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Circulating beta-hydroxybutyrate concentration may be a predictive measurement for young cows that have a greater probability to conceive at a fixed-time artificial insemination

J. D. Hobbs, S. R. Edwards, E. R. Cope, Z. D. McFarlane, K. G. Pohler, and J. T. Mulliniks[1]

Department of Animal Science, University of Tennessee, Knoxville 37996

ABSTRACT: Timing of conception, which has been indicated to be negatively influenced by metabolic dysfunctions, can influence lifetime productivity within the cow herd. Therefore, our objective was to analyze the association of milk production, serum metabolites as an indicator of nutrient status, cow BW and BW change, and calf BW with timing of pregnancy in 183 spring-calving beef cows. Cows were retrospectively classified by timing of pregnancy as cows that were diagnosed pregnant by timed AI (TAI; n = 118) or natural breeding (NAT; n = 65). In addition, cows were grouped by age to represent young (3 to 4 yr old), mature (5 to 6 yr old), and old (7 to 9 yr old) cows. Starting approximately d 30 postpartum, cow BW and BCS were recorded and blood samples were collected weekly through the end of breeding. Weekly serum samples were composited by cow within 2 production periods: 1) prebreeding and 2) TAI to end of NAT. Cow BW and BCS did not influence ($P \geq 0.40$) timing of pregnancy during the entire study. Similarly, calf BW at birth and weaning were not different ($P \geq 0.30$) between timing of pregnancy groups. However, calf BW at weaning and calf value the subsequent year of the study were greater ($P < 0.01$) for TAI cows than for NAT cows. An age group × treatment interaction ($P < 0.01$) occurred for serum β-hydroxybutyrate (BHB). Serum BHB concentrations for mature and old cows were similar regardless of timing of pregnancy. However, serum BHB concentrations for young NAT cows were greater than for young TAI cows. In addition, serum NEFA exhibited ($P = 0.04$) a timing of pregnancy × sampling period interaction. Prebreeding serum NEFA concentrations were greater for NAT cows than for TAI cows. In contrast, serum NEFA concentrations during the NAT season were similar regardless of timing of pregnancy. Area under the curve of the receiver-operating characteristic curve for young cows' circulating BHB concentrations (0.66) was an acceptable predictor for pregnancy by TAI ($P < 0.01$). Results from this study indicate that only the young, postpartum beef cows during early lactation were susceptible to the measured metabolic dysfunctions of elevated blood BHB concentrations, which may have caused a delay in the timing of pregnancy.

Key words: beef cow, beta-hydroxybutyrate, timing of pregnancy

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INTRODUCTION

Longevity of beef cows is imperative for sustainability and profitability within any cow–calf operation. However, there are no selection tools available for longevity. Timing of pregnancy and subsequent calving date has been shown to be a major influence on longevity and lifetime productivity (Cushman et al., 2013). Traditionally, BCS has been used as a tool for predicting reproductive competence (Selk et al., 1988). Mulliniks et al. (2012) suggests BCS has limitations in predicting reproductive competence. Mobilization and utilization capacity of body storage to meet energy demands during early lactation results in altered metabolic profiles, which may influence reproductive performance due to limited ability to oxidize fatty acids. With this in mind, increased β-hydroxybutyrate (**BHB**) concentrations prior to the breeding season have been linked to delayed timing of pregnancy in both beef (Mulliniks et al., 2013) and dairy (Pushpakumara et al., 2003; Walsh et al.,

[1]Corresponding author: jmulli@utk.edu
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2007) cows. Increased concentrations of BHB may act as a negative signal to suppress pulsatile LH secretion (Cope et al., 2016). Although each facet of reproductive control is coordinated within a complex system, evidence from these studies suggests that circulating BHB concentrations may be a nutritional indicator of reproductive status. Therefore, the hypothesis of our research was that an increase in metabolic load of lactation will cause a metabolic dysfunction resulting in elevated BHB concentrations and a delay in timing of pregnancy. Therefore, our objective was to analyze the association of milk production, serum metabolites, cow BW change, and calf performance with timing of pregnancy in spring-calving beef cows.

MATERIALS AND METHODS

All animal handling and experimental procedures were conducted according to the guidelines of the Institutional Animal Care and Use Committee of the University of Tennessee.

Animals

In a 2-yr study, 183 Angus-sired, spring-calving beef cows (3 to 9 yr old) were used to determine the association of milk production, serum metabolites, cow BW and BW change, and calf BW with time of pregnancy. This study was conducted at 3 locations: 1) the Highland Rim Research and Education Center (**HRREC**) in Springfield, TN; 2) the Plateau Research and Education Center (**PREC**) in Crossville, TN; and 3) the Middle Tennessee Research and Education Center (**MTREC**) in Spring Hill, TN. The average annual rainfall across the 3 locations is 1,354 mm, with tall fescue (*Schedonorus arundinaceus*) as the predominate grass species. From December to May in each year, cows at each location were fed either ad libitum corn silage (PREC; 9% CP and 47% NDF), orchard grass hay (MTREC; 17% CP and 48% NDF), or rye haylage (8% CP and 61% NDF) with 5% dried distiller's grain (HRREC; 30% CP). Forage samples were ground with a Wiley mill (Thomas Scientific, Swedesboro, NJ) before analysis was performed. Crude protein analysis was determined by combustion (Leco-NS2000; Leco Corp., St. Joseph, MI). Neutral detergent fiber concentrations were determined using a fiber analyzer vessel using methods described by ANKOM Technology (ANKOM A200; ANKOM Technology, Macedon, NY).

In April every year, all cows were synchronized using a controlled internal drug release (**CIDR**) device (Eazi-Breed CIDR; Zoetis Inc., Kalamazoo, MI) with a 7-d CO-Synch protocol. Cows were administered a single 2-mL intramuscular (**i.m.**) injection of GnRH (100 μg; Cystorelin; Merial, Merial Inc., Duluth, GA) and CIDR device on −9 d. On −2 d, the CIDR device was removed and cows were injected with 5-mL i.m. injection of $PGF_{2\alpha}$ (25 mg; Lutelyse; Zoetis Inc.). Approximately 66 h after CIDR device removal, all cows were given an i.m. injection of 2 mL GnRH (100 μg; Cystorelin) and concurrent timed AI (**TAI**) by an experienced technician. Fourteen days after TAI occurred, bulls were used for natural service with a cow-to-bull ratio of 1:20 at the PREC and 1:30 at the MTREC and HRREC. Bulls were removed from pastures after a 60-d natural breeding (**NAT**) season. In both years, TAI pregnancy diagnosis occurred 30 d after TAI and overall pregnancy diagnosis occurred approximately 84 d after the NAT season. Pregnancy diagnosis was determined at the PREC by circulating concentrations of pregnancy-associated glycoproteins greater than 0.15 ng/mL in serum (BioPRYN; Gold Standard Labs, Bowling Green, KY) and transrectal ultrasonography by scanning the uteri of cows at the HRREC and MTREC.

Experimental Groups

To determine the association of timing of pregnancy with predictive measurements, cows were retrospectively classified as diagnosed pregnant by TAI (n = 118) or during natural service period (NAT; n = 65). Timing of pregnancy was determined by pregnancy diagnosis and verified by back-calculating from the calving date of the subsequent year minus 285 d for gestation, and progeny were also DNA typed for paternity confirmation. To determine the effect of cow age on reproductive efficiency, cows were also classified by age groups as young (3 to 4 yr old; n = 73), mature (5 to 7 yr old; n = 65), and old (8 to 9 yr old; n = 45). The factorial arrangement of treatments resulted in 58 young, 46 mature, 25 old cows in the TAI group and 29 young, 23 mature, and 20 old cows in the NAT group.

Blood Sampling and Assays

Blood samples were collected weekly via coccygeal venipuncture (approximately 9 mL; Corvac; Sherwood Medical Co., St. Louis, MO) starting approximately 30 d postpartum until the end of the breeding season. Blood samples were cooled and then centrifuged at 2,000 × g at 4°C for 20 min. Serum was separated and stored in plastic vials at −20°C until further analysis. Weekly serum samples were composited by combining 50 mL of serum from each week's serum sample for each cow to make 2 production period composite samples: 1) prebreeding and 2) TAI to end of NAT. Composited serum samples were analyzed for insulin, glucose, NEFA, urea N (**SUN**), and BHB. Serum composite samples were analyzed using a 96-well microplate reader spectrophotometer with commercial kits for NEFA (Wako Chemicals USA, Inc., Richmond, VA; sensitivity of 0.01 mmol/L), glucose (Thermo Electron Corp., Waltham, MA; sensitivity of 0.3 mg/dL), and SUN (Thermo Electron Corp.; sensitivity of 2.0 mg/dL). Serum BHB concentrations were determined with the use of DL-β-hydoroxybutyric acid sodium salt, β-nicotinamide adenine dinucleotide hydrate, and 3-hydroxybutyrate dehydrogenase (Sigma-Aldrich, St. Louis, MO) as described by McCarthy et al. (2015). Serum BHB assay sensitivity was 0.025 mmol/L. Serum insulin concentrations were determined by RIA (EMD Millipore's Porcine Insulin RIA; EMD Millipore Corporation, Billerica, MA) using a Wizard^2 Gamma Counter (PerkinElmer, Inc., Waltham, MA) with a sensitivity of 0.06 ng/mL. The intra- and interassay CV were, respectively, 1.8% and 2.2% for serum NEFA, 3.3% and 3.8% for serum glucose, 3.2% and 4.6% for SUN, 4.0% and 4.2% for serum BHB, and 5.1% and 4.2% for serum insulin.

Cow and Calf BW

After calving, cows were weighed weekly until the termination of the breeding season. The number of days to BW nadir was calculated from the least BW after calving. Body condition scores (1 = emaciated and 9 = obese; Wagner et al., 1988) were assigned to each cow by visual observation and palpation weekly by 2 trained technicians. Calves were weighed at birth, approximately d 58 postpartum, and weaning each year. Calf weights at weaning were adjusted to a 205-d age constant BW without adjusting for sex of calf and age of dam. National average real prices (US$/kg) from 2000 to 2015 were collected for both steers and heifers in the following weight divisions: 227 to 272 kg and 273 to 319 kg. Calf value was then calculated for every calf weaned in the subsequent year of the study.

Milking Procedure

On approximately d 58 postpartum, all cows were milked using a modified version of weigh–suckle–weigh by a portable machine (Porta-Milker; The Coburn Company, Inc., Whitewater, WI) as described by Mulliniks et al. (2011). On the day of the milking, cows were gathered from their pasture and calves were removed. Ten minutes before milking, cows were administered an injection of oxytocin (20 IU; Vedco Inc., St. Joseph, MO) to facilitate milk ejection. Cows were milked until machine pressure could not extract any additional fluid, and the milk collected was subsequently discarded. After first milking, cows were kept separate from calves for approximately 4 h and then milked a second time following the same procedures as in the first milking. Milk weights were recorded and extrapolated to estimate 24-h milk production.

Statistical Analysis

Cow and calf performance data were analyzed as a completely randomized design with cow as the experimental unit using the Kenward–Roger degrees of freedom method. The MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) was used to test a model that included fixed effects of conception time, location, year, cow age group, calf sex, and their interaction. Serum metabolites were analyzed using the MIXED procedure of SAS with sampling period as the repeated factor and cow as the subject with "unstructured" as the covariance structure. The model included conception time, location, year, period, age group, calf sex, and their interaction. Separation of least squares means was performed by the PDIFF option of SAS when a significant ($P \leq 0.05$) effect of conception date classification was detected. Data was pooled when no significance of period was detected. The usefulness of each potential predictor variable for pregnancy at TAI (BHB, NEFA, cow BCS at TAI, cow BW at TAI, days to BW nadir, calf weaning weight, and 24-h milk yield) when used alone to predict pregnancy were compared using area under the curve of the receiver operating characteristic (**ROC**) curve for binary outcomes (MedCalc Software bvba, Ostend, Belgium).

RESULTS AND DISCUSSION

Management of multiparous beef cows from calving through breeding is a major factor of total operation productivity and efficiency (Short et al., 1990). The greatest opportunity for profit is getting the maximum percentage of cows rebred as early as possible during the breeding season (Spitzer et al., 1975). Calving date has been shown to positively influence calf BW at weaning and cow longevity (Marshall et al., 1990; Cushman et al., 2013). In addition, steers born earlier in the calving season have greater HCW and carcass values (Funston et al., 2011). Furthermore, lifetime productivity is increased in heifers that conceive to TAI than heifers conceiving to natural service after a fixed-time AI (**FTAI**) protocol (French et al., 2013). Therefore, minimizing the time from calving to pregnancy in beef cows potentially has tremendous impact on cow–calf productivity. Therefore, the objective of this study was to determine the association of milk production, serum metabolites, cow BW and BW change, and calf BW between cows that conceive to FTAI cows that conceive to natural service.

Calving date of the initial years of the study was not different ($P = 0.15$; Table 1) between TAI and NAT cows, which was expected due to initially selecting cows that were bred by TAI from the previous year. Due to classification of conception date groups, TAI cows did calve earlier ($P < 0.01$) during the calving season in the subsequent year of the study. Osoro and Wright (1992) reported

Table 1. Cow BW, BW change, and BCS for cows classified as becoming pregnant by timed AI (TAI) or natural service

	Timing of pregnancy[1]			
Measurement	TAI	NAT	SEM	*P*-value
n	118	65		
Calving date, Julian date				
Study year[2]	28	31	2	0.15
Subsequent year[3]	27	52	3	<0.01
BCS				
Initial	5.24	5.31	0.16	0.71
FTAI[4]	5.15	5.06	0.12	0.44
End of NAT	5.43	5.33	0.11	0.40
Cow BW, kg				
Initial	617	624	11	0.47
Nadir	572	578	11	0.61
FTAI	600	609	13	0.48
End of NAT	620	626	11	0.58
Cow BW change, kg				
Initial to nadir	−48	−47	4	0.86
Initial to FTAI	−17	−16	7	0.88
Initial to end of NAT	4	1	5	0.56
Days to BW nadir	73	75	2	0.40

[1]Timing of pregnancy was determined by ultrasound and calving date in the subsequent year. NAT = natural breeding.

[2]Calving date of the study year.

[3]Calving date of the subsequent year data was collected.

[4]FTAI = fixed-time AI.

Table 2. Milk production at 58 d and serum metabolites for cows classified as becoming pregnant by at timed AI (TAI) or natural service

	Timing of pregnancy[1]			
Measurement	TAI	NAT	SEM	*P*-value
Serum metabolites				
Glucose, mg/dL	60.16	62.34	1.82	0.23
Insulin, ng/mL	0.37	0.40	0.03	0.31
Urea N, mg/dL	13.42	13.42	0.38	0.99
24-h milk production, kg/d	9.85	9.70	0.49	0.75

[1]Timing of pregnancy was determined by ultrasound and calving date in the subsequent year. NAT = natural breeding.

spring-calving beef cows that calve earlier in the calving season are more likely to conceive earlier in the breeding season than those calving later. In the current study, BCS did not differ ($P \geq 0.40$; Table 1) between TAI and NAT cows at the initial, FTAI, and end of the NAT season time points. Also, no differences ($P \geq 0.47$) were detected for cow BW between TAI and NAT cows at initial, FTAI, end of NAT, or BW nadir time points. Likewise, cow BW change between TAI and NAT cows was not different ($P \geq 0.40$) during the entire course of this study. Body condition score, BW, and BW change are often used as an indicator of reproductive competence (Randel, 1990; DeRouen et al., 1994). However, BCS at calving in some management schemes and production systems also may not be a good indicator of pregnancy rates or days to resumption of estrus after calving (Mulliniks et al., 2012). In addition, Lake et al. (2005) reported no difference in first-service conception rates in BCS 6 compared with BCS 4 cows, but BCS 6 cows did have an increased overall pregnancy rate. Ciccioli et al. (2003) illustrated that BCS at calving had no effect on resumption of estrus, ovarian function, or reproductive performance. Therefore, within the given environment of the current study, BCS and BW were not adequate indicators of timing of pregnancy.

Glucose has been suggested as one of the most important metabolic substrates required for proper function of reproductive processes in beef cows (Short and Adams, 1988). Serum glucose concentrations were not different ($P = 0.23$; Table 2) between TAI and NAT cows. Garverick et al. (2013) reported an increase in circulating glucose concentrations in postpartum dairy cows that were pregnant at first AI compared with cows that failed to conceive at first AI. Similarly, Green et al. (2012) reported decreased plasma glucose concentration during the first 30 d postpartum in dairy cows that did not conceive at first AI when compared with pregnant dairy cows. However, Mulliniks et al. (2013) reported elevated serum glucose and reduced insulin concentrations with late conception cows compared with early conception cows. Within the current study, serum insulin concentrations were not different ($P = 0.31$) between TAI and NAT cows. Chagas (2003) reported increased serum insulin concentrations were associated with a positive effect on the restoration of LH pulse frequency. Vizcarra et al. (1998) reported no difference in insulin concentrations between primiparous beef cows with or without luteal activity. Circulating SUN concentrations have been indicted to influence fertility in lactating dairy cows (Ferguson et al., 1993; Butler et al., 1996). In the current study, SUN concentrations did not differ ($P = 0.99$) between TAI and NAT cows. Butler et al. (1996) reported pregnancy rate after AI was reduced in lactating dairy cows when plasma SUN concentrations were above 19 mg/d, which is significantly greater than SUN values reported by both conception groups in the current study.

Beta-hydroxybutyrate concentrations can accumulate in blood when the rate of acetate oxidation is inhibited by an inadequate supply of cellular oxaloacetate derived from serum glucose (Kaneko, 1997). Circulating BHB concentrations can increase when the rate of acetate supply is greater than the rate of acetate oxidation (Yamashita et al., 2001). In this study, an age group × conception breeding group interaction ($P < 0.01$; Table 3) occurred for serum BHB concentration. In mature and old cows, BHB concentrations did not differ by conception breeding group classification; however, young NAT cows had greater ($P < 0.01$) serum BHB concentrations when com-

Table 3. Concentrations of serum β-hydroxybutyrate (BHB) in young, mature, and old cows classified as becoming pregnant by timed AI (TAI) or natural service

	Timing of pregnancy[1]		
Measurement	TAI	NAT	SEM
Serum BHB, μmol/L			
Young[2]	221.83^{ax}	250.68^{by}	9.94
Mature[2]	243.70^{ay}	230.21^{axy}	10.17
Old[2]	226.40^{ax}	221.87^{ax}	11.74

[a,b]For each interaction within timing of sample, means in rows with different superscripts differ ($P < 0.05$).

[x,y]For each interaction within timing of sample, means in columns with different superscripts differ ($P < 0.05$).

[1]Timing of pregnancy was determined by ultrasound and calving date in the subsequent year. NAT = natural breeding.

[2]Cows were classified by age group: young were 3 to 4 yr old, mature were 5 to 7 yr old, and old were 8 to 9 yr old.

Table 4. Concentrations of serum NEFA during the prebreeding and breeding period in cows classified as becoming pregnant by timed AI (TAI) or natural service

	Timing of pregnancy[1]		
Measurement	TAI	NAT	SEM
Prebreeding[2] NEFA, mmol/L	671.89^{ay}	750.76^{by}	28.96
Breeding[2] NEFA, mmol/L	417.83^{ax}	427.73^{ax}	28.91

[a,b]For each interaction within timing of sample, means in rows with different superscripts differ ($P < 0.05$).

[x,y]For each interaction within timing of sample, means in columns with different superscripts differ ($P < 0.05$).

[1]Timing of pregnancy was determined by ultrasound and calving date in the subsequent year. NAT = natural breeding.

[2]Weekly serum samples were aliquoted into 2 separate composites: prebreeding, from approximately 30 d after calving to the week prior to TAI, and breeding, from TAI to the end of the NAT season.

pared with young TAI cows. Similarly, Mulliniks et al. (2013) reported that 2- and 3-yr-old cows classified as becoming pregnant late in the breeding season displayed an elevated whole blood BHB concentration prior to breeding. The similarity among old and mature NAT and old and mature TAI cows in the current study suggests that young postpartum cows may be more susceptible to the negative impact of elevated BHB concentrations due to metabolic imbalances. In addition, the lack of differences in mature and old cows may suggest that cows incapable of tolerating the metabolic load of lactation and calving earlier in life had been culled from the herd at a younger age due to decreased reproductive performance. Even though circulating BHB concentration was not different for mature and older cows between conception groups, the results of the current study and those from Mulliniks et al. (2013) may indicate that circulating BHB concentration during early lactation may be indicative of reproductive efficiency in young beef cows.

Circulating concentrations of NEFA have been shown to be negatively correlated with fertility due to its association with negative energy balance (Bell, 1995). In the current study, a sampling period × conception breeding group interaction ($P = 0.04$; Table 4) occurred for serum NEFA concentrations. During the prebreeding period, serum NEFA concentrations for NAT cows were greater ($P < 0.01$) than TAI cows. Conversely, during the breeding period, serum NEFA concentrations were similar regardless of timing of conception. In support, Wathes et al. (2007) reported NEFA concentrations 1 wk prior to calving to be negatively correlated with days from calving to conception in multiparous cows. In addition, Ospina et al. (2010) reported that NEFA concentrations had a greater negative association with reproductive performance than BHB in transition dairy cattle. Reist et al. (2000), however, reported no differences in serum NEFA concentrations in postpartum dairy cows that displayed first ovulation within the first 30 d postpartum compared with cows that ovulated between d 31 and 87 postpartum, whereas elevated BHB concentrations were reported in cows that ovulated after d 30 postpartum compared with those that ovulated during the first 30 d postpartum. Results from the current study indicate that NEFA concentrations could be an indicator of energy status; however, the variation from other studies implies that the consistency of NEFA impact on reproduction may depend on the ability of the animal to oxidize fatty acids.

Twenty-four hour milk production at d 58 postpartum did not differ ($P = 0.75$; Table 2) between TAI and NAT cows. Similarly, Pushpakumara et al. (2003) noted no differences in 24-h milk yield between dairy cows that were diagnosed as pregnant by early or late service. However, Edwards et al. (2017) reported a decreased TAI pregnancy rate in high milk producing multiparous beef cows compared with low and moderate milk producing multiparous beef cows.

Within the current study, calf BW at birth, 58 d, weaning, and 205 d adjusted BW did not differ ($P \geq 0.30$; Table 5) between TAI and NAT cows. These results were expected due to no differences in dam milk production or calving date the year of the study. Cows conceiving to TAI had greater ($P < 0.001$) calf BW at weaning in the subsequent year than NAT cows, which was expected due to becoming pregnant and calving earlier than NAT cows. An adjusted 205-d calf BW at weaning of the subsequent year of the study was not different ($P = 0.32$) between TAI and NAT cows. Therefore, calf BW at weaning of the subsequent year was influenced more by timing of pregnancy than by increased genetic potential from using AI sires. In addition, calf value at weaning of the subsequent year was greater ($P < 0.001$) for TAI cows than for NAT cows. French et al. (2013) reported yearling heifers that were pregnant by AI had greater lifetime revenue than those that conceived during NAT. Dunn and Kaltenbach (1980) concluded that allowing a

Table 5. Suckling calf BW and subsequent calf value of cows classified as becoming pregnant by AI or natural service

	Timing of pregnancy[1]			
Measurement	TAI	NAT	SEM	*P*-value
Calf BW, kg				
Birth	35	35	1	0.99
58 d	67	64	2	0.21
Weaning	291	286	4	0.30
205 d adjusted	270	267	2	0.60
Subsequent weaning[2]	299	270	5	<0.01
Subsequent 205 d adjusted	275	269	6	0.32
Subsequent calf value, US$	824.21	755.53	13.26	<0.01

[1]Timing of pregnancy was determined by ultrasound and calving date in the subsequent year. TAI = timed AI; NAT = natural breeding.

[2]Weaning weight the following year of the study.

Table 6. Univariable predictive ability of 7 variables for pregnancy after timed AI (TAI) of beef cows

Predictor variable	Pregnancy after TAI[1]	*P*-value
Serum BHB[2]	0.56	0.04
Young[3]	0.66	<0.01
Mature[3]	0.51	0.79
Old[3]	0.54	0.49
Prebreeding NEFA	0.54	0.38
BCS at TAI	0.52	0.58
Cow BW at TAI	0.53	0.45
Days to BW nadir	0.53	0.59
Calf weaning weight	0.53	0.55
24-h milk yield	0.56	0.22

[1]Area under the curve for receiver operating characteristic analysis.

[2]Circulating concentrations of β-hydroxybutyrate (BHB) pooled across all age groups.

[3]Cows were classified by age group: young were 3 to 4 yr old, mature were 5 to 7 yr old, and old were 8 to 9 yr old

greater number of females to be inseminated at one time results in earlier conception leading to older and heavier calves at weaning. Similarly, Garcia Paloma et al. (1992) concluded that an earlier date of calving contributed to greater concentrations of early calving in the subsequent calving seasons and an overall increase in production efficiency. In addition, Cushman et al. (2013) reported that calving date as a heifer influences calf weaning weights through multiple parturitions, suggesting there is a long-term effect of timing of conception in young cows.

Receiver-operating characteristic analysis can be a useful tool not only to predict pregnancy outcome but also as a selection tool for fertility (Holm et al., 2009). For all ages of cows, the area under the curve of the ROC curve for circulating BHB concentrations (0.56; $P = 0.04$; Table 6) and young cows circulating BHB concentrations (0.66; $P < 0.01$) both were acceptable predictors for pregnancy by TAI. Prebreeding circulating NEFA concentration, cow BCS at TAI, cow BW at TAI, days to BW nadir, calf weaning weight, and 24-h milk production were not acceptable predictors ($P \geq 0.22$), although prebreeding NEFA concentrations were different between conception groups. Young cows with circulating BHB concentrations less than or equal to 138.8 μmol/L were 95% likely to become pregnant by TAI, whereas young cows with circulating BHB concentrations greater than or equal to 301 μmol/L were 96% likely to not become pregnant by TAI. The ROC curve analysis for circulating BHB concentrations may illustrate how sensitive young, postpartum beef cows are to circulating BHB concentrations. In addition, this data may provide insight to selecting young cows that have an increased probability to conceive to a FTAI protocol.

The results from this study indicate that only young, postpartum beef cows during early lactation were susceptible to metabolic dysfunction, resulting in elevated BHB concentrations and delayed time to conception. Using handheld ketone meters, livestock producers could measure real-time circulating BHB concentrations at least 2 wk prior to the breeding season as indicated by Mulliniks et al. (2013). Hence, monitoring BHB concentrations postpartum may provide the opportunity to identify young cows that would have a delayed conception and manage them differently to decrease days from calving to conception and potentially provide the opportunity for an increase longevity and productivity. More research is needed to decide when to test cows and what the threshold values to use in a given environment and management system.

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