# TEMPERAMENT AND REPRODUCTION IN BEEF FEMALES

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For over a century, the word temperament has been used to define the fear-related behavioral responses of cattle when exposed to human handling (Fordyce et al., 1988). As cattle temperament worsens, their response to human contact or any other handling procedure becomes more excitable. Within the beef cattle industry, producers select cattle for temperament primarily for safety reasons. However, recent studies demonstrate that cattle temperament may also have productive and economic implications to beef operations.

## Is Excitable Temperament a Stress Response?

Stress response is defined as the reaction of cattle to internal and external factors that affect their well being, and animals that are unable to cope with these factors are classified as stressed. Examples are extreme temperatures, diseases, and injuries. Based on this concept, the agitated and/or aggressive responses expressed by cattle with excitable temperament when exposed to human handling can be attributed to their fear and consequent inability to cope with this situation; therefore, classified as a stress response. In addition to altered behavior, temperamental cattle may also experience changes in their body physiology, and the hormones produced during this fear-related stress reaction influence several aspects, such as growth, health, and reproduction.

One of the main hormones produced during a stress response is cortisol. Several studies reported that blood cortisol concentrations are greater in temperamental cattle compared to calm cattle (Table 1). This outcome validates that excitable temperament can be classified as a stress response, and is one of the reasons why cortisol is commonly considered paramount to the behavioral stress response.

## Assessment of Temperament in Beef Cattle

Cattle temperament can be visually evaluated by many methods, which can be categorized into non-restrained and restrained techniques (Burrow and Corbet, 2000). Within the non-restrained techniques, cattle temperament is evaluated by their fear or aggressive response to man when they are free to move within the evaluation area. Examples of these techniques are chute exit velocity and pen score. Exit velocity evaluates the speed of an individual animal immediately after it leaves the squeeze chute by measuring the time required for the animal to travel a pre-determined distance. This assessment can be expressed in actual speed measures (i.e., feet/second), or in a 1-5 scale, where 1 are the slowest and 5 are the fastest animals. The pen score evaluates the behavioral response of an individual animal when it enters a small pen and interacts with a person standing inside the pen. Typically in a 1-5 scale, the pen score increases as the animal response becomes more aggressive toward the person. The restrained techniques evaluate cattle temperament when these are physically restricted, such as in the squeeze chute. An example of the restrained techniques is the chute score, also denominated crush score. Cattle are individually restrained in the chute and scored in a 1-5 scale according to its behavior; where 1 = calm with no movement, 2 = restless movements, 3 = frequent movement with vocalization, 4 = constant movement, vocalization, shaking of the chute, and 5 = violent and

continuous struggling. This measurement can be taken in cattle that are squeezed or not in the chute. However, squeezed animals may not express their real temperament. Other methods to assess cattle temperament have also been reported; however, chute score, exit velocity, and pen score have been shown to be repeatable within animals and relatively simple to carry out during handling procedures. Additionally, these techniques are typically related to each other and with blood cortisol concentrations, indicating that these 3 measurements can similarly assess cattle temperament and denote the amount of behavioral stress that the animal is experiencing (Figure 1). To further increase the accuracy in temperament evaluation, producers can utilize more than one technique and combine the results into an overall temperament score, which typically relates better with blood cortisol concentrations compared to individual techniques (Figure 2).

## Factors that Influence Temperament in Beef Cattle

Cattle temperament is influenced by several factors such as sex, age, and horn status (Fordyce et al., 1988; Voisinet et al., 1997). However, none of these characteristics has been shown to affect cattle temperament as much as production system and breed type (Table 2). Cattle reared in extensive systems, such as the range cow-calf operations in the western states, are expected to have more excitable temperament compared to cattle reared in intensive operations because of less frequent interaction with humans (Fordyce et al., 1985). Further, cattle with high Brahman influence have more excitable temperament compared to *B. taurus* cattle (Fordyce et al., 1988; Voisinet et al., 1997). Therefore, cattle reared on extensive production systems, particularly if they have Brahman-influence, are potentially difficult to control and handle, which can pose significant management, economic, and productivity problems.

## Temperament and Reproduction in Beef Females

Excitable temperament is detrimental to the nutritional status of cattle, given that temperamental cattle have decreased feed intake compared to calm cohorts (Brown et al., 2004; Nkrumah et al., 2007). In addition, cattle with excitable temperament also have altered metabolism and partitioning of nutrients in order to sustain the behavioral stress response, which results in further decreases in nutrient availability to support body functions (Cooke et al., 2009a; Cooke et al, 2009b). Nutritional status largely determines reproductive performance in cattle; therefore, excitable temperament may indirectly impair reproduction in beef heifers and cows by decreasing nutritional balance.

Also, the hormones produced during a stress response, particularly cortisol, directly disrupt the physiological mechanisms that regulate reproduction in beef females, such as ovulation, conception, and establishment of pregnancy. As an example, cows with calm temperament have reduced cortisol and greater blood concentrations of luteinizing hormone, the hormone required for puberty establishment and ovulation, compared to temperamental cows. Accordingly, it was recently demonstrated that beef heifers with calm temperament reached puberty sooner than temperamental cohorts (Table 3). Brahman-influenced cows with excitable temperament had decreased chances of becoming pregnant during the breeding season compared to calm cohorts (Figure 3). Similar relationships were detected when blood cortisol concentrations were evaluated against puberty or pregnancy instead of temperament in those heifers and cows (Table 3 and Figure 3). In addition, Angus × Hereford cows with excitable temperament had reduced pregnancy rate, calving rate, weaning rate, and lbs of calf weaned/cow exposed compared to cows with adequate temperament (Table 4), indicating that excitable temperament not only impairs reproductive performance, but also overall production efficiency

in cow-calf systems. Therefore, management strategies that improve the overall temperament of the herd are imperative for optimal productivity of cow-calf operations (Plasse et al., 1970; Cooke et al. 2009a).

## Improving Temperament of Beef Cattle

One alternative to improve temperament and consequently benefit reproduction in beef females is to adapt them to human handling. Early studies reported that cattle accustomed to human handling had calmer temperament, reduced blood cortisol concentrations, and increased LH concentrations compared to non-acclimated cattle (Crookshank et al., 1979; Echternkamp, 1984; Fordyce et al., 1985). Further, replacement heifers exposed to an acclimation process to human handling for 4 weeks after weaning had improved temperament, reduced cortisol, and reached puberty and became pregnant earlier compared to non-acclimated cohorts (Table 4). However, no beneficial effects on temperament and reproduction were detected when mature cows were exposed to acclimation to human handling (Cooke et al., 2009a). Therefore, adapting beef females to human interaction early in their productive lives may be an alternative to improve their temperament and consequently hasten their reproductive development. Further, including temperament in culling/selection criteria might be the most appropriate alternative to improve the overall temperament and consequent reproductive performance of the adult cow herd.

## Conclusions

In summary, excitable temperament is a fear-related behavioral response that has detrimental effects on reproductive function of beef heifers and cows. Temperament is even a greater concern in extensive beef operations, particularly if they contain Brahman-influenced cattle. Temperament influences cattle reproduction indirectly by decreasing nutritional status, and directly by altering the physiological mechanism required for ovulation and conception. Beef producers can evaluate cattle temperament by visual assessments that can be conducted during common handling procedures, such as assessing chute score when cattle have to be handled for vaccination or weaning. Depending on the outcomes, producers can adopt management strategies to improve the overall temperament of the cow herd. Examples are acclimation to human handling and consideration of temperament in selection/culling decisions, which will bring benefits to the reproductive performance and consequent productivity of cow-calf operations containing temperamental cattle

## References

Brown, E. G., G. E. Carstens, J. T. Fox, M. B. White, T. W. Welsh, Jr., R.D. Randel, and J.W. Holloway. 2004. Relationships between temperament and performance traits of growing calves. In: 2004 Beef cattle research in Texas. Available at: http://animalscience.tamu.edu/ansc/beef/bcrt/2004/brown_erin.pdf. Accessed May, 2009.

Burrow, H. M., and N. J. Corbet. 2000. Genetic and environmental factors affecting temperament of zebu and zebu-derived beef cattle grazed at pasture in the tropics. Aust. J. Agric. Res. 51:155-162.

Cooke, R. F., J. D. Arthington, D. B. Araujo, and G. C. Lamb. 2009a. Effects of acclimation to human interaction on performance, temperament, physiological responses, and pregnancy rates of Brahman-crossbred cows. J. Anim. Sci. 87:4125-4132.

Cooke, R. F., J. D. Arthington, B. R. Austin, and J. V. Yelich. 2009b. Effects of acclimation to handling on performance, reproductive, and physiological responses of Brahman-crossbred heifers. J. Anim. Sci. 87:3403-3412.

Cooke, R. F., D. W. Bohnert, B. I. Cappellozza, C. J. Mueller, and T. DelCurto. 2012. Effects of temperament and acclimation to handling on reproductive performance of *Bos taurus* beef females. J. Anim. Sci. 90:3547-3555.

Crookshank, H. R., M. H. Elissalde, R. G. White, D. C. Clanton, and H. E. Smalley. 1979. Effect of transportation and handling of calves upon blood serum composition. J. Anim. Sci. 48:430-435.

Echternkamp, S. E. 1984. Relationship between LH and cortisol in acutely stressed beef cows. Theriogenology 22:305-311.

Fordyce, G., M. E. Goddard, R. Tyler, G. Williams, and M. A. Toleman. 1985. Temperament and bruising of Bos indicus cross cattle. Aust. J. Exp. Agric., 25:283-288.

Fordyce, G. E., R. M. Dodt, and J. R. Wythes. 1988. Cattle temperaments in extensive beef herds in northern Queensland. 1. Factors affecting temperament. Aust. J. Exp. Agric. 28:683.

Francisco, C. L., A. M. Jorge, F. D. Rezende, A. Schmidek, J. M. B. Benatti, M. H. Faria, E. Oba, and R. F. Cooke. 2012a. Effects of temperament on physiological responses, feedlot performance, and carcass characteristics of Nelore steers. J. Anim. Sci. 90 (E-Suppl. 3):427 (Abstr.)

Nkrumah, J. D., D. H. Crews, Jr, J. A. Basarab, M. A. Price, E. K. Okine, Z. Wang, C. Li, and S. S. Moore. 2007. Genetic and phenotypic relationships of feeding behavior and temperament with performance, feed efficiency, ultrasound, and carcass merit of beef cattle. J. Anim. Sci. 85:2382-2390.

Plasse, D., A.C. Warnick, and M. Koger. 1970. Reproductive behavior of *Bos indicus* females in a subtropical environment. IV. Length of estrous cycle, duration of estrus, time of ovulation, fertilization and embryo survival in grade Brahman heifers. J. Anim. Sci. 30:63-72.

Voisinet, B. D., T. Grandin, J. D. Tatum, S. F. O'Connor and J. J. Struthers. 1997. Feedlot cattle with calm temperaments have higher average daily gains than cattle with excitable temperaments. J Anim Sci. 75:892-896.

**Table 1. Blood cortisol concentrations of cattle with calm or excitable temperament.** [1]

| Item | Adequate | Excitable |
|---|---|---|
| *Bos indicus* | | |
| Steers | 16.7 | 19.6 |
| *B. indicus* × *B. taurus* | | |
| Heifers | 45.5 | 57.9 |
| Cows | 30.7 | 42.4 |
| *B. taurus* | | |
| Heifers | 32.1 | 41.8 |
| Cows | 17.8 | 22.7 |

[1] Cooke et al. (2009ab), Cooke et al. (2012a), and Francisco et al. (2012a).

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**Table 2. Factors that affect cattle temperament.** [1]

| **Item** | **Method of Assessment** [2] | **Mean** |
|---|---|---|
| Sex | | |
| Male | *Temperament Score; 1 – 5 scale* | 2.7 |
| Female | | 3.0 |
| Age | | |
| < 2 years | *Exit Velocity Score; 1 – 5 scale* | 3.1 |
| > 2 years | | 2.8 |
| Horn status | | |
| Horned | *Exit Velocity Score; 1 – 5 scale* | 2.7 |
| Polled | | 3.0 |
| Breed type | | |
| Brahman x Hereford | *Temperament Score; 1 – 5 scale* | 3.6 |
| Brahman x Angus | | 3.8 |
| Angus | | 1.7 |
| Simmental x Angus | | 1.8 |
| Human interaction | | |
| Frequent | *Crush Score; 1 – 7 scale* | 1.5 |
| Infrequent | | 2.1 |

[1] Adapted from Voisinet et al. (1997), Fordyce et al. (1985, 1988), and Cooke et al. (2009a).
[2] As score increases, exit velocity increases, and crush/temperament becomes more excitable.

**Table 3. Post-weaning temperament scores (1 = calm; 5 = excitable temperament) and blood cortisol concentrations of replacement heifers that attained or not puberty by 12 months of age. [1]**

| Item | Non-pubertal | Pubertal |
|---|---|---|
| Temperament score | 2.7 | 2.3 |
| Cortisol, ng/mL | 50.0 | 39.7 |

[1] Adapted from Cooke et al. (2009b).

**Table 4. Reproductive performance of Angus x Hereford beef cows according to temperament. [1]**

| Item | Adequate | Excitable |
|---|---|---|
| Pregnancy rate, % | 94.6 | 88.7 |
| Calving rate, % | 91.8 | 85.0 |
| Weaning rate, % | 89.9 | 83.9 |
| Calf weaning BW, lbs | 545 | 543 |
| Lbs of calf weaned/cow exposed to breeding | 490 | 455 |

[2] Adapted from Cooke et al. (2012).

**Table 5. Effects of acclimation to human handling on temperament, cortisol, and reproduction of replacement heifers. [1,2]**

| Item | Acclimated | Non-acclimated |
|---|---|---|
| *Brahman-influenced heifers* | | |
| Chute score, 1 – 5 scale | 1.4 | 1.9 |
| Cortisol, ng/mL | 37.8 | 50.5 |
| % of pubertal heifers by 12 months of age | 65. | 39 |
| % of pregnant heifers 30 days into breeding season | 50 | 32 |
| *Angus x Hereford heifers* | | |
| Exit velocity, feet/s | 7.0 | 8.6 |
| Cortisol, ng/mL | 26.1 | 32.8 |
| % of pubertal heifers by 12 months of age | 59.6 | 37.8 |

[1] Acclimated heifers were exposed to a handling process 3 times weekly for 4 weeks after weaning. Control heifers remained undisturbed on pasture.
[2] Adapted from Cooke et al. (2009b) and Cooke et al. (2012).

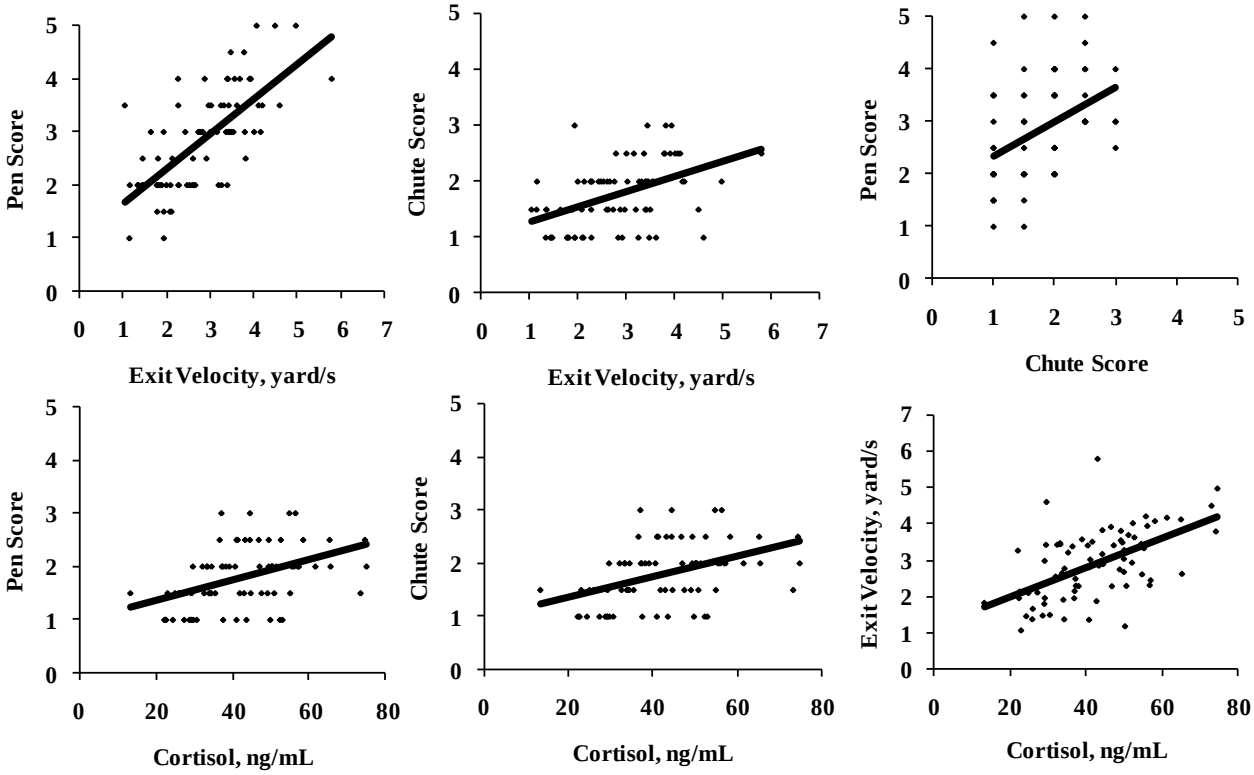


**Figure 1. Relationship among measurements of temperament and blood cortisol concentrations in beef heifers. Adapted from Cooke et al. (2009b).**

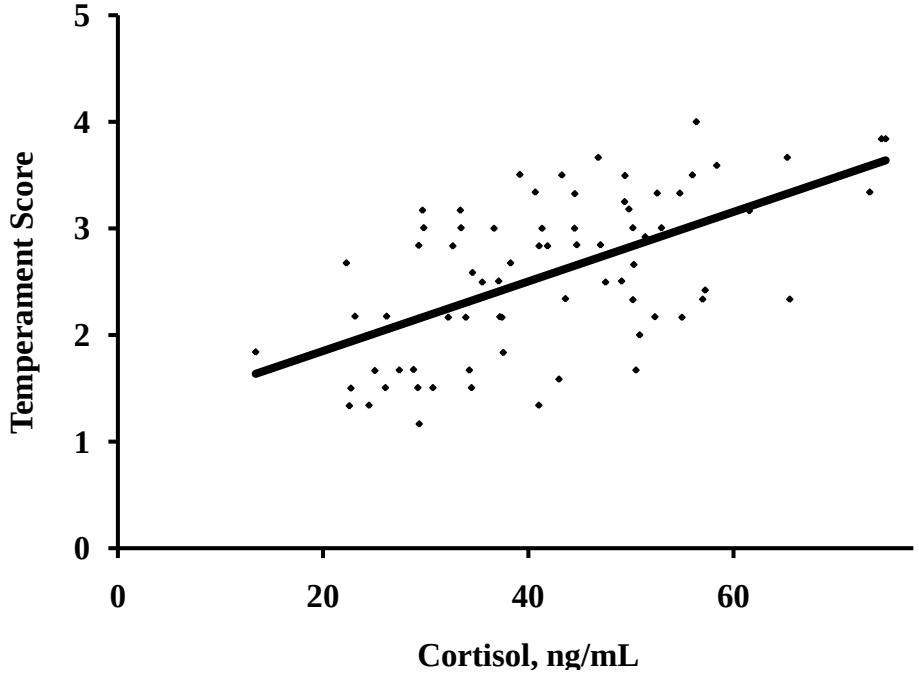


**Figure 2. Relationship between temperament score (1 = calm; 5 = excitable temperament) and blood cortisol concentrations in beef heifers. Adapted from Cooke et al. (2009b).**

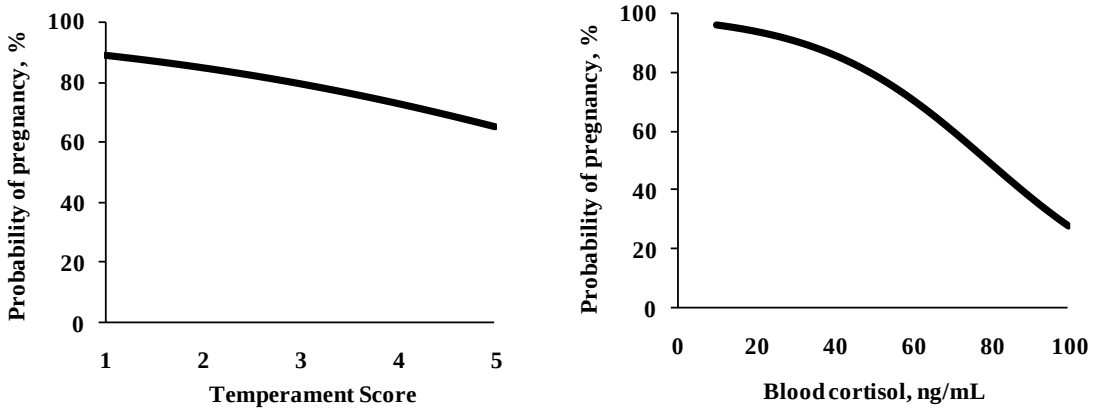


**Figure 3. Probability of beef cows to become pregnant according to temperament score (1 = calm; 5 = excitable temperament) and blood cortisol concentrations assessed at the beginning of the breeding season. Adapted from Cooke et al. (2009a)**

# APPENDIX

## Treatment with prostaglandin $F_{2\alpha}$ and an intravaginal progesterone insert in advance of gonadotropin-releasing hormone enhances response to estrus synchronization in mature beef cows

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An experiment was designed to evaluate treatments intended to enhance ovulatory response to gonadotropin-releasing hormone (GnRH; 100μg gonadorelin) administered at the start of an estrus synchronization protocol. We hypothesized that administration of prostaglandin $F_{2\alpha}$ (PG; 500μg cloprostenol) and an intravaginal progesterone-releasing insert (CIDR; 1.38g progesterone) would result in increased follicle size and maturity at GnRH, thereby enhancing response to GnRH and overall response to estrus synchronization. Postpartum suckled beef cows (n=190) were assigned to one of five treatments based on age, days postpartum, and body condition score. Cows in Treatment 1 (control) received the 7-d CO-Synch + CIDR protocol: administration of GnRH and CIDR insertion on Day -10, and administration of PG and CIDR removal on Day -3. Treatments 2-5 were designed as a two-by-two factorial. On Day -17, cows in Treatments 2-5 received a CIDR insert, either with (Treatments 2 and 3) or without (Treatments 4 and 5) administration of PG. On Day -10, all cows were administered GnRH, and CIDR inserts were either removed (Treatments 2 and 4) or remained in place until Day -3 (Treatments 3 and 5). On Day -3, PG was administered and estrus detection aids were applied. Estrus detection transmitters (Accubreed) were applied to a representative subset of cows in each treatment. Blood samples were collected on Days -27, -17, -10, -3, and 0 for determination of serum estradiol and/or progesterone concentrations via radioimmunoassay. Transrectal ovarian ultrasound was performed for a representative subset (n=104) to assess ovarian follicle size and presence of corpora lutea (CL) on Days -17, -10, -3, and 0. Fixed-time AI was performed on Day 0 at 66 h after PG administration in all treatments. Treatments with PG and CIDR on Day -17 (Treatments 2 and 3) resulted in increased diameter of the largest ovarian follicle present ($P<0.05$) and increased serum concentrations of estradiol ($P<0.05$) on Day -10. In addition, variation among cows in CL status tended to be decreased ($P=0.08$) on Day -3, with cows more likely to have a single CL rather than no CL or multiple CL. Lastly, estrous response prior to fixed-time AI tended ($P=0.08$) to be improved. Results support the hypothesis that administration of PG and treatment with a CIDR for 7 d prior to GnRH improves likelihood of GnRH-induced ovulation, enhancing uniformity in stage of cycle among mature cows undergoing estrus synchronization.

Key words: beef cows, estrus synchronization, presynchronization

# APPENDIX

## Pregnancy and hormonal response in suckled beef cows administered high-concentration prostaglandin $F_{2\alpha}$ in a fixed-time artificial insemination 5-day CO-Synch + CIDR program

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HighCon is a high-concentration [12.5 mg/mL] $PGF_{2\alpha}$ analogue (dinoprost tromethamine) that reaches a greater maximum plasma concentration and has a longer plasma half-life than conventional $PGF_{2\alpha}$ [5 mg/mL, dinoprost tromethamine]. The objective of this study was to compare the effects of a single dose of HighCon or two doses of conventional $PGF_{2\alpha}$ (2PG) on serum progesterone ($P_4$) concentration at timed-artificial insemination (TAI), and pregnancy per TAI in beef cows synchronized with a 5-day CO-Synch + controlled internal drug release (CIDR) program. Blood samples were collected from Angus-Hereford crossbred cows (n=200) seven days before (d -7) and the day of protocol initiation (d 0) to determine cyclicity status based on serum $P_4$ concentrations $\geq$ 1 ng/mL. Cows concurrently received GnRH (100 μg; i.m.) and a CIDR insert on d 0. Five days later (d 5), CIDR inserts were removed. Cows were stratified by BCS, BW, age, and days postpartum and assigned randomly to receive one dose of HighCon (25 mg; i.m.; n=100) or 2PG (25 mg; i.m.; n=100), 7 to 11 h apart on d 5. Estrual behavior was monitored from d 5 until TAI (d 8) using Estrotect patches and visual observation. On d 8, all cows were administered a second dose of GnRH and inseminated at a fixed time. A blood sample was collected from all animals on d 8 to measure serum $P_4$ concentration and determine luteolytic response. Pregnancy was confirmed via transrectal ultrasonography 48 or 57 d after TAI. Estrus expression, cyclicity status, pregnancy per TAI and luteolytic response data were analyzed by PROC LOGISTIC of SAS. Differences in $P_4$ concentrations on d -7, 0, and 8 between treatments were analyzed using PROC GLM. Percent of cows cyclic before protocol initiation did not differ between treatments ($P$ = 0.59). Similarly, no difference ($P$ = 0.18) in pregnancy per TAI was detected between HighCon and 2PG (62% vs. 71%, respectively). Although estrus expression was not different between treatments ($P$ = 0.16), pregnancy per TAI was greater ($P$ = 0.02) in cows detected in estrus before TAI (75%) than in cows that were not (58%). Incidence of luteolysis (defined as $P_4$ < 0.5 ng/mL at TAI) was greater ($P$ < 0.01) in 2PG (98%) than HighCon (80%) cows. In summary, 2PG was more effective at inducing luteolysis before TAI than one dose of HighCon in suckled beef cows, however, pregnancy per TAI between treatments was not different.

Key words: beef cow, pregnancy rate, progesterone, prostaglandin $F_{2\alpha}$

# APPENDIX

## Influence of BVDV infection on AI conception and breeding season pregnancy success in vaccinated beef herds

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Bovine Viral Diarrhea Virus (BVDV) causes reproductive and economic losses in cattle. The objective of this study was to evaluate the influence of BVDV infection on reproductive success (AI and breeding season conceptions). Well vaccinated cows (n=367) and heifers (n=540) from 9 different herds were synchronized using the 7-day CO Synch + CIDR protocol and were fixed-time AI (FTAI). On d 28 following insemination, blood samples were collected, and pregnancy status was determined by transrectal ultrasonography and the IDEXX Rapid Visual Pregnancy Test. Non-pregnant animals were resynchronized and FTAI a second time. In six herds bulls were comingled with females beginning 10-15 d after the second AI. Final pregnancy status was determined 33-80 d following the first pregnancy diagnosis. Blood samples were tested for the presence of BVDV antigen using the IDEXX BVDV PI X2 Kit. Positive samples were indicative of animals with an active infection. Herds were determined as being infected with BVDV by the presence of at least one animal having a positive test for active antigen (n=4 infected herds, n=5 non-infected herds). Statistical analyses were performed using the GLIMMIX procedure of SAS. Herds that were infected with BVDV during the breeding season had significantly decreased ($P$<0.01) first service AI conception rates compared to herds that had no exposure (34 ± 2.3% vs. 54 ± 2.3%). Additionally, breeding season pregnancy rates were decreased ($P$<0.01) in herds that had BVDV exposure compared to non-exposed herds (69 ± 3.4% vs. 80 ± 3.6%). There was no significant effect of BVDV infection on embryonic loss ($P$=0.42) or percentage of animals which lost a pregnancy and rebred by the end of the breeding season ($P$=0.63). In conclusion, infection of BVDV in well vaccinated herds still had a negative effect on both first service AI conception rate and overall breeding season pregnancy success.

Key words: AI, Bovine Viral Diarrhea Virus, pregnancy

# APPENDIX

## Evaluating strategies to improve pregnancy rates resulting from artificial insemination in beef heifers

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Split-time artificial insemination (STAI) was shown in previous studies to improve estrous response and pregnancy rates over fixed-time AI (FTAI) among beef heifers assigned to long-term progestin-based protocols (14-d CIDR®-PG and MGA®-PG). Two experiments were conducted to test hypotheses that STAI would improve pregnancy rates over FTAI among heifers assigned to a short-term CIDR®-based protocol (Experiment 1), and that extending the duration of CIDR® treatment in a long-term CIDR®-based protocol would improve pregnancy rates after STAI compared to a treatment of normal duration (Experiment 2). Yearling heifers in both experiments were assigned to balanced treatments based on weight and reproductive tract score. Heifers (n = 456) in Experiment 1 were assigned to FTAI or STAI treatments and subject to the 7-d CO-Synch + CIDR® protocol. Gonadotropin-releasing hormone (100 μg gonadorelin acetate; GnRH) was administered at CIDR® insertion (1.38g progesterone; Zoetis, Madison, NJ), and inserts were removed 7 d later. Prostaglandin $F_{2\alpha}$ (PG; 250 μg im cloprostenol sodium) was administered and estrus detection aids (Estrotect®) were applied at CIDR® removal. Heifers assigned to FTAI were inseminated 54 h after PG concurrent with GnRH administration. Heifers in the STAI treatment were inseminated 54 h (estrous at 54 h) or 78 h (non-estrous at 54 h) after PG dependent upon estrous status. Heifers that failed to exhibit estrus by 78 h received GnRH at AI. Estrous response ($P < 0.01$) was improved among heifers in the STAI treatment; however, pregnancy rates were not improved ($P = 0.4$) compared to heifers assigned to FTAI. Heifers (n = 842) in Experiment 2 were assigned to an 18 d or 14 d CIDR® treatment, with PG and estrus detection aids applied 16 d after CIDR® removal. Heifers that expressed estrus 66 h after PG were inseminated and heifers that were non-estrous at 66 h were inseminated at 90 h. Heifers that failed to express estrus by 90 h received GnRH at AI. Estrous response and pregnancy rate to AI did not differ ($P > 0.2$) between treatments. Collectively, these two experiments contribute to the current body of information pertaining to use of STAI in beef heifers. Results from Experiment 1 raise interesting questions pertaining to differences between long- and short-term progestin-based protocols for synchronizing estrous cycles of heifers, while results from Experiment 2 highlight flexibility in using long-term CIDR®-based protocols to facilitate STAI.

Key Words: artificial insemination, beef heifer, estrus synchronization

# APPENDIX

## Evaluation of the Variability among Bulls for CD9 and SERPINA5 on the Bovine Sperm Head

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Differences in fertility have been identified even among bulls that successfully pass a breeding soundness exam and quality control analysis. Several tests have been developed to attempt to try to explain lower fertility bulls; however, they have not added further information when compared to traditional morphology and progressive motility analysis. In addition to motility and morphology, proteins on the sperm may serve as fertility markers and further explain differences in fertility. Thus, to serve as a potential marker of fertility there must be variability in protein expression among animals. The proteins CD9 and SERPINA5 are associated with cell to cell interaction. Cell to cell interactions are a critical factor in the formation of the sperm reservoir in the female tract and in binding to the oocyte. The sperm reservoir plays a vital role in male fertility, as it impacts sperm longevity and supplies sperm to the site of fertilization. Therefore, the objective of this study was to characterize the variability of CD9 and SERPINA5 protein expression on sperm among bulls. Semen from 17 bulls of three different breeds (Angus, Simmental and Hereford) were fixed and evaluated for presence of CD9 and SERPINA5. All samples passed quality control for frozen-thawed semen and were from a CSS certified AI stud (total and progressive post-thaw motility were 32.6±12.5% and 19.0±8.3%, respectively). Aliquots containing ~500,000 sperm were incubated with 0.5 µg of anti-CD9 or anti-SERPINA5, and fluorescence intensity (FI) was evaluated on a minimum of 100 sperm per bull. Data were analyzed using the GLM procedure in SAS with bull as a fixed effect to determine if the variance in proteins was greater between bulls compared to within a bull. Both CD9 and SERPINA5 were localized to the sperm head. However, SERPINA5 was also detected on the proximal region of the sperm tail among all bulls (33.5±4.1% of sperm; range 4% to 61%). Variation in FI on the sperm head was greater for both CD9 ($P$<0.001; FI range 14.1±0.17 to 19.9±0.18) and SERPINA5 ($P$<0.001; FI range 12.9±0.35 to 19.0±0.36) among bulls (variance of 5.96 and 15.14, respectively) compared to within bulls (variance of 3.32 and 13.03, respectively). In summary, there was significant variation among bulls in both CD9 and SERPINA5. Furthermore, these sperm proteins might serve as a marker for fertility as they may be associated with the sperm reservoir and fertilization due to their role in cell to cell interaction.

Keywords: CD9, fertility marker, SERPINA5, sperm protein

# APPENDIX

## Presynchronization with Prostaglandin $F_{2\alpha}$ and prolonged exposure to exogenous progesterone impacts estrus expression and alters fertility in beef heifers

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To determine the effects of 2 presynchronization strategies in conjunction with delayed fixed-time artificial insemination (TAI) on pregnancy rates to TAI (PR/AI), 1,700 Angus beef heifers at 3 locations in South Dakota were enrolled in a completely randomized design with a 2 × 2 factorial arrangement of treatments. Within location, all heifers were randomly assigned to 1 of 4 treatments: 1) **PG54** ($n$ = 434), heifers received a 25-mg injection of prostaglandin $F_{2\alpha}$ (PGF) 7 d prior [d -14] to the initiation of the 7-d CO-Synch + controlled internal drug releasing (CIDR) protocol wherein they received a 100-µg injection of gonadotropin-releasing hormone (GnRH) and a CIDR insert on d -7, a 25-mg injection of PGF at CIDR removal on d 0, and a second injection of GnRH concurrently with TAI 54 ± 2 h later; 2) **PG72** ($n$ = 426), heifers were exposed to the same treatment as PG54, however, TAI was performed 72 ± 2 h after CIDR removal; 3) **PG-CIDR54** ($n$ = 422), same as PG54 but heifers received a CIDR insert on d -14 in addition to the injection of PGF; 4) **PG-CIDR72** ($n$ = 418), same as PG-CIDR54, however, TAI was performed 72 ± 2 h after CIDR removal. Estrus detection patches were applied to all heifers on d 0 and were evaluated for activation at TAI. Pregnancy was diagnosed via transrectal ultrasonography between 30 and 47 d after TAI. The percentage of heifers exhibiting estrus between d 0 and TAI was greater ($P < 0.001$) in the PG72, PG-CIDR54, and PG-CIDR72 treatments compared to the PG54 treatment (78.11, 86.59, and 91.09 vs. 31.05%, respectively). Furthermore, estrus response was greater ($P < 0.001$) in PG-CIDR72 heifers when compared to PG72. Pregnancy rates to TAI differed among treatments and were greater ($P < 0.05$) in the PG72 and PG-CIDR54 treatments when compared to PG-CIDR72 (48.8 and 50.4 vs. 38.4%, respectively), and were greater ($P = 0.034$) in PG-CIDR54 vs. PG54 (43.1%). Moreover, a tendency ($P = 0.097$) was determined on PR/AI between PG54 and PG72. In conclusion, presynchronization strategies and prolonged exposure to exogenous progesterone have the potential to alter estrus expression and improve fertility in replacement beef heifers.

Keywords: beef heifer, CIDR, presynchronization, prostaglandin $F_{2\alpha}$

# APPENDIX

## Impact of feeding management strategies on growth performance, rumen conditions, and semen quality of developing bulls

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Feeding high-energy diets to developing bulls is perceived to influence semen quality, yet little research has evaluated iso-caloric diets that differ in form or feeding management strategy in a real-world production scenario. The objectives of this experiment were to evaluate the effects of two feeding management strategies on growth performance and semen quality, and identify relationships between phenotypic traits and semen quality of developing bulls. Angus bulls (n = 48; 332 ± 47 d of age) were stratified into one of sixteen drylot pens before pens were randomly assigned to one of two iso-caloric diets: a total mixed ration (TMR; n = 8 pens) or a component- based ration (COMP; n = 8 pens) fed for 84 d. Rumen pH was continuously monitored via rumen boluses and dry matter intake (DMI) was measured daily. Subcutaneous fat thickness (SFT) and hoof conformation were measured on d 0 and 84, with body weight measured and breeding soundness examinations (BSE) conducted at 21-d intervals. Semen was assessed for collection volume, progressive motility, total sperm x collection$^{-1}$, and sperm cell morphology. Data were analyzed using JMP 13.0. Bulls fed the COMP ration had a greater DMI ($P < 0.01$) and ADG ($P< 0.01$), but similar feed efficiency ($P = 0.19$) when compared to TMR-fed bulls. Bulls fed the COMP ration had a lower mean rumen pH ($P < 0.01$) and higher probability of pH below 5.8 ($P< 0.01$) and 5.2 ($P < 0.01$). Additionally, COMP-fed bulls had greater SFT ($P < 0.01$) but similar hoof conformation ($P = 0.54$) when compared to TMR-fed bulls on d 84. Bulls fed the COMP ration provided more semen volume ($P < 0.05$), but similar progressive motility ($P = 0.34$), total sperm x collection$^{-1}$ ($P = 0.81$), and percent morphologically-normal sperm ($P = 0.34$) when compared to TMR-fed bulls. Change in scrotal circumference between d 0 and 84 was inversely related to percent morphologically-normal sperm ($R^2 = 0.07$; $P < 0.01$), but directly related to change in percent morphologically-normal sperm ($R^2 = 0.12$; $P < 0.05$). Change in SFT between d 0 and 84 was directly related to changes in semen volume ($R^2 = 0.10$; $P < 0.05$) and total sperm x collection$^{-1}$ ($R^2 = 0.12$; $P < 0.05$). Mean rumen pH tended to be directly related to changes in semen volume ($R^2 = 0.07$; $P < 0.10$) and total sperm x collection$^{-1}$ ($R^2 = 0.07$; $P < 0.10$). These results suggest feeding strategy and ruminal acidosis did not impact semen quality of developing bulls, and should not be expected to influence BSE outcomes.

Key words: breeding soundness exam, bull, pH, rumen, scrotal circumference, semen quality

# APPENDIX

## Cytokine supplementation improves the in vitro culture of bovine embryos

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Division of Animal Sciences, University of Missouri, Columbia, MO, 65211

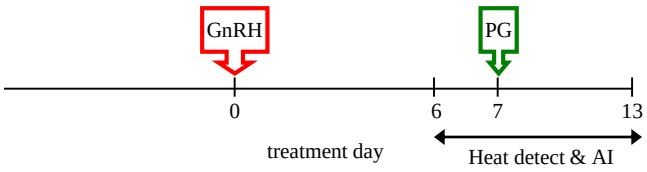
To improve in vitro production of bovine embryos we have identified three cytokines (FGF2, LIF, and IGF1 termed FLI) that play a role embryo development in vivo. Our hypothesis is that supplementation of culture medium with FLI improves preimplantation embryonic development in vitro. To test this hypothesis embryos were produced in vitro using abattoir-derived oocytes and fertilized with sperm from a single bull known to have high fertility. After an 18-20 h fertilization period, putative zygotes were cultured in synthetic oviductal fluid (SOF) with or without FLI for 8 days. The number of embryos that underwent at least one cellular division (cleavage), and the number of embryos that developed to the blastocyst stage was recorded on days 3 and 8 after insemination, respectively. There were 762 embryos supplemented with FLI, and 842 embryos in the control group across 8 replicates. The FLI-treated embryos had a higher ($P<0.05$) cleavage rate (88.1% ± 0.49) than the control group (85.7% ± 0.49), as well as a higher blastocyst rate (41.8% ± 1.2 versus 30.7% ± 1.2). Trophectoderm and inner cell mass cell number was determined after immunolocalization of the trophectoderm marker CDX2 and a nuclear stain using control (n=72) and FLI-treated (n=89) blastocysts. There was no difference in trophectoderm ($P=0.09$), inner cell mass ($P=0.93$), or total cell number ($P=0.26$) between the two groups. In a second experiment, blastocysts were cryopreserved by slow-freezing, thawed after at least a month of storage in liquid nitrogen, placed in SOF, and re-expansion was evaluated at 24, 48, and 72 h post thawing. The embryos supplemented with FLI had a greater ($P<0.05$) number of re-expanded embryos (81.8% ± 0.06) than the control group (38.6% ± 0.06) at 72 h post-thawing. Apoptosis was also analyzed by TUNEL staining and FLI-treated embryos had less ($P<0.05$) apoptotic cells (6.7% ± 1.3) than the controls (18.1% ± 1.3). These results suggest that FLI supplementation to a bovine in vitro embryo production system increases the number of embryos that develop to the blastocyst stage and improves embryo quality shown through higher resistance to a slow-freezing process. These cytokines have the potential to alleviate some of the challenges of the in vitro production of bovine embryos. Further research is required to elucidate the mechanisms by which FLI supplementation improves development in vitro. Supported by Food for the 21st Century and the Clifton Murphy scholarship fund.

Key Words: bovine embryo culture, cryopreservation, FGF2, LIF, IGF1, in vitro embryo development

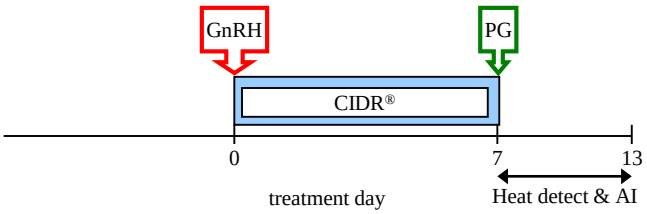
# BEEF COW PROTOCOLS - 2019

## HEAT DETECTION

### Select Synch

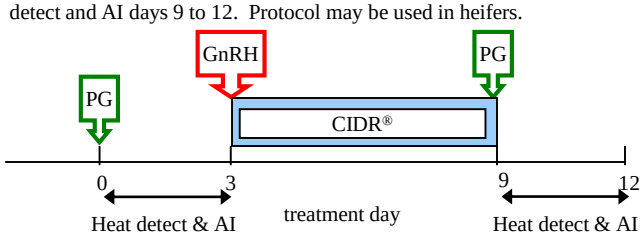



### Select Synch + CIDR®




### PG 6-day CIDR®

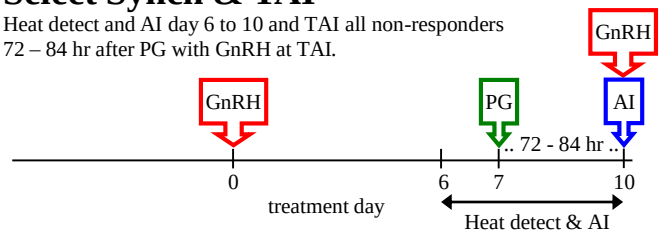
Heat detect and AI days 0 to 3. Administer CIDR to non-responders and heat detect and AI days 9 to 12. Protocol may be used in heifers.




## HEAT DETECT & TIME AI (TAI)

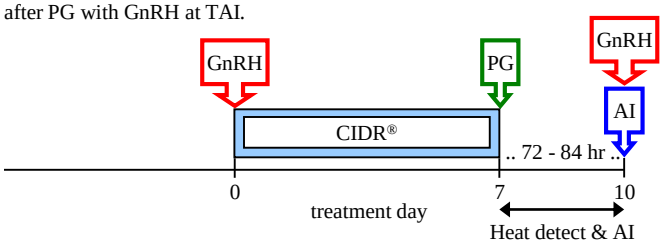
### Select Synch & TAI

Heat detect and AI day 6 to 10 and TAI all non-responders 72 – 84 hr after PG with GnRH at TAI.



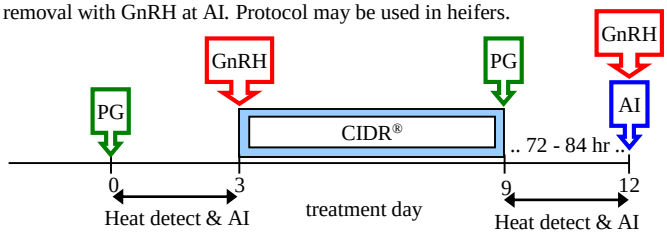

### Select Synch + CIDR® & TAI

Heat detect and AI day 7 to 10 and TAI all non-responders 72 - 84 hr after PG with GnRH at TAI.




### PG 6-day CIDR® & TAI

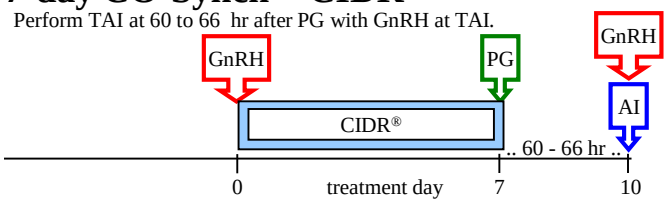
Heat detect & AI days 0 to 3. Administer CIDR to non-responders & heat detect and AI days 9 to 12. TAI non-responders 72 - 84 hr after CIDR removal with GnRH at AI. Protocol may be used in heifers.




## FIXED-TIME AI (TAI)*

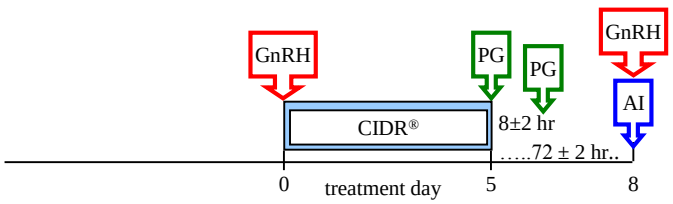
### 7-day CO-Synch + CIDR®

Perform TAI at 60 to 66 hr after PG with GnRH at TAI.




### 5-day CO-Synch + CIDR®

Perform TAI at 72 ± 2 hr after CIDR removal with GnRH at TAI.
Two injections of PG 8 ± 2 hr apart are required for this protocol.

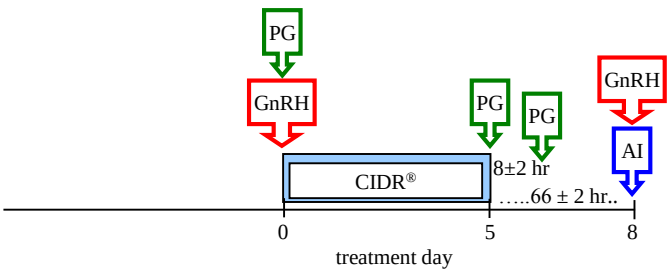



## FIXED-TIME AI (TAI)*

### for *Bos Indicus* cows only

### PG 5-day CO-Synch + CIDR®

Perform TAI at 66 ± 2 hr after CIDR removal with GnRH at TAI.
Two injections of PG 8 ± 2 hr apart are required for this protocol.




* The time listed for "Fixed-time AI" should be considered as the approximate average time of insemination. This should be based on the number of cows to inseminate, labor, and facilities.

These protocol sheets were assembled by the ***Beef Reproduction Task Force***. Programs are intended to promote sustainable food production systems by the beef industry through sound reproductive management practices for replacement heifers and postpartum cows. The Beef Reproduction Task Force recommends working with a licensed veterinarian for proper use and application of all reproductive hormones. **Approved 8-28-18**.

GnRH — Cystorelin®, Factrel®, Fertagyl®, OvaCyst®, GONABreed®

PG — estroPLAN®, Estrumate®, In-Synch®, Lutalyse®, Lutalyse® *HighCon*, ProstaMate®, SYNCHSURE™

# BEEF HEIFER PROTOCOLS - 2019

## HEAT DETECTION

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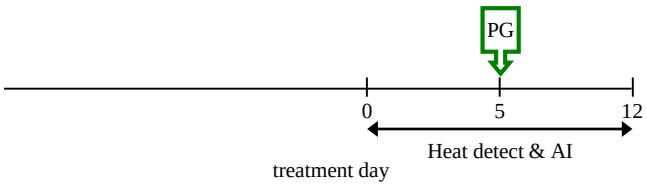
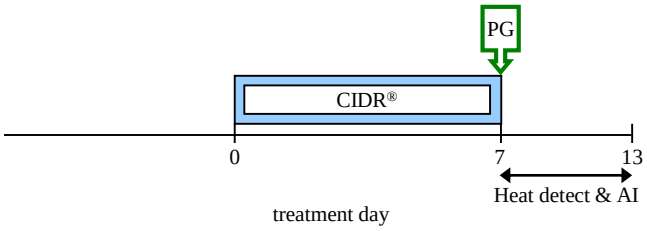
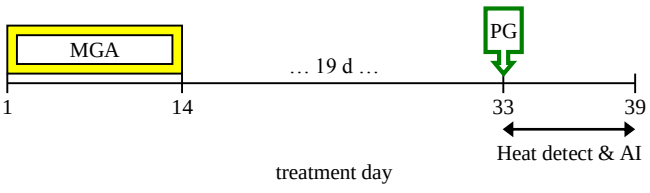



### 7-day CIDR®-PG



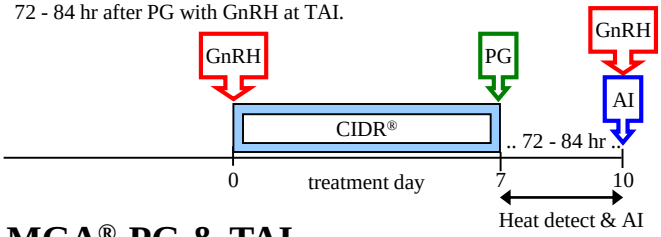

### MGA®-PG




## HEAT DETECT & TIME AI (TAI)

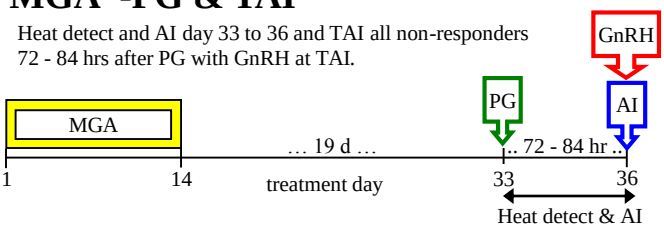
### Select Synch + CIDR® & TAI

Heat detect and AI day 7 to 10 and TAI all non-responders
72 - 84 hr after PG with GnRH at TAI.



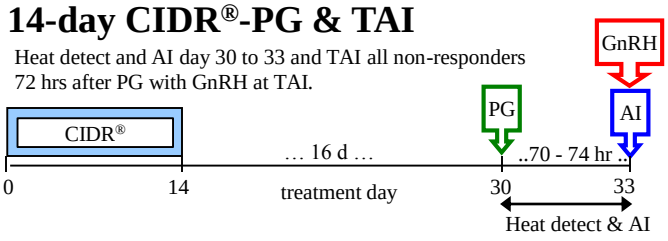

### MGA®-PG & TAI

Heat detect and AI day 33 to 36 and TAI all non-responders
72 - 84 hrs after PG with GnRH at TAI.




### 14-day CIDR®-PG & TAI

Heat detect and AI day 30 to 33 and TAI all non-responders
72 hrs after PG with GnRH at TAI.

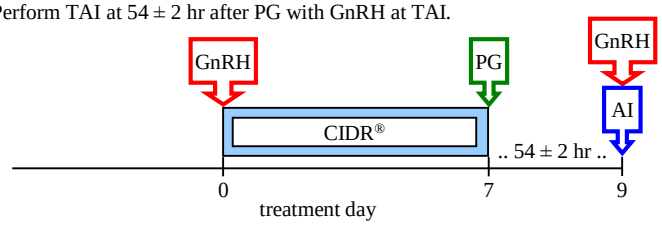



## FIXED-TIME AI (TAI)*

### Short-term Protocols

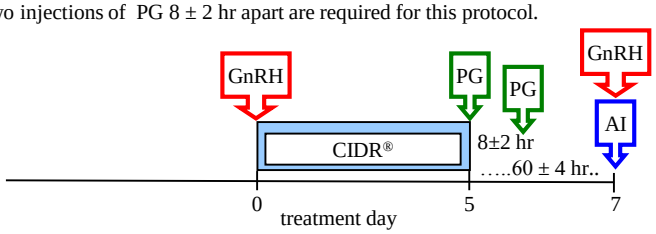
#### 7-day CO-Synch + CIDR®

Perform TAI at 54 ± 2 hr after PG with GnRH at TAI.




#### 5-day CO-Synch + CIDR®

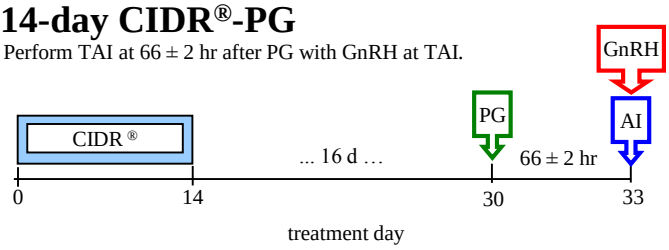
Perform TAI at 60 ± 4 hr after CIDR removal with GnRH at TAI.
Two injections of PG 8 ± 2 hr apart are required for this protocol.




### Long-term Protocols

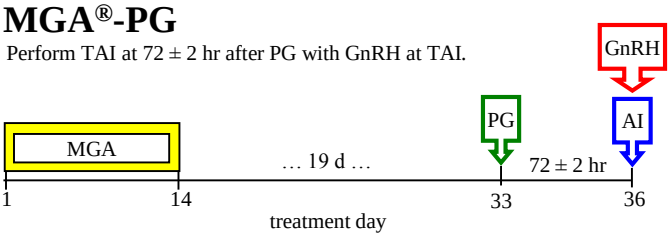
#### 14-day CIDR®-PG

Perform TAI at 66 ± 2 hr after PG with GnRH at TAI.




#### MGA®-PG

Perform TAI at 72 ± 2 hr after PG with GnRH at TAI.

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GnRH: Cystorelin®, Factrel®, Fertagyl®, OvaCyst®, GONABreed®

PG: estroPLAN®, Estrumate®, In-Synch®, Lutalyse®, Lutalyse® *HighCon*, ProstaMate®, SYNCHSURE™