Theriogenology 213 (2024) 59–65

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Evaluation of two beef cow fixed-time AI protocols that utilize presynchronization

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ARTICLE INFO

Keywords:
Beef cattle
Fixed-time artificial insemination
Pregnancy
Presynchronization
Synchronization of ovulation

ABSTRACT

Presynchronization was evaluated as a method to improve estrus response before fixed-time AI (FTAI). The objective was to compare FTAI results in beef cows from two different presynchronization approaches. Blood samples were collected on Day −14 (Day 0 = CIDR removal) to determine progesterone concentration (≥1 ng/mL = high, <1 ng/mL = low). In a subset (n = 1289), an additional blood sample was collected between Day −21 and −29 to determine cyclicity (if both the Day −14 and Day −21 to −29 samples were classified as low progesterone cows were classified as noncycling). Cows (n = 1388) from 30 herds were grouped by days postpartum (DPP) and age, and randomly assigned to either of two protocols. Cows assigned to the PG 6-day CIDR & FTAI protocol (PG6d) received prostaglandin $F_{2\alpha}$ (PG) on Day −9, CIDR insertion and GnRH on Day −6, and CIDR removal and PG on Day 0. Cows assigned to the 7&7 Synch protocol (7&7) were administered PG and CIDR insertion on Day −14, GnRH on Day −7, and CIDR removal and PG on Day 0. For both protocols, FTAI occurred concurrently with GnRH 66 h after second PG. Pregnancy was determined by transrectal ultrasonography 30–40 d after FTAI. The GLIMMIX procedure of SAS was used to detect differences in estrus response and pregnancy success with herd as a random variable. Estrus response (0–66 h) was analyzed with two models, one included cyclicity and another replaced cyclicity with progesterone concentration at Day −14. In both models, cows assigned to the 7&7 protocol had greater (P < 0.01) estrus response than cows assigned to the PG6d protocol. The model including cyclicity, estrus response was impacted by the cyclicity by DPP interaction (P = 0.03), cyclicity by protocol interaction (P = 0.04), and the tendency of BCS by protocol interaction (P = 0.08). In the estrus response model that included progesterone concentration at Day −14, significant variables included the protocol by progesterone concentration at Day −14 (P = 0.01), and BCS (P < 0.01), while DPP (P = 0.08) and progesterone concentration at Day −14 (P = 0.07) were tendencies. Pregnancy success was influenced by estrual status (P < 0.01), body condition score (P = 0.04), and cycling status (P = 0.02), but was not influenced by protocol (P = 0.75; PG6d = 38 ± 5% and 7&7 = 37 ± 5%). In conclusion, effectiveness of presynchronization method depended on a cows' physiological status, and the 7&7 protocol increased estrus response compared with PG6d, but there was no difference in pregnancy success.

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https://doi.org/10.1016/j.theriogenology.2023.09.017
Received 16 July 2023; Received in revised form 18 September 2023; Accepted 21 September 2023
Available online 22 September 2023

1. Introduction

Fixed-time artificial insemination (FTAI) protocols have been widely adopted by the beef industry due to their ability to provide every female the opportunity to conceive on the first day of breeding season, while also decreasing the time and labor associated with estrus detection. The average pregnancy rates of two commonly used cow FTAI protocols, the 7-day CO-Synch + CIDR (n = 10,701) and 5-day CO-Synch + CIDR (n = 2,189), are 58% and 62%, with ranges of 32–79% and 49–80%, respectively [1]. Although the average pregnancy rate demonstrates that more than half of the females can become pregnant to FTAI, the minimum pregnancy rates (32% and 49%), raise the question: Can pregnancy rates to FTAI protocols be improved?

Both the 7-day CO-Synch + CIDR and 5-day CO-Synch + CIDR protocols begin with administering gonadotropin releasing hormone (GnRH) to synchronize a follicular wave and induce luteal tissue formation. Typically, 66% of cows will ovulate to a single dose of GnRH, but the response is dependent on stage of the estrous cycle at the time of GnRH administration [2]. Periods of the estrous cycle that typically have a decreased ovulatory response to GnRH occur when dominant follicles begin to plateau or regress, or before dominant follicle selection [2]. Thus, pregnancy success to FTAI protocols that begin with administration of GnRH could potentially be improved by increasing the proportion of cows having a follicle capable of ovulating to the initial administration of GnRH.

The need for increased pregnancy success to AI in the dairy industry resulted in researchers developing several protocols that aimed to "presynchronize" estrous cycles and thus increase the ovulatory response to initial GnRH administration, resulting in improved follicular wave control [3]. Presynchronization often entails administering prostaglandin $F_{2\alpha}$ (PG) and/or GnRH before beginning a FTAI protocol. Presynchronization adds additional time, labor, and cost, but reportedly improves pregnancy rates and the proportion of cows that respond to the GnRH and PG within a FTAI protocol compared with FTAI protocols that do not utilize presynchronization [3,4].

The beef industry has also implemented presynchronization (PG 6-day CIDR & FTAI protocol [5–7]) to increase follicular wave control. Recently a new FTAI protocol that utilizes presynchronization (7&7 Synch protocol) has been reported [8–10]. The PG 6-day CIDR & FTAI protocol attempts to presynchronize estrous cycles by administering a single dose of PG 3 d before CIDR insertion and GnRH administration, thereby inducing luteolysis and resulting in a larger proportion of dominant follicles present at GnRH administration and CIDR insertion. By administering GnRH 3 d after PG, a greater proportion of cows should have a follicle present that is large/mature enough to respond to GnRH [6]. The 7&7 Synch protocol aims to presynchronize by purposefully creating a persistent follicle. This is done by administering PG 14 d before FTAI while also inserting a CIDR. Again, the administration of PG should regress any mature corpora lutea that are present, while the presence of sub-luteal progesterone concentrations delivered by the CIDR allows for continued follicular growth, but not ovulation of dominant follicles that presumably would have become atretic. When GnRH is administered 7 d after CIDR insertion, a new follicular wave should be initiated [11]. The objective of this experiment was to compare the two methods of presynchronization (regressing the CL to have low progesterone at time of GnRH compared with 7 d of low progesterone to try to induce a persistent follicle to be present at time of GnRH) to determine if cows of different physiological states would respond better to one form of presynchronization over the other. The authors hypothesized that anestrous and cycling cows would respond differently between the two forms of presynchronization.

2. Materials and methods

All procedures were approved by the Texas A&M AgriLife IACUC committee #2021-036A.

2.1. Experimental design

Bos taurus cows (n = 1,388) from 30 herds at 10 different locations (some locations implemented both fall and spring breeding seasons) in seven states were enrolled in a field trial (Table 1). Body condition scores (BCS; based on a one to nine scale; one = emaciated and nine = obese [12]) were assigned before or at FTAI. In addition, cow age and days postpartum (DPP; days from calving date to FTAI date) were calculated. Within herd, cows were grouped by DPP and age and then randomly assigned to either of two synchronization protocols (described in Section 2.2 Synchronization).

2.2. Synchronization

Cows assigned to the PG 6-day CIDR & FTAI protocol (PG6d; n = 693) received 25 mg dinoprost tromethamine (PG1; Lutalyse *HighCon*; Zoetis, Kalamazoo, MI) i.m. on Day −9. An intravaginal insert containing 1.38 g of progesterone (CIDR; Zoestis, Kalamazoo, MI) was inserted and 100 μg of gonadorelin (GnRH1; Factrel; Zoetis; Kalamazoo, MI) was administered i.m. on Day −6 (Fig. 1).

Cows assigned to the 7&7 Synch protocol (7&7; n = 695) were administered 25 mg of dinoprost tromethamine (PG1; Lutalyse *HighCon*; Zoetis, Kalamazoo, MI) i.m. on Day −14 coincident with CIDR (1.38 g progesterone; intravaginal insert; CIDR; Zoestis, Kalamazoo, MI) insertion. Administration of 100 μg of gonadorelin (GnRH1; Factrel; Zoetis; Kalamazoo, MI) occurred i.m. on Day −7 (Fig. 1).

On Day 0, all cows were administered 25 mg of dinoprost tromethamine (PG2; Lutalyse *HighCon*; Zoetis, Kalamazoo, MI) i.m., Estrotect patches (Estrotect; Estrotect Inc., Spring Valley, WI) were applied and CIDR removal occurred (Fig. 1). Cows were considered estrual when at the time of FTAI (66 h) ≥ 50% of the estrus detection aid was activated.

2.3. Artificial insemination

For both protocols, FTAI occurred coincident with i.m. administration of 100 μg of gonadorelin (GnRH2; Factrel; Zoetis; Kalamazoo, MI) 66 h after PG2 (Fig. 1). Sire was evenly distributed between protocols within herd. A subset of cows (n = 151) at one location were inseminated with sex-sorted semen (SexedULTRA 4 M™, Sexing Technologies, Navasota, TX) and were included in the estrus response analysis but removed from the pregnancy success analysis.

2.4. Blood sampling

Blood samples were collected on Day −14 (Fig. 1) via venipuncture into 10-mL vacutainer tubes to determine circulating progesterone concentration at the start of synchronization (≥1 ng/mL = high, <1 ng/mL = low). In a subset of cows (n = 1,289), an additional blood sample was collected between Day −21 and −29 to determine cyclicity. Circulating concentrations of progesterone were analyzed in plasma or serum samples by radioimmunoassay (RIA) in duplicate [13]. The intra- and inter-assay coefficients of variation (CV) were 5.24% and 7.20%, respectively, in 10 assays. Assay sensitivity was 0.08 ng/mL. A cow was classified as noncycling if neither blood sample had a progesterone concentration ≥1 ng/mL but was classified as cycling when either or both blood samples had a progesterone concentration ≥1 ng/mL.

2.5. Pregnancy diagnosis

Females were classified as pregnant when a conceptus was visualized by transrectal ultrasonography 30–40 d following FTAI.

2.6. Statistical analysis

All statistical analyses were conducted using SAS 9.4 software. Differences between protocols in BCS, DPP, age, and cycling status were analyzed by analysis of variance in SAS (PROC GLM). The statistical model consisted of the independent variable of protocol and the dependent variables tested (BCS, DPP, age, and cycling status). Mean separation was performed when the model was significant using least significant differences (Means ± SEM [standard error of the mean] [14]).

Estrus response and pregnancy success were analyzed with the GLIMMIX procedure in SAS using the binary distribution and the Kenward Rogers method for the denominator degrees of freedom. For statistical analysis of estrus response and pregnancy success, BCS classifications were restructured (<5, 5, 5.5, ≥6). In addition, females were classified into parity groups (primiparous and multiparous). Cows were also grouped by DPP (<45, 45 to 65, 66 to 85, 86 to 105, >105) for analysis of estrus response and pregnancy success.

To determine the final statistical model for estrus response, the independent variables of protocol, DPP, BCS, cycling status (noncycling or cycling), parity (primiparous or multiparous) and all two-way interactions were initially included. Backwards elimination ($P > 0.10$) was performed to determine the final statistical model for the dependent variable of estrus response which included the independent variables of protocol, DPP, BCS, cycling status, the protocol by BCS interaction, the protocol by cycling status interaction, and the DPP by cycling interaction.

In an additional model for estrus response, progesterone classification (high or low) on Day −14 replaced cycling status to further analyze the impact of physiological status. The independent variables initially included were protocol, DPP, BCS, progesterone concentration at Day −14, parity, and all two-way interactions. Backwards elimination ($P > 0.10$) was performed to determine the additional final statistical model for the dependent variable of estrus response which included the independent variables of protocol, DPP, BCS, progesterone classification on Day −14, and the protocol by progesterone classification on Day −14 interaction, with herd being included as a random variable.

A statistical analysis was then performed to determine the final statistical model for pregnancy success for cows receiving conventional semen. The independent variables of protocol, DPP, BCS, cycling status, progesterone classification (high or low) on Day −14, estrual status, parity (primiparous or multiparous) and all two-way interactions were initially included in the model. Backwards elimination ($P > 0.10$) was performed to determine the final model for the dependent variable of pregnancy success which included the independent variables of protocol, estrus status, cycling status, BCS, parity, and the BCS by age interaction, with herd being included as a random variable.

For all models, means separation was performed using least significant differences (Means ± SEM [standard error of the mean] [15]). Differences were considered to be significant when $P \leq 0.05$ and a tendency when $P > 0.05$ but $P \leq 0.10$. All data are reported as LSmeans ± (SEM).

3. Results

Overall, the dependent variables of DPP, age, BCS, cycling status, and the proportion of females that were classified as having high progesterone on Day −14 did not differ between protocols (Table 2). Within some herds, BCS (one herd; n = 47), progesterone concentration on Day −14 (two herds; n = 28 and n = 47) and cycling status (three herds; n = 30, n = 30, and n = 47) differed ($P \leq 0.10$) between protocols.

Table 1
Average days postpartum, age, body condition score, percent cycling and percent of females with high progesterone on day −14 by herd.

State	Location	Herd	n	DPP (range)[a]	Age (range)[b]	BCS (range)[c]	% Cycling[d]	% High P4[e]
AR	1	1	22	89 (32–241)	4 (2–7)	5 (4.5–6)	59	50
AR	1	2	20	70 (46–91)	5 (2–7)	6 (4.5–7)	70	70
GA	2	3	46	76 (32–128)	6 (2–12)	6 (5–6)	91	80
KS	3	4	119	81 (65–103)	7 (4–11)	5 (4–6.5)	28	28
MS	4	5	30	82 (40–101)	5 (2–16)	6 (5–7.5)	90	73
MS	4	6	30	82 (44–101)	5 (2–17)	6 (4–7.5)	87	73
MS	4	7	28	82 (49–101)	5 (2–11)	6 (5–8)	79	54
MS	4	8	30	80 (37–102)	5 (2–14)	6 (4–7)	80	60
MS	4	9	28	80 (42–102)	5 (2–11)	6 (5–8.5)	89	68
MS	4	10	30	81 (42–102)	5 (2–15)	6 (5–7)	73	60
NM	5	11	82	76 (39–99)	7 (4–10)	4 (3.5–5.5)	22	25
SD	6	12	137	80 (9–108)	4 (2–10)	5 (3.5–8.5)	86	80
SD	7	13	90	79 (45–117)	5 (3–11)	5 (4.5–6)	68	60
SD	8	14	102	92 (75–122)	3 (2–5)	5 (4–6.5)	84	48
SD	8	15	99	74 (57–98)	6 (3–13)	5 (5–7)	66	56
SD	8	16	50	64 (33–106)	6 (3–15)	5 (4–7)	48	46
TX	9	17	74	81 (58–128)	6 (2–12)	5 (4.5–6.5)	55	45
TX	9	18	28	74 (59–128)	6 (2–10)	5 (4.5–5.5)	57	46
TX	9	19	34	67 (59–78)	6 (2–12)	5 (4.5–6.5)	44	32
TX	9	20	24	66 (60–73)	7 (3–12)	5 (5–6)	54	33
TX	9	21	12	66 (61–72)	5 (3–9)	5 (5–5.5)	0	0
TX	9	22	30	68 (60–78)	7 (2–12)	5 (4.5–6.5)	30	30
TX	9	23	27	67 (60–75)	6 (2–10)	5 (5–6)	22	22
TX	9	24	18	65 (60–73)	7 (3–12)	5 (4–6)	39	44
TX	9	25	31	65 (58–73)	6 (2–11)	5 (4.5–6.5)	35	19
TX	9	26	48	69 (56–77)	6 (2–12)	5 (4–5.5)	33	25
TX	9	27	26	66 (53–74)	6 (2–12)	5 (4–6)	23	20
TX	9	28	13	64 (58–73)	7 (2–12)	5 (4.5–6)	23	23
TX	10	29	33	88 (52–111)	7 (3–18)	6 (3.5–7)	81	67
TX	10	30	47	90 (41–117)	6 (2–11)	6 (4.5–6.5)	81	70

[a] Days postpartum (DPP) were calculated as the number of days from the calving date to the date of FTAI.
[b] Years of age.
[c] A body condition score (BCS) was assigned to females before or at FTAI. A scale of one to nine (one = emaciated and nine = obese) was used.
[d] Females were considered cycling when at least one or both blood samples had a progesterone ≥1 ng/mL. Blood samples were taken on Day −14 and an additional blood sample was taken between Day −21 and −29 to determine cyclicity (%Cycling).
[e] Percent of females with high (≥1 ng/mL) progesterone (% High P4) on Day −14.

3.1. Estrus response

Cows assigned to the 7&7 protocol had a greater ($P < 0.01$; 75 ± 3% or 73 ± 3%) estrus response than cows assigned to the PG6d protocol (54 ± 4% or 57 ± 4%; Fig. 2) in the estrus response models that included progesterone concentration on d-14 or cycling status respectively.

Cycling status interacted ($P = 0.03$) with DPP as cycling females that were <45 DPP had a poorer ($P < 0.02$) estrus response compared with all other combinations of cycling status and DPP. Cycling status also interacted ($P = 0.04$) with protocol. The estrus response of noncycling (74 ± 4%) and cycling (72 ± 5%) cows assigned to the 7&7 protocol did not differ ($P = 0.68$). Cycling (48 ± 5%) cows assigned to the PG6d protocol had a poorer ($P < 0.01$) estrus response compared with both cycling and noncycling cows assigned to the 7&7 protocol. Noncycling (65 ± 5%) cows assigned to the PG6d protocol had a estrus response that did not differ ($P = 0.28$) compared to cycling cows assigned to the 7&7 protocol but a poorer ($P = 0.04$) estrus response compared with non-cycling cows assigned to the 7&7 protocol. Among cows assigned to the PG6d protocol, noncycling cows had a greater ($P = 0.01$) estrus response than cycling cows. Protocol tended ($P = 0.08$) to interact with BCS. Cows assigned a BCS <5 had a poorer ($P < 0.01$; 50 ± 6%) estrus response

Table 2
Average days postpartum, age, body condition score, percentage cycling, and percentage of females with high progesterone on Day −14 by protocol.

Protocol[a]	DPP[b]	Age	BCS[c]	% Cycling[d]	% High P4[e]
PG6d	78 ± 0.7 (n = 674)	5.6 ± 0.1 (n = 693)	5.3 ± 0.03 (n = 692)	58 ± 2 (405/693)	48 ± 2 (333/688)
7&7	78 ± 0.7 (n = 677)	5.7 ± 0.1 (n = 695)	5.3 ± 0.03 (n = 695)	61 ± 2 (423/695)	51 ± 2 (354/692)
P-value	0.98	0.60	0.53	0.36	0.31

[a] See Fig. 1 for protocol descriptions.
[b] Days postpartum (DPP) were calculated as the number of days from the calving date to the date of fixed time artificial insemination (FTAI).
[c] A Body condition score (BCS) was assigned to females before or at FTAI. A scale of one to nine (one = emaciated and nine = obese) was used.
[d] Females were considered cycling when at least one blood sample or if both blood samples had a progesterone ≥1 ng/mL. Blood samples were taken on Day −14 and an additional blood sample was taken between Day −21 and −29 to determine cyclicity (% Cycling).
[e] Percent of females with high (≥1 ng/mL) progesterone on Day −14 (% High P4).

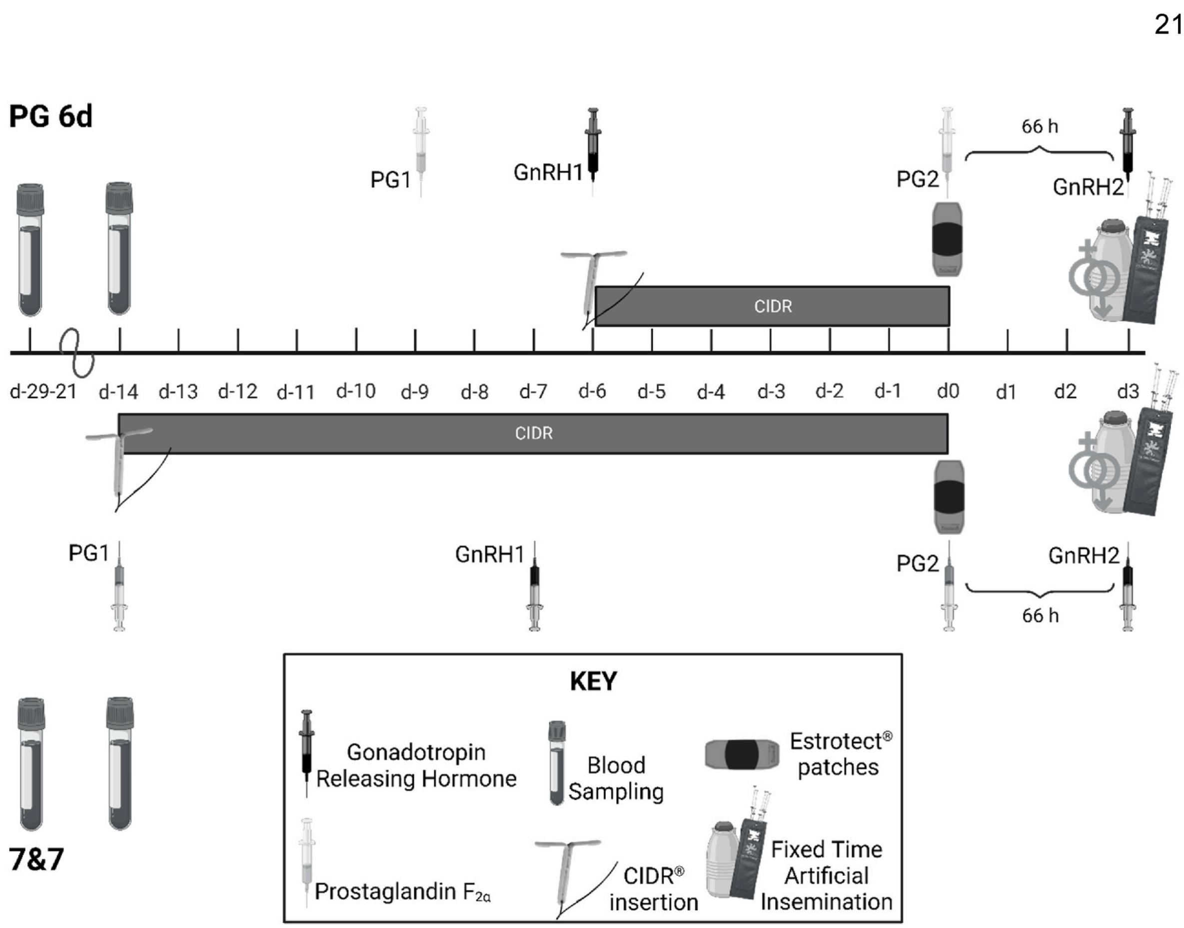

Fig. 1. Experimental design. Blood samples were collected on Day −14 and in a subset (n = 1,388), an additional blood sample was collected between Day-21 and -29 to determine cyclicity. Cows assigned to the 7&7 Synch protocol (7&7; below the timeline; n = 720) were administered 25 mg of dinoprost tromethamine (PG1; Lutalyse *HighCon*; Zoetis, Kalamazoo, MI) i.m. on Day −14 coincident with CIDR (1.38 g progesterone; intravaginal insert; CIDR; Zoestis, Kalamazoo, MI) insertion. Administration of 100 μg of gonadorelin (GnRH1; Factrel; Zoetis; Kalamazoo, MI) occurred i.m. on Day −7. Cows assigned to the PG 6-day CIDR & FTAI protocol (PG6d; above the timeline; n = 713) received 25 mg dinoprost tromethamine (PG1; Lutalyse *HighCon*; Zoetis, Kalamazoo, MI) i.m. on Day −9. An intravaginal insert containing 1.38 g of progesterone (CIDR; Zoestis, Kalamazoo, MI) was inserted and 100 mcg of gonadorelin (GnRH1; Factrel; Zoetis; Kalamazoo, MI) was administered i.m. on Day −6. On Day 0, all cows (PG6d and 7&7) were administered 25 mg of dinoprost tromethamine (PG2; Lutalyse *HighCon*; Zoetis, Kalamazoo, MI) i.m., Estrotect patches (Estrotect; Estrotect Inc., Spring Valley, WI) were applied and CIDR removal occurred. For both protocols, FTAI occurred coincident with i.m. administration of 100 mcg of gonadorelin (GnRH2; Factrel; Zoetis; Kalamazoo, MI) 66 h after PG2. A subset of cows (n = 151) at one location were inseminated with sex-sorted semen (SexedULTRA 4 M™, Sexing Technologies, Navasota, TX) and were removed from the pregnancy success analysis, while the remaining cows were inseminated with conventional semen and included in the pregnancy success analysis. Figure created with Biorender.com.

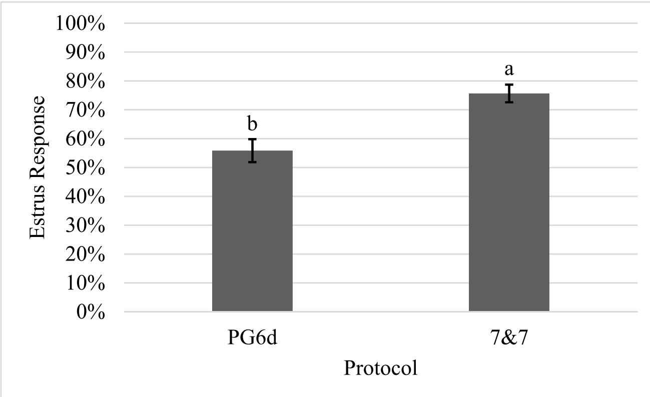

Fig. 2. Proportion of females that expressed estrus before fixed time artificial insemination by protocol. The proportion of females that expressed estrus was greater ($^{a,b}P < 0.0001$) in females assigned to the 7&7 protocol (76 ± 3%; n = 695) compared with females assigned to the PG6d protocol (56 ± 4%; n = 693).

than all other BCS groupings (>69%).

Protocol interacted ($P = 0.01$) with progesterone classification on Day −14 to impact estrus response. Cows assigned to the 7&7 protocol that had high progesterone on Day −14 had a greater ($P < 0.01$) estrus response (80 ± 3%) than all other cows. The estrus response of cows assigned to the 7&7 protocol that had low progesterone (69 ± 4%) on Day −14 was greater compared with cows assigned to the PG6d protocol that had high progesterone ($P < 0.01$; 53 ± 4%) and compared with cows assigned to the PG6d protocol with low progesterone ($P < 0.01$; 55 ± 4%). Estrus response did not differ ($P = 0.69$) among cows assigned to the PG6d protocol, regardless of progesterone classification. In addition, there tended ($P = 0.07$) to be a main effect of progesterone classification on Day −14 such that cows with high progesterone on Day −14 had a greater ($P < 0.01$; 68 ± 3%) estrus response than cows with low progesterone (62 ± 4%).

The main effect of DPP tended ($P = 0.08$) to impact estrus response as females <45 DPP (43 ± 10%) had the poorest estrus response. Cows assigned a BCS <5 had a poorer ($P < 0.01$; 51 ± 6%) estrus response than all other BCS groupings (>67%).

3.2. Pregnancy success

Among cows that received conventional semen, protocol did not significantly ($P = 0.66$; PG6d: 47 ± 5% vs 7&7: 46 ± 5%; Fig. 3) impact pregnancy success. Pregnancy success was significantly influenced by BCS ($P = 0.05$) such that females assigned a BCS <5 (30 ± 7%) had less pregnancy success than females assigned a BCS = 5 (53 ± 4%) or = 5.5 (54 ± 5%), with pregnancy success not different between BCS = 5 or BCS = 5.5. Females assigned a BCS ≥6 (50 ± 12%) had intermediate pregnancy success. Cycling status ($P = 0.03$; Cycling: 51 ± 5% vs Noncycling: 43 ± 5%; Fig. 4) and estrual status ($P < 0.0001$; Estrual: 59 ± 4% vs Nonestrual: 35 ± 4%; Fig. 5) impacted pregnancy success. There was a tendency for parity to interact ($P = 0.06$) with BCS with primiparous cows in a BCS <5 (14 ± 8%) having poorer pregnancy rates than all other BCS and parity combinations.

4. Discussion

The purpose of presynchronization in a FTAI protocol is to increase the proportion of females that are able to respond to GnRH1 to achieve better follicular wave control. Inducing luteolysis at CIDR insertion (Day −14) in the 7&7 protocol creates subluteal concentrations of exogenous progesterone that presumably increases the proportion of females that have a mature/persistent follicle present on Day −7 (GnRH administration; Day 0 = CIDR removal). This approach should increase the

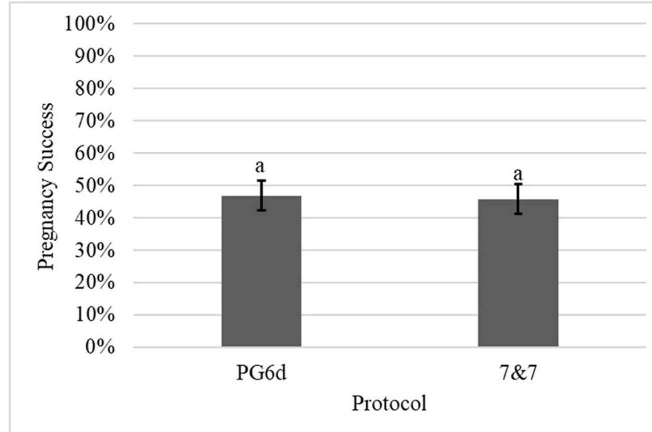

Fig. 3. Pregnancy success associated with protocol. Among females that received conventional semen, the proportion that became pregnant did not differ ($^{a}P = 0.66$) between cows assigned to the PG6d (47 ± 5%; n = 611) and the 7&7 (46 ± 5%; n = 626) protocol.

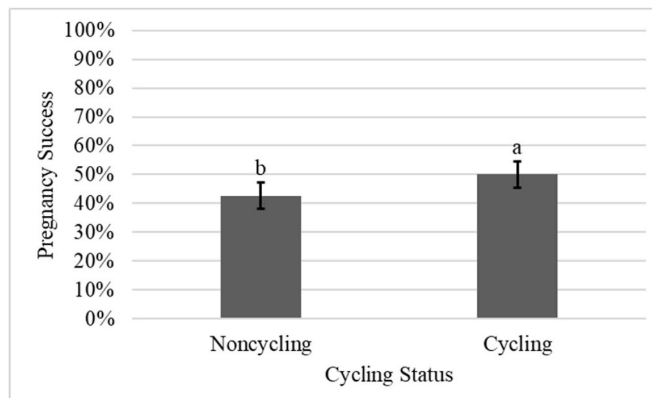

Fig. 4. Pregnancy success respective of cycling status within females receiving conventional semen. Pregnancy success was greater for females classified as cycling (n = 779) than noncycling (n = 458) cows ($^{a,b}P = 0.03$).

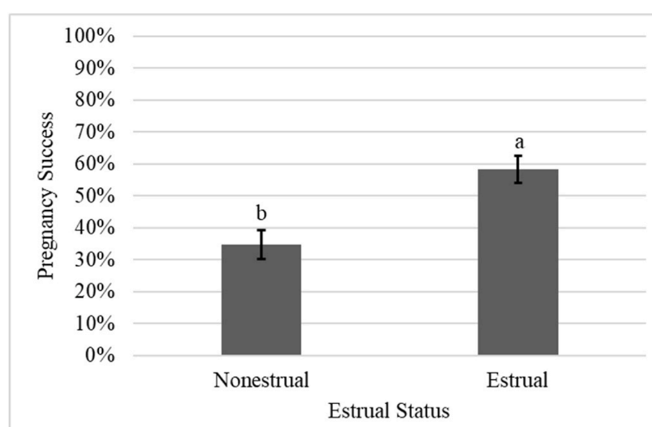

Fig. 5. Pregnancy success respective of estrual status. Females that expressed estrus (n = 833) had greater pregnancy rates than nonestrual (n = 357) females in females that received conventional semen ($^{a,b}P < 0.0001$).

likelihood of synchronizing follicular wave emergence following GnRH administration [9]; however, previous research has also reported that among anestrous females, low concentrations of progestin (MGA – [6]; CIDR – [16]) was not able to induce a persistent follicle. A different approach is taken by the PG6d protocol, in which luteolysis is induced by administering PG on Day −9 (Day 0 = CIDR removal) allowing circulating luteinizing hormone pulse frequency to increase [17–19], driving estradiol production [20] and thus preovulatory follicle development, resulting in a greater proportion of females that either express estrus or have a dominant follicle at GnRH1 administration [21]. Thus, the purpose of presynchronization in both the PG6d and 7&7 protocols is to increase the proportion of females capable of responding to GnRH1 and achieve better follicular wave control; however, anestrous cows will not respond to the initial administration of PG and thus among anestrous females variation in response to the GnRH1 administration on Day −6 will likely occur depending on the stage of follicular development.

The proportion of cows that expressed estrus before FTAI was greater for cows assigned to the 7&7 protocol compared with the PG6d protocol. Expression of estrus before FTAI protocols is increased when synchronization of a follicular wave is achieved [22]. Other studies [10,11] reported a greater estrus response for the 7&7 protocol compared with the 7-d CO-Synch + CIDR protocol, which does not implement presynchronization. Evidence to support increased follicular wave control in the 7&7 protocol compared with the 7-d CO-Synch + CIDR protocol includes presence of a larger dominant follicle and increased corresponding serum estradiol concentrations at GnRH1 [9]. In the current study, it is not clear why estrus response was greater following the 7&7 protocol compared with the PG6d protocol because ovulatory response to GnRH1 was not determined. Furthermore, circulating concentrations of estradiol at GnRH-induced ovulation, which have been reported to have a positive effect on the establishment of pregnancy [23,24], were not measured.

In the current study, a greater proportion of females were classified as pregnant when they expressed estrus (59 ± 4%) before FTAI compared with females that did not express estrus (35 ± 4%; Fig. 5). In FTAI protocols, heifers and cows that expressed estrus before FTAI had a 27% increase in pregnancy rate compared with those that did not express estrus [25]. The increase in pregnancy rates reported among females that are estrual before FTAI may be due to the role estradiol plays in induction of the preovulatory gonadotropin surge [26] thus allowing insemination to occur closer to ovulation, facilitation of sperm transport [27], as well as in uterine function [28]. However, in the present study, despite the 7&7 protocol resulting in a greater proportion of females expressing estrus, pregnancy rates did not differ. Therefore, even though estrus response differed between protocols, both forms of presynchronization resulted in acceptable pregnancy rates that did not differ. A possible hypothesis for increased estrus response but not increased pregnancy success could be the lack of turnover of the persistent follicle that was induced in the 7&7 protocol. Progesterone is capable of inhibiting ovulation by suppression of LH release [see review, 29] and increased concentrations of progesterone was associated with a decreased GnRH-induced LH surge on Day 6–8 of the estrous cycle [30]. Furthermore, following CIDR insertion there was a negative correlation between concentrations of progesterone at GnRH administration and area under the GnRH-induced LH curve and a tendency for a decrease in peak concentrations [31]. More specifically, females that had a CIDR inserted 48 h before administration of GnRH had a decreased ovulatory response compared with females that were administered GnRH at or before the time of CIDR insertion [31]. Thus, duration of progesterone exposure could decrease the efficacy of GnRH for follicular turnover in the 7&7 protocol. Presence of a persistent follicle at time of CIDR removal would increase concentrations of estradiol [32–34] and possibly the incidence of estrus expression, but would not increase the incidence of pregnancy success as ovulation of persistent follicles has been associated with low fertility [35].

Cycling status is known to impact pregnancy rates [36]. In this study, cycling status interacted with DPP such that cycling females that were <45 DPP had a poorer estrus response compared with all other combination of cycling status and DPP. In addition, cycling status also interacted with protocol such that regardless of cycling status, females assigned to the 7&7 protocol had a estrus response that did not differ. Among cows assigned to the PG6d protocol; however, noncycling cows had a greater estrus response than cycling cows. In addition, noncycling cows assigned to the PG6d protocol had a estrus response that did not differ compared to cycling cows assigned to the 7&7 protocol but a poorer estrus response compared with noncycling cows assigned to the 7&7 protocol. A meta-analysis that investigated factors that impact estrus response also reported that anestrous cows were more likely to express estrus than cycling cows [25]. This is possibly due to all anestrous cows being in the same stage of progesterone exposure when a protocol is initiated compared with the varying stages of that occur among cycling females. Cycling status also impacted pregnancy success such that cycling cows had greater pregnancy success than noncycling cows. Unlike in the estrus response model, there was not a protocol by cycling interaction in the pregnancy success model thus indicating that the increase in estrus response seen among noncycling females did not translate to increased pregnancy rates.

Females assigned a BCS <5 had a poorer estrus response and pregnancy success than females in greater BCS. Parity tended to interact with BCS to impact pregnancy success with primiparous cows assigned a BCS <5 having poorer pregnancy rates. Females that are still allocating nutrients to growth have fewer nutrients to partition towards reproduction, thus the poorer pregnancy rates among primiparous cows in poor BCS was expected as they are likely still in anestrous [37,38]. While DPP did not interact with cycling status nor BCS to impact pregnancy success, DPP interacted with cycling status to impact estrus response, which is known to contribute to pregnancy success.

5. Conclusion

Effectiveness of presynchronization method depends on a cow's physiological status at the beginning of the synchronization protocol. Although the 7&7 protocol increased estrus response compared with the PG6d, there was no difference in pregnancy success, thus utilization of both forms of presynchronization resulted in acceptable pregnancy rates.

Funding

This project was funded in part by the Multistate Hatch project 9835.

CRediT authorship contribution statement

Jaclyn N. Ketchum: Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Lacey K. Quail:** Investigation, Writing – review & editing. **Kaitlin M. Epperson:** Investigation, Writing – review & editing. **Chloey P. Guy:** Investigation, Writing – review & editing. **Jerica J.J. Rich:** Investigation, Writing – review & editing. **Saulo Menegatti Zoca:** Investigation, Writing – review & editing. **Adalaide C. Kline:** Investigation, Writing – review & editing. **Taylor N. Andrews:** Investigation, Writing – review & editing. **Julie A. Walker:** Investigation, Writing – review & editing. **Pedro Levy Piza Fontes:** Investigation, Writing – review & editing. **Sandy K. Johnson:** Investigation, Writing – review & editing. **Megan P.T. Owen:** Investigation, Writing – review & editing. **Douglas Eborn:** Investigation, Writing – review & editing. **Kelsey M. Harvey:** Investigation, Writing – review & editing. **Adam F. Summers:** Investigation, Writing – review & editing. **George A. Perry:** Conceptualization, Methodology, Validation, Formal analysis, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

There are no known competing interests.

Acknowledgements

The authors gratefully acknowledge Zoetis (Kalamazoo, MI) for supplying the CIDR cattle inserts, Lutalyse *HighCon*, and Factrel. In addition, the authors would like to acknowledge Estrotect Inc. (Spring Valley, WI) for generously providing estrus detection aids. The authors thank all of the collaborators and producers that contributed to the completion of this project.

References

[1] Beef Reproductive Task F. Protocols for synchronization of estrus and ovulation. 2009.
[2] Geary TW, Downing ER, Bruemmer JE, Whittier JC. Ovarian and estrous response of suckled beef cows to the select Synch estrous synchronization protocol. Prof Anim Sci 2000;16:1–5.
[3] Bello NM, Steibel JP, Pursley JR. Optimizing ovulation to first GnRH improved outcomes to each hormonal injection of ovsynch in lactating dairy cows. J Dairy Sci 2006;89:3413–24.
[4] El-Zarkouny SZ, Cartmill JA, Hensley BA, Stevenson JS. Pregnancy in dairy cows after synchronized ovulation regimens with or without presynchronization and progesterone. J Dairy Sci 2004;87:1024–37.
[5] Grant J, Abreu F, Hojer N, Fields S, Perry B, Perry G. Influence of inducing luteal regression before a modified controlled internal drug-releasing device treatment on control of follicular development. J Anim Sci 2011;89:3531–41.
[6] Perry GA, Perry BL, Krantz JH, Rodgers J. Influence of inducing luteal regression before a modified fixed-time artificial insemination protocol in postpartum beef cows on pregnancy success. J Anim Sci 2012;90:489–94.
[7] Bridges GA, Lake SL, Kruse SG, Bird SL, Funnell BJ, Arias R, et al. Comparison of three CIDR-based fixed-time AI protocols in beef heifers. J Anim Sci 2014;92:3127–33.
[8] Bonacker RC, Stoecklein KS, Ketchum JN, Knickmeyer ER, Locke JWC, Poock SE, et al. 299 Treatment with prostaglandin F2α and an intravaginal progesterone insert in advance of gonadotropin-releasing hormone enhances response to estrus synchronization in mature beef cows. J Anim Sci 2019;97:138–9.
[9] Bonacker RC, Stoecklein KS, Locke JWC, Ketchum JN, Knickmeyer ER, Spinka CM, et al. Treatment with prostaglandin F2α and an intravaginal progesterone insert promotes follicular maturity in advance of gonadotropin-releasing hormone among postpartum beef cows. Theriogenology 2020;157:350–9.
[10] Andersen CM, Bonacker RC, Smith EG, Spinka CM, Poock SE, Thomas JM. Evaluation of the 7 & 7 Synch and 7-day CO-Synch + CIDR treatment regimens for control of the estrous cycle among beef cows prior to fixed-time artificial insemination with conventional or sex-sorted semen. Anim Reprod Sci 2021:235.
[11] Bonacker RC, Gray KR, Breiner CA, Anderson JM, Patterson DJ, Spinka CM, et al. Comparison of the 7 & 7 Synch protocol and the 7-day CO-Synch + CIDR protocol among recipient beef cows in an embryo transfer program. Theriogenology 2020;158:490–6.
[12] Richards M, Spitzer J, Warner M. Effect of varying levels of postpartum nutrition and body condition at calving on subsequent reproductive performance in beef cattle. J Anim Sci 1986;62:300–6.
[13] Engel CL, Patterson HH, Perry GA. Effect of dried corn distillers grains plus solubles compared with soybean hulls, in late gestation heifer diets, on animal and reproductive performance. J Anim Sci 2008;86:1697–708.
[14] Snedecor GW, Cochran WG. Statistical methods. 1968.
[15] Snedecor GW, Cochran WG. Statist methods 1989:503.
[16] Rhodes F, Clark B, Day M, Macmillan K. Can persistent ovarian follicles be induced in young postpartum dairy cows? Proceeding of the annual conference-Australian Society for Reproductive Biology; 1997. p. 87.
[17] Ireland JJ, Roche JF. Growth and differentiation of large antral follicles after spontaneous luteolysis in heifers: changes in concentration of hormones in follicular fluid and specific binding of gonadotropins to follicles. J Anim Sci 1983;57:157–67.
[18] Leandro Henrique Cruppe B, Day ML, Steven Loerch Gustavo M Schuenemann Kichoon Lee Thomas W Geary AC. The effect of preovulatory concentration of estradiol and length of proestrus on pregnancy rate to timed AI and embryo transfer in beed cattle. [Doctoral dissertation; 2015.
[19] Kinder JE, Kojima FN, Bergfeld EGM, Wehrman ME, Fike KE. Progestin and estrogen regulation of pulsatile LH release and development of persistent ovarian follicles in cattle 1,2. J Anim Sci 1996;74:1424–40.
[20] Kaneko H, Terada T, Taya K, Watanabe G, Sasamoto S, Hasegawa Y, et al. Ovarian follicular dynamics and concentrations of oestradiol-17 beta, progesterone, luteinizing hormone and follicle stimulating hormone during the periovulatory phase of the oestrous cycle in the cow. Reprod Fertil Dev 1991;3:529–35.
[21] Perry GA, Perry BL, Krantz JH, Rodgers J. Influence of inducing luteal regression before a modified fixed-time artificial insemination protocol in postpartum beef cows on pregnancy success1. J Anim Sci 2012;90:489–94.
[22] Colazo MG, Mapletoft RJ. A review of current timed-AI (TAI) programs for beef and dairy cattle. Can Vet J 2014;55:772–80.
[23] Ciernia LA, Perry GA, Smith MF, Rich JJ, Northrop EJ, Perkins SD, et al. Effect of estradiol preceding and progesterone subsequent to ovulation on proportion of postpartum beef cows pregnant. Anim Reprod Sci 2021;227:106723.
[24] Jinks EM, Smith MF, Atkins JA, Pohler KG, Perry GA, MacNeil MD, et al. Preovulatory estradiol and the establishment and maintenance of pregnancy in suckled beef cows. J Anim Sci 2013;91:1176–85.
[25] Richardson BN, Hill SL, Stevenson JS, Djira GD, Perry GA. Expression of estrus before fixed-time AI affects conception rates and factors that impact expression of estrus and the repeatability of expression of estrus in sequential breeding seasons. Anim Reprod Sci 2016;166:133–40.
[26] Chenault JR, Thatcher WW, Kalra PS, Abrams RM, Wilcox CJ. Transitory changes in plasma progestins, estradiol, and luteinizing hormone approaching ovulation in the bovine. J Dairy Sci 1975;58:709–17.
[27] Hawk HW. Sperm survival and transport in the female reproductive tract. J Dairy Sci 1983;66:2645–60.
[28] Zelinski MB, Noel P, Weber DW, Stormshak F. Characterization of cytoplasmic progesterone receptors in the bovine endometrium during proestrus and diestrus. J Anim Sci 1982;55:376–83.
[29] Stormshak F, Bishop CV. Board-invited review: estrogen and progesterone signaling: genomic and nongenomic actions in domestic ruminants. J Anim Sci 2008;86:299–315.
[30] Colazo MG, Kastelic JP, Davis H, Rutledge MD, Martinez MF, Small JA, et al. Effects of plasma progesterone concentrations on LH release and ovulation in beef cattle given GnRH. Domest Anim Endocrinol 2008;34:109–17.
[31] Perry GA, Perry BL. Effect of the timing of controlled internal drug-releasing device insertion on the gonadotropin-releasing hormone-induced luteinizing hormone surge and ovulatory response. J Anim Sci 2009;87:3983–90.
[32] Zimbelman R, Smith L. Control of ovulation in cattle with melengestrol acetate. Reproduction 1966;11:193–201.
[33] Sirois J, Fortune JE. Lengthening the bovine estrous cycle with low levels of exogenous progesterone: a model for studying ovarian follicular dominance. Endocrinology 1990;127:916–25.
[34] Fortune J, Rivera G. Persistent dominant follicles in cattle: basic and applied aspects. Arq Fac Vet 1999;27:24–36.
[35] Mihm M, Baguisi A, Boland M, Roche J. Association between the duration of dominance of the ovulatory follicle and pregnancy rate in beef heifers. Reproduction 1994;102:123–30.
[36] Stevenson JS, Johnson SK, Milliken GA. Incidence of postpartum anestrus in suckled beef cattle: treatments to induce estrus, ovulation, and Conception121Presented at the managing reproduction in beef cattle symposium as a part of the 2002 midwest ASAS and ADSA regional meeting in des moines, IA in march 2002.2Contribution number 03-011-J from the Kansas agricultural experiment station, manhattan, KS. Prof Anim Sci 2003;19:124–34.
[37] Randel RD. Nutrition and postpartum rebreeding in cattle. J Anim Sci 1990;68:853–62.
[38] Short RE, Bellows RA, Staigmiller RB, Berardinelli JG, Custer EE. Physiological mechanisms controlling anestrus and infertility in postpartum beef cattle2. J Anim Sci 1990;68:799–816.