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Estrus response patches, timing for artificial insemination, and GnRH protocol in Split Timed AI beef cattle

Resposta do adesivo marcador do estro, inseminação artificial em tempo fixo e protocolo de GnRH em bovinos de corte na Inseminação artificial dividida

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Abstract
An estrous-detection patch was used to determine the optimum timing for STAI and the necessity of GnRH at STAI on a 7-day CO-Synch+CIDR protocol. Crossbred beef cows (n=216) were stratified into the following treatment groups: CTRL=TAI (n=67) at 72 h post CIDR removal, or TRT=STAI (n=149) at 72 or 84 h post CIDR removal. All females received GnRH (100 mcg) plus a CIDR on d0, $PGF_{2\alpha}$, CIDR removal, and an Estrotect estrous-detector patch on d7. At 72 h post-CIDR removal, a patch score was assigned (PS1<50% removed; PS2>50% removed) to all females. Cows in the CTRL group were administered a second GnRH (100 mcg) at 72 h TAI. Cows in the TRT group with PS2 were not administered GnRH. At 84 h, the remaining TRT group cows were given a second PS; cows with PS1 received a GnRH (100 mcg), and cows with PS2 were not administered. Results: The TAI pregnancy rates were similar (P=0.81) between the CTRL (45.6%) and TRT (44.8%) groups. Pregnancy rates tended to be higher (P=0.07) for cows with PS2 (50.3%) than for those with PS1 (29.4%). However, by extending TAI to 84 h in unresponsive cows, 82.0% of TRT cows did not receive a second injection of GnRH at TAI. It was concluded that the estrous detector patches reduced the percentage of cows that required GnRH at TAI without compromising pregnancy rates. The estrous detector patches significantly reduced the number of cows that received a second GnRH injection at TAI.
Keywords: Split-time artificial insemination; STAI; GnRH; Beef cattle; heat detector.

Resumo
Um adesivo de detecção de estro foi usado como ferramenta para determinar o momento ideal para a inseminação artificial em tempo dividido (STAI) e a necessidade de injeção de hormônio liberador de gonadotrofinas (GnRH) no 7º dia do protocolo CO-Sinc + CIDR sem comprometer as taxas de prenhez. As vacas eram cruzadas, multíparas e lactantes (Angus x Charolês, n=216) e foram estratificadas por idades (5,9 2.5 anos), BW (581 67kg), BCS (5,3 0,8; 1-9), intervalo entre partos (78,5 15,5 dias). O grupo de tratamento CTRL = IAT (n=67) foi inseminado após 72 h após a remoção do CIDR; já no grupo tratamento TRT= IATP (n=149) as vacas foram inseminadas 72 ou 84 h após a remoção do CIDR. Todas as fêmeas receberam GnRH (100 mcg I.M.), mais um CIDR (1,38 g de progesterone) no D0, no D7 foi realizado a retirada do CIDR, aplicação de $PGF_{2\alpha}$ (25 mcg I.M.) e colocação do adesivo detector de cio. Após 72 h da remoção do CIDR, uma pontuação foi atribuída ao adesivo (OS1<50% removido; OS2> 50% removido) de todas as fêmeas. As vacas do grupo CTRL receberam a segunda injeção de GnRH (100 mcg I.M.) às 72 h na IAT. Vacas do grupo TRT com OS2 não receberam GnRH. Às 84 h as vacas restantes do grupo TRT receberam um segundo OS, aquelas com OS1 receberam GnRH (100 mcg I.M.) e as vacas com OS2 não receberam GnRH. Amostras de sangue para concentração de progesterona foram coletadas nos D-11 e D-0 para determinar o percentual de vacas ciclando. Os dados foram analisados utilizando-se o Proc Genmod, tendo o tratamento e o técnico de IA como efeitos fixos, o touro como efeito aleatório e o BW, BCS, idade e IBP como covariáveis. Resultados: as taxas de gravidez da IAT foram semelhantes (P= 0,81) entre os grupos CTRL (45,6%) e TRT (44,8%). As taxas de prenhez tenderam a ser maiores (P=0,07) para vacas com OS2 (50,3%) do que para aquelas com OS1 (29,4%). No entanto, ao estender a IAT para 84 horas em vacas não responsivas, 82,0% das vacas TRT não receberam uma segunda aplicação de GnRH na IAT. Concluiu-se que os adesivos detectores de estro reduziram a porcentagem de vacas que necessitaram de GnRH na IAT sem comprometer as taxas de prenhez. Os adesivos de detecção de estro reduziram significativamente o número de vacas que receberam uma segunda injeção de GnRH na IAT.
Palavras-chave: inseminação em tempo dividido; STAI; GnRH; vaca de corte; detecção de cio.

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1. Introduction

Over the past decades, several assisted reproductive technologies (ART) have been developed that directly improve the reproductive performance of several domestic species. Artificial insemination (AI), one of the pioneering biotechnologies in the area of animal reproduction, has also contributed to the genetic improvement of farm animals [(1)], among other contributions. By 2016, technologies such as fixed-time AI (FTAI), split-time AI (STAI), semen and embryo cryopreservation, embryo transfer, and *in vitro* fertilization were widely available for producers. These technologies help maximize the production potential of farm animals while reducing the environmental impact [(2,3,4)] and shortening the interval between progenies [(5)].

Although AI has emerged as one of the most important reproductive biotechnologies, it has some limitations, which are mainly related to the fact that beef cattle producers have several herds over extensive ranges, where detecting estrus and managing animals in estrus for insemination is challenging. In recent decades, conventional AI has been significantly improved by inseminating animals at a predetermined time without the observation of estrus [(6, 7)], giving rise to FTAI.

Since then, many efforts have been made to improve the pregnancy rate through the application of hormonal protocols, particularly in animal protein farms. However, challenges arise in accurately observing estrus and ovulation, leading to difficulties in identifying the optimal timing for AI. As a consequence, AI pregnancy rates have been adversely affected [(8, 9)].

Due to the limitations in observing estrus in timed AI (TAI) in cattle, researchers have focused on developing techniques and products to induce AI in the period closest to ovulation to improve the reproductive indices. Among several measures applied, devices (patches) were developed for the upper part of the pelvis that help to predict the estrus status when an animal is mounted [(10)].

In addition to devices designed to detect estrus (marking of the animal's rump) and to provide AI only in animals that would have come into estrus before or on the scheduled day of the FTAI, FTAI protocols were developed in association with gonadotropin-releasing hormone (GnRH) administration. GnRH is intended to induce ovulation in animals that do not show estrus until the time of FTAI [(11,12)]. With the application of these protocols, it is possible to increase reproductive efficiency. In addition to these procedures, cows that had not come into estrus were inseminated hours after the expected FTAI day to synchronize the FTAI with ovulation [(13)] in a process called split-time AI (STAI) [(12, 14, 15)]. In STAI protocols, an increase in FTAI pregnancy rate has been observed [(12, 16)].

We hypothesized that animals in standing estrus with an activated patch do not require a second GnRH injection in STAI synchronized with a 7-day CO-Synch + controlled intravaginal drug release (CIDR) protocol for beef cattle. This study used estrous detection patches as simple and cost-effective reproductive management tools to identify animals which have been or are in standing estrus in STAI.

2. Materials and methods

The Institutional Animal Care and Use Committee at the Louisiana State University Agricultural Center approved the research protocols for all animal procedures (approval no. # A2016-07). This study was conducted at the LSU Hill Farm Research Station (Homer, Louisiana) (32.757330, -92.933410). The use of animals in this experiment was in accordance with proper humane animal handling procedures approved by the National Cattlemen's Beef Association and Louisiana Cattlemen's Beef Association.

2.1 Experimental design

Estrus was synchronized in multiparous Angus-crossbred (Angus and Charolais) beef cows (MEAN ± STD DEV) at the Hill Farm Research Station (n = 216, BW = 581 ± 67 kg, BCS = 5.3 ± 0.8, postpartum interval = 78.5 ± 15.5 days, age = 5.9 ± 2.5 years). Animals were stratified into two treatment groups by BCS [15], and BW was collected on days −11 and 0. Technicians who performed AI (two technicians) and AI sires (two sires) were pre-assigned to treatments based on BCS and BW to ensure that the treatments were not biased. Treatments for cows included a 7-day CO-Synch + CIDR (Figure 1) estrous synchronization protocol (CTRL; n = 67) with 72 h TAI or a 7-day CO-Synch + CIDR (Figure 2) estrous synchronization protocol with 72 or 84 h STAI (TRT; n =149). The study was performed in the breeding season of 2016.

All animals were managed on cool-season pastures (*Secale cereale* and *Lolium perenne*) through May and warm-season pastures (*Cynodon* dactylon) through October, and had *ad libitum* access to water, salt, and loose trace minerals throughout the experiment (Champion's Coice, Cargill – Mineapollis, MN, USA). All cows received the 7-day CO-Synch + CIDR (Eazi-Breed CIDR insert, 1.38 g progesterone; Zoetis, Madison, NJ, USA) protocol and included a CIDR insert + 100 µg (I.M.) GnRH (Cystorelin, Merial, Athens, GA, USA) injection was administered on d 0, followed by CIDR removal and 25 mg (I.M.) $PGF_{2\alpha}$ (Lutalyse, Zoetis) administered on d 7. An estrous detection aid (Estrotect, Rockway Inc., Spring Valley, WI, USA) was applied at CIDR removal/PG injection on d 7 for all cows in both treatment groups. All animals were assigned a patch score

(PS) of 1 (< 50% of the coating rubbed off the patch) or 2 (≥ 50% of the coating rubbed off the patch) 72 h post CIDR removal by the AI technician. Animals in the CTRL group were inseminated and administered GnRH at that time. The remaining animals in the TRT group were sorted and penned separately (without calves) for 12 h. At 84 h post CIDR removal, the remaining TRT animals were assigned a new PS and inseminated: those with a PS of 1 received a GnRH injection and those with a PS of 2 did not receive a second injection of GnRH. All cows were exposed to fertile bulls for 14 days after TAI. Diagnosis of TAI and final pregnancy was performed on d 43 and d 120 after TAI via transrectal ultrasonography (Aloka SSD-500v Ultrasound®, 5-Mhz, Corometrics, Wallingford, CT, USA).

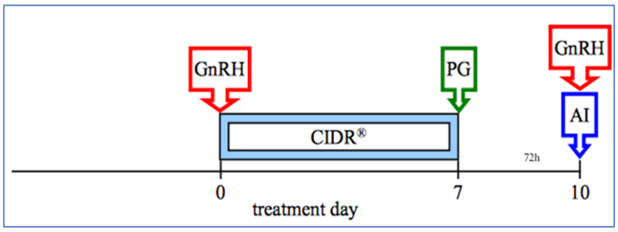

Figure 1. 7-day CO-Synch + CIDR estrous synchronization protocol in crossbred cows, TRT group (n = 149).

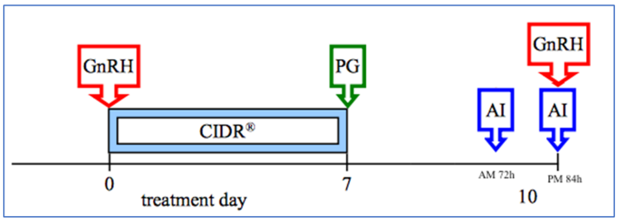

Figure 2. Diagram of the protocol administered to the group involving 7-day CO-Synch + CIDR estrous detection with estrous detection patches and split-time AI (TRT) in crossbred beef cows. Legend: CIDR (Eazi-Breed CIDR insert, 1.38 g progesterone; Zoetis, Madison, NJ, USA); GnRH (Cystorelin, Merial, Athens, GA, USA) 100 µg (I.M.); $PGF_{2\alpha}$ (Lutalyse, Zoetis) 25 µg (I.M.); and Estrous Detection Patch (Estrotect, Rockway Inc., Spring Valley, WI, USA).

Blood was collected via coccygeal venipuncture using an 18-gauge 2.54-cm collection needle (Vacuette, Greiner Bio-One GmbH, Kremsmünster, Austria) into 10-mL BD Vacutainer® Glass Serum tubes (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) for analysis of plasma progesterone. Blood samples were collected and placed on ice until they were centrifuged for 15 min at 4,235 *g* at 0 °C. plasma was pipetted into plastic vials before freezing until samples were analyzed for plasma progesterone levels via radioimmunoassay [17].

2.2 Statistical analysis

The treatment effects on the proportion of cows pregnant to TAI or final pregnancy rates were tested using the Proc GENMOD procedure (SAS Institute, Cary, NC) for binomial data. Fixed effects included treatment, AI technicians (two technicians), PS (1 = < 50% patch film removed or 2 = ≥ 50% patch films removed), PPI group (1 = ≤ 70 d postpartum or 2 = > 70 d postpartum), and cyclicity (cycling if plasma progesterone concentrations were ≥ 1 ng/ml) and their two-way interactions with treatment. Body weight, BCS, age, and PPI were included as covariates in all models, and backward elimination was used to identify if the variable remained in the final model, using a significance level set at $P < 0.05$. Cyclicity status and percentage of cows with a PS of 2 were included in the model as response variables, with fixed effects of treatment. The timed AI pregnancy rate was calculated as the proportion of females that were pregnant to AI 72 or 84 h following $PGF_{2\alpha}$ administration. Overall pregnancy was calculated as the proportion of females that were pregnant to TAI or natural services at the end of the breeding season. The significance of the main effects was determined using the Chi-squared test at $P < 0.05$, and tendencies were assessed at $0.10 > P > 0.05$.

3. Results and discussion

The current study evaluated the use of an estrous detection aid to determine if administration of GnRH during TAI in beef cows is required in a 7-day CO-Synch + CIDR protocol with STAI. There was no treatment by technician ($P = 0.78$), PPI group ($P = 0.15$), PS ($P = 0.28$), or cyclicity interaction ($P = 0.26$). Thus, interactions were removed from all the models. Breeding season (final) pregnancy rates tended to be different ($P = 0.05$) between the CTRL and TRT groups for cows (86.6% [58/67] vs. 76.4% [113/148], respectively; Figure 3). This study did not detect significant differences between pregnancy rates from the two AI technicians (28.2% [24/85] vs. 56.5% [74/131]; p = 0.07).

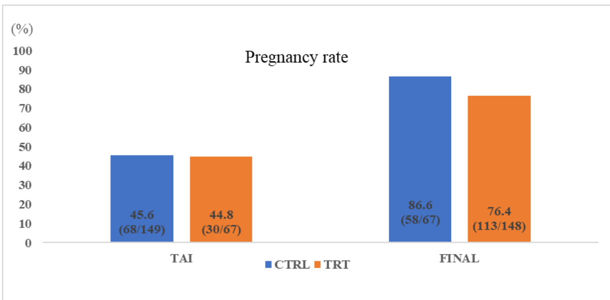

Figure 3. TAI and final pregnancy rates in crossbred beef cows. Treatments included: 7-day CO-Synch + CIDR estrous synchronization protocol (CTRL) with 72 h TAI or 7-day CO-Synch + CIDR estrous synchronization protocol with 72 or 84 h STAI (TRT). Pregnancy rates for TAI are measured by the percentage of animals pregnant at d 43 after TAI (P = 0.81). Final pregnancy rates are measured by the percentage of animals pregnant at d 120 after TAI (P = 0.05).

Pregnancy rates to TAI (Figure 3) were similar (*P* = 0.81) for cows in the TRT group (44.8% [68/149]), where 82.0% [122/149] of the cows did not receive GnRH at TAI because of an activated estrous detection patch, compared to cows in the CTRL group (45.6% [30/67]), where all cows received GnRH at TAI. There was a significant difference (*P* < 0.01) in the percentage of cows with a PS of 2 between the CTRL (64.2% [43/67]) and TRT (82.0% [122/149]) groups (Figure 4). However, pregnancy rates were not affected by this response in either group. Cyclicity did not differ (*P* = 0.14) between the CTRL and TRT groups (83.1% [54/65] and 74.0% [108/146], respectively; Figure 5).

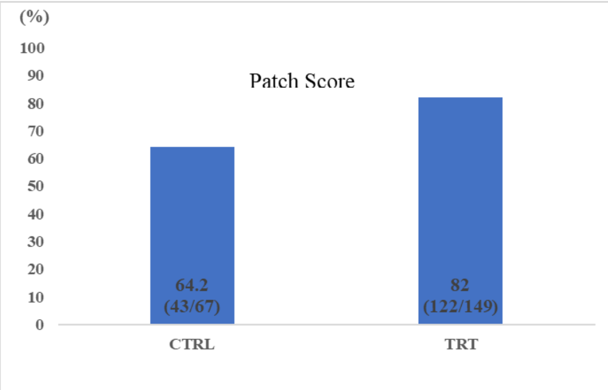

Figure 4. Percentage of cows with a PS of 2 within each treatment group. (P < 0.01). Treatments included: 7-day CO-Synch + CIDR estrous synchronization protocol (CTRL) with 72 h TAI or 7-day CO-Synch + CIDR estrous synchronization protocol with 72 or 84 h STAI (TRT).

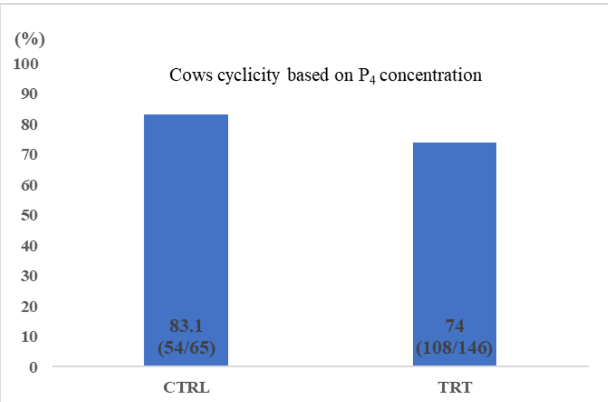

Figure 5. Percentage of cows cycling based on plasma progesterone concentration within each treatment group. Treatments for cows included: 7-day CO-Synch + CIDR estrous synchronization protocol (CTRL) with 72 h TAI or 7-day CO-Synch + CIDR estrous synchronization protocol with 72 or 84 h STAI (TRT). Cows were determined to be cycling if plasma progesterone concentrations were ≥ 1 ng/ml (P = 0.14).

TAI pregnancy rates based on PS tended to be different (*P* = 0.07) for cows assigned a PS of 2 when compared with those assigned a PS of 1 (50.3% [83/165] vs. 29.4% [15/51], respectively). However, final pregnancy rates were similar (*P* = 0.30; 81.8% [135/165] vs. 72.5% [37/51, respectively; Figure 6). TAI pregnancy rates were similar (*P* = 0.68) between anestrous cows (42.9% [21/49]) and cycling cows (46.9% [76/162]). However, final pregnancy rates tended to be higher (*P* = 0.06) in cycling cows than in anestrus cows (84.5% [137/162] vs. 67.3% [33/49], respectively; Figure 7).

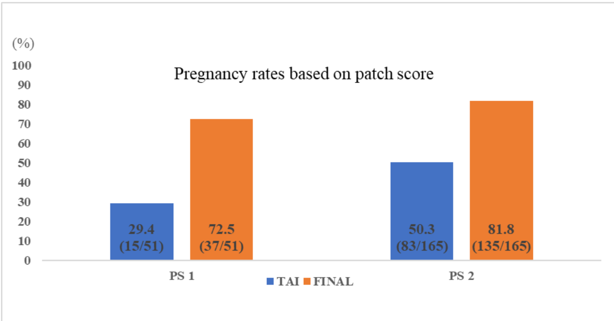

Figure 6. TAI (P = 0.07) and final (P= 0.30) pregnancy rates based on PS in crossbred beef cows. PSs were assigned 1 (< 50% patch film removed) or 2 (≥ 50% patch film removed).

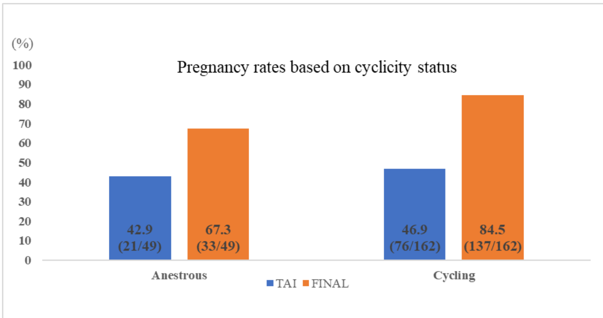

Figure 7. TAI (P = 0.68) and final (P = 0.06) pregnancy rates in cows based on cyclicity status. Cows were determined to be cycling if plasma progesterone concentrations were ≥ 1 ng/ml.

Bishop et al. [14] reported an increase in the estrus response in beef cows when TAI was delayed from 66 to 90 h after $PGF_{2\alpha}$ administration in a 7-day CO-Synch + CIDR protocol. In that study, Estrotect patches were applied at PG administration (day 7) with delayed TAI from 66 to 90 h post-PG administration according to estrus expression, and estrus was defined as having at least 50% of the coating rubbed off the Estrotect patch. GnRH was administered according to the estrous response (animals in estrus did not receive GnRH during TAI). However, there was no difference in pregnancy rates between the two groups when inseminated at 66 h, regardless of GnRH administration (57% and 58%, respectively). There was also no difference between TAI at 66 h with GnRH and TAI at 90 h with GnRH (44% and 49%, respectively). Pereira et al. [18] reported that animals that displayed estrus had a higher probability of becoming pregnant from TAI than those that did not display estrus (38.9% and 25.5%, respectively). It was also reported that the percentage of pregnancy loss in animals that conceived from TAI was lower in animals that displayed estrus than in those that did not display estrus (14.4% and 20.1%, respectively). Stegner et al. [19] reported higher pregnancy rates for cows inseminated at 72 h (64%) vs. those at 80 h (50%) synchronized with the MGA-Select protocol.

In the current study, there was no difference in TAI pregnancy rates between the CTRL and TRT groups that were assigned a PS of 1 or 2 at TAI (PS-1: 33.3 [8/24] vs. 25.99 % [7/27]; and PS-2: 51.2 [22/43] vs. 50 % [61/122], respectively), suggesting that GnRH may not have any beneficial effects on ovulation at TAI in beef cows that exhibit standing estrus at TAI. Prior to CIDR insertion on d 0, the cyclicity status of the herd was similar between the CTRL and TRT groups (83.1 [54/65] vs. 74.0% [108/146), which is even better than the usual 50% of cows cycling at the beginning of every synchronization protocol [20].

Pregnancy rates to TAI tended to remain the same in cows that had a PS of 2 and did not receive GnRH at TAI in the TRT group when compared to the CTRL group, in which all animals received GnRH at TAI (50.0% [61/122] vs. 51.2% 22/43], respectively). This result is consistent with that of existing studies [21, 22], indicating that GnRH is not needed at TAI if the animal is in standing estrus. Estrus detector patch loss was very low in this study (0.46%; 1 animal out of 216 lost its patch), indicating that the adhesive successfully held the patches. The data generated in this study suggest that cyclicity status has a major influence on pregnancy rates compared to GnRH administration at TAI. The CO-Synch protocol [23] is a modification of the Ovsynch protocol [24] to reduce the number of times animals are handled during the synchronization protocol. The addition of a CIDR insert in the CO-Synch protocol has been shown to increase the percentage of females displaying estrus within an 84-h period [25], supporting the objective of our study to inseminate at 72 and 84 h.

Several studies in the past decades have evaluated different estrous synchronization protocols aiming to increase pregnancy rates for TAI or FTAI in beef cows but have not focused on reducing costs. According to the National Animal Health Monitoring System (NAHMS, 2008), cost is still one of the main reasons why producers do not use these reproductive technologies.

The current study focused on evaluating a heat detection aid, the Estrotect Heat Detector patch, as a reproductive management tool to determine whether GnRH is needed at TAI. This study hypothesized that using a heat detector patch to determine the necessity of GnRH during TAI is an effective method to significantly reduce the number of animals that receive a second injection of GnRH during TAI, thus reducing costs without compromising pregnancy rates [14, 20-22, 26, 27]. Another important point in this context would be related to reducing the use of hormones in cattle reproduction protocols, aiming to reduce chemical residues in the environment as well as to obtain meat for a more natural diet [4].

4. Conclusion

It was concluded that 82% (122/149) of the cows in the treated group (STAI) did not receive GnRH at TAI, and only 18% (27/149) of these cows required GnRH at TAI, when using the Estrotect patch and delaying TAI to 84 h in non-responsive cows. The use of the heat detector patch reduced the number of animals that received the second dose of GnRH at TAI.

Declaration of conflict of interests

The authors declare that there is no conflict of interest

Author contributions

Conceptualization: D. Demeterco and R. S. Walker. *Data curation*: D. Demeterco and R. S. Walker. *Formal analysis*: D. Demeterco and C. C. Williams. *Investigation*: D. Demeterco and R. S. Walker. *Methodology*: D. Demeterco, R. S. Walker and A.K. Edwards. *Project administration*: D. Demeterco and R. S. Walker. *Supervision*: D. Demeterco, R. S. Walker and J. E. Anderson. *Visualization*: D. Demeterco. Funding acquisition: R. S. Walker. *Resources*: R. S. Walker. *Writing (original draft):* D. Demeterco and R. S. Walker. *Writing (review & editing):* L. E. Kozicki, V. Mohad Valle and C. C. Williams

References

1. Foote, RH. The history of artificial insemination: Selected notes and notables. Journal of Animal Science. 2002; 80, 1-10. (https://doi.org/10.2527/animalsci2002.80e-suppl_21a)

2. Dick, M, Silva, MA, Dewes, H. Mitigation of environmental impacts of beef cattle production in southern Brazil – Evaluation using farm-based life cycle assessment. Journal of Cleaner Production. 2015; 87, 58–67. (https://doi.org/10.1016/j.jclepro.2014.10.087)

3. Troy, SM, Duthie, CA, Ross, DW, Hyslop, JJ, Roehe, R, Waterhouse, A, Rooke, JA. A comparison of methane emissions from beef cattle measured using methane hoods with those measured using respiration chambers. Animal Feed Science Technology. 2016; 211, 227–240. (https://doi.org/10.1016/j.anifeedsci.2015.12.005)

4. Parlasca, MC, Qaim, M. Meat consumption and sustainability. Annual Review of Resources Economics. 2022; 14:17-41.

5. Burns, BM, Fordyce, G, Holroyd, RG. A review of factors that impact on the capacity of beef cattle females to conceive, maintain a pregnancy and wean a calf—Implications for repro-

ductive efficiency in northern Australia. Animal Reproduction Science. 2010; 122(1), 1–22. (https://doi.org/10.1016/j.anireprosci.2010.04.010)

6. Souza, AB, Segui, MS, Kozicki, LE, Weiss, RR, Bertol, MAF, Oliveira, DAM, Abreu, ACMR. Impact of Equine Chorionic Gonadotropin Associated with Temporary Weaning, Estradiol Benzoate, or Estradiol Cypionate on Timed Artificial Insemination in Primiparous Bos Indicus Cows. Brazilian Archive of Biology and Technology. 2016; 59. (https://doi.org/10.1590/1678-4324-2016150389)

7. Nebel, RL, Dransfield, MG, Jobst, SM, Bame, JH. Automated electronic systems for the detection of oestrus and timing of AI in cattle. Animal Reproduction Science. 2000; 60–61, 713–723. (https://doi.org/10.1016/s0378-4320(00)00090-7)

8. Baruselli, PS, Sales, JNS, Sala, RV, Vieira, LM, Sá Filho, MF. History, evolution and perspectives of timed artificial insemination programs in Brazil. Animal Reproduction. 2012; 9(3),139-152.

9. Lamb, GC, Mercadante, VRG, Henry, DD, Fontes, PLP, Dahlen, CR, Larson, JE, DiLorenzo, N. Invited Review: Advantages of current and future reproductive technologies for beef cattle production. The Professional Animal Scientist. 2016; 32(2), 162–171. (https://doi.org/10.15232/pas.2015-01455)

10. Hill, SL, Grieger, DM, Olson, KC, Jaeger, JR, Dahlen, CR, Bridges, GA, Dantas, F, Larson, JE, Muth-Spurlock, AM, Ahola, JK, Fischer, MC, Perry, GA, Larimore, EL, Steckler, TL, Whittier, WD, Currin, JF, Stevenson, JS.Using estrus-detection patches to optimally time insemination improved pregnancy risk in suckled beef cows enrolled in a fixed-time artificial insemination program. Journal of Animal Science. 2016; 94, 3703-3710. (https://doi.org/10.2527/jas.2016-0469)

11. Hill, SL, Grieger DM, Olson, KC, Jaeger, JR, Dahlen, CR, Crosswhite, MR, Negrin Pereira, N, Underdahl, SR, Neville, BW, Ahola, J, Fischer, MC, Seidel, GE, Stevenson, JS. Gonadotropin-releasing hormone increased pregnancy risk in suckled beef cows not detected in estrus and subjected to a split-time artificial insemination program. Journal of Animal Science. 2016; 94, 3722-3728. (https://doi.org/10.2527/jas.2016-0582)

12. Bishop, BE, Thomas, JM, Abel, JM, Poock, SE, Ellersieck, MR, Smith, MF, Patterson, DJ. Split-time artificial insemination in beef cattle: III. Comparing fixed-time artificial insemination to split-time artificial insemination with delayed administration of GnRH in postpartum cows. Theriogenology. 2017; 99, 48-52. (https://doi.org/10.1016/j.theriogenology.2017.04.046)

13. Stevenson, JS, Hill, SL, Grieger, DM, Olson, KC, Jaeger, JR, Ahola, JK, Seidel, GE, Kasimanickam, RK. Two split-time artificial insemination programs in suckled beef cows. Journal of Animal Science. 2017; 95, 5105-5111. (https://doi.org/10.4148/2378-5977.7557)

14. Bishop, BE, Thomas, JM, Abel, JM, Poock, SE, Ellersieck, MR, Smith, MF, Patterson, DJ. Split-time artificial insemination in beef cattle: Using estrous response to determine the optimal time(s) at which to administer GnRH in beef heifers and postpartum com Theriogenology. 2016 86(4),1102-1110. (https://doi.org/10.1016/j.theriogenology.2016.03.043)

15.Ketchum, JN, Bonacker, RC, Andersen,CM, Smith, EG, Stoecklein, KS, Spinka, CM, JM, Thomas, JM. Evaluation of later timepoints for split-time artificial insemination when using sex-sorted semen among beef heifers following the 14-d CIDR®-PG protocol. Animal Reproduction Science. 2021; 224, 106649

16. Knickmeyer, ER, Thomas, JM, Locke, JWC, Bonacker, RC, Ellersieck, MR, Poock, SE, Smith, MF, Patterson, DJ, Evaluation of split-time artificial insemination following administration of a long or short-term progestin-based estrus synchronization protocol in beef heifers. Theriogenology. 2019; 133, 179–186. https://doi.org/10.1016/j.theriogenology.2019.04.011.

17. Abraham, GE, Swerdloff, R, Tulchinsky, D, Odell, WD. Radioimmunoassay of Plasma Progesterone. The Journal of Clinical Endocrinol & Metabolism. 1971; 32(5), 619–624. (https://doi.org/10.1210/jcem-32-5-619)

18. Pereira, MHC, Wiltbank, MC, Vasconcelos, JLM. Expression of estrus improves fertility and decreases pregnancy losses in lactating dairy cows that receive artificial insemination or embryo transfer. Journal of Dairy Science. 2016; 99(3), 2237–2247. (https://doi.org/10.3168/jds.2015-9903)

19. Stegner, JE, Bader, JF, Kojima, FN, Ellersieck, MR, Smith, MF, Patterson, DJ. Fixed-time artificial insemination of postpartum beef cows at 72 or 80 h after treatment with the MGA® Select protocol. Theriogenology. 2004; 61(7-8), 1299–1305. (https://doi.org/10.1016/j.theriogenology.2003.07.023)

20. Lamb, GC, Larson, JE, Geary, TW, Stevenson, JS, Johnson, SK, Day, ML, Landblom, DG. Synchronization of estrus and artificial insemination in replacement beef heifers using gonadotropin-releasing hormone, prostaglandin F 2α, and progesterone. Journal of Animal Science. 2006; 84(11), 3000–3009. (https://doi.org/10.2527/jas.2006-220)

21. Bishop, BE. Timing GnRH administration with split-time artificial insemination following administration of CIDR-based protocols to synchronize estrus and ovulation in beef heifers and cows. Master's Thesis. Retrieved from University of Missouri – Columbia. 2015. (https://mospace.umsystem.edu/xmlui/handle/10355/48583)

22. Walker, RS., Buttrey, B, Gurie, J, Gentry, GT. Evaluating heat detector patch response to determine gonadotropin-releasing hormone use at timed artificial insemination on pregnancy rates in beef cattle. The Professional Animal Scientist. 2014; 30(6), 613–619. (https://doi.org/10.15232/pas.2014-01324)

23. Geary, TW, Whittier, JC. Effects of timed insemination following synchronization of ovulation using the Ovsynch or CO-Synch protocol in beef cows. The Professional Animal Scientist. 1998; 14, 217-220. (https://doi.org/10.15232/s1080-7446(15)31832-5)

24. Pursley, JR, Mee, MO, Wiltbank, MC. Synchronization of ovulation in dairy cows using $PGF_{2\alpha}$ and GnRH. Theriogenology. 1995; 44, 915-923. (https://doi.org/10.1016/0093-691x(95)00279-h)

25. Larson, JE, Lamb, GC, Stevenson, JS, Johnson, SK, Day, ML, Geary, TW, Kesler, DJ, DeJarnette, JM, Schrick, FN, DiCostanzo, A, Arsenau, JD. Synchronization of estrus in suckled beef cows for detected estrus and artificial insemination and timed artificial insemination using gonadotropin-releasing hormone, prostaglandin $F_{2\alpha}$ and progesterone. Journal of Animal Science. 2006; 84, 332-342. (https://doi.org/10.2527/2006.842332x)

26. Walker, RS, Burns, PD, Whittier, JC, Sides, GE, Zalesky, DD. Evaluation of Gonadotropin-Releasing Hormone and Insemination Time Using the CO-Synch Protocol in Beef Cows. The Professional Animal Scientist. 2005; 21(3), 190–194. (https://doi.org/10.15232/s1080-7446(15)31201-8)

27. Howard, JM, Falk, DG, Carnahan, KG, Dalton, JC, Chebel, RC, Ahmadzadeh, A. The Use of Gonadotropin- Releasing Hormone in a Progesterone-Based Timed Artificial Insemination Protocol in Replacement Beef Heifers. The Professional Animal Science. 2009; 25(6), 757–761. (https://doi.org/10.15232/s1080-7446(15)30785-3)