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Using estrus detection patches to optimally time insemination improved pregnancy risk in suckled beef cows enrolled in a fixed-time artificial insemination program[1,2]

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ABSTRACT: A multilocation study examined pregnancy risk (PR) after delaying AI in suckled beef cows from 60 to 75 h when estrus had not been detected by 60 h in response to a 7-d CO-Synch + progesterone insert (CIDR) timed AI (TAI) program (d −7: CIDR insert concurrent with an injection of GnRH; d 0: $PGF_{2\alpha}$ injection and removal of CIDR insert; and GnRH injection at TAI [60 or 75 h after CIDR removal]). A total of 1,611 suckled beef cows at 15 locations in 9 states (CO, IL, KS, MN, MS, MT, ND, SD, and VA) were enrolled. Before applying the fixed-time AI program, BCS was assessed, and blood samples were collected. Estrus was defined to have occurred when an estrus detection patch was >50% colored (activated). Pregnancy was determined 35 d after AI via transrectal ultrasound. Cows (n = 746) detected in estrus by 60 h (46.3%) after CIDR removal were inseminated and treated with GnRH at AI (Control). Remaining nonestrous cows were allocated within location to 3 treatments on the basis of parity and days postpartum: 1) GnRH injection and AI at 60 h (early-early = EE; n = 292), 2) GnRH injection at 60 h and AI at 75 h (early-delayed = ED; n = 282), or 3) GnRH injection and AI at 75 h (delayed-delayed = DD; n = 291). Control cows had a greater (P < 0.01) PR (64.2%) than other treatments (EE = 41.7%, ED = 52.8%, DD = 50.0%). Use of estrus detection patches to delay AI in cows not in estrus by 60 h after CIDR insert removal (ED and DD treatments) increased (P < 0.05) PR to TAI when compared with cows in the EE treatment. More (P < 0.001) cows that showed estrus by 60 h conceived to AI at 60 h than those not showing estrus (64.2% vs. 48.1%). Approximately half (49.2%) of the cows not in estrus by 60 h had activated patches by 75 h, resulting in a greater (P < 0.05) PR than their nonestrous herd mates in the EE (46.1% vs. 34.5%), ED (64.2% vs. 39.2%), and DD (64.8% vs. 31.5%) treatments, respectively. Overall, cows showing estrus by 75 h (72.7%) had greater (P < 0.001) PR to AI (61.3% vs. 37.9%) than cows not showing estrus. Use of estrus detection patches to allow for a delayed AI in cows not in estrus by 60 h after removal of the CIDR insert improved PR to TAI by optimizing the timing of the AI in those cows.

Key words: beef cattle, estrus detection, timed AI

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INTRODUCTION

Ovulation control programs are designed to maximize the opportunity for viable sperm and ova to interact in the oviduct after AI in beef cows. In AI systems that allow all cows to be inseminated at a predetermined time (fixed-time AI, **TAI**), the demand for time conformity of insemination may supersede optimal timing of semen placement relative to estrus or ovulation. Several different strategies have been employed to minimize the difference between actual and optimal timing by examining various single-insemination timings to produce the greatest probability of conception (Busch et al., 2008; Dobbins et al., 2009; Wilson et al., 2010). Allocating cows to optimal fixed times utilizing specific criteria, except for varying the time of AI for all cows (Dobbins et al., 2009), is largely unexplored. Although distribution of ovulation has not been quantified in TAI systems, hours to estrus following $PGF_{2\alpha}$ treatments concurrent with progesterone withdrawal has been investigated in the CO-Synch + CIDR program (Busch et al., 2008; Wilson et al., 2010). The reported distribution of estrus (36 through 120 h after removal of the CIDR insert), in addition to differences in identifiable groups based on parity and estrus-cycle status, indicates that multiple insemination times may increase pregnancy risk (**PR**) associated with TAI, particularly in heterogeneous groups of cows. The objective of this study was to test the hypothesis that allocating cows into 2 distinct insemination times on the basis of activated estrus detection patches would increase PR.

MATERIALS AND METHODS

All experimental procedures were approved by the respective Animal Care and Use Committees of the institutions participating in the study.

Experimental Design

A total of 1,611 mixed-parity beef cows at 15 locations in 9 states (CO, IL, KS, MN, MS, MT, ND, SD, and VA) were enrolled in the experiment. Characteristics of suckled beef cows enrolled by location including breed, percentage of 2-yr-old cows, days postpartum at AI, BCS, and proportion of cows having estrous cycles at the onset of the synchronization program (Table 1). All cows were treated with a 7-d CO-Synch + CIDR program (100 μg GnRH [2 mL Factrel, Pfizer Animal Health, Whitehouse Station, NJ] 7 d before and 60 or 75 h after 25 mg $PGF_{2\alpha}$ [d 0; 5 mL Lutalyse; Pfizer Animal Health]). A new controlled internal drug release (**CIDR**) insert (Pfizer Animal Health) containing 1.38 g progesterone was inserted per vagina at the time of the first GnRH injection (d −7). On d 0, concurrent with removal of the CIDR insert, estrus detection patches (Estrotect, Rockway Inc., Spring Valley, WI) were affixed to the tail head of each cow according to manufacturer's recommendation. Body condition scores (1 = thin; 9 = obese; Bellows et al., 1982) were assigned (d −17) before the start of the ovulation synchronization program by a trained evaluator (Fig. 1).

Treatment Assignment

Estrus detection patches were interpreted and treatment assignments were made at 60 h after CIDR insert removal. Cows were defined to have exhibited estrus (evidence of standing activity) when >50% of the gray coating was rubbed off or when the patch was missing (<10% were missing). Cows in estrus by 60 h had patches removed, received an injection of 100 μg GnRH, and were inseminated (Control). Cows within location that had not exhibited estrus by 60 h were balanced according to days postpartum and parity and then assigned randomly to 3 treatments: 1) injected with 100 μg GnRH and inseminated at 60 h (early-early; **EE**), 2) injected with 100 μg GnRH at 60 h but inseminated at 75 h (early-delayed; **ED**), or 3) injected with 100 μg GnRH and inseminated at 75 h (delayed-delayed; **DD**). At 75 h, all patches were evaluated and removed at 75 h from EE, ED, and DD cows to determine if estrus had occurred between 60 and 75 h (Fig. 1).

Pregnancy Diagnosis

Cows were either observed for estrus and inseminated on subsequent estrus or exposed to cleanup bulls beginning 10 to 12 d after TAI. At 35 ± 3 d after TAI, pregnancy was confirmed by transrectal ultrasonography (Aloka 500V, 5 MHz transrectal transducer, Wallingford, CT). A positive pregnancy outcome required presence of a corpus luteum and uterine fluid or an embryo with a heartbeat. A final pregnancy diagnosis was determined via transrectal ultrasonography approximately 35 d after the end of the breeding season (removal of natural service sires).

Estrus Cycle Status

Blood samples were collected via puncture of a caudal blood vessel from cows at 10 of the 14 locations on d −17 and −7. Concentrations of progesterone in serum 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

Table 1. Selected characteristics of suckled beef cows enrolled

Location[1]	Breed	n	2-yr-old cows, %	Mean (±SE) days postpartum at AI	Mean (±SE) BCS	Estrus cycle status,[2] %
CO-1	Angus × Hereford	93	28	74 ± 1.7	5.8 ± 0.07	NC[3]
KS-1	Angus × Hereford	178	0	93 ± 1.1	5.7 ± 0.03	96
KS-2	Angus × Hereford	18	0	74 ± 3.9	6.4 ± 0.14	44
KS-3	Angus × Hereford	57	0	67 ± 2.9	6.9 ± 0.11	74
KS-4	Angus, Hereford, Simmental	64	0	75 ± 1.7	4.8 ± 0.07	66
KS-5	Angus × Hereford	50	20	65 ± 1.6	4.5 ± 0.06	59
KS-6	Angus × Hereford	29	0	61 ± 4.2	6.8 ± 0.11	59
IL-1	Angus × Hereford	150	0	67 ± 1.0	5.2 ± 0.06	NC[3]
MN-1	Angus × Hereford	125	15	69 ± 2.3	4.9 ± 0.06	61
MS-1	Angus, Hereford	50	19	67 ± 2.8	5.4 ± 0.08	NC[3]
MT-1	Angus	95	35	73 ± 1.6	4.8 ± 0.08	NC[3]
ND-1	Angus × Hereford	173	21	71 ± 0.9	5.4 ± 0.05	NC[3]
SD-1	Angus × Hereford	102	28	81 ± 1.5	5.6 ± 0.04	32
VA-1	Angus × Simmental	211	15	79 ± 1.4	4.5 ± 0.06	62
VA-2	Angus × Simmental	216	16	75 ± 1.2	4.2 ± 0.04	52

[1]Cows at 15 locations in 9 states were enrolled.

[2]Status was based on progesterone concentrations measured in 2 blood samples collected 10 d apart before the onset of the experimental protocol. In 4 cows in which pregnancy outcome was determined, blood samples were missing.

[3]NC= blood samples were not collected to assess estrus cycle status.

for progesterone were 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 d −17 or −7 were defined to have resumed estrous cycles (Table 1). All other cows were considered to have been anestrous at the onset of the ovulation synchronization program.

Statistical Analyses

The GLIMMIX procedure (METHOD = LAPLACE; ILINK = LOGIT DIST = BINOMIAL SOLUTION ODDSRATIO) in SAS (SAS Inst. Inc., Cary, NC) was applied to analyze the binomial outcome variables of estrus cycle status, estrus expression, and PR associated with AI. The independent variables of BCS, days postpartum at AI, and parity (primiparous vs. multiparous) were included as fixed effects in all models. The median value of BCS (BCS = 5.0) was used to allocate cows into 2 BCS categories (<5.0 vs. ≥5.0). Treatment was tested as a fixed effect in the model that examined PR. Significance of treatment was tested by the random effect of treatment within location. Models included interactions of treatment with the fixed effects of parity, BCS, and the continuous variable days postpartum. When no significant interactions with treatment were detected, interactions with treatment were eliminated from the final model. The 2- and 3-way interactions among the variables days postpartum, BCS, and parity were then included in the model. Because a 3-way interaction was detected among parity, BCS, and days postpartum, days postpartum was grouped into quartiles and tested for interactions. Pregnancy risk changed when days postpartum were proportioned at 86 d, resulting in identification of the interaction. A separate model including total expression of estrus (excluded treatment) examined 2-, 3-, and 4-way interactions with BCS, days postpartum, parity, and estrus expression on the dependent variable of PR. No interactions with estrus expression were identified and were therefore removed from the model.

Models to examine expression of estrus were constructed for cows that exhibited estrus by 60 h, between 60 and 75 h, and by 75 h. The general models for estrus at each of the 3 times included estrus cycle status, BCS, and parity. The continuous variable, days postpartum, was converted to a binomial variable by dividing the range of days postpartum at its median (<75 vs. ≥75 d).

Effects of estrus cycle status were not significant when evaluated in any of the 3 models; therefore, estrus cycle status was eliminated to include cows for which that information was not available. The 2- and 3-way interactions of days postpartum, BCS, and parity were included in all estrus expression models. The effect of GnRH injection timing was evaluated in a model that included estrus expression between 60 and 75 h as the dependent variable; GnRH injection time of 60 or 75 h was treated as an independent fixed variable to determine the effect of prior GnRH exposure on subsequent expression of estrus. The GnRH timing model also included BCS, days postpartum, and parity as fixed effects. The random variable location was included as the testing term in all estrus expression models.

A subset of cows for which estrus cycle status information was available was analyzed in a separate GLIMMIX model with estrus cycle status as the

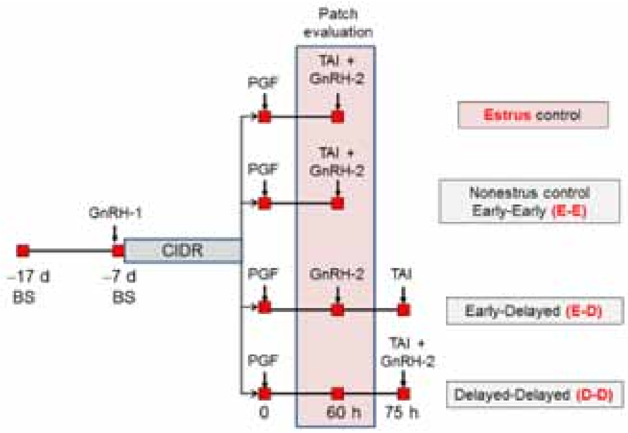

Figure 1. Experimental design of treatments. All cows received an injection of GnRH (GnRH-1) and an intravaginal controlled internal drug release (CIDR) insert containing 1.38 g of progesterone on study d −7, an injection of $PGF_{2\alpha}$ (PGF), and removal of the CIDR insert on d 0. Estrus detection patches were evaluated at 60 h to determine treatment allocation (activation was indicated when more than 50% of the covering material was removed). Cows (control) in estrus by 60 h had patches removed, received GnRH-2 injection, and were inseminated. Cows within location that had not exhibited estrus by 60 h were balanced according to days postpartum and parity and assigned randomly to 3 treatments: 1) GnRH-2 injection and AI at 60 h (early-early; EE), 2) GnRH-2 injection at 60 h but AI at 75 h (early-delayed; ED), or 3) GnRH-2 injection and AI at 75 h (delayed-delayed; DD). Patches in the latter 3 treatments were evaluated and removed at 75 h.

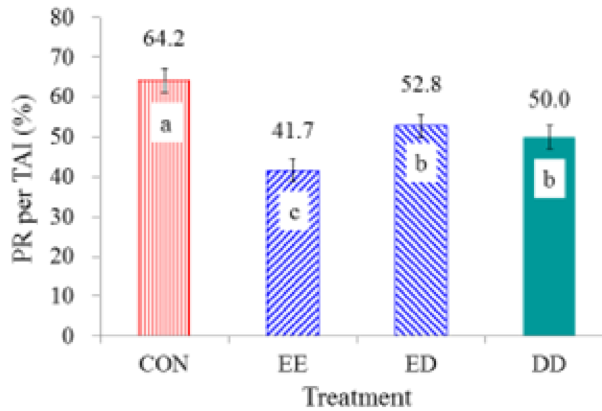

Figure 2. Least squares mean pregnancy risk (PR) per timed AI (TAI) by treatment. Control cows (CON; *n* = 746) were detected in estrus by 60 h, inseminated, and received GnRH at 60 h. The remaining cows were allocated to 3 treatments: 1) injected with GnRH and inseminated at 60 h (early-early; EE; *n* = 292), 2) injected with GnRH at 60 h but inseminated at 75 h (early-delayed; ED; *n* = 282), or 3) injected with GnRH and inseminated at 75 h (delayed-delayed; DD; *n* = 291). Bars with different letters differ ($P < 0.01$).

dependent variable. The independent fixed variables (BCS, parity, and days postpartum), all 2-and 3-way interactions among those variables, and the random effect of location were included in the model.

Final pregnancy risk and pregnancy loss (pregnancy loss between TAI and the final pregnancy diagnosis at the end of the breeding season) were both analyzed in models containing treatment or estrus expression and BCS, parity, and days postpartum. In all models differences were determined to be significant when $P \leq 0.05$.

RESULTS

Pregnancy Risk

Control cows had a greater ($P < 0.01$) PR than cows in the EE, ED, and DD treatments (Fig. 2). Cows in which AI was performed at 75 h (ED and DD) had greater ($P < 0.05$) PR than EE cows and did not differ ($P = 0.50$) from one another. The poorest PR was detected in EE cows (not detected in estrus but inseminated and treated with GnRH at 60 h). Cows that were not detected in estrus by 60 h but expressed estrus by 75 h (49.2% of cows not observed in estrus by 60 h were detected in estrus between 60 and 75 h) were more ($P < 0.05$) likely to become pregnant than nonestrous herd mates when they were in the EE (46.1% vs. 34.5%), ED (64.2 vs. 39.2%), and DD (64.8% vs. 31.5%) treatments, respectively. When assessing PR, no 2- and 3-way interactions were detected among treatments, BCS, and parity.

Final PR did not differ among treatments, parity, or days postpartum; however, cows with a BCS ≤ 5 had lower ($P < 0.01$) PR than cows with a BCS > 5 (89.5% vs. 94.1%, respectively). Final PR also was greater ($P = 0.001$) in cows that displayed estrus by either 60 or 75 h than those that did not display estrus (94.0% vs. 89.3%), respectively.

Pregnancy loss in cows pregnant after TAI was 1.3% ± 0.4%. Pregnancy losses associated with treatments (Control, DD, ED, and EE) were 1.0%, 0.2%, 2.1%, and 0.4%, respectively. Pregnancy loss was greater ($P = 0.04$) in ED than DD. Pregnancy loss did not differ between parity, estrus expression, days postpartum, or BCS. Furthermore, estrus cycle status before treatments were imposed did not affect pregnancy loss (cycling = 1.7% and not cycling = 1.5%).

Days postpartum at TAI were analyzed as a covariate in the model describing the effects of treatment on PR. Days postpartum were related positively ($P < 0.001$) with the PR at 35 d postbreeding, indicating that for every 10-d increase in days postpartum at AI, PR increased by 7.2% ± 1.6%. Pregnancy risk was not affected ($P = 0.30$) by BCS. In contrast, PR in multiparous cows was 1.43 times (adjusted odds ratio [**AOR**] = 1.43; CI = 1.03 to 1.98) more likely than in primiparous cows (56.7% vs. 47.8%). Cows that had resumed estrous cycles before the onset of the breeding season were 1.72 times (AOR = 1.72; CI = 1.29 to 2.29) more likely to become pregnant to AI than their anestrous herd mates (60.9% vs. 47.5%). Furthermore, cows detected in estrus by 75 h were 2.6 times (AOR = 2.60; CI = 1.95 to 3.46) more likely to become pregnant to AI than their herd mates that did not exhibit estrus (61.3% vs. 37.9%).

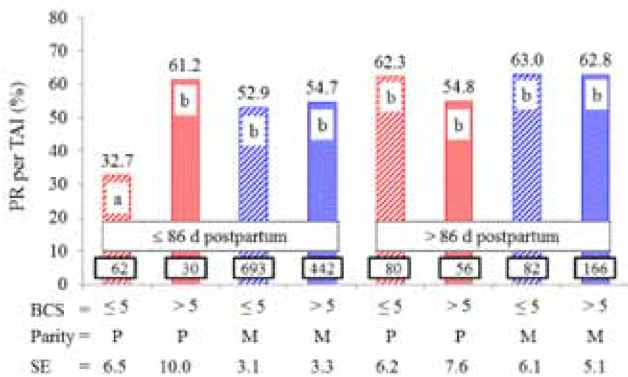

Figure 3. Pregnancy risk (PR) per timed AI (TAI) according to a 3-way interaction among BCS, parity (primiparous [P] and multiparous [M]), and days postpartum at AI. The values at the base of the bars reflect the number of cows in each subgroup. Bars with different letters differ ($P < 0.05$).

Effects of the independent variables, days postpartum, parity, and BCS on PR were analyzed separately, and a 3-way interaction was detected ($P = 0.05$; Fig. 3). Pregnancy risk was compromised in primiparous cows in poorer BCS and fewer days postpartum at AI compared with all other variable combinations of days postpartum, BCS, and parity.

Estrus Expression

A summary of when estrus was expressed (by 60 h or between 60 and 75 h) and the effects of BCS, parity, and days postpartum on expressed estrus is in Table 2. Overall, 46.3% of cows showed estrus by 60 h, 49.2% of the remaining cows showed estrus between 60 and 75 h, and 27.3% were not detected in estrus by 75 h after CIDR insert removal. Although days postpartum did not affect the expression of estrus, primiparous, better-conditioned cows were more ($P < 0.05$) likely to display estrus by 60 h than any other combination of parity and BCS (Table 2).

An interaction between parity and BCS was not detected in cows that displayed estrus between 60 and 75 h (Table 2). Neither days postpartum nor parity influenced expression of estrus during this 15-h interval, but more ($P < 0.05$) cows in better body condition showed estrus than those with poorer body condition (Table 2).

When estrus was expressed by 75 h, however, more ($P < 0.05$) primiparous cows with BCS > 5 were detected in estrus compared with all other combinations of parity and BCS (Table 2). Cows with more than 75 d since calving at AI did not differ ($P > 0.49$) from their herd mates with shorter postpartum intervals in expression of estrus by 75 h (Table 2).

When comparing occurrence of estrus between 60 and 75 h in the EE and ED cows, which received GnRH at 60 h, expression of estrus was not reduced ($P = 0.21$) subsequent to GnRH compared with that of the DD cows that received GnRH at 75 h (44.9% vs. 49.5%). Furthermore, for cows not detected in estrus between 60 and 75 h but inseminated at 75 h, PR did not differ ($P = 0.25$) between cows receiving GnRH at 60 and 75 h (46.0% vs. 39.1%).

Table 2. Estrus expression by 60 h, between 60 and 75 h, and by 75 h after controlled internal drug release insert removal as affected by BCS, parity, and days postpartum

Item	Days postpartum	BCS	Parity	*n*	Estrus, %
Estrus by 60 h					
Days postpartum	≤75	—	—	773	48.3
	>75	—	—	838	44.5
BCS × parity	—	>5	1	86	65.1[a]
	—	≤5	1	142	46.5[b]
	—	>5	2+	608	48.8[b]
	—	≤5	2+	775	42.2[b]
Estrus between 60 and 75 h					
Days postpartum	≤75	—	—	400	51.0
	>75	—	—	465	47.7
BCS	—	>5	—	341	51.9[a]
	—	≤5	—	524	47.5[b]
Parity	—	—	1	106	46.2
	—	—	2+	759	49.7
GnRH at 60 h	—	—	—	574	47.7
GnRH at 75 h	—	—	—	291	52.2
Estrus by 75 h					
Days postpartum	≤75	—	—	773	74.6
	>75	—	—	838	71.0
BCS × parity	—	>5	1	86	84.9[a]
	—	≤5	1	142	69.0[b]
	—	>5	2+	608	75.2[c]
	—	≤5	2+	775	70.2[b,c]

[a–c]Means within category with different superscript letters differ ($P \leq 0.05$).

Estrus Cycle Status

In a subset of cows (n = 1,046) factors affecting estrus cycle status at the beginning of the breeding season were analyzed. When all 2-way interactions among BCS, days postpartum, and parity were tested, an interaction of magnitude was detected between BCS and days postpartum. The combination of >75 d postpartum and BCS > 5 resulted in greater ($P < 0.006$) estrous cyclicity (78.2%) than the combinations of >75 d postpartum and BCS ≤ 5 (59.6%), ≤75 d postpartum and BCS > 5 (48.4%), or ≤75 d postpartum and BCS ≤ 5 (54.9%). The proportion of primiparous and multiparous cows having resumed estrous cycles before AI did not differ ($P = 0.37$; 64.4% vs. 57.7%), respectively.

DISCUSSION

Fixed-time AI is a strategy to reduce the variation in the timing of ovulation among females to maximize fertility after a single AI. Reproductive strategies that employ $PGF_{2\alpha}$, GnRH, and progestins have reduced the distribution of estrus in populations of cows subjected to AI compared with using no synchronization program. Ovulation after estrus is reported to be 31 ± 0.6 h in beef cows (White et al., 2002). The distribution of ovulation in beef cows exposed to a TAI system has not been quantified; however, numerous studies have shown increased proportion of cows becoming pregnant after TAI when they exhibit estrus at or before time of GnRH to induce ovulation (Perry et al., 2005; Busch et al., 2008; Hill et al., 2014). Perry et al. (2005) also reported that cows exhibiting estrus had greater subsequent concentrations of circulating estradiol and greater subsequent concentration of progesterone than nonestrous cows. Increasing the number of beef cows in estrus at the same time also increased the number of times each cow was mounted and the duration of estrus (Floyd et al., 2009) and may increase the possibility of identifying more cows with less overt estrus expression to inseminate them at a more ideal time in a split-time AI program.

Increased proportion of cows displaying estrus and becoming pregnant also is associated with greater conceptus size at d 19 of pregnancy and greater abundance of transcriptions for proteins in the endometrium that are favorable to pregnancy (Davoodi et al., 2016). Heifers inseminated after expressing estrus also had higher-quality embryos at a more advanced physiological stage than heifers not expressing estrus (Larimore et al., 2015). Acidification of the uterus (pH 7.0 to 6.7) occurs in cows that express estrus (Perry and Perry, 2008a,b), and prolonged viability of sperm has been associated with decreased intracellular sperm pH (Jones and Bavister, 2000).

It is clear that the uterine environment and endocrine milieu are more suitable for fertilization and embryo development in cows that express estrus when compared with those that do not. Recent research in beef cattle induced to ovulate immature follicles indicates that deficient uterine function is a major factor responsible for infertility in these cows (Bridges et al., 2013). Failure to produce adequate concentrations of estradiol before ovulation results in delayed effects of expression and localization of uterine genes and proteins that participate in regulating uterine functions during early gestation (Bridges et al., 2013).

Pregnancy risk was consistently greater in cows that expressed estrus up through 75 h (64.2% to 64.8%) except for the lower PR (46%) in the EE cows that expressed estrus. This lower PR could be attributed to GnRH induction of smaller follicles lacking insufficient estradiol to induce sexual behavior and estrus by 60 h but did not reduce subsequent estrus expression compared with cows that received GnRH at 75 h. In contrast, the ED cows expressing estrus also received GnRH at 60 h but had greater pregnancy risk. Therefore, lack of subsequent estradiol production and estrus may not explain the poorer PR, rather the timing of the insemination or insufficient uterine function because of inadequate estradiol associated with estrus expression (Bridges et al., 2013).

The classic work reported by Saacke (2008) illustrates the importance of insemination timing relative to the onset of estrus. In those studies, cows inseminated near the onset of estrus tended to have lower fertilization risk but greater embryonic quality compared with cows inseminated 24 h after the onset of estrus, in which fertilization risk was greater but embryo quality was compromised. Therefore, Saacke (2008) concluded that inseminating cows according the traditional a.m.-p.m. rule is a compromise to achieve maximal fertilization success and maintain embryo quality compared with inseminating very early or very late relative to the onset of estrus.

In light of those conclusions, perhaps the poorer PR in EE cows inseminated at 60 h was similar to PR in cows in which inseminations were made early in estrus that resulted in lower fertilization risk but greater embryo quality than that for ED cows, resulting in the difference in PR being related to more fertilization failure in the EE cows. Compared with beef heifers not exhibiting estrus before TAI, however, heifers that expressed estrus had improved embryo quality and advanced embryo stage on d 6 and increased the number of accessory sperm associated with the embryo (Larimore et al., 2015). Therefore, differences in PR between the EE and ED cows, which exhibited estrus, may be attributed to differences in uterine environment, fertilization risk, or embryo quality.

Strategies to increase the proportion of cows in estrus exposed to TAI systems may be valuable; however, the actual expression of estrus (79% to 85%) reported previously (Wilson et al., 2010) during 36 through 144 h after $PGF_{2\alpha}$ indicates that strategies to more closely synchronize estrus or ovulation and timing of insemination may have the greatest opportunity for success. In the current study we observed more than 75% of cows exhibiting estrus by 75 h after removal of the CIDR insert and $PGF_{2\alpha}$ injection, regardless of estrus cycle status, parity, or days postpartum. The recommended optimal insemination time of 66 h after CIDR removal is not ideal for up to 53.7% of the cows in the present study that had not displayed estrus by 60 h. The results of the current study indicate that primiparous cows display estrus earlier than multiparous cows. This observation corroborates results of an earlier study (Dobbins et al., 2009) in which younger cows were more fertile when inseminated at an earlier fixed time (≤56 h after removal of the CIDR insert) than multiparous cows. A possible bimodal distribution of estrus between primiparous and multiparous cows indicates that insemination at 2 distinct times determined by estrus detection would result in more cows being inseminated after estrus expression.

The distribution of estrus in cows reported elsewhere (Wilson et al., 2010) indicated that approximately 50% of cows exhibited estrus by 60 h after CIDR removal. We chose 60 h as the timing of the first AI and observed that 46% of cows expressed estrus by 60 h. We chose 75 h after CIDR insert removal for the second AI time to coincide with the 12- to 16-h interval from beginning of estrus expression to maximum conception risk in dairy cows (Stevenson et al., 2014). Although estrus expression by 75 h was not influenced by days postpartum, indicating that progestin-based synchronization programs are effective at inducing estrus and presumably ovulation in noncycling cows, parity-BCS interactions were detected, with better-conditioned primiparous cows having the greatest expression by 75 h. All cows that expressed estrus preceding AI had greater PR than their nonestrous herd mates, indicating that regardless of insemination time, it was preferable to inseminate after estrus. The EE cows that were inseminated at 60 h had lower PR than any other treatment, despite receiving GnRH before AI. Timing of GnRH injection at either 60 or 75 h did not affect either subsequent estrus expression or PR, indicating some flexibility in applying these procedures in commercial operations. The GnRH injection concurrent with insemination may be unnecessary in single fixed-time AI systems when estrus is detected (Perry and Perry, 2009). The importance of concurrent GnRH injection in cows displaying estrus has not been tested in a split timed AI system; however, increasing the number of cows inseminated after estrus has the potential to reduce the reliance on induced ovulation via exogenous GnRH treatment.

In a similarly conducted study in cows utilizing sex-sorted semen, increases in PR were noted when utilizing 2 AI times (Thomas et al., 2014a). In another study examining 2 AI times with conventional semen, no difference in PR was detected between early and later inseminated nonestrous cows (Thomas et al., 2014b). It is likely that the difference in results noted between the latter study and the current study is the choice of insemination times relative to removal of the CIDR insert. In the current study, it is probable that the second insemination was closer to the optimal time between semen placement and ovulation for cows that exhibited estrus between 60 and 75 h when compared with the second insemination timing of 86 h in the previous report (Thomas et al., 2014b).

In summary, delaying the insemination of cows to 75 h when not detected in estrus by 60 h increased the PR compared with that of like cows inseminated at 60 h regardless of whether GnRH was injected at 60 or 75 h. By delaying insemination of nonestrous cows to 75 h, the number of cows inseminated after exhibiting estrus was increased. Pregnancy risk was consistently greater in cows that had displayed estrus before TAI.

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