and it has been shown that improving postpartum nutrition, in this case through excess TDN, can help minimize the negative impact that poor energy balance during gestation can have on cow reproductive performance (Lalman et al., 1997). Also, providing excessive amounts of dietary protein has been shown to impact follicular growth and maturation by increasing ammonia concentration in follicular fluid and resulting in decreased blastocyst formation (Robinson et al., 2006). This is very important when considering superovulated females intended for embryo recovery, as medium sized follicles tend to be most affected by this increased ammonia concentration (Robinson et al., 2006). Thus, this phenomenon may not be as evident in cattle ovulating one dominant follicle.

Roberson et al. (1991) investigated changes in BW and its influence on LH and FSH in ovariectomized beef heifers. At 21 d post ovariectomy, cattle were fed to either increase or decrease BW over 8 wk periods and then reallocated to the opposing treatment. They reported that as average daily gain was increased, there was a greater frequency of LH pulse in a quadratic fashion ($P < 0.05$; $R^2 = 0.44$) and amplitude of LH pulses were decreased in a linear fashion ($P < 0.05$; $R^2 = 0.61$). Also, as BW increased, the mean concentration of FSH tended to decrease in a quadratic fashion ($P < 0.10$; $R^2 = 0.24$). At week 14, both treatment groups were similar in BW and those that were losing BW during the second 8 wk feeding period had elevated plasma urea nitrogen (PUN; $P < 0.05$) and decreased plasma glucose levels ($P < 0.05$) when compared with

those that were gaining weight during the second 8 wk feeding period. As previously mentioned, glucose is the primary metabolic fuel used by the central nervous system and inadequate glucose could result in decreased release of GnRH from the hypothalamus (Hess et al., 2005). Release of GnRH from the hypothalamus is a precursor for LH production and secretion (Hess et al., 2005). When cattle in this study were losing weight, they had decreased concentrations of glucose and decreased frequency of LH pulses. This would suggest that for cattle to have proper reproductive function, they must be maintaining or gaining weight.

Progesterone and estradiol

Another study, that was discussed previously, (Corah et al., 1974) was designed to look at the impacts of varying levels of energy fed during gestation and its impacts on plasma P₄ and estradiol concentrations. Twelve first calf heifers were either fed 11.4 or 17.6 Mcal DE/day from 100 d prior to their expected calving date until parturition. At calving, all cows were transitioned to a 28.7 Mcal DE/day diet. These authors noted that there were no differences in plasma P₄ and estradiol concentrations between prepartum nutritional treatments. However, they did discuss that plasma P₄ concentrations tended to be slightly elevated at d 3 and 5 postpartum for cows fed the 17.6 Mcal DE/day, diet when compared with those fed 11.4 Mcal DE/day prior to parturition. The lack of statistical significance may be due to the number of cows on study (n=12) and using more cows in this trial may have elicited a statistically significant response at d 3 and 5 postpartum.

Reproductive success in beef cattle is reliant on proper endocrine function and hormone concentrations. It has been shown that increased concentrations of P₄ and estradiol are linked with increased in pregnancy maintenance (Atkins et al., 2013). Nutrition can influence hormone

concentrations (Hess et al., 2005). Also, supplemental fats have been shown to increase concentrations of serum cholesterol (Lammoglia et al., 1996) and could then increase progesterone concentration as cholesterol serves as a precursor for P₄ production (Funston, 2004). Guedon et al. (1999) showed that cows with higher serum cholesterol concentrations from 2 wk prior to parturition until 4 wk postpartum had a decreased ($P < 0.05$) interval from calving to resumption of ovarian cyclicity. Yet, it has also been shown that feeding varying amounts of supplemental fats had no effect on P₄ concentrations (Carr et al., 1994).

BW and BCS associated with reproductive hormones

In studies investigating the impact of postpartum nutrition and BCS at calving on reproductive function, postpartum nutrition has been shown to influence (Ciccioli et al., 2003) and not impact (Lents et al., 2008) plasma concentrations of IGF-1. Ciccioli et al. (2003) noted that plasma IGF-1 concentrations were increased ($P < 0.01$) when cattle had increased postpartum BW gains (.90 vs .45kg/d) from a diet containing an increased energy density and calved at a BCS of 5; however, there were no differences ($P > 0.60$) in plasma IGF-1 concentrations, regardless of postpartum nutrition, when cattle calved at a BCS of 4. Ciccioli et al. (2003) also showed that when cattle that received an increased plane of nutrition for greater weight (0.90 kg/d) gains were moved to a maintenance diet, they had elevated ($P < 0.01$) plasma NEFA concentration in relation to cows that were targeted for lower gains (0.45kg/d). This indicates that the maintenance diet may not have provided adequate nutrition for cattle previously receiving an increased plane of nutrition to truly maintain their BW and BCS.

Luteinizing Hormone

Luetenizing hormone plays an important role in reproduction by assisting in the final maturation of the dominant follicle and inevitably leading to ovulation (Hess et al., 2005). A pulsatile release of GnRH is responsible for the production and release of LH. There has been interest in LH concentration in beef cattle and its response to GnRH. Whisnant et al. (1985) investigated the impact of varying amounts of dietary energy on serum LH concentrations in 10 crossbred primiparous beef cows from parturition to approximately 63 d postpartum. Diets were formulated to either provide 120 or 80% of recommended dietary energy from NRC. At 60 d postpartum, calves were separated from cows to trigger a LH release and 72 h later, cows were administered with 2 mL of GnRH to initiate another LH release. The LH response 24 h after calf removal was triggered sooner ($P < 0.01$) in cows receiving diets containing excess dietary energy when compared with those fed under their requirement. Also, cows receiving diets containing excess dietary energy also had an increased ($P < 0.01$) initial mean serum LH concentration due to the quicker response to calf removal. However, at 48 hr post calf separation, cows fed under their requirement had increased serum LH concentrations and became similar to those fed 120% NRC recommended dietary energy. Also, when cows were injected with GnRH, there was a greater ($P < 0.01$) LH response in cattle fed under their requirement for dietary energy, and thus suggesting that these cows had increased pituitary stores of LH when compared with cattle that were overfed energy. However, Kane et al. (2002) reported an enhanced ($P = 0.07$) LH response to GnRH in cattle that were fed the 335g of UIP/d compared with 180 and 165g of UIP/d. These inconsistent responses to GnRH induced LH responses offer justification for future research in this area. However, relying on calf removal to increase LH response should be benefited by increasing the plane of nutrition and energy content of the diet offered to cows.

Another study (Richards et al., 1991) investigated the impact of BCS and different planes of nutrition on reproductive hormones in 15 non-pregnant Hereford cows. At the onset of the study, all cows were in a moderate BCS (5.2 ± 0.3 ; using a 1 to 9 scale) and were then randomly allotted to treatments. Five cows received a diet that would maintain BW and BCS and 10 cows were limit-fed (2.5 kg prairie hay/ d in drylot) for 26 wk. Limit-fed cows became anestrus, whereas cattle fed to maintain BW and BCS displayed normal estrous cycles throughout the study. All cows were synchronized using 2 $\text{PGF}_{2\alpha}$ injections at 11 d apart. Five to 7 d after the onset of estrus from synchronization, 5 cows on maintenance diet and 5 cows on restricted diet were ovariectomized. On d -1, 0, 1, 2, 3, 4, 5, and 10 in relation to ovariectomy surgery, blood samples were taken for analysis of LH, P_4 , and IGF-1. The authors found that prior to ovariectomy procedure, cows that were fed to maintain BCS and BW had a numerically greater P_4 concentration (2.49 ± 0.49 ng/ml) than cows fed restricted diet (0.64 ± 0.10 ng/ml). Concentrations of IGF-1 were greater ($P < 0.05$) in cows that were fed to maintain BW and BCS when compared with cows that were limit fed to lose weight and condition. Another important observation reported by Richards et al. (1991) was the linear increase ($P < 0.01$), in relation to d post ovariectomy, seen in LH concentration of cows fed a maintenance diet but not seen in cows fed a restricted diet. The authors also reported a numerical increase in number of cows with a CL present at time of ovariectomy surgery and a trend ($P < 0.12$) for greater number of small follicles (≤ 3.9 mm) for the cows fed a maintenance diet in relation to those fed a restricted diet. This data suggests that prolonged dietary restriction has a negative impact on reproductive hormones and function. Cows that are underfed and lose BW could have decreased glucose (Roberson et al., 1991), and this could potentially decrease GnRH release from the hypothalamus and reduce LH concentration. Another study (Wiley et al., 1991) also reported that cattle that

were fed below their nutritional requirements had decreased amounts of insulin at 14 d postpartum. Insulin has been shown to increase LH binding activity (Wiley et al., 1991). LH concentrations were not affected by prepartum nutrition; however, LH pulses were of less magnitude for restricted fed cows than those that were fed their maintenance requirement during gestation.

Conclusion

Plane of nutrition impacts BW and BCS in beef cattle. Although results are variable, supplementing energy, protein, or lipids has been shown to improve reproductive efficiency. Also, nutrition can influence concentrations of serum and plasma hormones important in reproductive function. When cattle graze poor quality forage, that do not meet their requirements, hindrance of key hormones can reduce reproductive success. Excess dietary intake at the time of superovulation and prior to embryo recovery has been shown to decrease embryo quality. However, there is limited information on the impact of prepartum nutrition on superovulation as well as the recovery and quality of preimplantation embryos. Further research is needed to better understand the relationship between gestational plane of nutrition and embryo development and recovery in beef cattle.

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CHAPTER 2

EFFECT OF PREPARTUM PLANE OF NUTRITION DURING MID- OR LATE GESTATION ON COW BW, BCS, BLOOD HORMONE CONCENTRATIONS, AND PREIMPLANTATION EMBRYO

INTRODUCTION

The cow-calf sector of the beef industry has a constant challenge to ensure cattle have access to adequate nutrition to optimize reproductive success. Reproduction and nutrition are the two most important factors when considering profitability in the cow-calf industry (Hess et al., 2005). Feed costs account for 63% of the total annual cow cost, and nutritional management plays a major role in the financial viability of beef enterprises (Miller et al., 2001). Technologies, such as embryo transfer, are used to increase profitability and efficiency in the beef industry. Embryo transfer became a viable option for the North American beef industry during the early 1970s (Hasler, 2003). There is much evidence suggesting that increasing prepartum nutrition improves subsequent reproductive performance in beef cattle (Sasser et al., 1988; Selk et al., 1988; Lents et al., 2008). Prepartum nutrition affects BW and BCS (Stalker et al., 2006; Bohnert et al., 2013) and can manipulate circulating hormone concentrations (Wiley et al., 1991; Lalman et al., 1997) that are important in cattle achieving reproductive success. Also, postpartum nutrition has been shown to influence embryo quality (Nolan et al., 1998). Increasing postpartum maternal plane of nutrition can have negative effects on females involved in embryo production (Robinson et al., 2006). However, there has been minimal research investigating the impact that prepartum nutrition has on postpartum embryo production in beef cattle. Our hypothesis was

cattle fed below maintenance requirement during gestation will have decreased, and cattle fed to surpass maintenance requirement during gestation will have greater, BW, BCS, circulating hormones, and total embryo production compared with cattle fed to achieve maintenance requirement. Objectives were to evaluate the potential effect of prepartum plane of nutrition during mid- or late gestation on cow BW, BCS, blood hormone concentrations, and preimplantation embryo.

MATERIALS AND METHODS

All animal procedures were approved by the University of Illinois Institute of Animal Care and Use Committee and followed the guidelines recommended in the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 2010).

Experiment 1

Animal and Diet Management

Thirty-three multiparous Angus and Angus x Simmental crossbred cows (BW = 664 ± 78 kg) were used for this experiment at the University of Illinois Beef Cattle and Sheep Field Research Laboratory in Urbana, IL. Cows were housed in 3 sided barns open to the South. Pens were constructed of 5.08 cm galvanized steel tubing with partially slatted concrete floors and an area with a solid floor in which only calves were able to access. Both cow and calf areas were equipped with rubber matting. Pen dimensions were 10.36 m x 4.88 m. Cows were stratified by calf sire and BW and allotted to 9 pens. Pens were randomly assigned to treatments resulting in 3

replications of each treatment. There were 6 pens containing 4 animals per pen and 3 pens containing 3 animals per pen.

Three treatments were used to investigate the effects of maternal plane of nutrition during gestation on reproductive performance. Cow requirements were determined based on NRC values for actual group BW, BCS, and d pregnant with estimated calf BW and peak milk production (664 kg BW, BCS of 6, carrying a 36 kg calf, 9 kg of milk production during peak lactation, and 233 d pregnant). Cows were limit-fed the same diet (Table 1) to achieve: 100% NRC energy and protein requirement (9.1 kg DMI), 70% NRC requirement (6.4 kg DMI), or 130% NRC requirement (11.9 kg DMI). Cows were offered a free choice mineral while consuming the treatment diets. Diets were fed during late gestation (91 ± 4 to 8 ± 4 d prepartum). Upon completion of the treatment period, cows were fed a common diet (Table 1), that was formulated to meet NRC requirements, and offered free choice mineral. Cows were weighed 2 consecutive days, using a Flying W (Flying W Livestock Equipment, Watonga, OK) squeeze chute equipped with a Tru-Test (Tru-Test Incorporated, Mineral Wells, Texas) weighing system to determine BW, and a technician assigned a BCS (1 = emaciated to 9 = obese; (Wagner et al., 1988)) at time of breeding (76 ± 5 d postpartum).

Feed sampling and analysis

Dietary ingredient samples were collected every 14 d throughout the trial and composited over the course of the feeding period. Composited feed samples were dried at 55°C for 3 d and then ground using a Wiley mill (1mm screen, Arthur H. Thomas, Philadelphia, PA). Feed samples were analyzed for CP (Leco TruMac, LECO Corporation, St. Joseph, MI), crude fat (using the Ankom Technology Method 2; Ankom Technology, Macedon, NY), and ADF and

NDF (using Ankom Technology method 5 and 6, respectively; Ankom²⁰⁰ Fiber Analyzer, Ankom Technology). Individual feed ingredient compositions were used to construct overall diet composition.

Reproductive Procedures

Four cows were removed from the study after parturition due to twin births (n = 2) or calf mortality (n = 2). Twenty-nine cows were used for the remaining portion of the trial. Cows were assigned to 4 groups after parturition (3 groups of n = 7 and 1 group with n = 8) to minimize variation in days relative to calving. All reproductive procedures were performed on a single group at a given time to keep d postpartum similar across groups for each procedure. At 42 ± 5 d postpartum, cow reproductive tract examinations were performed. Cow ovaries and uteruses were examined via ultrasound imaging using Ibex Pro portable ultrasound (E.I. Medical Imaging, Loveland, Colorado) with L6.2 transducer (8-5MHz 66-mm linear array, 12 cm scan depth). For ultrasound procedures, the transducer was inserted into the rectum and placed directly over the broad ligament and uterine horns to scan the ovaries. Both left and right ovaries were scanned and frozen images were captured to determine the presence or absence of a follicle or corpus luteum (CL) as well as to measure follicular size. In order to determine ovarian response to hormones as well as follicular dynamics, ultrasound imaging was performed at 42, 56, 67, 76, and 84 ± 5 d postpartum (Figure 1).

On 42 and 56 ± 5 d postpartum, cows were pre-synchronized by administering prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$; 5 mL of Lutalyse, Zoetis, Florham, NJ). Cows were injected in the neck muscle using a 2.54 cm by 18 gauge needle. At 67 ± 5 d a progesterone controlled internal drug release (CIDR; Eazi-Breed CIDR, Zoetis, Florham, NJ) was inserted into the vagina of

cows and 2 mL of GnRH (Factrel, Zoetis, Florham, NJ) was administered. On d 71, 72, 73, and 74 ± 5 postpartum, while CIDR was in place, cows were administered FSH (Folltropin-V, Bioniche Animal Health, Belleville, Ontario, Canada) twice daily and 12 h apart to induce superovulation. Fifteen cows were administered with 200 mg Folltropin-V and 14 cows were administered with 160 mg Folltropin-V FSH. The change from 200 mg to 160 mg of FSH injections was prompted by unsatisfactory ovary stimulation observed in the initial 15 cows that were flushed. The CIDR was pulled at d 74 ± 5 postpartum and PGF_{2α} was administered to induce luteolysis. Forty-eight h after CIDR was removed, a second injection of GnRH was administered and 12 and 24 h post GnRH injection, cows were bred (AI) by 2 trained technicians. Cows were AI using 2 straws of semen per insemination for a total of 4 straws per cow. Cows were bred to generate Simmental x Angus crossbred calves. Therefore, Angus cows were bred to 1 purebred Simmental bull and Simmental x Angus cows were bred to 1 Simmental x Angus bull. All semen came from the same distributor (Select Sires, Plain City, OH).

At 7 d post-breeding (84 ± 5 d postpartum) preimplantation embryos were harvested (using procedures described by the International Embryo Transfer Society; (Robertson and Nelson, 2010). Cows were restrained in a Thorson (Thorson Manufacturing Co., West Des Moines, IA) squeeze chute for all flushing procedures. The hair on the animals' tailhead was clipped to 0.25 mm in length (using Andis clippers equipped with size 40 blades; Andis Company, Sturtevant, WI) and scrubbed with an iodine solution and rinsed with alcohol. Cows were given an epidural block by using 7 mL of lidocaine (2% solution) to reduce strain against palpation during the embryo collection procedure. Ultrasound imaging was performed on ovaries and images from both the left and right ovary were captured to analyze total number of CL present at time of flush as well as the presence of any follicles. The vulva was cleaned thoroughly using warm soapy

water before the insertion of a French Foley catheter (Agtech Inc., Manhattan, KS). The catheter was placed over a metal stylet and introduced into the vagina and passed through the cervix and placed in the first third of the uterine horn that contained the ovary with a greater number of CL present (identified by ultrasound imaging). Once in place, the cuff of the catheter was filled (using 15 to 20 mL of air) and the stylet was withdrawn. A sterile flushing medium (BioLife 'Advantage' Complete Flush Medium; BioLife Solutions, Bothell, WA) was used to flush embryos. Medium was suspended above the animal and a gravity based system was used to pass it through the catheter and drain into a filter. After the flush was completed, the cuff was deflated and the catheter was removed from the reproductive tract. A new catheter was used with identical procedure to flush the other uterine horn.

Embryo filter contents were transferred to a search disk by rinsing the filter with a holding and transfer medium (ViGro Holding Plus; Agtech Inc., Manhattan, KS) and contents were emptied into a search disk with a grid and placed under a dissecting microscope at 10X magnification to search for embryos. All embryo searching procedures were performed by the same technician. Total number of embryos recovered was recorded and then embryos were graded based on their morphology and stage of development. Embryos that were classified as freezable were transferred into a freeze medium (ViGro Ethylene Glycol; Agtech Inc., Manhattan, KS) to create an osmotic gradient and allow for cellular dehydration of embryos. Embryos were loaded into 0.25 mL straws and placed into a freezer (that froze at a controlled rate of 0.5°C/min) at a starting temperature of -6°C and freezing was complete at -34°C. Embryos categorized as code stage 4, 5, 6, and 7 as well as code quality 1 and 2 were frozen. Embryo stage and quality classification are outlined in Tables 2 and 3.

Blood samples were collected at 42, 56, 67, 74, 76, 80, and 84 ± 5 d postpartum. Blood collection was performed by venipuncture via the jugular or the tail vein or artery. Blood was collected using 10 mL vacutainer (Bectin, Dickinson and Company, Franklin Lakes, NJ). Blood tubes coated with a clot activator and gel, for serum separation, were used to obtain blood that was later spun down for serum collection. Blood tubes coated with K_2EDTA anticoagulant, to prevent blood from clotting, were used to obtain blood that was later spun down for plasma collection. Immediately after blood was collected, serum tubes were inverted 5 times to ensure proper mixing of blood and clotting activator; and plasma tubes were inverted 8 times to ensure proper mixing of blood and anticoagulant. Blood was allowed to clot at room temperature before spinning it down in a centrifuge at $1,300 \times g$ for 20 min for serum. Blood was placed on ice from the point of collection to the time of centrifuge ($1,300 \times g$ for 20 min) for plasma. After blood was spun in a centrifuge, both blood serum and plasma were then drawn out with a pipette and put into 2 mL microtubes (VWR International, Radnor, PA) and stored in a Symphony ultra-low temperature freezer (VWR International, Radnor, PA) set at $-80^\circ C$. Blood serum was used for progesterone (P_4) analysis. Blood plasma was later used for estradiol (E_2) and IGF-1 analyses.

A 96-well plate radioimmunoassay (Coat-A-Count kit; Siemens Healthcare Diagnostics Inc., Los Angeles, CA) was used to analyze blood serum concentrations of progesterone. Progesterone standards of: 0.3, 0.5, 1.0, 2.5, 5.0, 10.0 and 25.0 ng/mL were derived from the dilution of a 50.0 ng/mL stock solution with stripped bovine serum and were used to standardize each plate. Shoup (2014) validated the radioimmunoassay utilized for P_4 analysis. Standards and serum samples were allowed to thaw at room temperature and then vortexed and transferred into antibody-coated tubes that were provided in the kit. A ^{125}I progesterone tracer was added to the tubes and vortexed for 1 min. Tubes were allowed to set and incubate for 3 h at room

temperature. Upon conclusion of the incubation period, the liquid was aspirated out of the tubes, with the exception of the 2 tubes used for total count. Bound ^{125}I progesterone was then counted using a gamma counter (2470 Wizard2, PerkinElmer Inc., Waltham, MA). The inter-assay and intra-assay CV were 31.24% and 6.27%, respectively.

Serum concentrations of IGF-1 were determined using a competitive, liquid-liquid phase, double-antibody IGF-1 radioimmunoassay procedure as described previously by Lalman et al. (2000). Assay procedures and validation information were as follows. Serum samples were thawed, mixed thoroughly, and 10 μL of serum sample pipetted into individual wells of a 96 deep-well plate. Immediately thereafter, 400 μL of 1M glycine (pH 3.2) was added to acidify each sample followed by the addition of 500 μL of PABET+P (consisting of 0.1% gelatin, 0.01 M EDTA, 0.9% NaCl, 0.01 M PO_4 , 0.01% sodium azide, 0.05% Tween-20, 0.02% Protamine SO_4 , pH = 7.5). The acidified-diluted aliquots were then individually sealed within each well and incubated in a constant temperature oven at 37°C for 48 h. Thereafter, each acidified-diluted sample was neutralized by addition of 90 μL of 0.5 NaOH before being submitted to the IGF-1 assay. IGF-I assay procedures were adapted from those described by Holland et al. (1988). Recombinant human IGF-I was used for iodination and standards (UBI-01-141, Amgen Corp., Thousand Oaks, CA). Antiserum (UB3-189) was provided by the National Hormone and Pituitary Program and used at a final assay tube dilution of 1:10,000. Sample (40 μL of the acidified-diluted sample; in triplicate determinations), antisera, and PABET+P were combined (total volume balanced to 300 μL with PABET+P) and incubated at 4°C for 24 h. Iodine ^{125}I -labeled IGF-1 (^{125}I -IGF-1; 25,000 cpm) was then added and incubation continued at 4°C for an additional 16h. The antigen-antibody complex was then precipitated following a 15 min, 22°C incubation with 100 μL of a precipitated sheep-anti-rabbit second antiserum, by centrifugation at

3,000 × g for 30 min, and the supernatant discarded by aspiration. Assay tubes containing the precipitated antigen-1° antibody complex were counted for 1 min on a LKB1277 gamma counter (LKB Wallac, Turku, Finland). The IGF-1 standards and pooled aliquots of bovine serum extract were linear (log/logit transformation; $R^2 > 0.97$) and parallel over an IGF-1 mass of 1.5 to 15 ng/tube and an acid extracted serum volume of 2.5 to 100 µL per tube. Total specific binding was 39%, the minimum detectable concentration was 1.5 ng/tube, percentage recovery of mass was > 97% across the range of 2.5 to 100 µL of sample and the inter- and intra-assay CV were < 6%. Extraction recoveries were also assessed for concentrations of IGF-I in 2.5-, 5-, 7.5-, 10-, 25-, 40-, and 100 µL volumes of the acidified-diluted bovine serum sample and found to equal or exceed 99% over the 2.5 to 100 µL range tested. Parallelism was assessed and verified between standard concentrations of IGF-I and the acidified-diluted serum sample containing IGF-I in volumes ranging from 2.5 to 100 µL.

Concentrations of estradiol were measured using the procedures reported by Rozell and Keisler (1990) and later described by Kirby et al. (1997) with the substitution of a second antibody precipitation procedure in place of the charcoal extraction procedure. The estradiol assay was sensitive to 0.5 pg/mL, and had an intra-assay CV of 9% and an inter-assay CV of 11%.

Statistical Analysis

A final dataset including all the variables was constructed in SAS (SAS v9.3 Institute Inc., Cary, NC). Statistical analyses were performed using the MIXED, GLIMMIX, and FREQ procedures of SAS. A linear mixed model (MIXED procedure) was constructed to explore associations between plane of nutrition regimens and variables of interest (IGF-I, progesterone,

and estradiol). Body weight and BCS at time of breeding as well as BW and BCS change from 9 ± 6 d prior to parturition to breeding (71 ± 4 d postpartum) were analyzed. Treatment variable (plane of nutrition) was used in the model as a fixed effect. The covariate FSH (200 or 160mg) was left in the models whenever significant ($P < 0.10$). Repeated measures were used to analyze the effects of sampling day on cow parameters using the compound symmetry covariance structure. Variables were subjected to 5 covariance structures: compound symmetry, autoregressive order 1, autoregressive heterogeneous order 1, unstructured, and toeplitz. The covariance structure that yielded the lowest corrected Akaike information criterion was compound symmetry and used in the model (Littell et al., 2002). Pen was treated as a random effect. Degrees of freedom were adjusted by using the Kenward-Roger method (Littell et al., 2002). Residual distribution was evaluated for normality and homoscedasticity using the Univariate procedure in SAS. Progesterone concentration was dichotomized as cyclic or non-cyclic using a cut-off value of 1 ng/mL, as defined by Perry et al., (2004), to analyze cyclicity. Two measurements were used (56 and 67 d postpartum) to determine cyclicity and if progesterone concentration was at or over 1 ng/ml during at least one of these 2 time points then cows were considered to be cyclic. Progesterone values that were at or exceeded 3 standard deviations from the mean were considered outliers and discarded. Three outliers were removed for d 84 postpartum in progesterone analysis.

Secondly, multivariable logistic mixed models (GLIMMIX and FREQ procedures) considering the binary outcome variable cyclicity were constructed. Progesterone concentration was dichotomized as cyclic or non-cyclic using a cut-off value of 1 ng/mL, as defined by Perry et al. (2004), to analyze cow's cyclicity. Two measurements were used (56 and 67 d postpartum) to determine cyclicity and if progesterone concentration was at or over 1 ng/mL during at least one

of these 2 time points then cows were considered to be cyclic. Lastly, variables related to embryo and embryo quality (total follicles after superovulation, total CL at the time of flush, total embryos recovered, total embryos that received a quality score of good or excellent, total embryos with a code stage score of 5, 6 and 7, total embryos cleaved or degenerated, total embryos frozen, percentage of embryos recovered, and percent of embryos that were cleaved or degenerated) were analyzed using the Poisson distribution of the GLIMMIX procedure due to its count data characteristic. Estimated regression coefficients of the models were exponentiated and interpreted as a relative risk ratio (Dohoo et al., 2003).

Experiment 2

Animal and Diet Management

Thirty-five multiparous Angus and Angus Simmental crossbred cows (average initial BW = 601 ± 72 kg) were used for this experiment at the University of Illinois Beef Cattle and Sheep Field Research Laboratory in Urbana, IL. Cow housing was identical to Exp. 1. Cow requirements were determined based on NRC values for actual group BW, BCS, and d pregnant with estimated calf BW and peak milk production (601 kg BW, BCS of 5.7, carrying a 36 kg calf, 9 kg of milk production during peak lactation, and 153 d pregnant). Diet was identical to Exp. 1 (Table 1) with cows receiving the following: 7.4 kg DMI for cows fed REQ, 5.2 kg DMI for cows fed 70%REQ, and 9.7 kg DMI for cows fed 130%REQ. Cows were offered a free choice mineral while consuming the treatment diets. Nutritional treatments were applied during mid-gestation (195 to 112 ± 4 d prepartum). Upon completion of the treatment period, cows were fed a common diet (Table 1) formulated to meet NRC requirements and offered free choice

mineral. Stratification, allotment, and assignment to treatments were the same as Exp. 1. There were 8 pens containing 4 animals per pen and 1 pen containing 3 animals per pen.

Feed sampling and analysis

Dietary ingredient samples were collected every 14 d throughout the trial and composited over the course of the feeding period. Feed sample analysis and procedures were identical to Exp. 1.

Reproductive Processes

Four cows were removed from the study after parturition due to undesirable temperament ($n = 1$) or calf mortality ($n = 3$). Thirty-one cows were used for the remaining portion of the trial. Cows were assigned to 6 groups after parturition (3 groups of $n = 5$, 2 groups of $n = 6$, and 1 group with $n = 4$) to minimize variation in days related to calving. All reproductive procedures were performed on a single group at a given time to keep d postpartum similar across groups for each procedure. At 44 ± 4 d postpartum, cow reproductive tract examinations were performed. All procedures and equipment used for reproductive tract examinations were the same as Exp.1. Ultrasound imaging was performed at 44, 58, 69, 78, and 86 ± 4 d postpartum (Figure 2).

On 44 and 58 ± 4 d postpartum, cows had their estrus cycles pre-synchronized using the same procedure as Exp. 1. A CIDR was inserted into the vagina and 2 mL of GnRH (Factrel, Zoetis, Florham, NJ) was administered into the neck muscle of each cow on 69 ± 4 d postpartum. Superovulation was induced by injecting 160 mg of FSH (Folltropin-V, Bioniche Animal Health, Belleville, Ontario, Canada) to all cows on d 73, 74, 75, and 76 ± 4 postpartum. The CIDR was removed from cows on 76 ± 4 d postpartum. All AI procedures, matings, BW, and BCS collection were identical to Exp. 1.

At 7 d post-breeding (86 ± 4 d postpartum) preimplantation embryos were harvested (using procedures described by the International Embryo Transfer Society; (Robertson and Nelson, 2010). All procedures for embryo recovery, grading, and freezing are identical to Exp. 1.

Blood was collected at 44, 58, 69, 76, 78, 82, and 86 ± 4 d postpartum. All blood collection and hormone analysis are the same as presented in Exp. 1.

Statistical Analysis

All statistical procedures were performed as explained in Exp. 1, with the exception that FSH was not used as a covariate in Exp. 2. All variables analyzed for Exp. 2 are the same as stated in Exp. 1.

RESULTS

Exp. 1

Body weight and BCS

When cows were fed different planes of nutrition during late gestation, there was a trend ($P = 0.06$) for cows fed 130%REQ to have greater BW at time of breeding (71 ± 4 d postpartum) than cows fed the REQ diet and cows fed 70%REQ had intermediate body weights (Table 4). Cow BCS were not different ($P = 0.19$) across nutritional treatments applied to cows during late gestation. As the plane of nutrition increased, BW and BCS change from pre-calving (9 d prepartum) until breeding were not different ($P \geq 0.37$) among cows.

Cyclicality

Plane of nutrition fed to cows during late gestation did not affect ($P = 0.78$) the percentage of cows cycling by 67 ± 4 d postpartum (Table 5). Also, nutritional treatments did not affect ($P \geq 0.67$) the percentage of cows with a CL or follicle present at the time of CIDR insertion at 67 ± 4 d postpartum (Table 5).

Hormones

There was no interaction ($P \geq 0.11$) of nutritional treatment applied during late gestation and day postpartum for P_4 (Figure 3), E_2 (Figure 4), IGF-1 (Figure 5) plasma concentrations, or $E_2:P_4$ ratio (Figure 6). Cows that were fed 130%REQ during late gestation tended ($P = 0.09$) to have greater average P_4 concentrations than cows that were fed REQ and cows fed 70%REQ were intermediate. As expected, P_4 concentrations were different ($P < 0.01$) over time (Figure 3). Also, the plane of nutrition applied during late gestation did not affect ($P \geq 0.44$) blood plasma concentrations of estradiol and IGF-1. However, both estradiol (Figure 4) and IGF-1 (Figure 5) concentrations were different ($P < 0.0002$) over time. The plane of nutrition applied during late gestation did not affect ($P = 0.13$) the $E_2:P_4$ ratio; however, the $E_2:P_4$ ratio was different ($P < 0.01$) over time (Figure 6).

Embryo Data

The plane of nutrition fed to cows during late gestation did not impact ($P = 0.41$) the amount of follicles present after superovulation (76 ± 4 d postpartum; Table 6). Also, nutritional treatment did not affect ($P = 0.76$) the total number of CL present at the time of flush. There was a tendency ($P = 0.07$) for cows that were fed 70%REQ and REQ to have greater number of total embryos recovered when compared to cows that were fed 130%REQ diet during late gestation.

Cows that were fed 70%REQ and REQ tended ($P = 0.06$) to have increased number of embryos that were degenerated or cleaved when compared to cows that were fed 130%REQ. The total number of embryos frozen was not affected ($P = 0.91$) by nutritional treatments. Also, the number of embryos that received a quality score of excellent or good was not different ($P \geq 0.53$) across nutritional treatments. There was no difference ($P = 0.69$) in number of embryos that received a code stage score of 5, 6, and 7 across nutritional treatments. Plane of nutrition fed during late gestation did not affect ($P \geq 0.66$) the percentage of embryos recovered or the percentage of embryos that were cleaved or degenerated.

Exp. 2

Body weight and BCS

When cows were fed different planes of nutrition during mid-gestation, cows receiving 130%REQ and REQ had greater ($P = 0.02$) BW at breeding when compared with cows fed 70%REQ (Table 7). There was also a trend ($P = 0.06$) for cows fed 130%REQ to have greater BCS at breeding than cows fed the 70%REQ and cows fed REQ were intermediate. Body weight and BCS changes from pre-calving (21 d prepartum) until breeding were not different ($P \geq 0.54$) for cows receiving different planes of nutrition during mid-gestation.

Cyclicity

Plane of nutrition fed to cows during mid-gestation did not affect ($P = 0.92$) the percentage of cows cycling by 69 ± 5 d postpartum (Table 8). Also, nutritional treatments did not affect ($P \geq 0.94$) the percentage of cows with a CL or follicle present at the time of CIDR insertion at 69 ± 5 d postpartum (Table 8).

Hormones

There was no interaction ($P \geq 0.08$) of nutritional treatment applied during mid-gestation and day postpartum for progesterone, estradiol or IGF-1 plasma concentrations. Plane of nutrition during mid-gestation did not affect ($P \geq 0.23$) P_4 , E_2 , or IGF-1 concentration in this experiment. As expected, P_4 (Figure 7), E_2 (Figure 8), and IGF-1 (Figure 9) concentrations were different ($P < 0.0001$) over time. Also, the plane of nutrition applied during late gestation did not affect ($P = 0.40$) the $E_2:P_4$ ratio (Figure 10).

Embryo Data

The plane of nutrition fed to cows during mid-gestation did not impact ($P \geq 0.17$) the number of follicles present after superovulation or of CL present at the time of flush (Table 9). The nutritional plane fed during mid-gestation did affect ($P = 0.03$) the total number of embryos recovered. Cows fed 70%REQ and 130%REQ had greater ($P = 0.03$) number of embryos recovered when compared with cows receiving REQ. The total number of embryos that were cleaved or degenerated was not affected ($P = 0.13$) by maternal plane of nutrition during mid-gestation. Cows that received 70%REQ and 130%REQ had greater embryos that were cleaved or degenerated when compared with cows fed REQ. The total number of embryos that were frozen were not affected ($P = 0.41$) by nutritional treatments. Also, the number of embryos that received a quality score of excellent or good as well as the number that received a code stage score of 5, 6, and 7 was not different ($P \geq 0.27$) across nutritional treatments. Plane of nutrition fed during mid-gestation did not affect ($P \geq 0.61$) the percentage of embryos recovered or the percentage of embryos that were cleaved or degenerated.

DISCUSSION

Experiment 1

BW and BCS

In Exp. 1, cows fed 130%REQ tended to have increased BW at the time of breeding when related to cows fed REQ and cows fed 70%REQ had intermediate BW. Other studies have found that cows fed greater planes of nutrition prepartum, have increased BW and BCS at parturition (Corah et al., 1974; Stalker et al., 2006; Winterholler et al., 2012) as well as at the time of breeding (Winterholler et al., 2012). There were no differences in BCS in Exp. 1; however, all cows were moderate BCS (5.9, 6.2, and 6.4 for cows fed 70%REQ, REQ, and 130%REQ, respectively).

Cyclicality

Plane of nutrition fed to cows during late gestation did not affect the percentage of cows cycling by 67 ± 4 d postpartum. Also, nutritional treatments did not affect the percentage of cows with a CL or follicle present at the time of CIDR insertion at 67 ± 4 d postpartum. Corah et al. (1974) also reported that when cows were fed increasing amounts of dietary energy during gestation, there was not a reduction in postpartum interval (PPI) and resumption to estrus. Also, supporting results of Exp. 1, another study (Stalker et al., 2006) investigated the impact of varying amounts of protein in the diet and its impact on reproduction and reported that increased protein content in the diet did not reduce PPI. However, Lents et al. (2008) reported that cows grazing tallgrass prairie and supplemented 115 d prepartum with 1.4 kg of a 42% CP supplement per d and targeted to calve at a BCS of 5 (1 to 9 scale; (Wagner et al., 1988) had a reduced interval from parturition to estrus, as opposed to cows being fed 0.7 kg of the same supplement

per d and targeted to calve at a BCS of 4. However, in Exp. 1, all cows calved with a BCS greater than 4. The percentage of cows cycling, determined via P₄ concentrations, was approximately 10% for all cows fed during late gestation. There was a much greater percentage (78 to 100%) of cows that had a CL present. The CL produces progesterone; and, thus, with the number of CL that were detected with ultrasound measurements, there should be a greater percentage of cows that had P₄ concentrations great enough to be considered as cycling. However, the CL detected via ultrasound could have been non-functioning CL or corpus albicans. This would lead to the presence of a CL without an increase in P₄ concentrations.

Hormones

Plane of nutrition fed to cows during late gestation did not affect estradiol and IGF-1 concentrations. Estradiol concentrations differed over time. Estradiol concentrations were the greatest at 12 h prior to breeding, which cows should have corresponded to cow estrus, and then declined at the time of breeding and 4 d post breeding. Corah et al. (1974) also did not report any differences in estradiol concentrations associated with varying amounts of energy fed during the prepartum period. Ciccioli et al. (2003) reported that plasma IGF-1 concentrations were increased when cows had increased postpartum BW gains. In Exp. 1, BW change from pre-calving until breeding was not significantly different across nutritional treatments. This lack of difference may explain why IGF-1 concentrations were not different for cows fed diverging planes of nutrition during late gestation.

However, cows that were fed 130%REQ tended to have greater P₄ concentrations compared with cows fed REQ and cows fed 70%REQ were intermediate. Corah et al., (1974) reported that cows that were fed increased amounts of dietary energy during gestation also

tended to have elevated amounts of P₄ at 3 and 5 d postpartum. Although the day postpartum that P₄ amounts were measured are different for Exp. 1 and Corah et al. (1974), the results are in agreement that cows fed increasing amounts of dietary energy during gestation tend to have increased P₄ concentrations postpartum. It has been shown that increased concentrations of P₄ and estradiol are linked with an increase in pregnancy maintenance (Atkins et al., 2013). Guedon et al. (1999) found that cows with higher serum cholesterol concentrations from 2 wk prior to parturition until 4 wk postpartum had a decreased interval from calving to resumption of ovarian cyclicity. Cholesterol serves as a precursor for P₄ synthesis (Funston, 2004), and P₄ plays a major role in embryo implantation and survival (Grummer and Carroll, 1988). Progesterone concentration was different over time. During the administration of the first PGF_{2α} injection, P₄ concentrations were low and increased by the second PGF_{2α} injection and, as expected, continued to rise throughout CIDR insertion.

Embryo Data

The plane of nutrition did not affect total number of follicles after superovulation or the total number of CL at the time of flush. However, it does become more challenging to count structures when there are more than 10 structures present on each ovary. The hormone data helps reinforce the lack of difference in the ovarian structures after superovulation and at the time of flush and vice versa. Steroidogenesis of follicular cells by FSH and LH are supported by IGF-1 (Zulu et al., 2002). Also, IGF-1 can increase the sensitivity of follicular cells to FSH and LH, which would promote follicular growth and maturation (Zulu et al., 2002). There was no difference in IGF-1 concentration across plane of nutrition fed during late gestation. The total number of embryos recovered tended to be increased in cows fed 70%REQ and REQ compared with cows fed 130%REQ. This could be attributed to 130%REQ fed cows being transitioned

onto a diet that offered less consumable nutrients (130% NRC requirement vs. 100% NRC requirement). If these cows were experiencing a negative energy balance, then NEFA concentrations could have been elevated. Increased NEFA concentrations have been associated with negative energy balance in cows, and cows that are in a negative energy balance have been shown to have reduced concentrations of LH. Neither NEFA, nor LH concentrations were measured in Exp. 1. However, these 2 factors could help explain the poor embryo recovery rate in cows fed 130%REQ. Spitzer et al. (1995) reported that cows that were fed for increased BW gain during the postpartum period had increased pregnancy rates by d 20, 40, and 60 of the breeding season. Although BW change from pre-calving to breeding were not significantly different, cows fed 70%REQ had numerically greater BW and BCS gain compared with cows fed 130%REQ. Also, nutritionally derived stress would be limited for cows fed REQ diet because both the treatment and common diets were formulated to meet 100% NRC requirement.

Progesterone concentrations during synchronization (42, 56, and 67 d postpartum) were numerically lower in cows fed 130%REQ. Guedon et al. (1999) showed that cows with greater serum cholesterol concentrations from 2 wk prior to parturition until 4 wk postpartum had a decreased interval from calving to resumption of ovarian cyclicity. Guedon et al. (1999) did not measure P_4 ; however, cholesterol serves as a precursor for P_4 synthesis (Funston, 2004) and the decreased PPI is most likely attributed to increased P_4 amounts associated with increased cholesterol. This would suggest that cows fed 130%REQ were the least likely to have normal reproductive function and generate embryos. Another contributor could be that the numerically greater $E_2:P_4$ in cows fed 70%REQ and REQ when compared to those fed 130%REQ. Increased $E_2:P_4$ suggests that these cows had a greater number of dominant follicles producing E_2 when compared with cows fed 130%REQ; however, follicle count did not differ at superovulation. If

cows fed 70%REQ and REQ had a greater number of functional dominant follicles, then they would have a greater number of total embryos produced and recovered, and numerically they did. It is possible that follicles were unable to be counted properly because there were so many of them. The number of cleaved and degenerated embryos in this study was greater than expected and may be attributed to the decreased amount of cows cycling at the beginning of the synchronization process. Cows were synchronized in winter months of February and March which had an average temperature of -1.28 and 1.33°C, respectively. The high and low temperatures in February were 12.28 and -15.61°C, respectively and in March 16.50 and -9.44°C, respectively. This fluctuation in temperature could have caused environmental stress on the cows. Williams et al. (2005) reported that calving season and environment can have an impact on cow cyclicity.

The total number of embryos cleaved or degenerated tended to be higher in cows fed REQ and 70%REQ when compared with cows fed 130%REQ. However, there were no differences in the percent cleaved; and the greater number of cleaved embryos observed in cows fed REQ and 70%REQ, when compared with cows fed 130%REQ, is most likely a function of more embryos to start with. With similar trends in the number of cleaved or degenerated embryos and the total number of embryos recovered, it is as expected that the total number of embryos frozen were not different (only embryos with quality 1 or 2 were frozen). It has been shown that cows undergoing short term nutrient restriction ($9.6 \text{ Mcal}\cdot\text{kg}^{-1}\cdot\text{head}^{-1}\cdot\text{d}^{-1}$) during the time of flush have a numerical increase in the number of CL and total transferrable embryos when related to cows that were fed a high plane ($28.6 \text{ Mcal}\cdot\text{kg}^{-1}\cdot\text{head}^{-1}\cdot\text{d}^{-1}$) of nutrition during the time of embryo recovery (Nolan et al., 1998). However when cows were fed diverging planes of nutrition during late gestation in Exp. 1, nutrition did not affect embryo quality or the total

number of CL at the time of flush. Nolen et al. (1998) also reported that cows fed a low plane of nutrition had significantly greater concentrations of P_4 during the last 3 d that the CIDR was inserted. Progesterone is crucial for embryo implantation and survival (Grummer and Carroll, 1988) and maternal recognition of the pregnancy must occur to continue CL lifespan and P_4 production to suppress GnRH release (Hess et al., 2005). Increased P_4 concentrations reported in cows fed a low plane of nutrition could explain the increase in transferrable embryos recovered from cows receiving the low plane of nutrition that is seen in the Nolen et al, (1998) study. In contrast, in Exp. 1, there was a trend for cows fed a greater plane of nutrition to have increased amounts of P_4 concentration and no difference in the quality of embryos across nutritional planes fed during late gestation.

At d 56 postpartum (time at which second $PGF_{2\alpha}$ injection was administered), cows fed 70%REQ and REQ had numerically greater P_4 concentrations when compared with cows fed 130%REQ. Progesterone can exert a positive priming effect on the brain that can enhance the response to estradiol after P_4 concentrations are reduced (Senger, 2012). Greater amounts of P_4 prior to estrus can amplify the intensity and duration of estrus (Senger, 2012). This could have a potential effect on the pool of oocytes that were recruited for the next follicular wave. If cows that were fed 130%REQ had a reduced sensitivity to estradiol due to this decrease in P_4 concentration prior to the onset of estrus, follicular dynamics of the dominant follicles could have been different in these cows when compared with cows fed 70%REQ and REQ. This is also supported by cows fed 130%REQ having the lowest $E_2:P_4$ ratio; which would suggest that these cows had smaller or fewer dominant follicles after superovulation.

Prepartum nutrition has been shown to impact (Sasser et al., 1988; Selk et al., 1988; Marston et al., 1995) and not affect (Corah et al., 1974; Stalker et al., 2006; Winterholler et al.,

2012) postpartum reproductive performance in beef cows. Marston et al. (1995) reported that when cows were supplemented with increased energy amounts (7.0 vs 3.6 Mcal ME/d), cows had increased pregnancy rates. However, Corah et al. (1974) reported results that are more similar to what was observed in this study, in that increasing prepartum energy (17.6 vs 11.4 Mcal DE/d) resulted in greater BW gains; but, it did not affect cow cyclicity or pregnancy rates.

All treatment groups (70%REQ, REQ, and 130%REQ) in this study were in a moderate BCS (5.9 to 6.4). Previous literature has shown that cows in moderate BCS (5 to 6) have increased conception rates (Lents et al., 2008; Bohnert et al., 2013) and decreased PPI (Lents et al., 2008) when compared with cows that are in poor BCS (4.3 to 4.4). In Exp. 1, all cows were in a moderate BCS and, thus, may not have been stressed enough, from an energy and nutritional stand point, to compromise reproductive function. Morrison et al. (1999) managed cows to move from a BCS of 4 to a 5, move from a BCS of 7 to a 5, or maintain a BCS of 5 for 180 d prepartum. All cows, regardless of their initial BCS calved in a moderate BCS (5 to 6) and there was no difference in cyclicity or pregnancy rates. This suggests that weight change during the last trimester of pregnancy towards a moderate BCS at calving is favorable for reproductive performance in beef cows. Cows in this study were in a moderate BCS at calving (Wilson and Shike, 2014) which may help explain the lack of difference in embryo quality and number of embryos frozen.

Experiment 2

BW and BCS

In Exp. 2, cows fed REQ and 130%REQ had increased BW at time of breeding when compared with cows fed 70%REQ. Also, BCS tended to be greater in cows fed 130%REQ

compared with cows fed 70%REQ, cows fed REQ were intermediate. Other studies have illustrated that when cows received greater planes of nutrition prepartum, they had increased BW and BCS at parturition (Corah et al., 1974; Stalker et al., 2006; Winterholler et al., 2012) as well as at the time of breeding (Winterholler et al., 2012). Both BW and BCS were numerically similar to that seen in Exp. 1, as all cows were in moderate BCS and BW ranged from 675 to 722 kg in Exp. 2 while BW ranged from 686 to 739 kg in Exp. 1. Also, the change in BW and BCS data from pre-calving to breeding for Exp. 2 is similar to what is reported in Exp.1. We anticipated that the treatments may realign BW and BCS over time but, from calving until breeding, that did not happen.

Cyclicality

Cows in Exp. 2 had a greater number of cows cycling by 69 d postpartum when compared with Exp. 1. Circulating concentrations of estradiol, nutritional status, and calving season are critical to cows' ability to return to estrus (Williams, 2005). Perhaps the increased percentage of cows cycling in Exp. 2 could be attributed to all cows in Exp. 2 were gaining BW and BCS during late gestation (Wilson and Shike, 2014). Marston et al. (1995) reported that cows supplemented with dietary energy during the last trimester of pregnancy had increased BW gains and pregnancy rates when related to cows fed protein supplementation during late gestation. This suggests that cows receiving greater planes of nutrition and gaining weight during the last trimester of pregnancy have greater potential to cycle and conceive. Freetly et al. (2000) reported that cows that lost BW during the 2nd trimester of pregnancy but regained that BW during the 3rd trimester of pregnancy had similar pregnancy rates when related to cows that maintained BW throughout the entire prepartum period. This supports data represented in Exp. 2, as cyclicality was similar regardless of prepartum nutrition. Cows in Exp. 2 calved later in the year

and experienced warmer weather during the synchronization process when compared with cows in Exp. 1. Cows in Exp. 2 calved during April (average temperature of 10.33 °C) and May (average temperature of 18.06 °C) and experienced temperatures that were on average 18.06 and 21.83°C during synchronization; whereas, cows in Exp. 1 calved during January (average temperature of -1.28 °C) and experienced temperatures that were on average 1.33 and 10.33 °C during the synchronization process. Mature beef cows consuming a maintenance diet have a thermoneutral zone between -15 and 28°C (Federation of Animal Science Societies, 2010). More favorable average temperatures and less fluctuation in temperature during Exp. 2 may attribute to the increase in number of cows cycling prior to CIDR insertion when compared with Exp. 1. Also, the amount of cows that were cycling by 69 d postpartum is consistent with the number of cows that had a CL present at 69 d postpartum in Exp. 2.

Hormones

The plane of nutrition during mid-gestation did not affect P₄, E₂, or IGF-1 concentrations in Exp. 2. This data is supported by Corah et al. (1974) that reported varying amounts of prepartum energy did not affect plasma P₄ and estradiol concentrations. The mechanisms for E₂ and IGF-1 concentrations are discussed before in Exp. 1. Progesterone concentration was different over time. After breeding, P₄ concentrations increased due to the development of CL and increased P₄ production resulting from those structures. Estradiol concentrations were different over time. Estradiol concentrations were the highest at 12 h prior to breeding, which would be when cows should have been exhibiting estrus, and then declined at the time of breeding and 4 d post breeding.

Embryo Data

The plane of nutrition fed during mid-gestation did not affect total number of follicles after superovulation or the total number of CL at the time of flush. The plane of nutrition during mid-gestation did affect the total number of embryos recovered; with cows fed 70%REQ and 130%REQ flushing a greater number of embryos than cows that were fed REQ. Cows that were fed 70%REQ and 130%REQ also had the greatest numerical numbers of CL at the time of flush and the greatest numerical concentrations of P₄. Steroidogenesis of follicular cells by FSH and LH are supported by IGF-1 (Zulu et al., 2002). Also, IGF-1 can increase the sensitivity of follicular cells to FSH and LH, which would promote follicular growth and maturation (Zulu et al., 2002). There was no difference in IGF-1 concentration across nutritional plane of nutrition fed during mid-gestation. However, from 12 h prior to breeding through the time of flush, cows fed 130%REQ had the numerically greatest IGF-1 concentrations. This could have led to increased sensitivity of follicular cells to FSH and LH and resulted in more follicles, CL, and embryos produced. Increased IGF-1 could justify the increased number of embryos recovered from cows fed 130%REQ. Also, although not significantly different across nutritional treatments, cows that were fed 70%REQ had the greatest E₂ concentrations and E₂:P₄ ratio at 12 h prior to breeding. Increased E₂:P₄ would suggest that cows fed 70%REQ had a greater number of dominant follicles that were producing E₂ and thus could have developed into functional CL and generated more total embryos. More functional CL is supported by cows fed 70%REQ had a numerically greater count of follicles after superovulation and CL at the time of flush. Nolen et al. (1998) reported that cows fed a low energy diet (9.6 Mcal·kg⁻¹·head⁻¹·d⁻¹) during embryo recovery had numerical increases in CL, P₄, and total transferrable embryos recovered when compared with cows fed a high energy diet (28.6 Mcal·kg⁻¹·head⁻¹·d⁻¹). Also, cows in Exp. 2 had

a greater number of embryos recovered than cows in Exp. 1. Increased embryos recovered could be attributed to the greater number of cows cycling by the time of CIDR insertion in Exp. 2 when compared with cows in Exp. 1. Although the count of embryos that were cleaved or degenerated was not significantly different, the numerical pattern follows the total number of embryos recovered with 70%REQ and 130%REQ having a greater count of embryos cleaved or degenerated when compared with cows fed REQ. Perhaps, prepartum nutrition had an impact on uterine environment. Uterine dysfunction is most commonly seen when cows do not have sufficient concentrations of E_2 prior to ovulation or do not have a rapid increase in P_4 after ovulation; and these hormone insufficiencies could result in embryo mortality (Bridges et al., 2013). As stated previously, Nolen et al. (1998) reported that nutrition affected P_4 concentrations in cows. Oversupplying protein during the time of flush has been shown to decrease embryo viability (Rhoads et al., 2006). Robinson et al. (2006) reported that increased plane of nutrition can have negative effects on superovulated animals involved in embryo production. Dietary excess of RDP could lead to elevated concentrations of ammonia in follicular fluid and reduce blastocyst formation (Robinson et al., 2006). Uterine environment may have been altered through prepartum nutrition and could contribute to the increased number of cleaved and degenerated embryos. Embryo quality, stage of development, and total number of embryos frozen were not significantly different among treatments in Exp. 2. The previous literature and mechanisms involved with the explanation of the lack of significance in embryo quality, development, and count of embryos that were frozen is the same as that reported in Exp. 1. Also, there was no difference in the percent recovery. This would suggest that plane of nutrition during mid-gestation did not significantly impact CL function. Also, there was not a difference in the

percent of embryos cleaved or degenerated; which would suggest that the plane of nutrition during mid-gestation did not impact embryo viability or survivability.

Conclusion

In conclusion, BW and BCS at breeding tended to increase as the plane of nutrition was increased during either mid- or late gestation. The plane of nutrition during mid- or late gestation did not affect cyclicity, blood hormone concentrations, or embryo quality. However, when cows were fed diverging planes of nutrition during late gestation in Exp. 1, cows fed 70%REQ and REQ tended to have greater number of embryos recovered and embryos cleaved or degenerated when compared with cows that were fed 130%REQ. When cows were fed diverging planes of nutrition during mid-gestation in Exp. 2, cows fed 70%REQ and 130%REQ flushed a greater number of embryos when compared with cows fed 100%REQ. Feeding diverging planes of nutrition in either mid- or late gestation did not impact the number of embryos that were frozen.

Tables

Table 1. Composition (DM basis) of diets fed to cows in Exp. 1 and Exp. 2

Item	Inclusion, % DM	
	Treatment Diet ^{1,2,6}	Common Diet ^{3,4,5}
Ingredient, %		
Soy Hulls	23.6	--
Corn Silage	52.8	87.0
Alfalfa Haylage	23.6	--
Modified Wet Distillers Grains with Solubles	--	13.0
Total	100.0	100.0
Analyzed Nutrient Content		
CP	11.6	11.8
NDF	47.0	43.7
ADF	34.3	22.7
Ether Extract	2.2	2.9

¹ Cows fed the treatment diet during late gestation (Exp.1) received the following DMI: 6.4 kg DMI for cows fed to receive 70% of their NRC requirement (**70%REQ**), 9.1 kg DMI for cows fed to receive 100% of their NRC requirement (**REQ**), and 11.9 kg DMI for cows fed to receive 130% of their NRC requirement (**130%REQ**).

² Cows fed the treatment diet during mid-gestation (Exp. 2) received the following DMI: 5.2 kg DMI for cows fed 70%REQ, 7.4 kg DMI for cows fed REQ, and 9.7 kg DMI for cows fed 130%REQ.

³ Cows in Exp. 1 were fed 9.5 kg DMI of the common diet during the postpartum period.

⁴ Cows in Exp. 2 were fed 8.5 kg DMI of the common diet during late gestation.

⁵ Cows in Exp. 2 were fed 9.5 kg DMI of the common diet during the postpartum period.

⁶ Free choice mineral offered during treatment period consisted of: (0.09% Ca, 0.11% P, 0.01% K, 5.00 mg/kg Cu, 0.01% S, 20.00 ppm Fe, 30.00 ppm Zn, 0.15 ppm Se, 0.99 ppm I, 0.83 mg/kg Vit A, 0.02 mg/kg Vit D)

⁷ Free choice mineral offered while cows were fed the common diet consisted of: (0.13% Ca, 0.08% P, 0.01% K, 42.49 mg/kg Cu, 0.02% S, 43.49 ppm Fe, 55.52 ppm Zn, 0.28 ppm Se, 1.15 ppm I, 1.22 mg/kg Vit A, 0.03 mg/kg Vit D)

Table 2.IETS Evaluation system used to grade Embryo Development

Score	Description
4	Morula (d 6)
5	Early Blastocyst (d 7)
6	Blastocyst (d 7 to 8)
7	Expanded blastocyst (d 8 to 9)

Table 3. IETS Evaluation System used to grade Embryo Quality

Score	Description
1	(Excellent) perfect for the stage of the embryo, compact blastomeres, no debris
2	(Good) trivial imperfections, such as oval zona, few small loose blastomeres
3	(Fair) definite but not severe problems such as moderate numbers of loose blastomeres
4	(Poor) partly degenerated, with vesiculated cells and greatly varying sizes of cells
5	(Very Poor) severely degenerated
6	Unfertilized

Table 4. Effect of plane of nutrition during late gestation on BW and BCS in Exp. 1

Item	Plane of Nutrition			SEM	P-value
	70%REQ ¹	REQ ²	130%REQ ³		
BW at breeding, kg	687 ^{xy}	686 ^y	739 ^x	18	0.06
Change in BW from pre-calving to breeding, kg	-1.8	-40.1	-18.5	19.5	0.37
BCS at breeding	5.9	6.2	6.4	0.2	0.19
Change in BCS from pre-calving to breeding	0.2	0.1	0.1	0.2	0.80

^{x,y} Within a row, means without common superscripts tend to differ ($0.05 < P < 0.10$)

¹ Cows received 70% of their NRC requirement during late gestation

² Cows received 100% of their NRC requirement during late gestation

³ Cows received 130% of their NRC requirement during late gestation

Table 5. Effect of plane of nutrition during late gestation on cyclicity in Exp. 1

Item	Treatment			SEM	P-value
	70%REQ ¹	REQ ²	130%REQ ³		
Percentage cycling at 67 d postpartum ⁴	11	20	10	-	0.78
Percentage of cows with CL present at 67 d postpartum	78	100	90	32	0.89
Percentage of cows with follicles present at 67 d postpartum	33	60	30	24	0.67

¹ Cows received 70% of their NRC requirement during late gestation

² Cows received 100% of their NRC requirement during late gestation

³ Cows received 130% of their NRC requirement during late gestation

⁴ percentage cycling represents cows with serum P4 concentration ≥ 1 ng/mL

Table 6. Effect of plane of nutrition during late gestation on ovarian structures and embryo recovery in Exp. 1

Item	Plane of Nutrition			SEM	P-value
	70%REQ ¹	REQ ²	130%REQ ³		
Count total follicles after superovulation	11.9	10.5	12.9	1.4	0.41
Count total CL ⁴ at time of flush	9.6	9.7	10.7	1.2	0.76
Count total embryos recovered	6.9 ^x	6.4 ^x	3.1 ^y	1.3	0.07
Count total embryos with quality score excellent	4.7	3.3	2.9	2.5	0.81
Count total embryos with quality score good	1.3	2.0	3.5	1.3	0.53
Count total embryos with code stage score 5, 6, & 7 ⁵	0.6	1.1	1.2	0.6	0.69
Count total embryos cleaved/degenerated	4.3 ^x	4.5 ^x	1.6 ^y	0.9	0.06
Count total embryos frozen	2.1	1.9	1.5	1.0	0.91
Percent recovered ⁶	62	73	35	30	0.66
Percent cleaved ⁷	67	57	47	31	0.88

^{x,y} Within a row, means without common superscripts tend to differ ($0.05 < P < 0.10$)

¹ Cows received 70% of their NRC requirement during late gestation

² Cows received 100% of their NRC requirement during late gestation

³ Cows received 130% of their NRC requirement during late gestation

⁴ Corpus Luteum

⁵FSH was used in the model as a covariate

⁶ Total embryos recovered / total number of CL at the time of flush

⁷ Total number embryos cleaved or degenerated / total number of embryos recovered

Table 7. Effect of plane of nutrition during mid-gestation on BW and BCS in Exp. 2

Item	Plane of Nutrition			SEM	P-value
	70%REQ ¹	REQ ²	130%REQ ³		
BW at breeding, kg	675 ^a	710 ^b	722 ^b	11.68	0.02
Change in BW from pre-calving to breeding, kg	-53	-52	-48	15.33	0.97
BCS at breeding	5.8 ^x	6.3 ^{xy}	6.6 ^y	0.20	0.06
Change in BCS from pre-calving to breeding	-0.19	0.14	0.06	0.23	0.54

^{a,b} Within a row, means without common superscripts differ ($P < 0.05$)

^{x,y} Within a row, means without common superscripts differ ($0.05 < P < 0.10$)

¹ Cows received 70% of their NRC requirement during mid-gestation

² Cows received 100% of their NRC requirement during mid-gestation

³ Cows received 130% of their NRC requirement during mid-gestation

Table 8. Effect of plane of nutrition during mid-gestation on cyclicity in Exp. 2

Item	Treatment			SEM	P-value
	70%REQ ¹	REQ ²	130%REQ ³		
Percentage cycling at 69 d postpartum ⁴	70	75	78	-	0.92
Percentage of cows with a CL present at 69 d postpartum	67	67	78	29	0.95
Percentage of cows with follicles present at 69 d postpartum	78	92	89	31	0.94

¹ Cows received 70% of their NRC requirement during mid-gestation

² Cows received 100% of their NRC requirement during mid-gestation

³ Cows received 130% of their NRC requirement during mid-gestation

⁴ percentage cycling represents cows with serum P4 concentration ≥ 1 ng/mL

Table 9. Effect of plane of nutrition during mid-gestation on ovarian structures and embryo recovery in Exp. 2

Item	Plane of Nutrition			SEM	P-value
	70%REQ ¹	REQ ²	130%REQ ³		
Count total follicles after superovulation	14.3	10.6	12.8	1.3	0.38
Count total CL ⁴ at time of Flush	12.7	9.2	10.2	1.4	0.17
Count total embryos recovered	15.1 ^a	6.6 ^b	12.4 ^a	2.4	0.03
Count total embryos with quality score excellent	1.3	1.1	2.8	1.0	0.28
Count total embryos with quality score good	0.7	0.4	0.9	0.6	0.79
Count total embryos with code stage score 5, 6, & 7	2.3	1.6	3.6	1.3	0.38
Count total embryos cleaved or degenerated	12.2	4.8	8.1	3.3	0.13
Count total embryos frozen	2.3	1.6	3.6	1.3	0.41
Percent recovered ⁵	97	81	111	35	0.82
Percent cleaved ⁶	76	64	56	29	0.88

^{a,b} Within a row, means without common superscripts differ ($P < 0.05$)

¹ Cows received 70% of their NRC requirement during mid-gestation

² Cows received 100% of their NRC requirement during mid-gestation

³ Cows received 130% of their NRC requirement during mid-gestation

⁴ Corpus Luteum

⁵ Total recovered / total number of CL at the time of flush

⁶ Total number embryos cleaved or degenerated / total number of embryos recovered

Figures

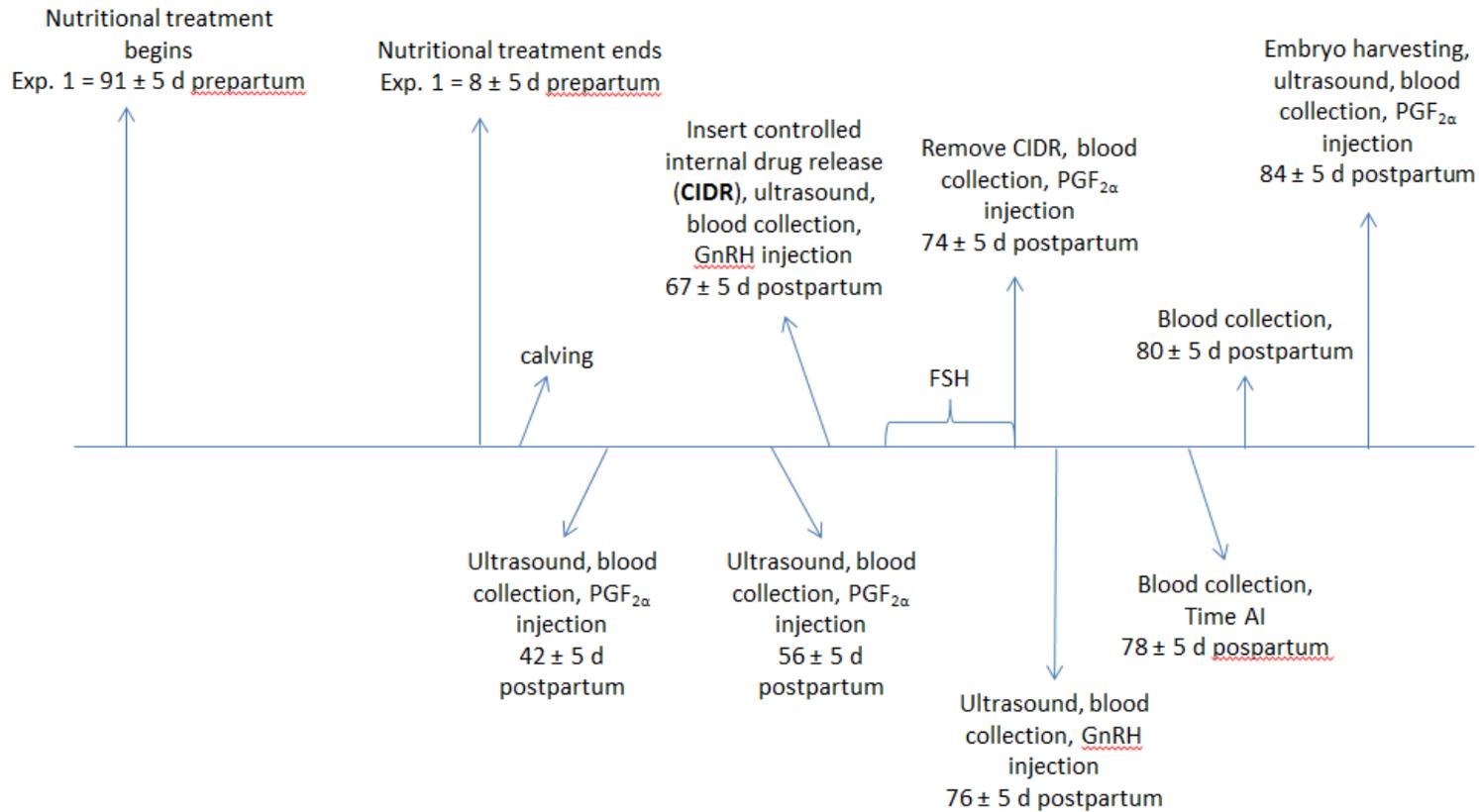


Figure 1. Exp. 1 Timeline

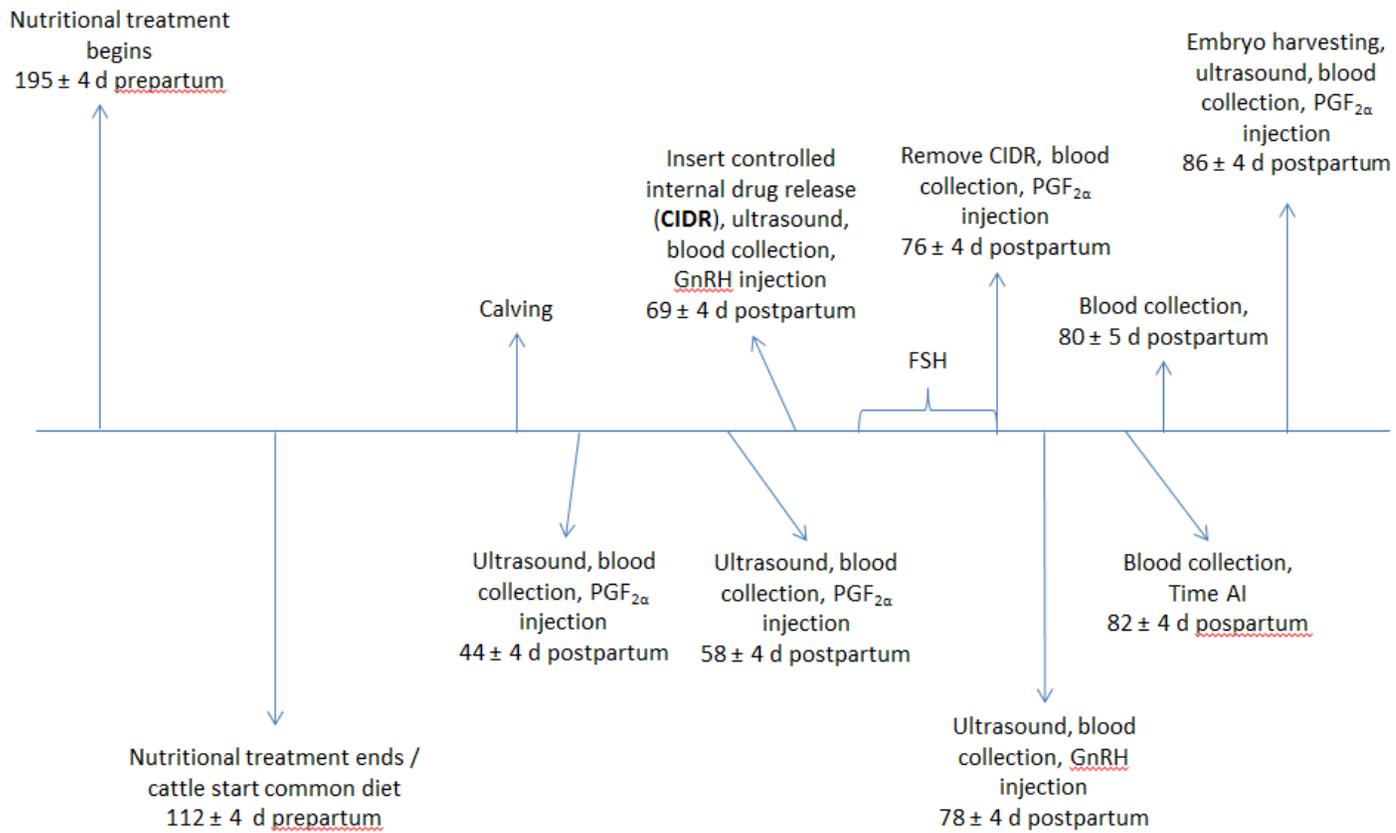


Figure 2. Exp. 2 Timeline

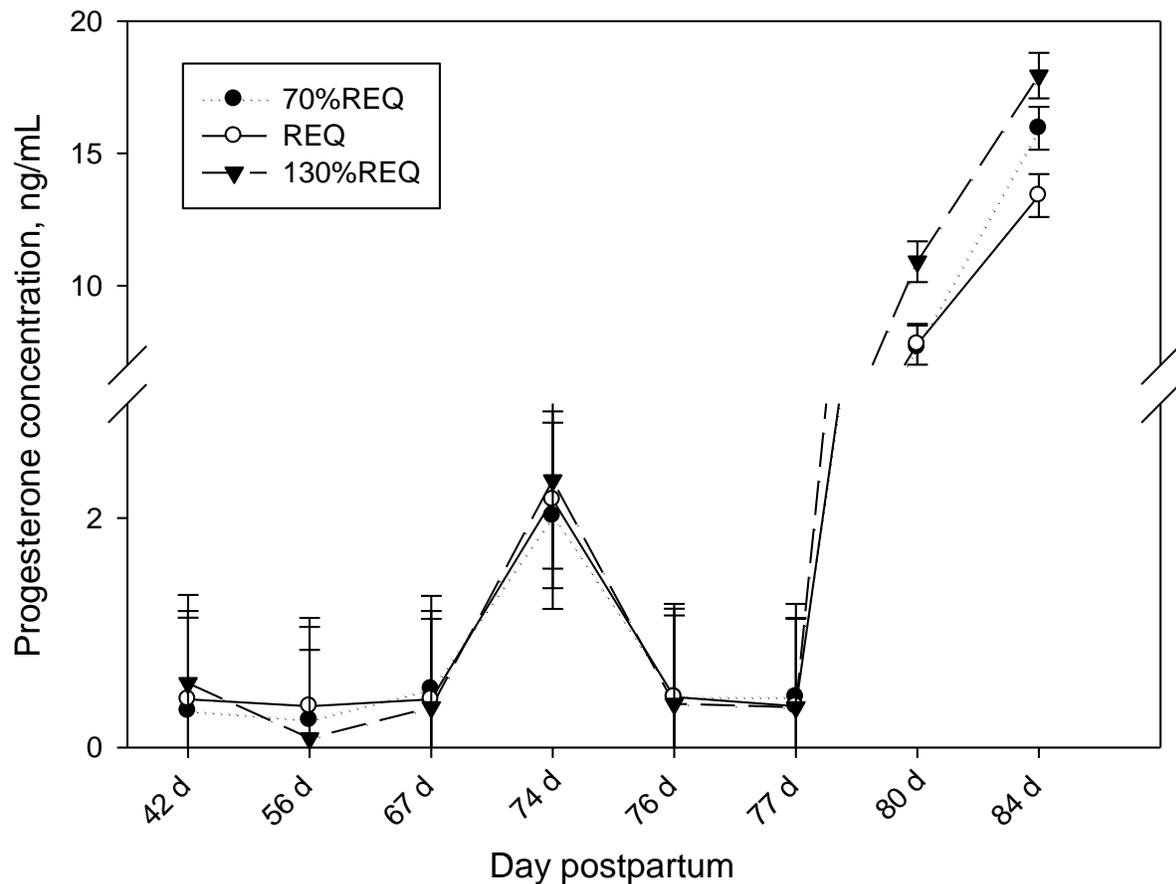


Figure 3. Effect of plane of nutrition during late gestation on progesterone concentration over time in Exp. 1. Cows were fed 70% their NRC requirement for 70%REQ, cows were fed 100% of their NRC requirement for REQ, and cows were fed 130% of their NRC requirement for 130%REQ. There was no interaction ($P = 0.11$) for plane of nutrition and day postpartum. There was a trend ($P = 0.09$) for plane of nutrition during late gestation to affect progesterone concentrations. Progesterone concentrations were different ($P < 0.01$) over time. Follicle stimulating hormone (FSH) was included in the statistical model as a covariate for progesterone concentration. At 42 d postpartum cows were administered the first injection of $\text{PGF}_{2\alpha}$. At 56 d postpartum cows were administered the second injection of $\text{PGF}_{2\alpha}$. At 67 d postpartum, CIDR were inserted. At 74 d postpartum, CIDR were removed. At 76 d postpartum (12 h prior to breeding), cows were given GnRH injection. At 77 d postpartum, cows were AI. At 80 d postpartum, represents the halfway point between breeding and the time of flush. At 84 d postpartum, cows were flushed.

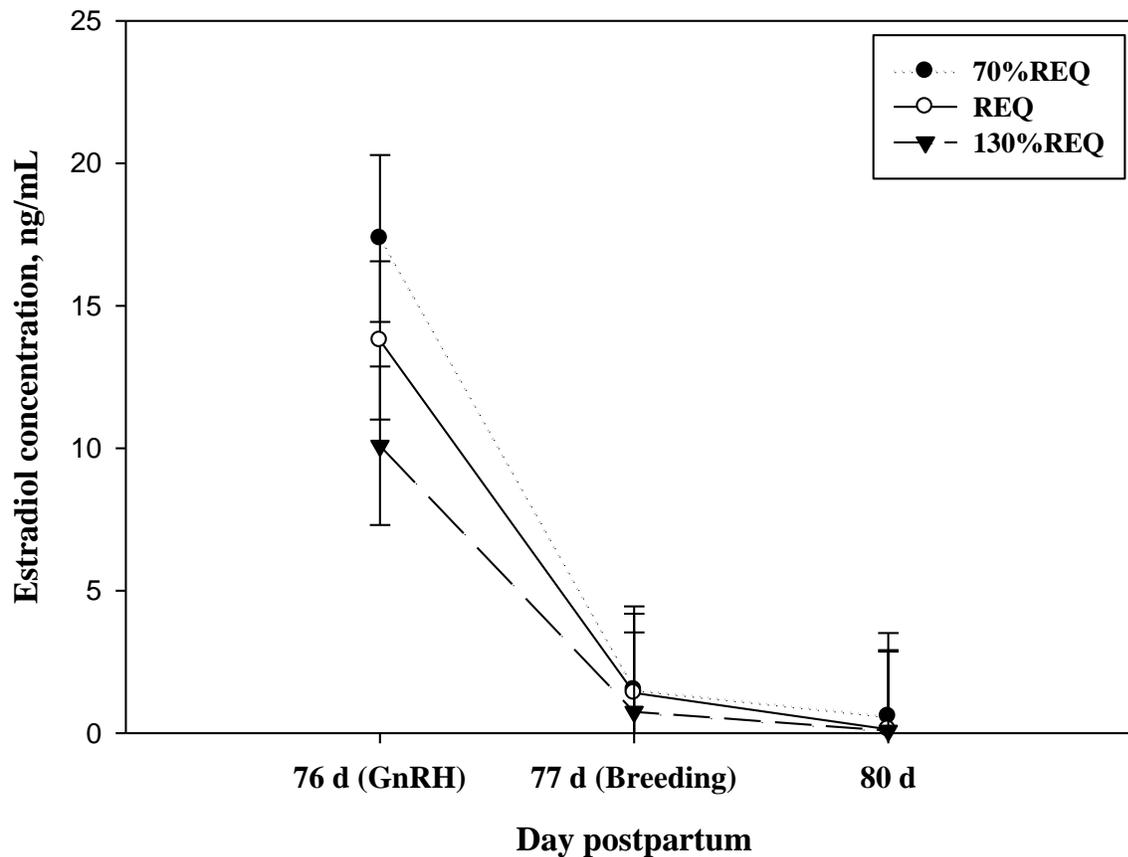


Figure 4. Effect of plane of nutrition during late gestation on estradiol concentrations over time in Exp. 1. Cows were fed 70% their NRC requirement for 70%REQ, cows were fed 100% of their NRC requirement for REQ, and cows were fed 130% of their NRC requirement for 130%REQ. There was no interaction ($P = 0.79$) between plane of nutrition and day postpartum. The plane of nutrition during late gestation did not affect ($P = 0.44$) estradiol concentrations. Concentrations of estradiol were different ($P < 0.0001$) over time. Follicle stimulating hormone (FSH) was used in the statistical model as a covariate. At 76 d postpartum (12 h prior to breeding), cows were given GnRH injection. At 77 d postpartum, cows were AI. At 80 d postpartum, represents the halfway point between breeding and the time of flush.

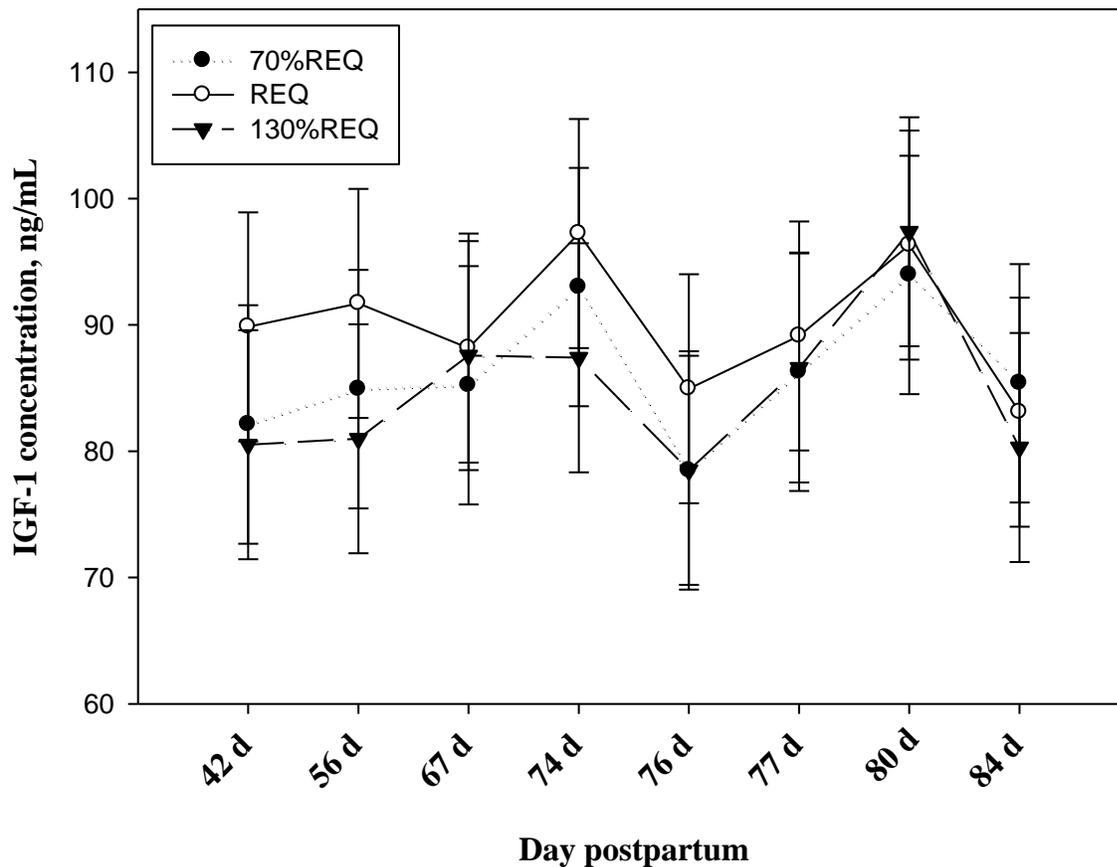


Figure 5. Effect of plane of nutrition during late gestation on IGF-1 concentrations over time in Exp. 1. Cows were fed 70% their NRC requirement for 70%REQ, cows were fed 100% of their NRC requirement for REQ, and cows were fed 130% of their NRC requirement for 130%REQ. There was no interaction ($P = 0.97$) between plane of nutrition and day postpartum. The plane of nutrition offered during late gestation did not affect ($P = 0.90$) IGF-1 concentrations. Concentrations of IGF-1 were different ($P = 0.0002$) over time. At 42 d postpartum cows were administered with the first injection of $\text{PGF}_{2\alpha}$. At 56 d postpartum cows were administered with the second injection of $\text{PGF}_{2\alpha}$. At 67 d postpartum, CIDR were inserted. At 74 d postpartum, CIDR were removed. At 76 d postpartum (12 h prior to breeding), cows were given GnRH injection. At 77 d postpartum, cows were AI. At 80 d postpartum, represents the halfway point between breeding and the time of flush. At 84 d postpartum, cows were flushed.

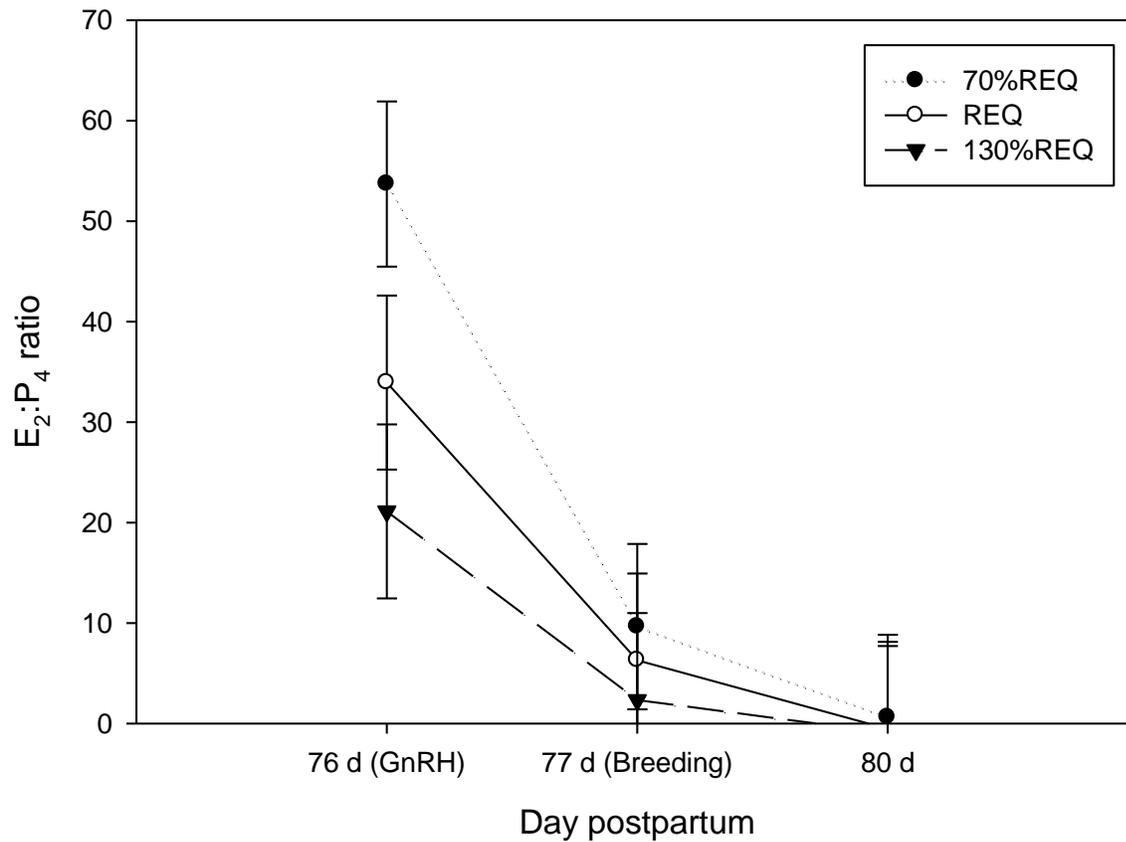


Figure 6. Effect of plane of nutrition during late gestation on estradiol:progesterone ratio in Exp. 1. Cows were fed 70% their NRC requirement for 70%REQ, cows were fed 100% of their NRC requirement for REQ, and cows were fed 130% of their NRC requirement for 130%REQ. There was no interaction ($P = 0.46$) between plane of nutrition and day postpartum. The plane of nutrition during late gestation did not affect ($P = 0.13$) the $E_2:P_4$ ratio. The $E_2:P_4$ ratio was different ($P < 0.01$) over time. FSH was used in the statistical model as a covariate. At 76 d postpartum (12 h prior to breeding), cows were given GnRH injection. At 77 d postpartum, cows were AI. At 80 d postpartum, represents the halfway point between breeding and the time of flush.

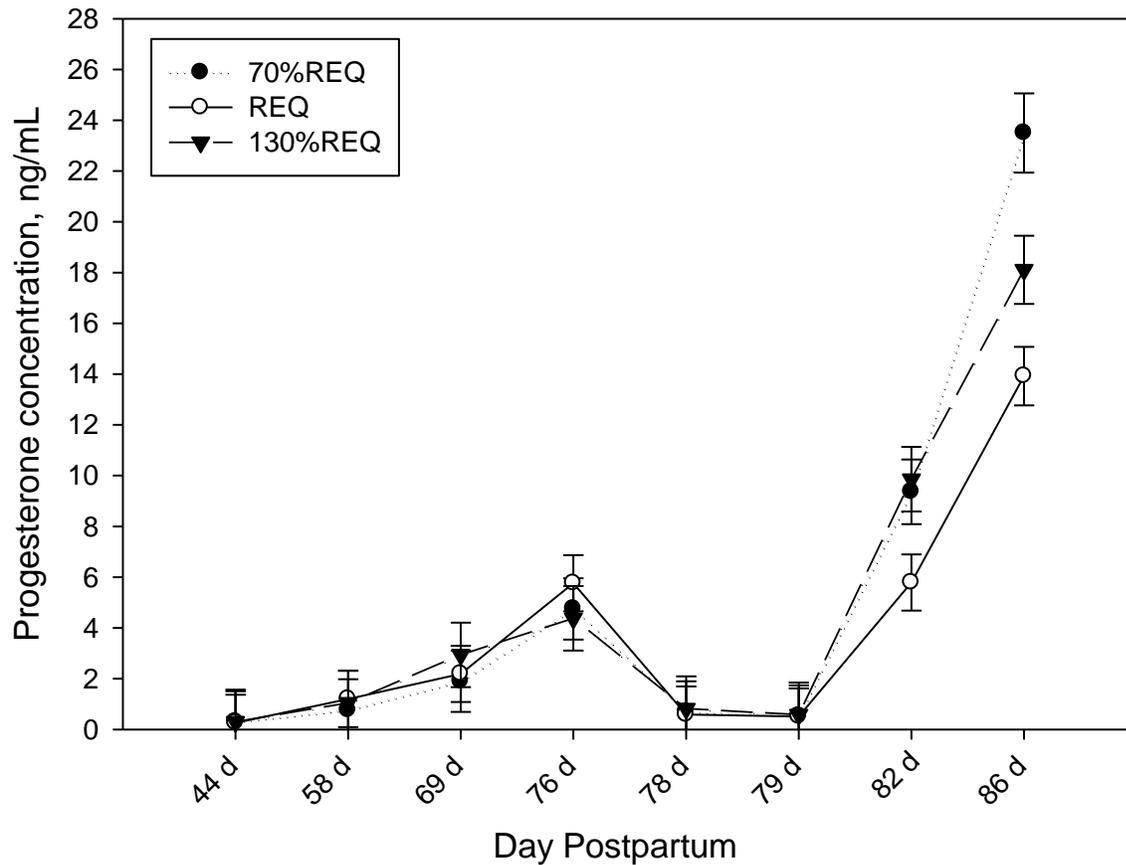


Figure 7. Effect of plane of nutrition during mid-gestation on progesterone concentration over time in Exp. 2. Cows were fed 70% their NRC requirement for 70%REQ, cows were fed 100% of their NRC requirement for REQ, and cows were fed 130% of their NRC requirement for 130%REQ. There was no interaction ($P = 0.08$) for plane of nutrition and day postpartum. The plane of nutrition did not affect ($P = 0.23$) progesterone concentration. Progesterone concentrations were different ($P < 0.01$) over time. At 44 d postpartum cows were administered with the first injection of $\text{PGF}_{2\alpha}$. At 58 d postpartum cows were administered with the second injection of $\text{PGF}_{2\alpha}$. At 69 d postpartum, CIDR were inserted. At 76 d postpartum, CIDR were removed. At 78 d postpartum (12 h prior to breeding), cows were given GnRH injection. At 79 d postpartum, cows were AI. At 82 d postpartum, represents the halfway point between breeding and the time of flush. At 86 d postpartum, cows were flushed.

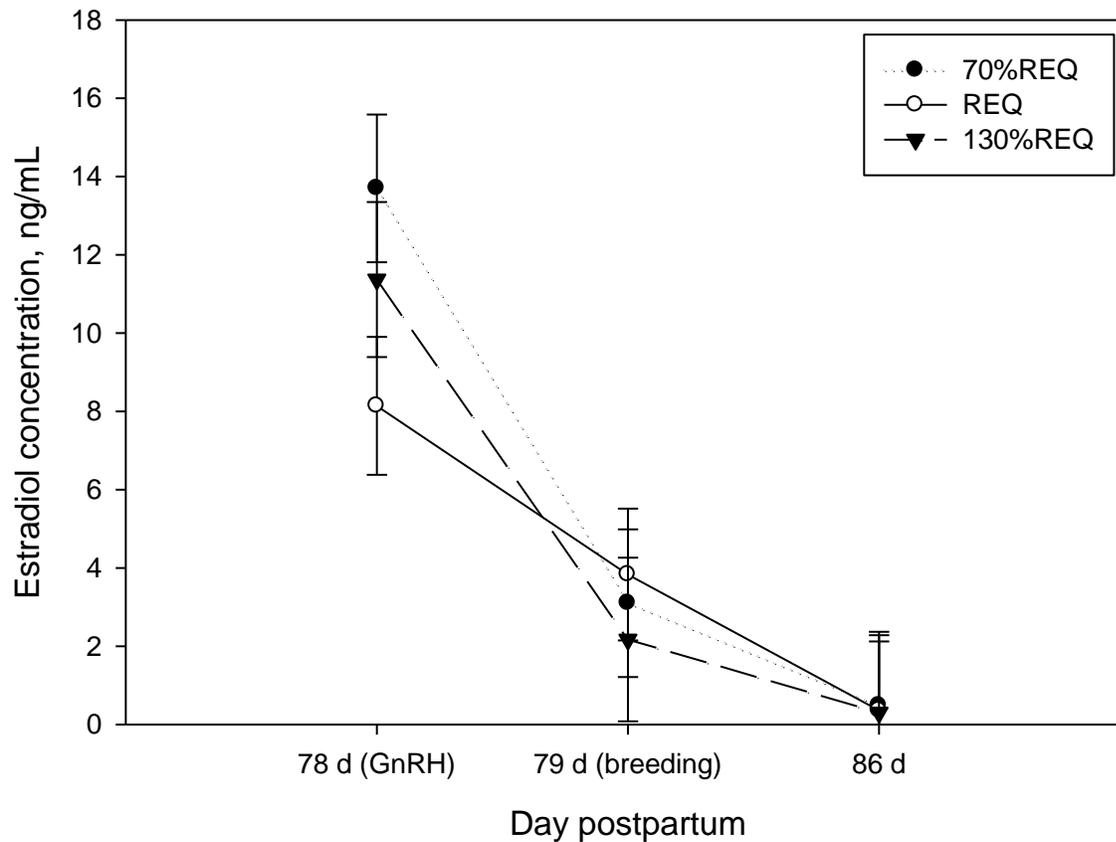


Figure 8. Effect of plane of nutrition during mid-gestation on estradiol concentrations over time in Exp. 2. Cows were fed 70% their NRC requirement for 70%REQ, cows were fed 100% of their NRC requirement for REQ, and cows were fed 130% of their NRC requirement for 130%REQ. There was no interaction ($P = 0.36$) between plane of nutrition and day postpartum. The plane of nutrition during late gestation did not affect ($P = 0.63$) estradiol concentrations. Concentrations of estradiol were different ($P < 0.01$) over time. At 78 d postpartum (12 h prior to breeding), cows were given GnRH injection. At 79 d postpartum, cows were AI. . At 82 d postpartum, represents the halfway point between breeding and the time of flush.

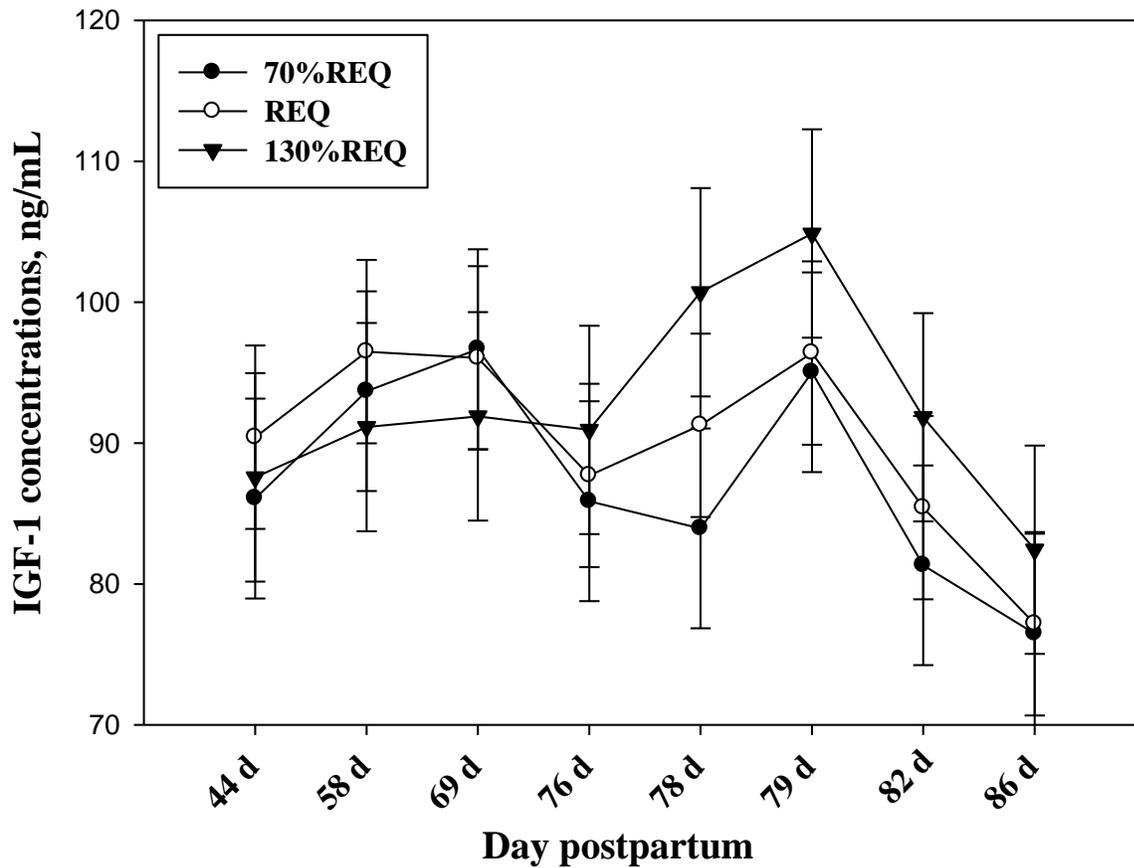


Figure 9. Effect of plane of nutrition during mid-gestation on IGF-1 concentrations over time in Exp. 2. Cows were fed 70% their NRC requirement for 70%REQ, cows were fed 100% of their NRC requirement for REQ, and cows were fed 130% of their NRC requirement for 130%REQ. There was no interaction ($P = 0.17$) between plane of nutrition and day postpartum. The plane of nutrition offered during late gestation did not affect ($P = 0.81$) IGF-1 concentrations. Concentrations of IGF-1 were different ($P < 0.01$) over time. At 44 d postpartum cows were administered with the first injection of $\text{PGF}_{2\alpha}$. At 58 d postpartum cows were administered with the second injection of $\text{PGF}_{2\alpha}$. At 69 d postpartum, CIDR were inserted. At 76 d postpartum, CIDR were removed. At 78 d postpartum (12 h prior to breeding), cows were given GnRH injection. At 79 d postpartum, cows were AI. . At 82 d postpartum, represents the halfway point between breeding and the time of flush. At 86 d postpartum, cows were flushed.

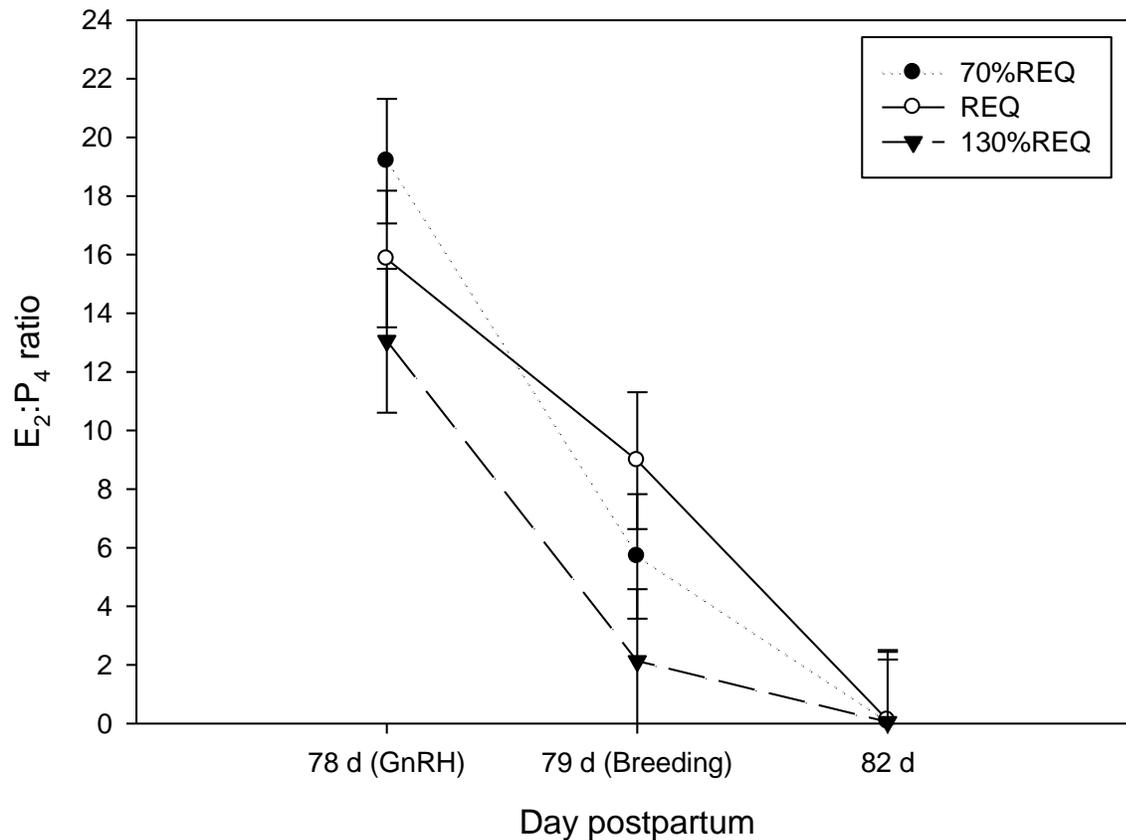


Figure 10. Effect of plane of nutrition during mid-gestation on estradiol:progesterone ratio in Exp. 2. Cows were fed 70% their NRC requirement for 70%REQ, cows were fed 100% of their NRC requirement for REQ, and cows were fed 130% of their NRC requirement for 130%REQ. There was no interaction ($P = 0.54$) between plane of nutrition and day postpartum. The plane of nutrition during late gestation did not affect ($P = 0.40$) the $E_2:P_4$ ratio. The $E_2:P_4$ ratio was different ($P < 0.01$) over time. At 78 d postpartum (12 h prior to breeding), cows were given GnRH injection. At 79 d postpartum, cows were AI. . At 82 d postpartum, represents the halfway point between breeding and the time of flush.

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CHAPTER 3

CONCLUSIONS

There is evidence suggesting that prepartum nutrition affects subsequent postpartum reproduction in beef cattle. There is also evidence that cow nutrition at the time of flush can impact embryo development. These experiments were designed to fill a gap in knowledge on how prepartum plane of nutrition affects beef cow postpartum reproduction and embryo production. In Exp. 1, cows fed 130%REQ had greater BW at breeding when compared with cows fed REQ and cows fed 70%REQ were intermediate; whereas, in Exp. 2, cows fed 130%REQ and REQ had greater BW at breeding compared with cows fed 70%REQ. There were no differences in BW change from 7 to 14 d pre-calving until breeding across treatments, thus, suggesting that differences in cow BW were maintained from parturition to calving. Cow BCS for cows in Exp. 1 and Exp. 2 were all considered moderate (BCS ranging from a 5 to 6). These experiments indicate that the stage of gestation in which diverging planes of nutrition were fed had different effects on total embryos produced. In Exp. 1, cows fed 70%REQ and REQ tended to have greater number of embryos recovered and embryos cleaved or degenerated when compared with cows that were fed 130%REQ. When cows were fed diverging planes of nutrition during mid-gestation in Exp. 2, cows fed 70%REQ and 130%REQ flushed a greater number of embryos when compared with cows fed REQ. Also, there were numerical differences in circulating hormone concentrations that coincide with the embryo data. In Exp. 1, the numerical decrease in P_4 concentrations observed in cows fed 130%REQ during the pre-synchronization process and the numerically greater $E_2:P_4$ in cows fed REQ and 70%REQ support the differences in total embryos recovered by each respective treatment. In Exp. 2, the numerically greater IGF-

1 concentrations in cows fed 130%REQ during estrus and the numerically greater $E_2:P_4$ in cows fed 70%REQ support that cows fed 70%REQ and 130%REQ flushed a greater number of total embryos when compared with cattle fed REQ. These hormonal and embryo differences could be attributed to the stage of gestation in which cows were fed diverging planes of nutrition.

However, environment could also be a confounding factor. Cows in Exp. 1 calved, on average, in January and cows in Exp. 2 calved, on average, in April. Cows in Exp. 1 would have experienced colder temperatures not only during the calving and early lactation period, but also during the time of breeding and flush. Cows in Exp. 1 had numerically decreased cyclicity when compared with cows in Exp. 2, which, could also be partially attributed to environmental stress.

There are still questions to be answered and much potential for further research to investigate the effects of prepartum nutrition on embryo development and production in beef cattle. As stated previously, cows in these studies were all in moderate BCS and this could have masked any potential effects prepartum nutrition could have on postpartum embryo production. Feeding diverging planes of nutrition during gestation to cows that are closer to a threshold BCS (BCS = to 4 or 5) could help illustrate the effects of prepartum nutrition when cattle are truly stressed from a nutrient standpoint. Also, cows selected for intensive embryo production can become overconditioned ($BCS \geq 7$) because they are not entering a normal production cycle and do not lose BW and BCS during lactation, when requirements are highest. Thus, there is application for feeding diverging planes of nutrition to a set of cows that are at a greater starting BCS (approximately 7). Understanding the possible effects of overfeeding and underfeeding cows that are in thin or excessive BCS on embryo production and development could be very applicable to producers that use embryo transfer (**ET**). Along with manipulating the starting BCS

of cows, to better understand the effect of gestational nutrition on embryo production, the feeding period could be modified. In these experiments, single trimesters were used to investigate the effects of prepartum nutrition on cow performance, circulating hormone concentrations, and preimplantation embryo. Targeting 2 different stages of gestation decreased statistical power by decreasing the number of cows in each study. Instead of feeding during two different stages of gestation, all cows could be fed during the last trimester of pregnancy and in result there will be increased statistical power due to an increase in experimental units. Another possibility is to continue to target 100%, 70%, or 130% of cow maintenance requirement from gestation throughout breeding. Feeding cows diverging planes of nutrition during the last trimester of pregnancy until the time of breeding could be a possibility to better understand the effect of long term nutrient surplus or insufficiencies on cow performance, circulating hormone concentrations, and embryo production.

These experiments measured P_4 , E_2 , and IGF-1 concentrations to investigate hormonal differences associated with prepartum nutrition and to help explain their impact on embryo production. However, there are other variables that can be measured to better understand the mechanisms involved with embryo production. Non-esterified fatty acids (**NEFA**) were not measured in these experiments, but could be a key component to understand the metabolic and hormonal mechanisms that result in the embryo data that is presented in Exp. 1 and Exp. 2. Increases in NEFA are seen when cattle enter a negative energy balance and there is potential for an inverse relationship with NEFA and luteinizing hormone. Also, measuring NEFA will give a better understanding of when cattle are truly in a negative energy balance during the prepartum and postpartum periods. There are also alternative methods to ET to evaluate embryo

characteristics. Much like ET, In-vitro fertilization (**IVF**) is another reproductive technology that has gained popularity in the beef industry to increase the number of offspring from elite genetic lines. However, it is a slightly different procedure than embryo transfer because IVF uses harvested oocytes and semen to generate embryos in a petri dish. Oocytes are collected using follicular aspiration and matured in a petri dish, thus, oocyte quality can be analyzed and reported using IVF. All embryos would be cultured in the same medium and, thus, there would be no differences in the environment that embryos would be developing in. Potential differences in cow uterine environment could affect embryo development when using ET, and those differences would not be present with IVF. Also, with IVF, there is potential to investigate the implantation rate of embryos developed using IVF in a contemporary group of cows to see if prepartum nutrition has an effect on embryo implantation.

APPENDIX

Uterine Biopsies were taken for future analysis of progesterone receptors and estrogen receptor- α . In Exp. 1, at 42 and 84 ± 5 d postpartum uterine biopsies were taken using J0116JW Uterine Biopsy Forceps that was 60 cm long (Jorgensen Labs, Loveland, Colorado). The forceps was covered with a sanitary disposable sheath (Agtech Inc. USA, Manhattan, KS) before insertion into the vagina. The sheath was retracted prior to entering the cervix and the forceps were passed through the cervix into the uterine body. Endometrial tissue samples were collected from the uterine body. These samples were immediately placed into a 2.0 mL cryogenic vial (Corning Inc, Corning, NY) and placed into liquid nitrogen. Samples were later transferred into Symphony ultra-low temperature freezer (VWR International, Radnor, PA) set at -80°C . In Exp. 2, all procedures and equipment used for uterine biopsy collection were identical to exp. 1 and performed at 44 and 86 ± 4 d postpartum.

All embryos that were frozen during this experiment were stored in liquid nitrogen at the University of Illinois beef and sheep research unit.