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Effects of twice daily compared with split-time estrous detection on pregnancy percentage in recipient beef cows

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ABSTRACT

Objectives were to compare pregnancy percentages per embryo transfer (P/ET) in recipient beef cows following twice daily compared with split-time estrous detection and to determine associations of dominant follicle diameter, CL volume and progesterone concentrations. All cows (n = 695) were treated to synchronize time of estrus among cows using a CIDR + Select-Synch treatment regimen and randomly assigned to twice-daily or split-time estrous detection (CS-DEET or CS-SEET, respectively). Cows in the CS-DEET group were observed twice daily (eight times) for estrus until 96 h after the time of PGF2α administration, whereas cows in the CS-SEET group were observed twice (64 and 84 h after PGF2α). In 280 recipient cows, blood sampling (for progesterone) and ultrasonographic assessment of dominant follicle diameter were conducted 48 h after the time of PGF2α administration. At 7 d after estrus, the CL was imaged and there was transfer of a frozen-thawed embryo into cows with a CL ≥ 1.5 cm. There were positive correlations between follicle diameter and CL volume (r_s = 0.827; $P < 0.001$) and CL volume and progesterone concentration (r_s = 0.680; $P < 0.001$). Progesterone and CL volume differed between cows in CS-SEET and CS-DEET groups $P < 0.05$), however, percentage P/ET for cows in the CS-SEET and CS-DEET groups did not differ ($P > 0.1$). Dominant follicle diameter, CL volume and progesterone concentrations were greater in pregnant compared with nonpregnant cows. In conclusion, percentage P/ET did not differ when there was twice daily and split-time estrous detection highlighting the value of this approach in beef enterprises.

1. Introduction

The goal of an embryo transfer (ET) program is to increase the number of progeny from genetically valuable cows. The overarching goal is for donor cows to produce a large number of high-quality embryos and after transfer of embryos into recipient cows there be maintenance of pregnancy throughout a gestational period, parturition without assistance, and providing of the maternal resources necessary for calf development to weaning. Success of an ET program requires proper management of both donor and recipient cows.

Transfer of embryos to estrous-synchronized recipient cows was most effective when embryos were transferred 6–8 d after the detected estrus (Bó et al., 2002). The initial estrous synchronization treatment regimen for ET included administration of PGF2α to

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induce luteolysis; however, the lack of efficiency in synchronizing the time of estrus and large cost of recipient maintenance limited widespread application and success of ET (Bó et al., 2002). Subsequently, gonadotropin-releasing hormone (GnRH), estradiol, progestogen-releasing devices and PGF2α have been used to synchronize timing of ovarian follicular and luteal dynamics a as well as ovulation among females, enabling transfer of embryos at a fixed time (FTET) without estrous detection (Bó et al., 2002; Baruselli et al., 2010, 2011). The use of some of these FTET treatment regimens have resulted in acceptable pregnancy/ET percentages.

To further improve ET outcomes, the focus is now directed on preovulatory follicle growth, estradiol concentrations and effects on estrous expression, embryo growth and pregnancy. When there is expression of estrus in females used for fixed time artificial insemination (FTAI) programs, there is an improved P/AI percentage in both *Bos taurus* (Kasimanickam et al., 2016; Richardson et al., 2016) and *Bos taurus* × *Bos indicus* beef cattle (Bó et al., 2017). Furthermore, diameter of dominant follicle at the time of FTAI, size of subsequently developed CL and blood progesterone concentrations during the ensuing luteal phase in beef cattle (Lamb et al., 2001; Perry et al., 2007; Sá Filho et al., 2010, 2011) also affected P/AI percentages. When beef cows were inseminated based on time of standing estrus, however, there was no effect of ovulatory follicle diameter on pregnancy outcomes (Perry et al., 2007), indicating that when there is relatively greater estradiol production by and secretion from the ovulatory follicle there are greater P/AI percentages (Perry et al., 2014). It is plausible that maturation of an estrogen-producing dominant follicle may affect fertility by having effects on the: oocyte and embryo development; ovarian follicular cells and subsequent luteinization; and/or the uterine milieu and subsequently establishment and maintenance of pregnancy. Similarly, when there is expression of estrus in recipient cows there is a greater P/ET percentage when there is use of FTET treatment regimens for synchronization of time of estrus among recipient cows (Atkins et al., 2010a, b).

With use of the various types of reproductive management programs, there are differences in relative economic costs for imposing the program (e.g., on-farm labor costs; Olynk and Wolf, 2008). For example, visual estrous detection requires more labor per cow and, therefore, there is a relatively greater cost of labor than when there is a FTAI treatment regimen imposed. Because of the greater labor costs when there is detection of estrus as part of an embryo transfer program, when all other costs are constant, reproductive management programs in which FTAI was a component gained favor because of having a greater expected net value (Olynk and Wolf, 2008).

The objective of the present study, therefore, was to compare pregnancy per ET (P/ET) percentages in recipient beef cows following twice daily (until 96 h after PGF2α administration in CIDR + Select-Synch program) compared with split-time estrous detection (twice, at 64 and 84 h after PGF2α administration when there was use of a CIDR + Select-Synch treatment regimen) when imposing a progesterone-based treatment regimen for ET. Furthermore, the goal was to determine the association among values for dominant follicle diameter, CL volume and progesterone concentrations in recipient beef cows. The primary objective in the present study was to compare P/ET percentages when there was twice daily estrous detection for 96 h (eight times in total) and estrous detection at two times (at 64 and 84 h) in recipient cows. The hypothesis for conducting this experiment was that P/ET percentages would not be different when using these two estrous detection methods.

2. Materials and methods

This study was performed in accordance with the ethics, standard operating procedure, handling and use of animals, collection and use of biomaterials for research.

2.1. Cows and estrous synchronization

Angus-cross beef cows (n = 733) from three locations, in moderate to good (5–7) body condition (BCS: 1, emaciated; 9, obese) (Bellows et al., 1982) that had calved at least 60 d prior to initiation of estrous synchronization treatment regimen were assigned a temperament score (1 = slow exit, walk; calm temperament; 2 = jump, trot or run; excitable temperament) (Kasimanickam et al., 2014). All cows had been dewormed (LongRange®, 50 mg of eprionomectin/mL, Merial Inc., Duluth, GA, USA) and vaccinated (Bovi-Shield Gold FP5 VL5®; Zoetis Animal Health, Parsippany, NJ, USA) as part of routine herd health management.

Cows were fed mixed alfalfa or grass hay from 3 mo before to 2 mo after the time of ET and were then moved to a pasture, or grazed Bermuda grass and were supplemented with corn silage as well as a corn soybean-meal concentrate mixture. Cows were administered a slow-release mineral bolus before ET and had *ad libitum* access to chelated minerals during the period when there was pasture grazing throughout the year. Cows were fed to meet National Research Council (NRC) recommendations [Nutrient requirements of beef cattle: eighth revised edition (2016)].

The CIDR + Select-Synch treatment regimen was imposed on all cows to synchronize the time of estrus among these cows (Fig. 1). Briefly, all cows were administered a 1.3 g progesterone intravaginal insert (CIDR, Eazi-Breed™ CIDR® Cattle Insert; Zoetis Animal Health), plus 100 μg of gonadorelin diacetate tetrahydrate (GnRH, 2 mL; Cystorelin®, Merial Inc., Duluth, GA, USA) im and 7 d later CIDR devices were removed, estrous detection aids (Estrotect™, Western Point Inc., Apple Valley, MN, USA) were applied and 25 mg of dinoprost (PGF2α; 5 mL; im; Lutalyse® sterile solution; Zoetis Animal Health) were administered. Cows were randomly assigned to CS-DEET (CIDR + Select-Synch Daily Estrus ET) or CS-SEET (CIDR + Select-Synch, Split-time Estrus ET). Cows in CS-DEET groups were observed twice daily (eight times in total) for standing estrus and evaluations of an estrous detector aid status were made until 96 h after PGF2α administration. Cows in CS-SEET group were observed only twice (64 and 84 h after PGF2α administration) for standing estrus and estrous detector aid status. In both groups, cows were considered to be expressing estrus (Day 0) if a cow was visually observed to stand for mounting by herd-mates or if a cow had an activated, lost (with mount marks) or partially activated (> 50 %) estrous detector aid.

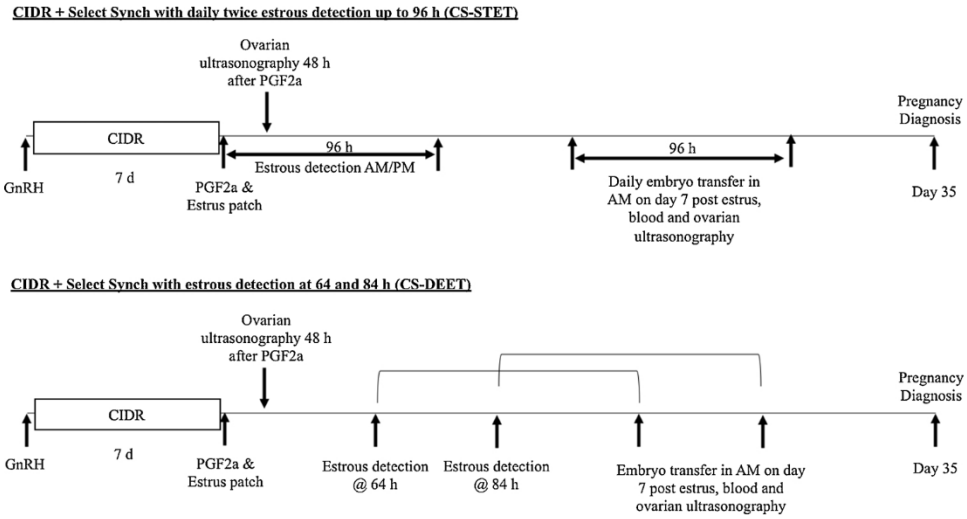

Fig. 1. Schematic presentation of embryo transfer treatment protocol. There was synchronization of estrus in all cows (n = 733) using a CIDR + Select-Synch treatment regimen. Briefly, all cows received CIDR for 7 days, GnRH on day 0, and PGF2α and estrous detection aids on day 7. Cows were randomly assigned to CS-DEET (CIDR + Select-Synch Daily Estrus ET) and CS-SEET (CIDR + Select-Synch, Split-time Estrus ET) treatment groups. Cows in CS-DEET groups were observed twice daily until 96 h after PGF2α administration and cows in CS-SEET group were observed at 64 and 84 h after PGF2α administration for standing estrus and evaluation of estrous detector aid status. Forty-eight hours after PGF2α administration, ovarian ultrasonography was performed by one clinician in a subset of cows (n = 280; 140 in each treatment group) to determine diameter of dominant follicle. Cows (n = 695) that expressed estrus and had an acceptable corpus luteum (CL, ≥1.5 cm in diameter) at ET were selected as embryo recipients and received a frozen-thawed embryo non-surgically 7 day after estrus to the uterine horn ipsilateral to the CL. Blood samples from 280 cows (140 cows per treatment group, balanced across age, BCS and temperament) were collected by coccygeal venipuncture at the time of ET for determination of serum progesterone concentrations. All cows were examined using transrectal ultrasonography for pregnancy status 35 d after ET.

2.2. Embryo transfer

All cows were subjected to transrectal palpation and ultrasonography (7.5 MHz linear-array transducer, Sonoscape S8, Universal Diagnostic Solutions, Oceanside, CA, USA). Cows (n = 695) that expressed estrus and had a CL ≥ 1.5 cm in diameter were selected as embryo recipients. There were 202, 233, and 260 cows, respectively, at Locations 1, 2 and 3 that were included in this study, with 38 cows being excluded as a result of not being detected in estrus and/or that had a CL ≤ 1.5 cm in diameter. Cows were administered caudal epidural anesthesia (2.5–4.0 mL, 2% lidocaine; MWI Animal Health, Boise, ID, USA) and the perineum area was washed and dried before conducting the ET procedures. Frozen-thawed embryos (in 1.5 M ethylene glycol) were non-surgically transferred on Day 7 after estrus, to the uterine horn ipsilateral to the CL. In the CS-DEET group, there was ET in cows on the morning of the Day 7 (four groups) subsequent to estrous detection that were detected in estrus in the morning and evening of Day 0 (Day of estrous detection). In the CS-SEET group (first group), there was ET on the morning of Day 7 after estrous detection in cows that were detected in estrus within 64 h (Day 0) after PGF2α administration. In the cows of the other group (Group 2 CS-SEET), that were detected in estrus between 64 and 84 h (Day 0) after PGF2α administration, there was ET on Day 7 after estrous detection. Classification stages of transferred embryos were 4 (morula), 5 (early blastocyst) and 6 (blastocyst) and quality of transferred embryos were Grades 1 (excellent/good) and 2 (fair) based on International Embryo Transfer Society (IETS) guidelines for classification of cattle embryos (Bo and Mapletoft, 2012). Immediately after ET, all recipient cows were administered injectable flunixin meglumine (Banamine® Injectable Solution; (1.1 mg/kg) BW; 1 mL/45 kg; im; Merck Animal Health; Madison, NJ, USA). Embryos were transferred by three highly experienced personnel. Embryo transfer difficulty score (1–3) was assigned, as follows: 1) transfer with minimal difficulty, requiring minimal manipulation of the genital tract, with the embryo being deposited in the upper-third of the uterine horn; 2) transfer with moderate difficulty, requiring moderate manipulation of the genital tract, with the embryo being deposited in the upper third of the uterine horn; or 3) transfer with maximum difficulty, requiring extreme manipulation of the genital tract, with the embryo being deposited in the upper half to third of the uterine horn.

2.3. Blood samples and assays

Blood samples from 280 recipients (140 per treatment group, balanced across age, BCS and temperament score) were collected by coccygeal venipuncture at 48 h after PGF2α administration. Samples were collected into vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ, USA). Blood samples were stored at 4 °C for ~6 h and centrifuged at 1500 × g for 20 min at 4 °C to separate serum, which was removed and stored at −20 °C. Serum progesterone concentrations were determined in duplicate using a validated ELISA (Enzo Life Sciences, Farmingdale, NY, USA), according to manufacturer's instructions (Mutinati et al., 2013). All samples were analyzed in

single assays, to eliminate inter-assay variation. Assay sensitivity was 0.02 ng/mL and intra-assay variation was 7.40 %.

2.4. Ultrasonography

2.4.1. Ovarian ultrasonography

2.4.1.1. Dominant follicle diameter. At 48 h after PGF2α administration, ovarian ultrasonography (Sonoscape S8, Universal Imaging, Bothell, WA, USA) was conducted using a 7.5 MHz linear-array transducer by one clinician in a subset of cows (n = 280; 140 per treatment group) to determine dominant follicle diameter.

2.4.1.2. CL volume. On day of ET, transrectal ultrasonographic examinations of ovaries of all cows were performed immediately prior to ET by the same clinician using a 7.5 MHz, linear-array transducer (Sonoscape S8, Universal Diagnostic Solutions). Three cross-sectional images of the CL were evaluated. In a CL with cavity, area of the CL cavity was assessed separately and subtracted from total CL area (Stevenson and Pulley, 2012; Carvalho et al., 2015; Ricci et al., 2017). Volume of the CL (cm^3) was calculated as follows:

$$\text{CL volume} = \frac{4}{3} \times \pi \times \left(\frac{\frac{\text{Height of CL}}{2} + \frac{\text{Width of CL}}{2}}{2} \right)^3$$

2.4.2. Pregnancy diagnosis

Cows were examined using transrectal ultrasonography for pregnancy status 35 d after ET and confirmed 90 d after ET using a 5 MHz, linear-array transducer (Sonoscape S8, Universal Diagnostic Solutions). A positive pregnancy outcome was based on visualization of the uterus and its contents, including a viable embryo.

2.5. Statistical analyses

Data were analyzed using a statistical software program (SAS Version 9.4 for Windows, SAS Institute, Cary, NC, USA). For all analyses, mean values were considered to be different when $P \leq 0.05$. Differences in mean body condition score, age of recipient cows, dominant follicle diameter and CL volume among treatments were analyzed using one-way ANOVA (PROC GLM of SAS). Datasets were tested for normality distribution by Komogorov-Smirnov test and were log-10 or arcsine transformed as needed; transformed values were back-transformed before being reported. Correlation between the values for dominant follicle diameter and CL volume and between CL volume and progesterone concentrations were tested using the Spearman method to determine the correlation coefficients on the subset of cows (n = 280).

The PROC GLIMMIX of SAS procedure were used to test for differences in percentage pregnancy rates. Fixed variables included in the model were treatment (CS-DEET compared with CS-SEET), temperament (calm or excitable), BCS (5, 6 or 7), age (3, 4–6 or > 6), difficulty with ET (1, 2 or 3), embryo quality (1 or 2), embryo stage (4, 5 or 6) and difficulty of ET score by treatment, temperament score by treatment, embryo quality by treatment, embryo stage by treatment, embryo quality by difficulty score and embryo developmental stage by difficulty score interactions. Location and ET personnel nested in location was included as a random effect and percentage pregnancy rate was the dependent variable. Furthermore, to evaluate effects of follicle diameter, CL volume and progesterone concentration on percentage pregnancy rates in the subset of cows, the PROC LOGISTIC program of SAS was used.

3. Results

Mean (± SEM) age, BCS, dominant follicle diameter and ET difficulty score did not differ between cows of the CS-SEET and CS-DEET ($P > 0.1$). Mean CL volume was ~ 10.3 % smaller for cows of the CS-SEET compared to CS-DEET ($P < 0.05$; Table 1). Percentages of cows with a temperament score of 1 (rapid chute exit) at the three locations were 20.3 (41/202), 41.2 (96/233) and 34.6 (90/260) ($P < 0.001$). Developmental stage classifications for transferred embryos were morula [Stage 4; 30.4 % (211/695)], early blastocyst [Stage 5; 35.4 % (246/695)] and blastocyst [Stage 6; 34.2 % (238/695)]. Regarding embryo quality, 52.1 % (362/695) were classified excellent/good (Grade 1) and the remaining 47.9 % (333/695) were fair (Grade 2).

3.1. Association among dominant follicle diameter, corpus luteum volume and progesterone concentration

There were positive correlations between dominant follicle diameter and CL volume (r_s = 0.827; $P < 0.001$; Fig. 2), and CL volume and progesterone concentrations (r_s = 0.680; $P < 0.001$; Fig. 3). Progesterone concentrations and CL volume differed between cows in CS-SEET and CS-DEET groups, 5.23 ± 1.6 and 8.41 ± 1.0 ng/mL and 12.5 ± 0.22 and 16.8 ± 0.3 cm^3, respectively ($P < 0.05$). Dominant follicle diameter, CL volume and progesterone concentrations differed between pregnant and non-pregnant cows, 16.9 ± 0.7 and 14.2 ± 0.9 mm, 16.2 ± 1.1 and 13.3 ± 0.8 cm^3 and 8.67 ± 0.8 and 6.59 ± 0.7, respectively ($P < 0.05$). The logistic regression equation was Pregnancy = 3.698 + 0.223 dominant follicle diameter + 0.293 CL volume + 0.358 serum progesterone concentrations ($P < 0.05$); area under curve was 0.769 ($P < 0.001$).

Table 1
Mean (± SEM) characteristics of embryo recipient cows, based on treatment.

End point	Treatment*	
	CS-SEET group§	CS-DEET‡
n	354	341
Age (y)	3.72 ± 0.09[a]	3.61 ± 0.11[a]
Body condition score[1]	6.46 ± 0.06[a]	6.67 ± 0.09[a]
Dominant follicle diameter (mm)	17.2 ± 2.1[a]	16.3 ± 2.8[a]
CL volume (cm^3)	12.5 ± 0.22[a]	16.8 ± 0.3[b]
ET Difficulty score[2]	1.74 ± 0.04[a]	1.78 ± 0.06[a]
Progesterone conc. (ng/mL)	5.23 ± 1.6[a]	8.41 ± 1.0[b]

[a,b]Variables without a common superscript differed between treatment groups ($P < 0.05$).

* Experimental design is described in Fig. 1.

§ CS-SEET group, CIDR + Select-synch Split-time estrus ET; Cows in estrus (Day 0) at 64 and 84 h after PGF2α administration received a frozen-thawed embryo 7 d after estrus (64 h + 7 d and 84 h + 7 d, respectively).

‡ CS-DEET group, CIDR + Select-Synch daily estrus ET; Cows observed for estrus daily and in cows that were in estrus (Day 0) there was a transfer of a frozen-thawed embryo 7 d after estrus.

[1] Body condition score, 1–9 (1, emaciated; 9, obese).

[2] ET Difficulty score (1, easy embryo transfer; 3, difficult).

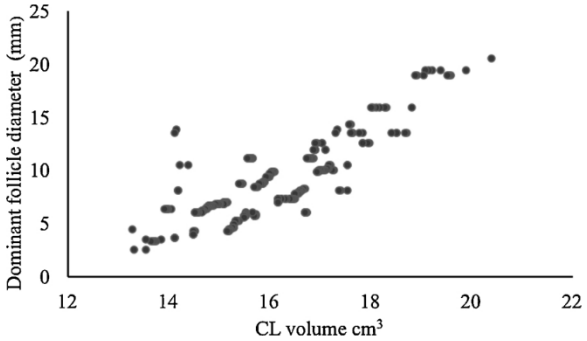

Fig. 2. Association of dominant follicle diameter (48 h after PGF2α administration*) and CL volume at embryo transfer in recipient beef cows. Spearman correlation coefficient, $r_s = 0.827$; $P < 0.001$. *Refer Fig. 1 for treatment protocol.

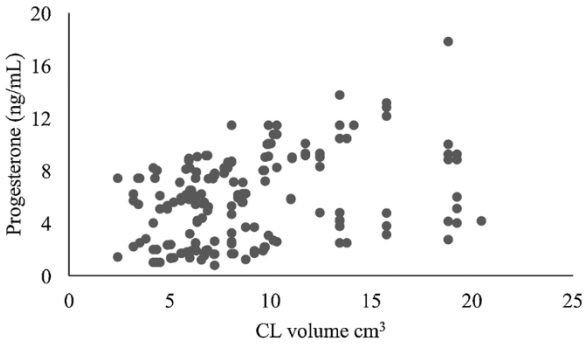

Fig. 3. Association of CL volume and circulating progesterone concentrations at embryo transfer in recipient beef cows. Spearman correlation coefficient, $r_s = 0.680$; $P < 0.001$.

3.2. Reproductive performance after embryo transfer

Treatment for synchronization of estrus, temperament, ET difficulty score, age of the dam, BCS of dam, embryo quality and developmental stage, estrous synchronization treatment by temperament score and treatment by embryo quality grade, synchronization treatment by embryo stage, treatment for synchronization of estrus by ET difficulty score; embryo quality by ET difficulty score and embryo stage by ET difficulty score did not affect P/ET percentages ($P > 0.05$; Table 2).

The proportion of cows in estrus by 64 h after PGF2α administration was not different between the cows of the CS-SEET (68.9 %) and CS-DEET (71.8 %) groups ($P > 0.1$; Table 3). Similarly, proportion of cows in estrus by 84 h after PGF2α administration was not different between CS-SEET (81.9 %) and CS-DEET (79.8 %) groups ($P > 0.1$; Table 2). The cumulative estrous response between

Table 2
Effects of various factors on embryo transfer (ET) percentage pregnancy rate following daily or split-time estrous detection subsequent to imposing a progesterone-based estrous synchronization treatment regimen* in beef cows (n = 1145).

Effect	DF†	F Value	$P > F$
Estrous synchronization treatment (CS-SEET§ and CS-DEET‡)	2	0.91	0.322
Temperament score[1]	1	0.82	0.516
ET difficulty score[2]	2	0.74	0.592
Age of dam[3]	4	0.76	0.541
BCS of dam[4]	2	0.87	0.490
Embryo quality grade[5]	1	1.46	0.191
Embryo developmental stage at time of transfer[6]	2	1.09	0.301
Treatment by temperament	2	2.47	0.091
Treatment by embryo quality grade	2	2.14	0.118
Treatment by embryo stage	2	0.85	0.523
Treatment by ET difficulty score	4	1.28	0.295
Embryo quality by ET difficulty score	2	1.18	0.312
Embryo stage by ET difficulty score	4	1.36	0.278

Covariance parameter estimates: Location, 0.002276 ± 0.000982; ET personnel (Location), 0.003134 ± 0.000788; Residual 0.1611 ± 0.01973; Fit statistics - BIC = 726.52; -2 Res log likelihood = 718.13.

* Experimental design described in Fig. 1.

§ CS-SEET group, CIDR + Select-synch Split-time estrus ET; There was a transfer of a frozen-thawed embryo 7 d after estrus (64 h + 7 d and 84 h + 7 d, respectively into cows in estrus (Day 0) at 64 and 84 h after PGF2α administration).

‡ CS-DEET group, CIDR + Select-Synch daily estrus ET; There was transfer of a frozen-thawed embryo into recipient cows 7 d after detection of estrus into cows observed for estrus daily and cows in estrus (Day 0).

[1] Temperament score - 0, calm, 1, excitable.

[2] ET difficulty score - 1–3, easy to difficult.

[3] Age of dam - 3, 4–6 and > 6 y.

[4] Body condition score - 5, 6, and 7; (BCS, 1–9; 1, emaciated; 9, obese).

[5] Embryo quality, 1, Excellent/good; 2, Fair; (Grade 1–4; 1, Excellent/good; 2, fair; 3, poor; 4, unfertilized/dead/degenerate).

[6] Embryo stage, 4, Morula; 5, Early blastocyst and 6, Blastocyst; (Stage 1–9; 1, 1-cell embryo and 9, expanding hatched blastocyst).

† Degrees of freedom.

Table 3
Estrous expression rate, conception and percentage pregnancy/ET in beef recipient cows following daily or split-time estrous detection after imposing a progesterone-based estrous synchronization treatment regimen* in Angus cross beef cows.

End point	CS-SEET group§	95 % confidence interval	CS-DEET ET‡	95 % confidence interval
N	354		341	
Estrus (%)†				
Up to 64 h	68.9; (244/354)	(63.4, 74.4)	71.8 (245/341)	(66.9, 76.7)
Up to 84 h	81.9 (290/354)	(75.2, 88.6)	79.8 (272/341)	(72.0, 87.6)
Total (until 96 h)	–	–	83.3 (284/341)	(74.5, 92.1)
Non-responders	18.1 ± 1.6 (64/354)	(16.5, 19.7)	16.7 ± 1.6 (57/341)	(15.1, 18.3)
Conception/ET (%)				
64 h	61.5 (150/244)	(53.6, 69.4)	56.7 (139/245)	(50.5, 62.9)
84 h	52.2 (24/46)	(48.5, 55.9)	55.9 (152/272)	(49.8, 62.0)
Total	60.0 (174/290) – 84 h	(54.6, 65.4)	56.3 (160/284) – 96 h	(50.7, 61.9)
Pregnancy/ET (%)	49.2 (174/354)	(44.3, 54.1)	46.9 (160/341)	(42.7, 51.1)

* Refer Fig. 1 for treatment regimen.

§ CS-SEET group, CIDR + Select-synch Split-time estrus ET; There was transfer of a frozen-thawed embryo into recipient animals 7 d after estrus (64 h + 7 d and 84 h + 7 d, respectively into cows in estrus (Day 0) at 64 and 84 h after PGF2α administration.

‡ CS-DEET group, CIDR + Select-Synch daily estrus ET; There was transfer of a frozen-thawed embryo into recipient cows 7 d after detection of estrus (Day 0) into cows observed for estrus daily and cows in estrus.

† A cow was designated in estrus if observed to stand for mounting by other herd mates or if cow had an activated (> 90 % of grey patch was red colored), lost (with mount marks) or partially-activated (50%–90% of grey patch was red colored) estrous detection aid.

cows of the CS-SEET (by 84 h) and CS-DEET (by 96 h) groups did not differ ($P > 0.1$), being 81.9 % (290/354) and 83.3 % (284/341), respectively (Table 3).

The percentage P/ET for CS-SEET group [49.2 % (174/354)] and CS-DEET group [46.9 (160/341)] did not differ ($P > 0.1$). The percentage conception/ET for cows in the CS-SEET and CS-DEET were 60.0 % (174/290) and 56.3 % (160/284), respectively ($P > 0.1$). The percentage of P/ET for cows with a temperament score of 1 (rapid chute exit) [44.9 % (102/227)] and 0 (slow chute exit) [49.6 % (232/468)] were not different ($P > 0.1$). The percentage P/ET was not affected by: difficulty scores in conducting the ET [48.5 % (79/163), 48.2 % (132/274) and 47.7 % (123/258) for Scores 1, 2 and 3; $P < 0.01$], cow age [49.6 % (116/234), 47.3 % (148/313) and 47.3 % (70/148), for ages 3, 4–6 and > 6 y, respectively, $P > 0.1$], BCS [46.3 % (67/143), 49.4 % (165/334) and

46.8 % (102/218) for BCS 5, 6 and ≥7 respectively, $P > 0.1$]; embryo developmental stages [49.8 % (105/211), 47.6 % (117/246) and 47.1 % (112/238) for morula, early blastocyst and blastocyst; $P > 0.1$]; or embryo quality [48.6 % (176/362) and 47.4 % (158/333), for Grades 1 (excellent/good) and 2 (fair); $P > 0.1$]. The interactions between values for ET difficulty score by treatment, temperament score by treatment, embryo quality by treatment, embryo stage by treatment, embryo quality by ET difficulty score and embryo stage by ET difficulty score were not significant ($P > 0.05$). When CL volume was included in the model, ET difficulty score did not affect pregnancy rate ($P > 0.1$). The percentage P/ET among locations was not different ($P > 0.1$), being 47.0 % (95/202), 48.1 % (112/233) and 48.8 % (127/260) for Locations 1, 2 and 3, respectively.

4. Discussion

Treatment regimens that are effective for synchronization of time of estrus and ovulation have been beneficial for producers to increase the use of FTAI in beef heifers and cows, rather than performing AI after estrous detection. Consequently, AI is performed at a predetermined time following PGF2α administration. Acceptable pregnancy rates can result with use of several types of FTAI treatment regimens; however, a proportion of females on which estrous synchronization treatment regimens are imposed do not express estrus prior to FTAI. To account for these non-estrous animals, all cows and heifers are usually administered GnRH at the time of FTAI to ensure that ovulation is induced. The endocrine milieu associated with estrous expression, however, are known to positively affect fertility, and pregnancy rates are on average 27 % less among females that fail to express estrus prior to FTAI (Perry et al., 2007). Consequently, a split-time approach was developed to manage timed insemination of cows and heifers that have not expressed estrus prior to fixed-time AI; it involves delaying insemination of non-estrous females until 20–24 h after the scheduled time (Thomas et al., 2014a, b; Bishop et al., 2016; Richardson et al., 2016). With use of the split-time AI approach, there was an improvement in pregnancy rates when there was FTAI among beef replacement heifers following a 14-d CIDR-PG treatment regimen (Thomas et al., 2014b) and among mature beef cows following a 7-d CO-Synch + CIDR treatment regimen (Thomas et al., 2014a; Bishop et al., 2016). An additional advantage is that insemination can be performed without GnRH administration for cows and heifers that have expressed estrus prior to the standard time of FTAI, as well as for cows and heifers that expressed estrus before the second AI period when there is use of split-time AI approach. There has been a series of experiments conducted to evaluate different estrous synchronization treatment regimens that are used for FTAI to synchronize time of estrus among recipient cows subjected to ET, with or without estrous detection. In the current study, there was imposing of a progesterone-based treatment regimen with split-time estrous detection being conducted and comparison to daily estrous detection of embryo recipients. The percentage P/ET between cows in CS-SEET [49.2 % (174/354)] and CS-DEET [46.9 % (160/341)] groups did not differ ($P > 0.1$). Furthermore, total estrous response between cows of the CS-SEET and CS-DEET groups did not differ ($P > 0.1$), being 81.9 % (290/354) and 83.3 % (284/341), respectively.

In several studies, there was use of various approaches for FTET with inconsistent results. The percentage P/ET as a result of embryo transfers into recipients 7 day after a detected estrus when there was use of a PGF2α treatment regimen for estrous synchrony, were lower [31.3 % (97/130); $P < 0.05$] compared with that in the Ovsynch treatment group without estrous detection [39.1 % (104/266)] (Hinshaw, 1999; Baruselli et al., 2000; Zanenga et al., 2000; Bó et al., 2002). Pregnancy rates were not different ($P > 0.1$) among recipient cows as a result of ET 6–8 day after an observed estrus following administration of GnRH on Day 0 and PGF2α on Day 7 [39.6 % (67/169)] as compared with when there was ET 7 d after the second GnRH injection when there was imposing of the Ovsynch treatment regimen without [44.0 % (72/165)] or with [49.7 % (82/165)] a progestogen implant (Ovsynch + P) without estrous detection (Beal, 1999). Percentage pregnancy rates, as a result of ET, were greater in recipient cows treated with a combination of a progesterone device, injectable estradiol, injectable progesterone, eCG, and estradiol benzoate when there was transfer on Day 17 without estrus detection (with a palpable CL at ET) as compared with the percentage in the control group (Bó et al., 2002; Tribulo et al., 2002). When there was administration of eCG, there was an increased CL diameter (18.5 compared with 17.7 mm) in this previous study. In the present study, progesterone concentrations and CL volume differed between cows in CS-SEET and CS-DEET groups ($P < 0.05$).

Pregnancy rates were compared when there was evaluation of two split-time AI programs in suckled beef cows (Stevenson et al., 2017). Cows that expressed estrus were inseminated at either 55 or 65 h, whereas cows that were not detected in estrus in both treatment groups were administered GnRH at 55 or 65 h and were inseminated 20 h later (75 or 85 h, respectively). Percentages of estrous response in this study were similar to when there was AI using the split-time approach at the same timepoints of 65 and 85 h, respectively.

Bó et al. (2002) reported transfer of embryos to estrous-synchronized embryo recipient cows was most effective when embryos were transferred 6–8 d after detected estrus. In the current study, percentage conception/ET for cows in estrus by 64 h after PGF2α treatment was not different between CS-SEET (61.5 %, with one time estrous detection and one time ET) and CS-DEET (56.7 % with five times estrous detection and three times ET) groups ($P > 0.1$). Similarly, percentage conception/ET for cows in estrus by 84 h was not different when there was imposing of the CS-SEET (52.2 %, one time estrous detection and one time ET) and CS-DEET (55.9 %, with two times estrus detection and one time ET) treatment regimens ($P > 0.1$). Considering this 6–8 day time-period, transfer of embryos, following imposing of the CO-Synch + CIDR treatment regimen, on Day 10 after PGF2α treatment (72 h after PGF2α + 7 d), would have been more effective in cows that expressed estrus from 48 to 96 h after PGF2α administration. Similarly, with the CS-DEET treatment regimen, this time-period for ET would be effective for cows expressing estrus up to 120 h (96 + 24) and in cows of the CS-SEET group it would be effective for cows expressing estrus up to 108 h after PGF2α administration. When there was this additional 12 h period before performing ET in cows of the CS-SEET group, there was not an increase in percentage P/ET probably because there were very few females expressing estrus during this time period.

Considering this 6–8 day time-period when imposing the split-time ET treatment regimen, ET at 64 h after administration of PGF2α would be an optimal time for ET in cows expressing estrus from 40 to 88 h and ET at 84 h would be an optimal time for ET in cows expressing estrus from 60 to 108 h after administration of PGF2α. Even though the conception rate results in the present study did not indicate so, it is possible that in recipient cows expressing estrus within 40 h after PGF2α administration there was an asynchrony in uterine function and time of embryo development at the time of ET. When imposing the SEET treatment regimen, it would be interesting to investigate adjusting the time of ET to 36 and 84 h after administration of PGF2α. With the SEET treatment regimen, the first ET at 36 h after PGF2α administration would be an optimal time for the first ET in cows expressing estrus from 12 to 60 h and the optimal time for the second ET would be at 84 h for cows expressing estrus from 60 to 108 h after PGF2α administration.

Several factors, including diameter or maturity of the follicle from which ovulation occurs (Lamb et al., 2001; Vasconcelos et al., 2001; Kastelic et al., 2004; Perry et al., 2005), and content of endocrine milieu (Pelican et al., 2010; Wen et al., 2010; Var et al., 2011), as well as animal age, body weight and adiposity content (Scheffer et al., 1999; Souter et al., 2011) and CL function (Vasconcelos et al., 2001; Perry et al., 2005; Busch et al., 2008) all have effects on establishment and maintenance of pregnancy. In the present study, there were positive correlations between follicle diameter and CL volume and between CL volume and progesterone concentrations. Furthermore, dominant follicle diameter, CL volume and progesterone concentrations were greater in pregnant compared with nonpregnant cows. Optimal progesterone concentrations post ovulation are important for establishment and maintenance of pregnancy (Mann and Lamming, 1999). The capacity of a CL to produce progesterone depends on size and number of granulosa cells in the ovulatory follicle from which the CL developed (McNatty and Sawers, 1975). Plasma progesterone concentrations were correlated positively with total CL or ovulatory follicle volume, indicating that CL size and function were affected by diameter of the follicle of origin (Echternkamp et al., 2009). Follicles ≤11 mm in diameter from which ovulation was induced with GnRH administrations in beef cows resulted in relatively lesser pregnancy rates and greater late embryonic mortality (Perry et al., 2007). This lesser fertility was associated with lesser blood estradiol concentrations on the day of insemination and decreased blood progesterone concentrations. Beef cows in which there were ovulations from a follicle > 19 mm had a larger CL than those in which there were ovulations from a follicle < 15 mm in diameter. Cows with a follicle from which there ovulations that was13 to 15 mm had a greater pregnancy rate than when there ovulations from follicles of other sizes (Pfeifer et al., 2012). When there are ovulations from relatively larger follicles, there is development of a larger CL and consequently there is greater progesterone production. When, however, there are ovulations from follicles with an optimal diameter (13–15 mm), there may be a greater pregnancy rate in cows as a result of FTAI when estrous synchrony treatment regimens result in sub-luteal concentrations of progesterone (Pfeifer et al., 2012).

In the present study, the CL volume and progesterone concentrations were less for cows in the CS-SEET group compared with cows of the CS-DEET group. With the split-time ET approach, the uterine functions at the time when ET occurred would be less precisely synchronized with stage of embryo development at the time of ET. In some cows, the ET might have occurred slightly earlier and for some later when there was the split-time approach for ET, therefore, there may have been a different mean CL volume in these cows with there being a smaller CL volume in cows in which ET occurred relatively earlier and a larger CL volume in cows in which there was a later ET relative to stage of the estrous cycle. In addition, variation in time from GnRH treatment to follicle wave emergence (range, 1.5–5 days; Martínez et al., 2003) results in variations in dominant follicle size, CL volume and progesterone concentrations.

Ovarian follicular contents of donor cows affect fertilization rate and embryonic survival, however, maintenance of pregnancy in recipient cows is dependent on follicle size from which there is ovulation and the resulting hormone production from the follicle and resulting CL (Atkins et al., 2013). In this previous study, the probability that an embryo collected on Day 7 was viable was enhanced if there was a larger follicle from which ovulation occurred, greater serum progesterone concentration on Day −2, and a follicle that grew more slowly from Days −2 to 0. Serum progesterone concentrations in recipient cows at the time of ET (Day 7) was greater ($P < 0.10$) when there were greater concentrations of estradiol on Day 0 and progesterone on Day −2 and when there was a greater follicle diameter on Day 0. When all results from this previous study were considered, these effects explained 21 % of the variation in serum progesterone on Day 7. Embryonic development was obviously enhanced by embryo viability and to a lesser extent by growth rate of the follicle from which there was ovulation and serum progesterone concentration on Day 7. The conclusion from the present study was that maintenance of pregnancy after Day 7 was dependent on diameter of the follicle from which there was ovulation and subsequent estradiol as well as progesterone production in recipient cows. In the present study, probability of pregnancy, when there was transfer of an embryo on Day 7, was greater when there was a larger follicle from which ovulation occurred, greater CL volume and progesterone concentration in recipient cows. Estradiol concentrations were not determined in the present study. An important consideration in the present study, is that there was selection of recipient cows based on a CL size of ≥ 1.5 cm; thus, mean dominant follicle diameter in this study was presumably greater than in previous studies.

5. Conclusion

In conclusion, percentage P/ET following twice daily or split-time estrous detection when there was a progesterone-based ET treatment regimen in recipient beef cows did not differ. The split-time ET program was an effective alternate for daily estrous detection when there was imposing of a progesterone-based treatment regimen to conduct ET. Dominant follicle diameter, CL volume and progesterone concentrations were greater in pregnant compared with nonpregnant cows.

Declaration of Competing Interest

The authors declare no conflict of interest

CRediT authorship contribution statement

Ramanathan Kasimanickam: Conceptualization, Resources, Methodology, Investigation, Formal analysis, Software, Supervision, Writing - review & editing, Funding acquisition. **Vanmathy Kasimanickam:** Conceptualization, Methodology, Resources, Investigation, Formal analysis, Software, Writing - review & editing. **John Kastelic:** Methodology, Writing - review & editing. **Shelbey Nagle:** Methodology, Investigation, Formal analysis. **Aliasgar Kapi:** Methodology, Investigation, Formal analysis.

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