Effects of prepartum plane of nutrition during mid- or late gestation on beef cow body weight, body condition score, blood hormone concentrations and preimplantation embryo

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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 × Simmental cows (n = 33; BW = 664 ± 78 kg) were fed diets formulated to provide three 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). Cows fed 130% REQ tended to have greater BW (p = 0.06) at breeding, greater progesterone concentrations (p = 0.09), and fewer total embryos recovered at 84 ± 5 d postpartum (p = 0.07) when compared with cows fed REQ. In Exp. 2, multiparous Angus and Angus × Simmental cows (n = 35; BW = 601 ± 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. Cows fed 70% REQ and 130% REQ during mid-gestation had a greater (p = 0.03) count of total embryos recovered at 86 ± 4 d postpartum when compared with cows fed REQ. In conclusion, while prepartum nutritional treatments tended to affect cow BW and BCS at breeding, effects on embryo production differed depending on the stage of gestation at which nutritional treatments were applied.

Introduction

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 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 improve 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). Considerable evidence exists which implicates that prepartum nutritional management will improve 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 alter circulating hormone concentrations (Wiley et al. 1991; Lalman et al. 1997) that are important in cattle achieving reproductive success. Moreover, the influence of postpartum nutritional management has been reported to influence embryo quality (Nolan et al. 1998). A high postpartum plane of nutrition can have negative effects on embryo production in females (Robinson et al. 2006). However, there has been minimal research investigating the collective effects of prepartum nutrition on postpartum embryo production in beef cattle. Our hypothesis was that BW, BCS, circulating hormones and total embryo production in cattle fed a plane of nutrition below maintenance requirements during gestation would be negatively impacted relative to cattle fed a plane of nutrition to achieve maintenance requirement, and positively impacted in cattle fed a plane of nutrition to surpass maintenance requirement during gestation. 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 quality characteristics.
Materials and methods

Experiment 1-animal and diet management

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). Thirty-three multiparous Angus and Angus × Simmental crossbred cows (BW = 664 ± 78 kg) were used in Exp. 1 at the University of Illinois Beef Cattle and Sheep Field Research Laboratory in Urbana, IL. Cows were housed in three 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 × 4.88 m. Cows were stratified by calf sire, calf sex and BW and allotted to nine pens. Pen was used as the experimental unit and pens were randomly assigned to treatments resulting in three replications of each treatment. There were six pens containing four animals per pen and three pens containing three 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). All cows were limit-fed the same diet reported in Table 1 during late gestation (91 ± 4 to 8 ± 4 d prepartum) in varying quantities to achieve: 100% of NRC requirement (9.1 kg DMI), 70% of NRC requirement (6.4 kg DMI), or 130% of NRC requirement (11.9 kg DMI). Upon completion of the treatment diets, cows were fed a common diet (Table 1), that was formulated to meet NRC requirements. Cows had access to free choice mineral at all times. Cows were weighed on two consecutive days, using a Flying W (Flying W Livestock Equipment, Watonga, OK) squeeze chute equipped with a Tru-Test (Tru-Test Incorporated, Mineral Wells, TX) 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 analysed 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 Analyser, 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; 70% REQ, 1, REQ = 1) or calf mortality (n = 2; 70% REQ = 2). Twenty-nine cows were used for the remaining portion of the trial. Cows were assigned to four groups after parturition (3 groups of n = 7 and 1 group with n = 8) to minimize

| Ingredient, % | Treatment diet<sup>a,b</sup> | Common diet<sup>c,d,e,g</sup>
|--------------|----------------|----------------|
| Soybean hulls | 23.6 | – |
| Corn silage | 52.8 | 87.0 |
| Alfalfa haylage | 23.6 | – |
| Modified wet distillers grains with solubles | – | 13.0 |

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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± d postpartum, cow reproductive tract examinations were performed. Cows ovaries and uteri were examined via ultrasound imaging using Ibex Pro portable ultrasound (E.I. Medical Imaging, Loveland, CO) 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± d postpartum.

On 42 and 56± d postpartum, cows were pre-synchronized by administering prostaglandin F2α (PGF2α; 25mg; 5 mL of Lutalyse, Zoetis, Florham, NJ). At 67± d a progesterone controlled internal drug release device (CIDR; Eazi-Breed CIDR, Zoetis, Florham, NJ) was inserted into the vagina of cows and 2 mL of GnRH (100ug; Factrel, Zoetis, Florham, NJ) was administered. On d 71, 72, 73, and 74± d postpartum, cows were administered FSH (Folltropin-V, Bioniche Animal Health, Belleville, Ontario, Canada) twice daily (12 h apart) to induce superovulation. Fifteen cows were administered 200 mg Folltropin-V FSH (which subsequently was determined to yield unsatisfactory ovarian stimulation) therefore the remaining 14 cows were administered 160 mg Folltropin-V FSH. The CIDRs were removed on d 74± d postpartum and cows were given 25mg PGF2α to induce luteolysis. Forty-eight h after CIDR removal cows were given a second injection of GnRH (100ug) and 12 and 24 h thereafter cows were bred (AI) by two trained technicians. Cows were inseminated using two straws of semen per insemination for a total of four straws per cow. Cows were bred to one Simmental × Angus crossbred bull.

On 7 d post-breeding (84± d postpartum) preimplantation embryos were harvested using procedures described by the International Embryo Transfer Society; (Robertson & Nelson 2010). Ultrasound imaging was performed on ovaries and images from both the left and right ovary were captured to analyse total number of CL present at time of embryo recovery as well as the presence of any follicles. 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 10× 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 categorized as code stage 4 (morula), 5 (d 7 early blastocyst), 6 (d 7 to 8 blastocyst), and 7 (d 8 to 9 expanded blastocyst) as well as code quality 1 (excellent) and 2 (good with trivial imperfections) were classified as freezeable.

Blood samples were collected by venipuncture from the jugular or tail vein using 10 mL vacutainer tubes (Becton, Dickinson and Company, Franklin Lakes, NJ) at 42, 56, 67, 74, 76, 80 and 84± d postpartum. Serum tubes contained a clot activator and gel for serum separation while plasma tubes contained K2EDTA. Immediately following blood collection, serum tubes were allowed to clot at room temperature for 2h, while plasma tubes were gently inverted eight times to ensure proper mixing of blood and anticoagulant and placed in an ice bath for 2h. All samples were centrifuged at 1,300 × g for 10 min; then sample was harvested and aliquoted into 2 mL microtubes (VWR International, Radnor, PA) and stored in a Symphony ultra-low temperature freezer (VWR International, Radnor, PA) set at −80 °C. Blood serum was used for progesterone (P4) analysis. Blood plasma was later used for estradiol (E2) and IGF-1 analyses.

A radioimmunoassay (Coat-A-Count kit; Siemens Healthcare Diagnostics Inc., Los Angeles, CA) was used to analyse blood serum concentrations of progesterone and was validated using the method described in Shoup et al. (2015). The inter-assay and intra-assay CV were 31.2% and 6.3%, 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). The IGF-1 standards and pooled aliquots of bovine serum extract were linear (log/logit transformation; R² > 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 2.5–100 µL of sample and the inter- and intra-assay CV were <6%.

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-1, 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 analysed. Treatment variable (plane of nutrition) was used in the model as a fixed effect. The dichotomized variable FSH (200 or 160 mg) was left in the models whenever significant (p < 0.10). Repeated measures were used to analyse the effects of sampling day on cow parameters using the compound symmetry covariance structure. Variables were subjected to five 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 as a random effect. Degrees of freedom were adjusted by using the Kenward-Roger method (Littell et al. 2002). Pen was the experimental unit and included in the model 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.

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 analyse 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 two 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. 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, or 7, total embryos cleaved or degenerated, total freezable embryos, percentage of embryos recovered, and percent of embryos that were cleaved or degenerated) were analysed 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). Statistical significance was declared at p ≤ 0.05 and trends will be discussed at p > 0.05 to ≤ 0.10.

**Experiment 2-animal and diet management**

Thirty-five multiparous Angus and Angus × Simmental crossbred cows (average initial BW = 601 ± 72 kg) were used for Exp. 2 and housed identically 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 formulation 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. Pen was used as the experimental unit.

**Feed sampling and analysis**

Feed sample collection and analysis were identical to Exp. 1.

**Reproductive processes**

Four cows were removed from the study after parturition due to undesirable temperament (n = 1; 70% REQ) or calf mortality (n = 3; 70% REQ = 1, REQ = 1, 130% REQ = 1). 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. On 44 and 58 ± 4 d postpartum, cows had their oestrus 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 at 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 at 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 & Nelson 2010). All procedures for embryo recovery and grading were 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 were 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 included as a dichotomized variable in Exp. 2.

Table 2. Effect of plane of nutrition during late gestation on BW, BCS, cyclicity, ovarian structures and embryo recovery in Exp. 1.

| Item                                             | 70% REQa | REQb | 130% REQc | SEM  | p Value |
|--------------------------------------------------|----------|------|-----------|------|---------|
| BW at breeding, kg                               | 687g     | 686h | 739g      | 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    |
| Percentage of cows cycling at 67 d postpartumd  | 11       | 20   | 10        | −    | 0.78    |
| Percentage of cows with CL present at 67 d postpartum| 78      | 100  | 90        | −    | 0.89    |
| Percentage of cows with follicles present at 67 d postpartum| 33      | 60   | 30        | −    | 0.67    |
| Count total follicles after superovulation       | 11.9     | 10.5 | 12.9      | 1.4  | 0.41    |
| Count total CL at time of flush                  | 9.6      | 9.7  | 10.7      | 1.2  | 0.76    |
| Count total embryos recovered                    | 6.9g     | 6.4h | 3.1h      | 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| 0.6      | 1.1  | 1.2       | 0.6  | 0.69    |
| Count total embryos cleaved/degenerated          | 4.3g     | 4.5g | 1.6h      | 0.9  | 0.06    |
| Count total embryos freezable                    | 2.1      | 1.9  | 1.5       | 1.0  | 0.91    |
| Percent recoveredd                               | 62       | 73   | 35        | −    | 0.66    |
| Percent cleavedd                                 | 67       | 57   | 47        | −    | 0.88    |

aCows received 70% of their NRC requirement during late gestation.
bCows received 100% of their NRC requirement during late gestation.
cCows received 130% of their NRC requirement during late gestation.
dPercentage cycling represents cows with serum P4 concentration ≥1 ng/mL.
eTotal embryos recovered/total number of CL at the time of flush.
fTotal number embryos cleaved or degenerated/total number of embryos recovered.
g,hWithin a row, means without common superscripts tend to differ (0.05 < p < 0.10).

**Results**

**Experiment 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; while cows fed 70% REQ had intermediate body weights (Table 2). Neither cow BCS at breeding nor cow change in BCS from pre-calving to breeding differed with regard to late gestation nutritional treatments (p = 0.19).

**Reproductive hormones**

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 2). Also, nutritional treatments did not affect (p ≥ 0.67) the percentage of cows with a CL or follicle present at the time of CIDR insertion at 67 ± 4 d postpartum (Table 2). There was no interaction (p ≥ 0.11) of nutritional treatment applied during late gestation and day postpartum for P4 (Figure 1), E2 (Figure 2), or IGF-1 (Figure 3) concentrations. Cows that were fed 130% REQ during late gestation tended (p = 0.09) to have greater average P4 concentrations than cows that were fed REQ and cows fed 70% REQ were intermediate. As expected, P4...
concentrations were different ($p < 0.01$) over time. Also, the plane of nutrition applied during late gestation did not affect ($p/C21 0.44$) blood plasma concentrations of estradiol and IGF-1. However, both estradiol and IGF-1 concentrations were different ($p < 0.0002$) over time.

### Embryo data

The plane of nutrition fed to cows during late gestation did not impact ($p = 0.41$) the number of follicles present after superovulation ($76 ± 4$ d postpartum; Table 2). Also, nutritional treatment did not affect ($p = 0.76$) the total number of CL present at the time of flush. There was a tendency ($p = 0.06$) for cows that were fed 130% REQ to have greater number of total embryos recovered when compared to cows that were fed 70% 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 freezable embryos 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 ($p/C21 0.53$) among nutritional treatments. There was no difference ($p = 0.69$) in number of embryos that received a code stage score of 5, 6, or 7 among nutritional treatments. Plane of nutrition fed during late gestation did not affect ($p/C21 0.66$) the percentage of embryos recovered or the percentage of embryos that were cleaved or degenerated.

### Experiment 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 3). 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 change from pre-calving ($21 ± 14$ d prepartum) until breeding did not differ ($p/C21 0.54$) among nutritional treatments during mid-gestation.
Reproductive hormones

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 3). Also, nutritional treatments did not affect \( p = 0.94 \) the percentage of cows with a CL or follicle present at the time of CIDR insertion at 69 ± 5 d postpartum (Table 3). 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.

Embryo data

The plane of nutrition fed to cows during mid-gestation did not impact \( p = 0.17 \) the number of follicles present after superovulation or the number of CL present at the time of flush (Table 3). However, 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. The total number of embryos that were freezable 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, or 7 was not different \( p = 0.27 \) across nutritional treatments. Plane of nutrition fed during mid-gestation did not affect \( p = 0.61 \) the percentage of embryos recovered or the percentage of embryos that were cleaved or degenerated.

Discussion

Experiment 1

The observed trend for plane of nutrition during late gestation to affect BW is similar to other reported
differences in BW and BCS at parturition (Corah et al. 1974; Stalker et al. 2006; Winterholler et al. 2012; Shoup et al. 2015) as well as at the time of breeding (Winterholler et al. 2012). Although there were no treatment differences of BCS in Exp. 1, all cows were in moderate BCS (5.9, 6.2 and 6.4 for cows fed 70% REQ, REQ and 130% REQ, respectively).

The percentage of cows cycling, determined via concentrations of P4, was not different among treatments but was very low (10–20%). There was a much greater percentage (78–100%) of cows that had a CL present. The CL produces progesterone, thus with the number of CL that were detected with ultrasound measurements, there should have been 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 could explain the presence of a CL or CL like structures without an increase in concentrations of P4.

Corah et al. (1974) reported that cows that were fed increased amounts of dietary energy during gestation tended to have elevated concentrations of P4 at 3 and 5 d postpartum. While P4 concentrations were measured at different days postpartum between 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 greater concentrations of P4 postpartum. Progesterone can exert a positive priming effect on the brain that can enhance the response to estradiol after concentrations of P4 decline (Senger 2012). Greater amounts of P4 prior to oestrus can amplify the intensity and duration of oestrus (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 P4 prior to the onset of oestrus, follicular dynamics of the dominant follicles could have been different in these cows when compared with cows fed 70% REQ and REQ.

Plasma concentrations of estradiol were the greatest at 12 h prior to breeding, which corresponds to when the cows were in oestrus, and then declined at the time of breeding and 4 d post breeding; however, there were no differences among treatments. Corah et al. (1974) also reported no differences in concentrations of estradiol associated with varying amounts of energy fed during the prepartum period.

There was no difference in concentrations of IGF-1 due to plane of nutrition fed to cows during late gestation. Ciccioli et al. (2003) reported that plasma concentrations of IGF-1 were greater when cows had increased postpartum BW gains. In Exp. 1, BW change from pre-calving until breeding was not different across nutritional treatments. This lack of difference may explain why concentrations of IGF-1 were not different for cows fed diverging planes of nutrition during late gestation.

The total number of embryos recovered tended to be greater 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 lower plane of nutrition (130% NRC requirement vs. 100% NRC requirement). 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.

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 average monthly temperatures of −1.3 and 1.3 °C, respectively. The high/low temperatures in February and March were 12.3−15.6 °C and 16.5−9.4 °C, respectively. This fluctuation in temperature could have caused environmental stress on the cows. Williams (2005) reported that calving season and environment can have an impact on cow cyclicity.

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 greater total number of embryos. 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 freezable embryos were not different. It has been shown that cows undergoing short term nutrient restriction (9.6 Mcal·kg−1·cow−1·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 Mcal·kg−1·cow−1·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 (Wilson & Shike 2014). 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. More favourable average temperatures and less fluctuation in temperature during Exp. 2 may have contributed to the increase in number of cows cycling prior to CIDR insertion when compared with Exp. 1. Cows in Exp. 2 calved during April (average temperature of 10.3 °C) and May (average temperature of 18.1 °C) and experienced temperatures that were on average 18.1 and 21.8 °C during synchronization; whereas, cows in Exp. 1 calved during January (average temperature of −1.3 °C) and experienced temperatures that were on average 1.3 and 10.3 °C during the synchronization process.

The lack of difference in concentrations of P4, E2, or IGF-1 across nutritional planes fed during mid-gestation in Exp. 2 are supported by the observations of Corah et al. (1974) in which they reported varying amounts of prepartum energy did not affect concentrations of P4 or estradiol. Progesterone differed over time. After breeding, P4 increased due to the development of CL and the increased P4 production resulting from those structures. Estradiol also differed over time. Concentrations of E2 were the highest at 12 h prior to

Previous literature supports that cows in moderate BCS (5–6) have increased conception rates (Lents et al. 2008; Bohnert et al. 2013) and decreased postpartum interval (Lents et al. 2008) when compared with cows that are in poor BCS (4). In Exp. 1, all cows were in a moderate BCS and, thus, may not have been nutritionally stressed enough to compromise reproductive function. The authors acknowledge that greater number of cows on each treatment is necessary to detect small differences in embryo data.

Experiment 2

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.

There were a greater number of cows cycling by 69 d postpartum in Exp. 2 when compared with Exp. 1. Circulating concentrations of estradiol, nutritional status, and calving season are critical to cows’ ability to return to oestrus (Williams 2005). Perhaps the increased percentage of cows cycling in Exp. 2 could be attributed to all cows in Exp. 2 gaining BW and BCS during late gestation (Wilson & Shike 2014). 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. More favourable average temperatures and less fluctuation in temperature during Exp. 2 may have contributed to the increase in number of cows cycling prior to CIDR insertion when compared with Exp. 1. Cows in Exp. 2 calved during April (average temperature of 10.3 °C) and May (average temperature of 18.1 °C) and experienced temperatures that were on average 18.1 and 21.8 °C during synchronization; whereas, cows in Exp. 1 calved during January (average temperature of −1.3 °C) and experienced temperatures that were on average 1.3 and 10.3 °C during the synchronization process.

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breeding, which would be when cows should have been exhibiting oestrus, and then declined at the time of breeding and 4 d post breeding.

The plane of nutrition during mid-gestation did affect the total number of embryos recovered; cows fed 70% REQ and 130% REQ had a greater number of embryos than cows that were fed REQ. Cows that were fed 70% REQ and 130% REQ also had the greatest number of CL at the time of flush. 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 were no differences in concentrations of IGF-1 across 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 concentrations of IGF-1. 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.

Nolan et al. (1998) reported that cows fed a low energy diet (9.6 Mcal-kg⁻¹-cow⁻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-cow⁻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. Embryo quality, stage of development, and total number of freezable embryos were not different among treatments in Exp. 2. Also, there was no difference in the percent recovery or percent of embryos cleaved or degenerated. The previous literature and mechanisms involved with the explanation of the lack of differences in embryo quality, development, and count of freezable embryos was discussed previously.

**Conclusions**

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, concentrations of blood hormone, 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 REQ. Feeding diverging planes of nutrition in either mid- or late gestation did not impact the number of freezable embryos.

**Disclosure statement**

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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