Serum and plasma metabolites associated with postpartum ovulation and pregnancy risks in suckled beef cows subjected to artificial insemination¹

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ABSTRACT: Two experiments were conducted to determine relationships of blood metabolite concentrations, BW, BCS, and rump fat depth with postpartum ovulation and pregnancy risks, as well as their utility in predicting those outcomes in suckled beef cows. In experiment 1, plasma glucose collected 10 and 3 d before AI of suckled beef cows at seven locations did not differ between cows that had resumed estrous cycles (ovulated) before AI compared with anovulatory cows, whereas plasma glucose 3 d before AI was greater ($P < 0.01$) in cows that became pregnant compared with nonpregnant cows. Serum beta-hydroxybutyrate (BHB) tended ($P = 0.09$) to be less in ovulatory cows compared with anovulatory cows 10 d before AI, but was unrelated to pregnancy status. Receiver-operator derived true-positive (sensitivity) and false-positive (1—specificity) risks were determined for plasma glucose and serum BHB as predictors for postpartum ovulation and pregnancy status. Serum BHB 3 d before AI produced true-positive and false-positive risks of 82% and 37%, respectively, when predicting ovulatory status before AI. Serum BHB 10 d before AI produced a true-positive and false-positive risks of 92% and 25%, respectively, when predicting pregnancy status. In experiment 2, blood

was collected weekly for 12 wk from multiparous suckled beef cows to assess blood metabolite concentrations in addition to concurrent weekly assessments of BW, BCS, and rump fat. When blood metabolites and physical measures were normalized to parturition reflecting changes occurring during the first 6 wk after calving, we observed reduced ($P < 0.05$) concentrations of serum BHB and NEFA, and greater ($P < 0.05$) rump fat and BCS in cows that ovulated before first AI, whereas reduced ($P < 0.05$) plasma glucose was characteristic of cows that became pregnant. When blood metabolites and physical measures were normalized to the onset of the AI program reflecting changes during 6 wk before AI, ovulatory cows had increased ($P < 0.05$) BCS and lower ($P < 0.05$) NEFA from 3 to 6 wk before the onset of the AI program compared with anovulatory cows. With all predictor variables in regression models, some multiple correlation coefficients (R^2) exceeded 50% when predicting postpartum ovulatory status, but those for predicting pregnancy risk were less than 25%. Although measures of BCS and BHB during 6 wk after calving were related to postpartum ovulation risk, rump fat, glucose, BCS, and NEFA were associated with cows that were ovulatory and pregnant.

Key words: artificial insemination, blood metabolites, ovulation risk, pregnancy risk, suckled beef cows

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INTRODUCTION

Estrus, ovulation, and pregnancy risk after AI of suckled beef cows varies with parity, BCS, and days postpartum (Stevenson et al., 2003). Nutrition

and BCS are causative factors related to pregnancy outcomes (Short and Adams, 1988). Visual appraisals of BCS (Bellows et al., 1982) effectively assess adipose tissue stores, but do not indicate current energy partitioning (Short et al., 1990). Although energy partitioning may determine pregnancy success to AI (Short et al., 1990), insufficient information is available to identify the metabolic priority of reproductive processes in beef cows.

Blood metabolites are likely candidate signals to hypothalamic control centers to indicate current nutritional status as well as potential candidates for regulating reproductive outcomes. Brain, erythrocytes, kidney medulla, and mammary tissue have obligatory requirements for glucose as a substrate (Mayes, 1996). Metabolism of adipose tissue increases serum concentrations of NEFA and prolonged postpartum intervals to estrus and ovulation in beef cows are linked to prepartum NEFA concentration (Guedon et al., 1999). Beta-hydroxybutyrate (BHB) is one of three major ketones produced by the liver during incomplete fatty acid oxidation (Duffield, 2000) and lesser serum BHB concentrations were indicative of improved reproductive performance in young beef cows in natural mating systems (Mulliniks et al., 2013). Moreover, blood urea nitrogen (BUN) may be indicator of negative energy balance (Fenwick et al., 2008).

Two experiments were conducted with the objective to determine relationships of blood metabolites (glucose, BHB, NEFA, and BUN) and BW, BCS, and rump fat depth with postpartum ovulation and pregnancy risks as well as their utility in predicting those outcomes in beef cows. We hypothesized that indicator traits of metabolic status in suckled postpartum beef cows will be predictive of ovulatory response and pregnancy risk in a fixed-timed AI system.

MATERIALS AND METHODS

Experiment 1

Experimental procedures were approved by the Kansas State University Institutional Animal Care and Use committee (protocol no. 3392). Mixed parity ($n = 602$; range of 1 through 13 parities; mean $\pm$ SD = 3.3 ± 2.1) suckled beef cows from seven locations in Kansas during two spring breeding seasons were enrolled. Characteristics of suckled beef cows enrolled at each location included: breeds of Angus ($n = 210$), Hereford ($n = 11$), Simmental ($n = 21$), and Angus $\times$ Hereford crossbred ($n = 360$) cows (15.5% were primiparous cows); days postpartum

at AI averaging 79 ± 37 (mean $\pm$ SD); and BCS at the onset of ovulation synchronization program averaging 5.6 ± 0.9 (mean $\pm$ SD). Body condition scores (1 = thin; 9 = very fat; Bellows et al., 1982) were assigned by the same two experienced evaluators (each evaluating cows at the same location during both years).

Blood samples were collected via puncture of a caudal blood vessel at 20 and 10 d before insemination (day 0) for later assessment of serum progesterone using 10-mL vacutainers containing no additives (Pulmolab, Northridge, CA). In addition, blood samples also were collected at days -10 and -3 to assess concentrations of plasma glucose and serum BHB. Blood for plasma glucose analyses was collected into 10-mL vacutainers containing 143 units of sodium heparin (Covidien LLC, Mansfield, MA). Blood tubes were inverted several times after collection, stored on ice, transported to the lab, immediately centrifuged at $1,000 \times g$, and plasma was frozen at -20°C . Blood samples to harvest serum were stored on ice after collection, allowed to clot at 5°C , and centrifuged at $1,000 \times g$ to harvest serum. Serum was immediately frozen at -20°C for later analyses.

On day -10 , cows were treated i.m. with 100 μg GnRH (2 mL Factrel; Zoetis, Florham Park, NJ) and received a progesterone-impregnated controlled internal drug release (CIDR; Zoetis) insert per vagina containing 1.38 g progesterone. On day -3 , 25 mg PGF_{2 α} (5 mL Lutalyse; Zoetis) were administered i.m. and the CIDR insert was removed. At 66 to 72 h after removal of the CIDR insert, cows were inseminated artificially by experienced technicians using frozen-thawed semen from 10 different sires.

Pregnancy diagnosis. After AI, cows were either observed for estrus and reinseminated (purebred cows) or exposed to natural-service bulls (crossbred cows) no sooner than 10 to 12 d after AI. At 35 ± 3 d after AI, pregnancy was confirmed by transrectal ultrasonography (Aloka 500V, 5 MHz transrectal transducer, Wallingford, CT). A positive pregnancy outcome required the presence of an embryo with a heartbeat, or in rare occurrences, in the absence of visualizing the embryo, presence of a corpus luteum > 25 mm and uterine fluid consistent with that stage of pregnancy.

Assays. Serum concentrations of progesterone from blood samples collected from cows on days -20 and -10 were measured in duplicate by direct quantitative (nonextracted) RIA using Coat-A-Count progesterone kits (Siemens Medical Solutions Diagnostics, Los Angeles, CA) previously validated for bovine serum (Stevenson et al.,

2012). Intra- and interassay CV for progesterone was 8.9% and 6.1%, respectively. Assay sensitivity was 14.1 ± 1.6 pg/mL. Progesterone concentrations were categorized as high (≥ 1 ng/mL) or low (all other samples). Cows with a high progesterone status at either days -20 or -10 were defined to have resumed estrous cycles (ovulatory), whereas remaining cows were considered to be anovulatory.

Glucose was measured in duplicated plasma samples by an enzymatic assay using a 96-well plate and an Autokit Glucose (Cat# 439–90901, Wako Chemicals, Richmond, VA). Samples were read in a plate reader at 490 nm. Concentrations of serum BHB were determined in duplicate using an enzymatic reactivity assay (Pointe Scientific, Ann Arbor, MI). The color reactivity was measured in a plate reader at 490 nm. When CV for duplicates were greater than 10%, the samples were reassayed. Intra- and interassay CV for these assays were less than 5%.

Statistical analyses. Concentrations of plasma glucose and serum BHB at days -10 and -3 before AI were analyzed by using separate general linear models (MIXED) in SAS 9.4 (SAS Inst. Inc., Cary, NC). The model included the fixed effects of either ovulatory status at day -10 or pregnancy risk assessed at 35 d after AI, parity (primiparous vs. multiparous) and their interaction, location nested within year, and random effects of BCS and days postpartum at AI. The threshold for significance of all comparisons was $P < 0.05$ with tendencies set at $0.05 < P < 0.10$.

Blood concentrations of glucose and BHB were analyzed and receiver operator curves (ROC) were generated by using procedure LOGISTIC in SAS. The event was the result of the pregnancy or ovulation diagnosis. For purposes of analyses, glucose and BHB concentrations in samples collected on days -10 and -3 were evaluated as tests for predicting ovulatory status and subsequent pregnancy outcome. Sensitivity was the proportion of true-positive responders (response = 1) that had a positive test result (test = 1) as correctly identified for a given glucose or BHB concentration (cut points; i.e., in other words, the number of pregnant cows above or below the selected cut points for pregnancy status divided by the total number of pregnant cows). For ovulation detection, the ratio represented the number of ovulating cows above or below the selected cut points for ovulation divided by the total number of ovulating cows.

Specificity is the proportion of true negative responders (response = 0) that have a negative test result (test = 0). The false-positive risk is equal to

1—specificity (i.e., in other words, pregnant cows incorrectly diagnosed as pregnant or ovulating cows incorrectly diagnosed as ovulating). Glucose and BHB concentrations for each of the two time points for pregnancy or for each of two time points for ovulation were tested independently in separate analyses. The receiver operator curve plots (data not shown) were created by applying a macro described in the SAS Knowledge Base (Sample 25018: Plot ROC curve with cut point labeling and optimal cut point analyses; <http://support.sas.com/kb/25/018.html>). Cut points were selected that had the greatest accuracy in which the greatest sensitivity (true-positive risk) and greatest specificity occurred in the ROC plot as predicted by the Youden index. True-positive (sensitivity) and false-positive risks (1—specificity) are reported with the accuracies of the respective predictive risks for ovulatory and pregnancy status.

Experiment 2

Experimental procedures were approved by the Kansas State University Animal Care and Use committee (protocol no. 3392). Multiparous Hereford $\times$ Angus crossbred suckled beef cows ($n = 38$; ranged 2 through 9 parities; mean $\pm$ SD = 5.0 ± 2.2) at a single location in Kansas were enrolled in a 12-wk collection study before the initiation of the ovulation synchronization and AI program. Cows grazed a native pasture consisting primarily of big bluestem (*Andropogon gerardii*), little bluestem (*Schizachyrium scoparium*), and Indian grass (*Sorghastrum nutans*). During the dormant season (week 1 through 8), cows were supplemented daily (1 kg per cow [as fed]) with a 16% CP supplement and free-choice, low quality (CP $< 10\%$) prairie hay. New growth of native grasses was 7 cm in height at study week 8 of the experiment and supplementation was discontinued thereafter. Greater than 5 cm rainfall occurred during study week 9 of the experiment and pasture conditions were excellent during the remainder of the study.

Cows were either postpartum (< 3 wk; $n = 21$) or prepartum ($n = 17$) at the onset of the study. Every 7 d during a period of 12 wk (between 0800 and 1100 h) until day -10 (initiation of the ovulation-synchronization program), cows were weighed (no feed restriction), assigned a BCS (Bellows et al., 1982) by two experienced evaluators, and assessed for rump fat depth at the same shaved thurl location via ultrasound (Aloka 500V, 3.5 MHz linear transducer, Wallingford, CT; Odhiambo et al., 2009). At the same time, blood samples were collected via

puncture of a caudal blood vessel and processed as described in experiment 1 for later analyses of serum concentrations of NEFA, BHB, BUN, and progesterone. Blood samples for plasma concentrations of glucose analyses were collected and processed as described in experiment 1.

At the conclusion of the sample collection period (day -10), each cow was treated i.m. with 100 µg GnRH (2 mL Factrel, Zoetis) and received per vagina a new controlled internal drug release (CIDR; Zoetis) insert containing 1.38 g progesterone. Seven days later, CIDR inserts were removed and cows were treated i.m. with 25 mg PGF_{2α} (5 mL Lutalyse; Zoetis). Concurrent with removal of the CIDR insert, estrus-detection patches (Estroject, Rockway, Inc., Spring Valley, WI) were affixed to the tail head of each cow according to manufacturer's recommendation. Cows were inseminated using frozen-thawed semen from two sires by one experienced technician at either 60 or 75 h after CIDR insert removal depending on when estrus was detected by estrus-detection patch activation (patch was more than 50% colored). Cows not detected in estrus by 60 or 75 h after removal the CIDR insert were treated with 100 µg GnRH and inseminated. All cows were exposed to natural service bulls beginning 10 to 12 d after AI. At 35 ± 3 d after AI, pregnancy status was confirmed as described in experiment 1.

Ovulatory status. Concentrations of progesterone in blood serum were assayed in duplicate in each weekly sample by direct quantitative (nonextracted) RIA using ImmuChem Double Antibody progesterone ¹²⁵I kits (Catalog # 07-170105; MP Biomedicals LLC, Orangeburg, NY) and validated for bovine serum (Hill et al., 2016). Intra-assay CV for two assays for progesterone averaged 7.5%. Interassay CV for the low pool (1.26 ± 0.3 ng/mL) was 7.7% and for the high pool (3.68 ± 0.3 ng/mL) was 7.3%. Assay sensitivity was 27 ± 16 pg/mL. First postpartum ovulation was determined to have occurred during the week before the first weekly blood serum concentration of progesterone exceeded 1 ng/mL.

Metabolite assays. Plasma concentrations of glucose and serum BHB were analyzed in duplicate as described in experiment 1. Nonesterified fatty acids were quantified in serum samples using an acylation of coenzyme A (Wako Chemicals, Richmond, VA; Morey et al., 2011) and the color saturation was read at 490 nm. To determine BUN concentrations, 1-mL serum samples were processed in duplicate by an AutoAnalyzer III (Seal Analytical Inc., Mequon, WI; Marsh et al., 1965). The serum samples for the BUN assay were read in a plate

reader at 520 nm. When CV for sample duplicates was greater than 10%, the samples were reassayed. Intra- and interassay CV for BUN and NEFA concentrations were less than 6%.

Statistical analyses. Metabolite and physical measures of each cow that could be associated with postpartum ovulatory status and pregnancy outcome were defined as predictor variables: blood concentrations of glucose, BHB, BUN, and NEFA, in addition to BCS, BW, and rump fat. Less than five missing measurements were estimated by interpolation of the value preceding and following the missing value.

Two different analyses were conducted based on the normalization of variables to parturition and to the onset of the AI program. Normalized weekly assessed metabolic and physical traits were used to create two reproductive status categories: 1) postpartum ovulatory status before the initiation of the ovulation synchronization and AI program (d -10); and 2) pregnancy status after AI. Postpartum ovulatory (ovulatory [$n = 20$] vs. anovulatory [$n = 14$]) and pregnancy (pregnant [$n = 18$] vs. nonpregnant [$n = 16$]) statuses were compared for: 1) all variables normalized to calving, which included weekly assessments during the first 6 wk after calving; and 2) all variables normalized to the onset of AI program, which included weekly assessments during the 6 wk preceding the onset of the AI program. Weekly values for each variable were analyzed in repeated-measure models using the MIXED procedure in SAS (METHOD = REML). The type of covariance matrix that yielded the smallest Akaike's coefficient was used for the final model. Dependent variables were ovulatory and pregnancy status in separate analyses. Independent fixed variables included reproductive status (ovulation or pregnancy), cow, week and the week × status interaction. Status was tested using the cow within status split-plot error, whereas the effect of week or the interaction of status by week was tested by the whole-plot (residual) error. A third repeat-measure analysis in procedure MIXED also was conducted with the same dependent variables using a similar model with the independent fixed effects of four classification variables that combined reproductive statuses of cows: 1) nonpregnant anovulatory ($n = 7$); 2) nonpregnant ovulatory ($n = 9$); 3) pregnant anovulatory ($n = 7$); and 4) pregnant ovulatory ($n = 11$). Least squares means were separated using a Dunnett t -test to compare means with a designated control (nonpregnant anovulatory cows). The threshold for significance of all comparisons was $P < 0.05$ with tendencies set at $0.05 < P < 0.10$.

Stepwise regression models were constructed in attempts to predict postpartum ovulation and pregnancy risks using the predictor variables. Procedure LOGISTIC was applied to the dependent variables of ovulatory and pregnancy risk using a stepwise selection approach allowing variables to enter ($P = 0.15$) and remain ($P = 0.15$) in the models. The independent variables were the seven predictor variables (blood concentrations of glucose, BHB, BUN, and NEFA, BCS, BW, and rump fat). The model was executed for each of the first 6-wk variable values normalized to parturition and for each of the 6-wk variable values normalized to the initiation of the AI program. In addition, procedure RSQUARE was executed for each week to determine the multiple coefficients of variation (R^2) for the best one-variable model and for a model that included all seven predictor variables.

Partial correlations among predictor variables were determined by multivariate analysis of variance (MANOVA). A separate model was constructed using the general linear model (GLM) procedure in SAS with all seven predictor variables included. The MANOVA option was utilized to determine partial correlations among variables, and the significance of each predictor variable to estimate ovulation and pregnancy risks. A separate GLM model was executed for each week of the 6 wk before the onset of the AI program. Results for each of the weekly determined correlations and their associated P -values were then summarized. The week with the most significant correlation between each pair of variables also was noted.

RESULTS

Experiment 1

Postpartum ovulation risk. Ovulation risk after calving ranged from 45% to 96% by location, with an average of 64%. Concentrations of plasma glucose (Fig. 1A) and serum BHB (Fig. 1B) at days -10 and -3 were compared between cows that ovulated and those that failed to ovulate before the initiation of the AI program. Plasma glucose concentrations did not differ ($P > 0.77$) between ovulatory statuses on either day. In contrast, at day -10, concentrations of BHB tended ($P = 0.09$) to be less in cows that had previously ovulated compared with anovulatory cows. No differences in BHB concentration were detected ($P = 0.69$) at day -3 after cows had been treated with GnRH and a progesterone intravaginal insert.

Figure 1. Concentrations (least squares mean $\pm$ SE) of plasma glucose (panel A) and serum beta-hydroxybutyrate (BHB; panel B) in suckled beef cows collected either 10 or 3 d before AI (experiment 1). Comparisons of concentrations were made between cows that had previously ovulated ($n = 76$ to 385) or were yet anovulatory ($n = 132$ to 213; first postpartum ovulation risk) and between cows that failed to conceive ($n = 153$ to 300) or were diagnosed pregnant at 35 d after AI ($n = 54$ to 352).

Receiver operator curves were produced to identify key cut points in glucose and BHB that might be predictive of ovulatory status. Cut points for glucose at day -10 (58.7 mg/dL) and day -3 (63.4 mg/dL) produced true-positive risks (sensitivity) for ovulation in the range of 42% to 71% with false-positive risks of 48% to 49% (Table 1). In other words, 71% and 42% of the ovulating cows would be diagnosed correctly at days -10 and -3, respectively, in cows with glucose greater than the selected cut points. The same cut points would incorrectly diagnose 48% to 49% of anovulatory cows as ovulating. At the selected cut point for day -10, it was 2.6 times more ($P < 0.05$) likely that ovulation risk of cows diagnosed correctly as ovulating was greater than the ovulation risk of anovulatory

Table 1. Receiver operator-derived true-positive and false-positive risks for concentrations of plasma glucose and serum BHB in suckled beef cows at 10 and 3 d before AI to predict postpartum ovulatory status (experiment 1)

Item	Accuracy ¹ (%)	True positive ²	False positive ³	Ovulatory status ⁴	
				Anovulatory ⁵	Ovulatory ⁶
Glucose (day -10)	64.0	274/385	104/213	111/220	274/378
		(71.2)	(48.8)	(50.4)	(72.5)
		66.4–75.6 ⁷	41.9–55.7 ⁷	Referent	2.59 ⁸ (1.83–3.66)
Glucose (day -3)	47.6	32/76	65/132	44/111	32/97
		(42.1)	(49.2)	(39.6)	(33.0)
		30.9–54.0 ⁷	40.4–58.1 ⁷	Referent	0.75 ⁸ (0.42–1.32)
BHB (day -10)	33.1	41/97	108/148	56/96	41/149
		(42.3)	(73.0)	(58.3)	(27.5)
		32.3–52.7 ⁷	65.1–79.9 ⁷	Referent	0.27 ⁸ (0.16–0.47)
BHB (day -3)	69.7	62/76	49/132	14/97	62/111
		(81.6)	(37.1)	(14.4)	(55.9)
		71.0–89.6 ⁷	28.9–46.0 ⁷	Referent	7.50 ⁸ (3.80–14.8)

* $P < 0.05$.

¹Correct classification rate is the proportion of observations for which the test result and actual response agree.

²Proportion of true-positive risk (sensitivity [%]). Number of anovulatory cows below the selected cut points for either glucose or BHB divided by the total number of ovulatory cows. Selected cut points for glucose (day -10 = 58.7 or day -3 = 63.4 mg/dL) and for BHB (day -10 = 566 or day -3 = 775 μ M), respectively.

³Proportion of false positive (1 – specificity [%]). Number of anovulatory cows below the selected cut points for either glucose or BHB divided by the total number of ovulatory cows. Selected cut points for glucose (day -10 = 58.7 or day -3 = 63.4 mg/dL) and for BHB (day -10 = 566 or day -3 = 775 μ M), respectively.

⁴Fewer samples were collected for glucose on day -3 and for BHB on days -10 and -3 than for glucose on day -10.

⁵Postpartum ovulatory status for anovulatory cows based on the selected cut point.

⁶Postpartum ovulatory status for ovulatory cows based on the selected cut point.

⁷95% confidence intervals for true-positive or false-positive values.

⁸Odds ratio and 95% confidence interval for comparison of postpartum ovulatory status

cows (Table 1). In contrast, because of the relatively poor predictability of glucose on day -3 for ovulatory status, it was 0.75 times less ($P < 0.05$) likely that the ovulation risk of cows diagnosed correctly as ovulatory was greater than the ovulation risk of anovulatory herd mates (Table 1).

Similar results were detected for predicting ovulation risk based on BHB concentration cut points. True-positive risks on day -10 (cut point = 566 μ M) was 42%, with a high false-positive risk of 73%. On day -10, it was less ($P < 0.05$) likely that ovulation risk of cows diagnosed correctly as ovulating was greater than the ovulation risk of anovulatory cows (Table 1). In contrast, selected cut point of BHB concentration on day -3 (775 μ M) had a true-positive risk of 82% and a modest false-positive risk of 37% (Table 1). On day -3, it was 7.5 times more ($P < 0.05$) likely that ovulation risk of cows diagnosed correctly as ovulating was greater than the ovulation risk of anovulatory cows (Table 1).

Postpartum pregnancy risk. Pregnancy risk after AI ranged from 37% to 67% by location, with an average of 50%. Glucose concentration on day -10 did not affect subsequent pregnancy risk but was greater ($P < 0.01$) in pregnant than in

nonpregnant cows on day -3 (Fig. 1A). In contrast, concentration of BHB did not differ between pregnancy statuses when assessed on either day.

Receiver operator curves produced cut points for glucose at day -10 (57.5 mg/dl) and day -3 (71.8 mg/dl) and true-positive risks for pregnancy in the range of 52% to 71% with false-positive risks of 33% to 47% (Table 2). At the selected cut points at days -10 and -3, it was 2.2 to 2.9 times more ($P < 0.05$) likely that the pregnancy risk of cows diagnosed correctly as pregnant was greater than the pregnancy risk of nonpregnant cows.

The cut point for BHB predicted by receiver operator curves (794 μ M) produced true-positive risk of 92% for samples collected on day -10 with a false-positive risk of 25%. At the selected cut point for BHB at day -10, it was 32.2 times more ($P < 0.05$) likely that the pregnancy risk of cows diagnosed correctly as pregnant was greater than the pregnancy risk of nonpregnant cows. The predictive power of BHB was somewhat less for samples collected on day -3 (cut point = 751 μ M) with 74% true-positive and 39% false-positive risks. At the selected cut point for BHB at day -10, it was 4.4 times more ($P < 0.05$) likely that the pregnancy

Table 2. Receiver-operator-derived true-positive and false-positive risks for plasma concentrations of glucose and serum BHB in suckled beef cows at 10 and 3 d before AI to predict pregnancy risk (experiment 1)

Item	Accuracy ¹ (%)	True positive ²	False positive ³	Pregnancy risk ⁴	
				Not pregnant ⁵	Pregnant ⁶
Glucose (day -10)	62.4	216/302	140/300	86/246	216/356
		(71.5)	(46.7)	(35.0)	(60.7)
		66.1–76.6 ⁷	40.9–52.5 ⁷	Referent	2.87** (2.05–4.02)
Glucose (day -3)	62.8	28/54	51/153	26/128	28/79
		(51.8)	(33.3)	(20.3)	(35.4)
		37.8–65.7 ⁷	25.9–41.4 ⁷	Referent	2.15** (1.15–4.05)
BHB (day -10)	79.6	66/72	44/173	6/135	66/110
		(91.7)	(25.4)	(4.4)	(60.0)
		82.7–96.9 ⁷	19.1–32.6 ⁷	Referent	32.2** (13.1–79.6)
BHB (day -3)	64.2	40/54	60/153	14/107	40/100
		(74.1)	(39.2)	(13.1)	(40.0)
		60.4–85.0 ⁷	31.4–47.4 ⁷	Referent	4.43** (2.22–8.83)

* $P < 0.05$.

¹Correct classification rate is the proportion of observations for which the test result and actual response agree.

²Proportion of true-positive risk (sensitivity [%]). Number of nonpregnant cows below the selected cut points for either glucose or BHB divided by the total number of pregnant cows. Selected cut points for glucose (day -10 = 57.5 or day -3 = 71.8 mg/dL) and for BHB (day -10 = 794 or day -3 = 751 μ M), respectively.

³Proportion of false positive (1 – specificity [%]). Number of nonpregnant cows below the selected cut points for either glucose or BHB divided by the total number of pregnant cows. Selected cut points for glucose (day -10 = 57.5 or day -3 = 71.8 mg/dL) and for BHB (day -10 = 794 or day -3 = 751 μ M), respectively.

⁴Fewer samples were collected for glucose on day -3 and for BHB on days -10 and -3 than for glucose on day -10.

⁵Pregnancy risk for cows not pregnant based on the selected cut point.

⁶Pregnancy risk for pregnant cows based on the selected cut point.

⁷95% confidence intervals for true-positive or false-positive values.

⁸Odds ratio and 95% confidence interval for comparison of pregnancy risk.

risk of cows diagnosed correctly as pregnant was greater than the pregnancy risk of nonpregnant cows at the selected cut point for BHB at day -10.

Experiment 2

The 38 multiparous cows enrolled in the study had an overall first postpartum ovulation risk during the 12-wk study period of 57.9%. The pregnancy risk associated with timed AI determined 35 d after AI was 47.4%.

Postpartum ovulatory status. Cows that ovulated before the initiation of the AI program had greater ($P = 0.004$) BCS and lesser ($P = 0.017$) concentrations of BHB during the first 6 wk postpartum compared with anovulatory cows (Table 3). Although BW, rump fat, BUN, NEFA, and glucose concentrations did not differ between ovulatory statuses, interactions of status by week were detected for NEFA ($P = 0.015$; Fig. 2) and rump fat ($P = 0.001$; Fig. 3). Concentrations of serum NEFA in ovulatory cows decreased from 0 to 6 wk postpartum following a pattern described by a third-order polynomial curve ($\text{NEFA} = -3.84 \times \text{wk}^3 + 56.2 \times \text{wk}^2 - 278.8 \times \text{wk} + 890.7$; $R^2 = 0.87$), whereas serum NEFA concentrations in anovulatory cows

were greater at week 0 and decreased thereafter in a pattern described by a fourth-order polynomial curve ($\text{NEFA} = 7.54 \times \text{wk}^4 - 123.9 \times \text{wk}^3 + 676.7 \times \text{wk}^2 - 1456 \times \text{wk} + 1,655$; $R^2 = 0.99$; Fig. 2). Concentrations of serum NEFA differed ($P < 0.05$) between ovulatory statuses during week 2 and tended ($P < 0.10$) to differ during week 3 through 5 (Fig. 2). Rump fat depth was similar between ovulatory statuses until week 6 postpartum when it increased ($P < 0.05$) substantially in ovulatory cows compared with anovulatory cows (Fig. 3).

When predictor variables were normalized to the initiation of the AI program, ovulatory cows had greater ($P = 0.001$) BCS, greater glucose concentrations ($P = 0.002$), but lesser serum concentrations of NEFA ($P = 0.001$) and BHB ($P = 0.037$) compared with anovulatory cows (Table 4). Furthermore, an interaction of ovulatory status and serum NEFA concentration was detected ($P = 0.001$; Fig. 4). Serum concentrations of NEFA were less ($P < 0.01$) in ovulatory than anovulatory cows during week -6 through -3 before the onset of the AI program. Serum concentrations of NEFA in ovulatory cows followed a decreasing pattern described by a fourth-order polynomial curve ($\text{NEFA} = 4.73 \times \text{wk}^4 - 71.1 \times \text{wk}^3 + 351 \times$

$\text{wk}^2 - 718 \times \text{wk} + 942$; $R^2 = 0.94$) and were consistently less than those in anovulatory cows until 1 wk before the onset of the AI program. The pattern of serum NEFA concentrations in anovulatory cows followed a decreasing pattern described by a fifth-order polynomial curve ($-1.77 \times \text{wk}^5 + 38.4 \times \text{wk}^4 - 294 \times \text{wk}^3 + 940 \times \text{wk}^2 - 1,237 \times \text{wk} + 1,254$; $R^2 = 0.99$).

Pregnancy status. The relationship between predictor variables assessed during the first 6 wk postpartum and eventual pregnancy status after AI was limited to two significant variables (Table 3). Cows that became pregnant tended ($P = 0.08$) to have greater rump fat depth and lesser ($P = 0.008$) concentrations of glucose. When predictor variables were normalized to calving, no interactions were detected ($P > 0.15$) between pregnancy status and week.

When predictor variables were normalized to the onset of the AI program, only rump fat depth tended ($P = 0.08$) to be greater in cows that became pregnant compared with nonpregnant cows (Table 4).

Combined ovulatory-pregnancy statuses. Comparisons were made with the designated control (nonpregnant anovulatory cows) for the 6 wk preceding the onset of the AI program. Cows of all other reproductive statuses had greater ($P = 0.001$) BCS and lesser ($P = 0.001$) serum concentrations of NEFA (Table 5), whereas no differences were detected in BW or concentrations of BUN and BHB. Glucose was greater ($P < 0.05$) in pregnant ovulatory cows than in nonpregnant anovulatory cows. Rump fat depth tended ($P = 0.06$) to be greater in pregnant cows regardless of ovulatory status compared with nonpregnant anovulatory

Table 3. Physical and metabolic trait least square means ($\pm$ SE) in suckled beef cows during 6 wk postpartum according to postpartum ovulatory and subsequent pregnancy status (experiment 2)

Trait ³	Estrus-cycle status before AI ¹			Pregnancy status after AI ²		
	Anovulatory	Ovulatory	<i>P</i> -value	Not pregnant	Pregnant	<i>P</i> -value
Cows, number	14	20		16	18	
BW, kg	477 $\pm$ 17	495 $\pm$ 15	0.428	480 $\pm$ 15	497 $\pm$ 16	0.456
BCS	4.4 $\pm$ 0.1	4.7 $\pm$ 0.1	0.004	4.5 $\pm$ 0.1	4.6 $\pm$ 0.1	0.612
Rump fat ³ , mm	2.0 $\pm$ 0.04	2.1 $\pm$ 0.03	0.181	2.0 $\pm$ 0.03	2.1 $\pm$ 0.03	0.081
BUN ⁴ , mg/dl	24.7 $\pm$ 1.1	24.6 $\pm$ 0.9	0.919	24.4 $\pm$ 1.0	24.9 $\pm$ 1.0	0.760
NEFA ⁵ , μ M	523 $\pm$ 49	468 $\pm$ 41	0.407	465 $\pm$ 44	520 $\pm$ 45	0.394
BHB ⁴ , μ M	447 $\pm$ 22	375 $\pm$ 21	0.017	423 $\pm$ 21	387 $\pm$ 21	0.236
Glucose, mg/dl	50.0 $\pm$ 1.8	48.4 $\pm$ 1.5	0.507	51.9 $\pm$ 1.5	45.9 $\pm$ 1.5	0.008

¹Ovulatory status was based on when weekly concentrations of progesterone exceeded 1 ng/mL.

²Based on transrectal ultrasonography at 35 d after AI.

³Interaction ($P < 0.05$) between ovulatory status and week (see Fig. 3).

⁴BUN = blood urea nitrogen; BHB = beta-hydroxybutyrate.

⁵Interaction ($P < 0.05$) between ovulatory status and week (see Fig. 2).

Figure 2. Serum concentrations of NEFA (least squares mean $\pm$ SE) during 6 wk after calving according to postpartum first ovulatory status (OV) in 14 cows that were anovulatory compared with 20 cows that were ovulatory (experiment 2).

Figure 3. Rump fat depth (least squares mean $\pm$ SE) during 6 wk after calving according to postpartum first ovulatory status (OV) in 14 cows that were anovulatory compared with 20 cows that were ovulatory (experiment 2).

Table 4. Physical and metabolic trait least squares means ($\pm$ SE) in suckled beef cows during 6 wk before the initiation of the AI program according to postpartum ovulatory and subsequent pregnancy status (experiment 2)

Trait ³	Estrus-cycle status before AI ¹			Pregnancy status after AI ²		
	Anovulatory	Ovulatory	P-value	Not pregnant	Pregnant	P-value
Cows, number	14	20		16	18	
BW, kg	469 $\pm$ 17	472 $\pm$ 14	0.893	462 $\pm$ 16	478 $\pm$ 15	0.489
BCS	4.3 $\pm$ 0.1	4.8 $\pm$ 0.1	0.001	4.5 $\pm$ 0.1	4.7 $\pm$ 0.1	0.357
Rump fat, mm	2.1 $\pm$ 0.05	2.1 $\pm$ 0.04	0.169	2.0 $\pm$ 0.04	2.2 $\pm$ 0.04	0.081
BUN ³ , mg/dL	25.0 $\pm$ 1.1	25.3 $\pm$ 0.9	0.839	24.9 $\pm$ 1.0	25.5 $\pm$ 0.9	0.670
NEFA ⁴ , μ M	446 $\pm$ 31	295 $\pm$ 25	0.001	393 $\pm$ 33	323 $\pm$ 31	0.129
BHB ³ , μ M	457 $\pm$ 19	402 $\pm$ 16	0.037	433 $\pm$ 19	415 $\pm$ 18	0.493
Glucose, mg/dL	55.5 $\pm$ 1.4	61.4 $\pm$ 1.1	0.002	58.4 $\pm$ 1.4	59.6 $\pm$ 1.3	0.555

¹Ovulatory status was based on when weekly concentrations of progesterone exceeded 1 ng/mL.

²Based on transrectal ultrasonography at 35 d after AI.

³BUN = blood urea nitrogen; BHB = beta-hydroxybutyrate.

⁴Interaction ($P = 0.002$) between ovulatory status and week (see Fig. 4).

Figure 4. Serum concentrations of NEFA (least squares mean $\pm$ SE) during 6 wk before the initiation of the AI program according to postpartum first ovulatory status (OV) in 14 cows that were anovulatory compared with 20 cows that were ovulatory (experiment 2).

cows. The only interaction between reproductive-ovulatory status and week was detected for serum concentrations of NEFA. The pattern of serum NEFA concentrations was elevated in anovulatory cows during week -6 through -3 before the onset of the AI program compared with the ovulatory cows regardless of pregnancy status (Fig. 5).

Multiple regression models. Relationships of predictor variables with postpartum ovulatory and pregnancy status are summarized in Table 6 for variables normalized to parturition. Multiple variables accounted for 30% to 58% of the variation in postpartum ovulatory risk. Body condition score was significant in regression models for all but weeks 3 and 4 postpartum. Concentrations of serum BHB were significant during 2, 4, 5, and 6 wk, glucose in 2, 3, and

6 wk, and serum BUN and rump fat only in week 2. One-trait best-fitting models producing the greatest multiple coefficients of determination (R^2) included BCS during 0, 1, and 5 wk, serum NEFA during 2 and 3 wk, and serum BHB during 4 and 6 wk, with R^2 values ranging from 0.13 to 0.29.

When variables were normalized to the onset of the AI program to predict pregnancy risk, no predictor traits were significant ($P = 0.15$) during the 6-wk period preceding the onset of AI program (data not shown). Rump fat during weeks 5 and 6 and serum NEFA in week 2 tended ($P < 0.10$) to be important; however, in no week was there more than 1 variable retained in the model ($P > 0.15$). During weeks 3, 1, and 0 before the onset of the AI program, no variable was retained in the models. One-trait best-fitting models produced R^2 values that ranged from 0.4 to 0.11, and when all traits were retained in the models, R^2 values ranged from 0.06 to 0.24.

Partial correlations. Weekly partial correlations among the seven variables during 6 wk before the onset of the AI program were determined with the greatest correlation ($r = 0.73$) detected between rump fat and BCS at week 4 before the onset of the AI program. Other highly correlated ($r > 0.60$; $P \leq 0.05$) variables were BW and BCS at week 3 ($r = 0.61$), BW and RF at week 5 ($r = 0.64$), and serum BHB and NEFA concentrations at week 6 ($r = 0.67$). Partial correlations determined for week 0 detected BW to be correlated positively ($P < 0.05$) with BCS ($r = 0.51$) and rump fat ($r = 0.43$); and BCS with rump fat ($r = 0.47$). Concentrations of BUN were correlated negatively ($P < 0.05$) with BHB ($r = -0.38$).

Table 5. Physical and metabolic trait least squares means ($\pm$ SE) in suckled beef cows during 6 wk before the initiation of the AI program according to subsequent pregnancy and previous first postpartum ovulatory status before the onset of AI program (experiment 2)¹

Trait ²	Not pregnant		Pregnant		P-value
	Anovulatory	Ovulatory	Anovulatory	Ovulatory	
Cows, number	7	9	7	11	
BW ² , kg	436 $\pm$ 24	484 $\pm$ 21	502 $\pm$ 24	462 $\pm$ 19	0.224
BCS	4.1 $\pm$ 0.2	4.8 $\pm$ 0.1 ^a	4.5 $\pm$ 0.1 ^a	4.7 $\pm$ 0.1 ^a	0.001
Rump fat, mm	1.9 $\pm$ 0.06	2.1 $\pm$ 0.06	2.2 $\pm$ 0.06	2.2 $\pm$ 0.05	0.060
BUN ² , mg/dL	24.4 $\pm$ 1.6	25.1 $\pm$ 1.4	25.4 $\pm$ 1.6	25.4 $\pm$ 1.3	0.957
NEFA ³ , μ M	521 $\pm$ 43	303 $\pm$ 39 ^a	378 $\pm$ 42 ^a	288 $\pm$ 34 ^a	0.001
BHB ² , μ M	465 $\pm$ 29	416 $\pm$ 25	455 $\pm$ 28	390 $\pm$ 23	0.155
Glucose, mg/dL	55.2 $\pm$ 1.9	60.0 $\pm$ 1.7	54.9 $\pm$ 1.9	62.6 $\pm$ 1.5 ^a	0.007

^aDifferent ($P < 0.05$) from control (not pregnant and anovulatory).

¹Ovulatory status was based on weekly concentrations of progesterone and pregnancy status was accessed at 35 d after AI.

²BUN = blood urea nitrogen; BHB = beta-hydroxybutyrate.

³Interaction ($P \leq 0.05$) between statuses and week (see Fig. 5).

Figure 5. Serum concentrations of NEFA (least squares mean $\pm$ SE) during 6 wk before the initiation of the AI program according to postpartum ovulation and subsequent pregnancy status: NOT PREG + ANOV ($n = 7$) nonpregnant anovulatory cows; NOT PREG + OV ($n = 9$) nonpregnant ovulatory cows; PREG + ANOV ($n = 7$) pregnant anovulatory cows; and PREG + OV ($n = 11$) pregnant ovulatory cows (experiment 2). ^{ab}Means differ ($P \leq 0.05$) from the control (NOT PREG + ANOV).

DISCUSSION

Ovulatory Status

Researchers have identified estimations of stored adipose tissue (BCS) in beef cows and its association with reproductive performance (Bellows et al., 1982). The reproductive measure of importance was the postpartum interval to resumption of estrous cycles because AI programs utilized in that early research relied on detection of estrus before insemination. Our research corroborates previous findings in which cows with a greater BCS had a greater risk of resuming estrus after calving. Furthermore, either BW or rump fat were correlated positively with BCS during 4 of the

6 wk that preceded the onset of the AI program in experiment 2.

Short et al. (1990) proposed that until a critical amount of body adipose reserve is satisfied, no energy is partitioned for the establishment of pregnancy. Cows that calved with a body condition score of 5 or greater had shorter postpartum intervals to first estrus. In the current study, cows that ovulated before initiating the AI period had greater BCS than cows that had not yet ovulated. In experiment 2, BCS was one of the most consistent and accurate indicators of postpartum ovulatory status.

Variability and subjectivity of BCS assessment and the inability to detect subtle changes in BCS has prompted the search for more precise measures of energy status. Nutritional status indicators

Table 6. Multiple regression models to predict postpartum ovulation risk based on physical (BCS, rump fat, and BW) and metabolic traits (NEFA, glucose, BUN, and BHB) assessed during the first 6 wk postpartum (experiment 2)

Stepwise multiple regression models			Regression models		
Postpartum week	Trait ¹	P-value ²	Best 1-trait model	R ²	All seven traits R ²
0	BCS	0.010	BCS	0.139	0.306
	BW	0.045			
1	BCS	0.001	BCS	0.194	0.453
	BW	0.018			
2	Glucose	0.011	NEFA	0.136	0.532
	NEFA	0.021			
	BCS	0.030			
	BHB	0.048			
	BUN	0.074			
	Rump fat	0.103			
3	NEFA	0.015	NEFA	0.166	0.361
	Glucose	0.069			
4	BHB	0.045	BHB	0.155	0.304
5	BCS	0.018	BCS	0.148	0.333
	BHB	0.051			
6	BCS	0.020	BHB	0.289	0.584
	BHB	0.021			
	Glucose	0.078			

¹BUN = blood urea nitrogen; BHB = beta-hydroxybutyrate.

²Adjusted probability values of remaining traits in the model after stepwise regression (entry criterion [$P = 0.15$] or retention criterion [$P = 0.15$]).

that have continuous numerical distributions could provide more precise information than subjective discrete assessments of body condition. First postpartum ovulation in beef cows is difficult to quantify in typical pasture management systems; however, when natural service sires or AI utilizing estrus detection is the method by which cows are identified for insemination, the estrus that precedes ovulation is essential for reproductive success.

Rump fat did not increase in ovulatory compared with anovulatory cows until the week 6 after calving. In contrast, BCS during 6 wk after calving and during 6 wk before the onset of the AI program were greater in ovulatory than anovulatory cows and was a significant source of variation in postpartum ovulation risk in 5 of 6-wk postpartum multiple regression models in addition to being the most important single model variable in 3 of the 6-wk models.

Acute nutritional stress is identified by greater concentrations of BHB when inadequate dietary nutrients are available and metabolism of adipose tissues ensues. In experiment 1, BHB concentrations tended to be less in cows that had ovulated by 10 d before insemination. Despite that trend, receiver operator curves indicated that BHB concentration was a better retrospective predictor of ovulation risk at 3 d before insemination (sensitivity = 81.6%) than at 10 d before AI (sensitivity = 42.3%). Lesser

concentrations of serum BHB also were indicative of improved reproductive performance in young cows in natural mating systems (Mulliniks et al., 2013) and characteristic of earlier postpartum ovulation based on patterns of BHB during 6 wk postpartum and during 6 wk before the onset of the AI program (experiment 2). Timing of BHB sampling relative to parturition and/or improved range conditions to which cows were exposed was important because serum BHB in experiment 2 accounted for significant variation in predicting ovulation risk at 2 through 6 wk postpartum based on multiple regression modeling.

Volatile fatty acids in the ruminant are the primary energy sources for many bodily functions. Milk synthesis in the lactating female, in contrast, is dependent on glucose availability (Mayes, 1996). Reproductive processes and tissues including oocyte development in sheep also require glucose (Berlinguer et al., 2012). A decrease in plasma concentrations of glucose in early postpartum dairy cows indicates an energy partitioning priority that favors lactation over reproduction (Lucy et al., 2013). Although beef cows produce less milk than dairy cows, energy partitioning priorities are likely similar. When early postpartum lactating dairy cows were infused with glucose in stepwise increasing doses, the physiological upper limit of plasma glucose concentration was accompanied by marked

and rapid decreases in serum NEFA and BHB concentrations (Lucy et al., 2013). In experiment 1, in which only 2 pre-AI samples were collected to estimate plasma glucose concentrations, no difference in glucose was noted between cows that ovulated before insemination and those that did not. In experiment 2, however, with multiple collections over several weeks, cows with greater plasma concentrations of glucose during the 6 wk preceding the onset of the AI program were more likely to have ovulate, whereas that relationship was not true when examining plasma glucose patterns during the first 6 wk postpartum.

Ruminants including beef cows use propionate and acetate as their primary energy source (Harmon, 1992). During periods when energy supply is inadequate to meet nutritional requirements, such as during early lactation, stored lipids are mobilized as serum NEFA for conversion to energy sources by the liver (Beever, 2006). In confined dairy cows, in which negative energy balance is easier to quantify than in beef cows because of management differences (dairy cows are fed necessary nutrients in a total mixed diet rather than grazing for nutrients), early lactation is associated with decreased blood concentrations of glucose and insulin and increased serum concentrations of NEFA (Overton and Waldron, 2004). In agreement with the dairy data, Lake et al. (2006) reported an inverse relationship between insulin and NEFA in postpartum beef cows. Although mean serum concentrations of NEFA did not differ between ovulatory and anovulatory cows during 6 wk postpartum in experiment 2, we observed greater serum concentrations of NEFA in anovulatory cows during 2 through 5 wk postpartum compared with cows that ovulated before the onset of the AI program. Furthermore, anovulatory cows had greater serum NEFA during 3 through 6 wk before the onset of the AI program compared with ovulatory cows.

Beef heifers infused with VFA responded with greater blood concentrations of insulin and lesser NEFA (DiCostanzo et al., 1999). Primiparous beef cows with greater concentrations of NEFA before calving had longer intervals of postpartum anestrus than primiparous cows with lesser NEFA concentration (Guedon et al., 1999). When dairy cows reached the nadir of energy balance and available energy exceeded requirements, NEFA concentrations started to decrease (Gross et al., 2011). Decreasing serum NEFA in experiment 2 was consistent with the previous observation in dairy cows.

Reproductive management systems in beef cattle that employ timed AI have demonstrated that

pregnancy can be established in anestrous cows (Stevenson et al., 2003). Inducing ovulation as part of ovulation synchronization, however, lessens the importance of presynchronization ovulation and consequently may marginalize the importance of indicators of ovulation previously discussed. Moreover, indicators of pregnancy success in a timed AI system may be different than ovulation indicators that parallel pregnancy success in a natural mating system.

Other predictor measures (BUN and BW) were not related to postpartum ovulation risk when ovulatory and anovulatory cows were compared, although both measures were significant sources of that variation in 1- and 2-wk postpartum multiple regression models, respectively. Blood urea nitrogen is traditionally a biological marker for CP or RDP status rather than energy. It may reflect energy deficiency, however, when energy is lacking in the rumen to capture ruminal ammonia for microbial metabolism. Blood BUN often fluctuates around calving in dairy cows (Wathes et al., 2007) and may be another indicator of negative energy balance because it can denote insufficient energy or excess protein. When microbes in the rumen metabolize protein, ammonia is released, and in cases of a negative energy balance, a tendency was reported for excess of ammonia in dairy cows in severe negative energy balance (Fenwick et al., 2008). Both low energy and high protein feeds fed to cattle are associated with a negative energy balance (Simili da Silva et al., 2014). Without enough available energy, the microbes are unable to convert the abundant ammonia to amino acids and protein, so the ammonia builds up in the rumen (Hammond, 2013).

Change in BW may be more important than absolute BW because lactating dairy cows that lost or maintained BW between calving and 3 wk postpartum had the greatest serum NEFA concentrations at calving and thereafter to week 3 (Carvalho et al., 2014). Blood urea nitrogen can reflect insufficient energy or excess protein when microbes in the rumen metabolize protein and release ammonia. In cases of a negative energy balance, excess of ammonia is detected (Simili da Silva et al., 2014). Although blood urea nitrogen was not related to postpartum ovulation risk in our study, mean concentrations of BUN exceeded 25 mg/dl and likely reflect negative energy balance.

Pregnancy Status

Glucose is likely more sensitive to nutritional status and nutrient availability than measures of

stored adipose tissue such as BCS. When beef cows were sampled for glucose concentration 31 and 61 d after calving, plasma concentrations of glucose did not differ between cows with BCS of 4 vs. 6 (Lake et al., 2006). Contrary to a report in dairy cows (Garverick et al., 2013), Mulliniks et al. (2013) reported that beef cows having greater plasma glucose concentrations were less likely to become pregnant early in the breeding season. Samples in the latter study were collected during 60 d before calving compared with day 3 postpartum in the dairy cow study (Garverick et al., 2013). Based on changes in plasma glucose during the first 6 wk postpartum in experiment 2, mean plasma glucose was less in cows that later conceived to AI compared with those that did not conceive. In contrast, mean plasma glucose concentration 3 d before AI in cows that became pregnant in experiment 1 was 25% greater than in cows that failed to conceive to AI suggesting that plasma glucose eventually increased with increasing days postpartum in those cows that eventually conceived after AI. The predictability of pregnancy status by established cut points of plasma glucose at either 10 or 3 d before AI, was poor to good (sensitivities 52% to 71%). The somewhat conflicting results of the earlier published studies and that in our study may be attributed to time of collection relative to feeding as well as other factors relating to energy balance. Furthermore, temporal differences in glucose concentration, depending on the time of collection relative to feeding also have been noted (Lake et al., 2006). Daily variations in glucose concentrations increase difficulty in accurately interpreting glucose results. In addition, rapid changes in glucose concentration after feeding and other physiological events complicate interpretation of glucose status. Nevertheless, the predictive power of glucose on pregnancy risk warrants further examination. Quantifying the relationship between glucose and reproductive success could lead to an inexpensive and effective chute-side, prebreeding reproductive fitness test.

When dairy cows had serum blood concentrations of BHB > 10 mg/dL during the first 2 wk after calving, pregnancy outcomes were compromised compared with cows with lesser BHB (Ospina et al., 2010). In experiment 1, the established cut point (794 μ M) accurately predicted pregnancy risk 79.6% of the time 10 d before timed AI with a high sensitivity (91.7%) and fairly low false-positive risk (25.4%). All cows had a decrease in serum NEFA concentration that was associated with improving range conditions in experiment 2 (starting 3 to 4 wk before the onset of AI program). The earlier

decrease in serum NEFA noted in pregnant cows may indicate greater nutritional efficiency and be a good indicator of increasing energy balance and resumption of estrus before the breeding season. Another study also indicated that dairy cows with serum NEFA concentration > 0.70 mEq/L during the first 2 wk of lactation were more likely to have lesser pregnancy risk and milk yield than herd mates with lesser NEFA (Ospina et al., 2010). A larger more geographically diverse study (Chapinal et al., 2012) indicated milk yield was reduced in dairy cows with elevated serum concentrations of NEFA during the first 2 wk postpartum but no effect was detected on subsequent pregnancy risk.

In contrast to the strong relationship of BCS with ovulation risk, neither BCS nor BW differed between cows that became pregnant to AI and those that did not. The magnitude of postpartum BW loss in lactating dairy cows was consistent across lactations and this trait is associated with a reduction in days to conception (Zachut and Moallem, 2017). Furthermore, in that previous study, the degree of BW loss postpartum, regardless of genetic merit for milk production, seemed to be an intrinsic trait that represents the variation in trade-off toward tissue mobilization between cows.

Body condition score at calving was not related to future reproductive performance in young *Bos taurus* cows grazing in New Mexico (Mulliniks et al., 2012). Furthermore, BCS at calving was an inadequate indicator of breeding competence in primiparous beef cows studied in Montana (Roberts, 2008). An effect of poor BCS (≤ 2.50 ; 1 = thin and 5 = obese) near AI on fertility of dairy cows was detected, but change in BCS during the first 3 wk postpartum had a more profound effect on pregnancy risk at first timed AI (Carvalho et al., 2014).

Rump fat during 6 wk after calving and during 6 wk before the onset of the AI program, however, tended to be greater in cows that conceived to AI and may be a surrogate for changes in BCS. In a study investigating postpartum changes in BCS and RF, cows that maintained or gained fat reserves had greater pregnancy risk than cows that were thinner (Looper et al., 2010). Ultrasound measurements of fat deposition are repeatable among technicians (Brethour, 1992) and have the potential to accurately monitor subtle changes in body composition in field conditions (Odhiambo et al., 2009).

Concentrations of serum BUN exceeded 20 mg/dL in experiment 2 and were not related to pregnancy risk. High dietary protein (nitrogen) intake resulting in BUN or MUN of greater than 19 to

20 mg/dL has been associated with an altered uterine environment and decreased fertility in lactating dairy cows (Ferguson et al., 1993; Rajala-Schultz et al., 2001). In contrast, average first-service pregnancy risk in 1,047 beef cows averaged 55.6% and regardless of age and nutritional management scheme, concentrations of BUN were not associated with pregnancy risk (Gunn et al., 2016).

Combined Ovulation and Pregnancy Status

In experiment 2, cows that ovulated and became pregnant to a fixed-time AI tended to have more rump fat, greater BCS, less serum NEFA, and greater plasma glucose than nonpregnant anovulatory cows during 6 wk before the onset of the AI program. Cows that ovulated had lesser serum NEFA 3 to 6 wk before the onset of AI program and those that ovulated and became pregnant had serum concentrations of NEFA that were 44% less than those in nonovulatory, nonpregnant cows. Ultrasound-determined rump fat measurements are more precise and objective than BCS, perhaps allowing detection of subtle changes in fat deposition associated with energy balance. These results are consistent with expectations that cows becoming more fit as evidenced by increasing rump fat, BCS, and decreased NEFA are more likely to ovulate earlier after calving and conceive at first insemination.

Overall Conclusions

In summary, the complexity of the nutritional impact on the establishment of pregnancy is highlighted in our results. The measures of BCS and BHB during 6 wk after calving predicted earlier first postpartum ovulation, whereas fat reserves as indicated by RF and BCS, were predictive of pregnancy success to timed AI programs for cows that ovulated before the breeding season. When considering both prebreeding ovulatory and pregnancy status, however, BCS, rump fat, serum NEFA, and plasma glucose were consistently different from values in nonovulatory, nonpregnant cows. Although nutritional status is not likely to be characterized by a static measure of any trait at a single time point, concentrations of plasma glucose and serum NEFA during the early postpartum period probably indicate the nutritional status of the cow. Relationships exist between these metabolic indicators and ovulatory-pregnancy success. More research is warranted to identify the most accurate physical indicators and specific early predictive value of metabolic indicators, but those with the greatest sensitivity

and specificity will be the most accurate predictors of ovulatory status, pregnancy status, or both.

Conflict of interest statement. None declared.

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