Review Report

Estrus and ovulation synchronization strategies in beef cattle

Ramanathan Kasimanickam,[a] Quinlan Harting,[a] Rachel Hanson,[b] Randa Boler[a]
[a]Veterinary Clinical Sciences, Washington State University, Pullman, WA, USA
[b]Animal Health Clinic, Blackfoot, ID, USA

Abstract

Various technologies in bioveterinary medicine offer beef producers unique opportunities to improve overall herd genetics. Research and technology have greatly enhanced our understanding of cattle reproductive physiology, facilitating induction and synchronization of estrus and/or ovulation in replacement heifers and postpartum cows. These improvements assist beef producers to increase the use of artificial insemination (AI) and facilitate mass breeding at predetermined times. In addition to improving genetics, this helps to increase uniformity (genetics and body weight) at weaning. Pregnancy rates following implementation of these approaches are acceptable and generally comparable to breeding after detecting estrus. This review explains dynamics of synchronization, treatment regimens for various protocols, and factors that need to be considered while implementing protocols to achieve greater success.

Keywords: Beef cattle, estrus, ovulation, synchronization, artificial insemination

Introduction

Sustainable beef production targets long-term health of the environment, maintains economic viability of the beef enterprise, and addresses consumer concerns. The economic advantage of a cow-calf operation is realized by a sensible and achievable production goal, 1 calf/cow/year, with a mean calving interval of 365 days. Approximately 285 days of pregnancy allows only 80 days for the cow to become pregnant again. During this interval, the cow must recover from calving, resume cyclicity, and have 2 or 3 opportunities to become pregnant.

Synchronization of estrus and/or ovulation is a reproductive management tool to increase efficiency and profitability in beef production. Although it is to shorten breeding and calving intervals, other benefits include optimizing labor and time and facilitating AI.

In the USA, > 66% of dairy cows are AI bred; however, only 7.9% beef operations use estrus synchronization and 7.6% of operations use AI.[1,2] Time and labor are the primary reasons producers indicate as barriers to using AI in commercial beef cows and heifers.[1,2]

There are many options with various treatment regimens for synchronization of estrus and/or ovulation in beef cattle. Before selecting an approach for mass breeding, it is imperative to assess key elements, including status of cattle intended for synchronization. Consideration of key traits such as body weight/condition, pubertal status, pelvic size, and temperament of heifers; and in cows, body condition, days postpartum and temperament, will help improve success. Evaluation of resources, including facilities, availability of labor, prior experience, and budget will help to select an appropriate synchronization protocol. In addition, duration of the protocol, number of animal handlings, ability to successfully provide treatments, and proper AI techniques (compliance) are other determining factors for a successful outcome. These key elements are discussed.

Calf crop and estrus synchronization

$$\text{Percentage annual calf crop} = \frac{\text{\# of calves weaned}}{\text{\# of females exposed}} \times 100 .$$

Although 90-95% of calf crop is achievable in a year, economic benefits are determined by pregnancy rate and pregnancy early in the breeding season which translates to calving rate and calving early in the calving season.[1-3] This goal may be attained more easily with estrus synchronization and AI programs. Realistically, > 50% of eligible beef females become pregnant to 1 AI after implementation of effective estrus/ovulation synchronization and AI programs.[3-5] Estrus synchronization optimizes labor and time and facilitates AI;[3] the latter allows access to superior genetics, hastens genetic

CONTACT Ramanathan Kasimanickam **ramkasi@wsu.edu**

Citation: Clinical Theriogenology 2025, 17, 11650, http://dx.doi.org/10.58292/CT.v17.11650

improvement within a herd, and is frequently cheaper than natural service (NS).[3-5]

Synchronized females: 1. express estrus at a controlled time; 2. intensify calf uniformity; 3. calve earlier in the season; and 4. wean calves that are older and heavier, all of which can increase economic return. Economic return is calculated as: weight of calves at sale (lbs) × calf crop = lbs of beef produced/cow exposed. The following AI and NS combinations (Table 1) were used in a trial (n = 1,249) involving 12 cow-calf operations and clearly indicated benefits of using AI in beef operations.

There was an increase in pregnancy rate by 5-9% across the 12 locations, fewer assisted births (1.3 versus 2.9%), lower death loss (3.5 versus 5.5%) and overall, 10% more calves when dams and daughters were bred by AI/AI combination compared to NS/NS combination (personal communication, William Whittier, December 23, 2024). Increased age at weaning, improved pregnancy rates, and potential for increased growth due to improved genetics resulted in reported weaning weight increases (20-40 lbs) for the entire calf crop.[6]

Estrous cycle

Estrous cycle consists of follicular and luteal phases. The follicular phase includes the interval from corpus luteum (CL) regression to ovulation, including proestrus and estrus stages of the cycle. During the follicular phase, the dominant ovarian structure is a mature and estrogenic follicle that releases estrogen, the predominant hormone. Physiological events duing the follicular phase include sexual receptivity, preparation of the dominant follicle for ovulation, gonadotropin release from the anterior pituitary, and ovulation. The luteal phase includes the interval from ovulation (CL formation) to CL regression, including metestrus and diestrus. During the luteal phase, the dominant ovarian structure is a CL that produces progesterone, the dominant hormone. Physiological events during the luteal phase include formation of a CL, progesterone production by the CL, uterine quiscence, release of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) from the endometrium, and lysis of the CL.

Table 1. AI and NS combinations in beef cattle

Dam bred by	Daughter bred by
AI	AI
AI	NS
NS	AI
NS	NS

Control of follicular and lutal phases

To synchronize estrus and/or ovulation in all eligible cows, the follicular phase is controlled by initiating emergence of a new follicular wave, whereas the luteal phase is controlled by initiating and/or prolonging the lifespan of the CL by gonadotropin releasing hormone (GnRH) treatment to induce ovulation and CL formation, or by supplementing exogenous progesterone (with or without a CL). Liming factors, principles and options to control follicular and luteal phases in beef females with varying physiological status are illustrated in Table 2.

Drugs and dosages

Prostaglandin $F_{2\alpha}$

Intramuscular dinoprost tromethamine	:	25 mg
Intramuscular cloprostenol	:	500 µg
Intramuscular GnRH	:	100 µg

Progesterone

Controlled Internal Drug Release

(CIDR) inravaginal insert	:	1.38/1.55/1.9 gram for 5-14 days
Oral melengestrol acetate (MGA)	:	0.5 mg/head/day mixed in feed

Oral supplementation of MGA is approved for estrus suppression in heifers only (Federal Register, 1997). Use of MGA as part of any estrus synchronization protocol in beef cows constitutes an extra-label use of medicated feed that is prohibited by the animal medicinal drug use and clarification act and Regulation 21 CFR 530.11(b). Food and Drug Administration (FDA) approved pharmacuticals used for synchronization in cattle are given in Table 3.

Estrus synchronization treatment regimens

Synchronization of estrus in beef females for mass breeding involves: 1. shortening the luteal phase by inducing premature luteolysis or 2. prolonging the luteal phase by maintaining

Table 2. Principles, liming factors and options to control follicular and luteal phases

Criteria	Follicular phase	Luteal phase
How could you control?	Initiating a new follicular wave	Shortening (or) prolonging CL lifespan
What is the limiting factor?	Presence of a dominant follicle	Shortening - presence of CL and CYCLICITY are needed Prolonging - presence of CL and CYCLICITY do not matter
How do you do this?	Removing dominant follicle	Shortening - lyse CL Prolonging - supplement progesterone
What methods could be employed?	Giving GnRH/LH/hCG), estradiol (varies with country) Remove follicle with ultrasound-guided aspiration	Shortening – give $PGF_{2\alpha}$ – need a responsive/active CL Prolonging – induce CL and/or give intravaginal or oral progesterone

Citation: Clinical Theriogenology 2025, 17, 11650, http://dx.doi.org/10.58292/CT.v17.11650

Table 3. Available FDA-Approved drugs to control and synchronize estrous cycles in cattle (refer animal drugs @ FDA for specific information about each drug)

Regimen	Drug name (active ingredient)	Application number and manufacturer	Sequential use with another drug
Gonadorelin-prostaglandin	Factrel® Injection (gonadorelin injection)	NADA 139-237 Zoetis Inc.	Lutalyse® or Lutalyse® *HighCon* (Dinoprost tromethamine)
	Fertagyl® (gonadorelin)	ANADA 200-134 Intervet, Inc.	Estrumate® (Cloprostenol sodium)
	GONAbreed® (gonadorelin acetate)	ANADA 200-541 Parnell Technologies Pty. Ltd.	Cloprostenol sodium
	CYSTORELIN® (gonadorelin)	NADA 098-379 Boehringer Ingelheim Animal Health, Inc.	Cloprostenol sodium
Progestin only	EAZI-BREED™ CIDR® (progesterone intravaginal insert)	NADA 141-200 Zoetis Inc.	-
Progestin-prostaglandin	EAZI-BREED™ CIDR® (progesterone intravaginal insert)	NADA 141-200 Zoetis Inc.	Lutalyse® or Lutalyse® HighCon
Prostaglandin only	Lutalyse® or Lutalyse® HighCon Injection	NADA 108-901 & NADA 141-442 Zoetis Inc.	-
	Estrumate®	NADA 113-645 Intervet, Inc.	-
	ProstaMate™ (dinoprost tromethamine)	ANADA 200-253 Bimeda Animal Health Ltd.	-
	estroPLAN® (cloprostenol sodium)	ANADA 200-310 Parnell Technologies Pty. Ltd.	-
	Synchsure (cloprostenol sodium)	ANADA 200-310 Boehringer Ingelheim Animal Health	-

NADA: new animal drug applications; ANADA: abbreviated new animal drug applications

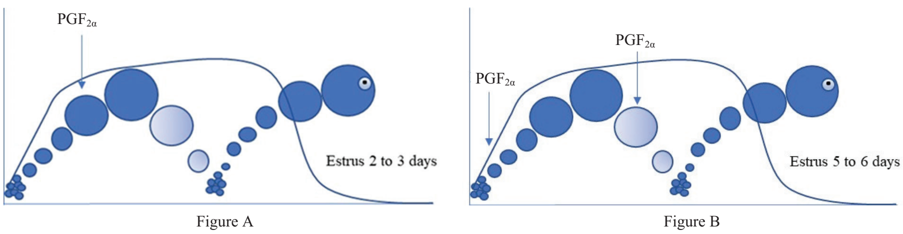

Figure 1. Schematic presentation of interval from $PGF_{2\alpha}$ treatment and estrus expression based on follicle size and phase of the follicular wave in cattle

circulating progesterone concentrations via supplementation (daily oral, slow-release injectable (ear implant), or vaginal inserts). The injectable method is uncommon in cattle in North America.

Shortening luteal phase

Luteolytic dose of $PGF_{2\alpha}$ or its analog given to eligible beef females (with a mature active CL) results in luteolysis, and estrus[7,8] occurs within 2-6 days (Figure 1),[9] depending on follicle diameter and phase of the follicular wave. When $PGF_{2\alpha}$ is given during growing and static phases, estrus occurs soon (Figure 1A), whereas estrus occurs later if $PGF_{2\alpha}$ is given during the regressing phase (Figure 1B) as it takes time for a new dominant follicle to emerge and reach preovulatory size. $PGF_{2\alpha}$ causes luteolysis, with subsequent surges in estrogen and LH, with ovulation occuring ~ 24 hours after the LH surge.

$PGF_{2\alpha}$ programs

$PGF_{2\alpha}$ is generally inexpensive; single, double, or biweekly programs are effective only in cycling females when a CL is mature/responsive.[10] CL becomes responsive to $PGF_{2\alpha}$ ~ 7 days after ovulation. There are several $PGF_{2\alpha}$ protocols, according to management needs (Figures 2-4). However, implementation of a successful estrus detection program is essential to achieve good results with $PGF_{2\alpha}$ programs. The AI is performed following the AM-PM rule (i.e. a cow should have AI 12 hours after first being observed in estrus).[11,12] If a cow is noticed in standing estrus in the AM, AI is performed that PM, whereas cows observed in standing estrus in the PM should AI is performed the following AM. The AM-PM rule requires twice daily AI. However, recent studies recommended that AI should be performed 6-18 hours after first observation of standing estrus.[13] Conception rate to AI following implementation of $PGF_{2\alpha}$ program is generally similar to AI following spontaneous estrus.

Weekly Monday morning program

On Monday morning, all eligible cows receive an injection of $PGF_{2\alpha}$, followed by estrus detection for the remainder of the week and cows detected in estrus have AI following the AM-PM rule (Figure 2). Approximately 65% of cows are expected to exhibit estrus. Cows not detected in estrus receive $PGF_{2\alpha}$ the following Monday morning along with new group of eligible cows and the same procedure is followed every week. Cows given weekly doses of prostaglandin had a 30% higher pregnancy rate than those receiving prostaglandin based on transrectal palpation of a CL.[14]

Biweekly Monday morning or double $PGF_{2\alpha}$ program

On Monday morning, all eligible cows receive an injection of $PGF_{2\alpha}$, with[6] or without AI for cows exhibiting estrus after $PGF_{2\alpha}$ treatment. Two weeks later (Monday), cows that did not have AI[15] or all cows, receive another dose of $PGF_{2\alpha}$, followed by detection of estrus for the remainder of the week, with AI based on the AM-PM rule (Figure 3). Approximately 85-90% of cows should exhibit estrus. This program requires weekly or biweekly estrus detection.

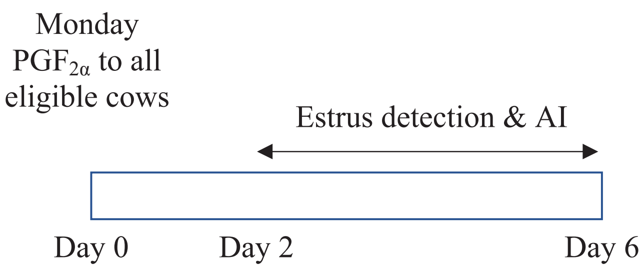

Figure 2. Schematic presentation of weekly monday morning program in cattle

There are several $PGF_{2\alpha}$ programs, including $PGF_{2\alpha}$ treatment for nonpregnant cows with an active CL at pregnancy diagnosis or repeated biweekly $PGF_{2\alpha}$ treatment as a postpartum reproductive management tool[16] at 25-32, 39-46, and 53-60 days, with first insemination following the last injection. Cows not inseminated after $PGF_{2\alpha}$ injection between 53-60 days are given $PGF_{2\alpha}$ 14 days later.

$PGF_{2\alpha}$ programs are popular in dairy operations. However, some beef operations implement $PGF_{2\alpha}$ program before/after exposing cows to bulls (Figure 4). This apporach generally resulted in more pregnancies and more calves compared to no $PGF_{2\alpha}$ program before or after bull introduction.

Inducing or prolonging luteal phase

Luteal phase can be achieved by induction of ovulation with GnRH and subsequent CL formation and/or by withdrawal of progesterone following supplementation for 5 or 7 days and a concommitant luteolytic dose of $PGF_{2\alpha}$ will result in a decline in progesterone concentration to basal values and estrus.

Select Synch programs

Several Select Synch protocols are available for use in AI or NS breeding programs, including GnRH + $PGF_{2\alpha}$, CIDR + $PGF_{2\alpha}$, and MGA + $PGF_{2\alpha}$.[17-19]

GnRH and $PGF_{2\alpha}$

GnRH treatment on day 0 (random stages of the estrous cycle) is to control follicular wave emergence and/or to induce CL formation; $PGF_{2\alpha}$ is given on days 6 or 7, observe for estrus days 2-6 after $PGF_{2\alpha}$, and AI cows that express estrus (AM-PM rule) (Figure 5). Conception rate is similar to AI following spontaneous estrus and $PGF_{2\alpha}$-induced estrus. These programs are ideal for smaller beef operations.

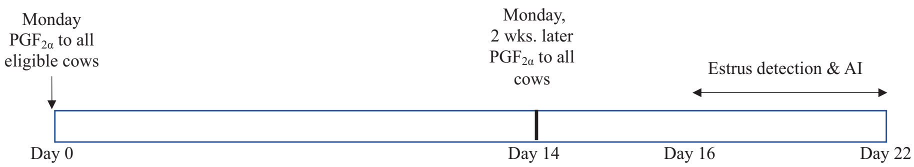

Figure 3. Schematic presentation of biweekly Monday morning program: in cattle

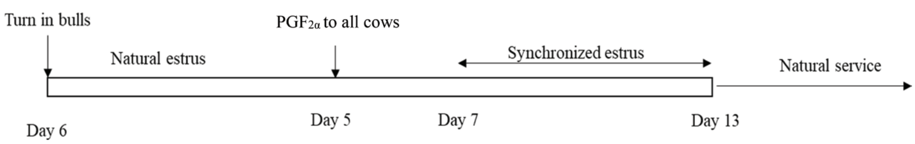

Figure 4. $PGF_{2\alpha}$ program before/after exposing beef females to bulls

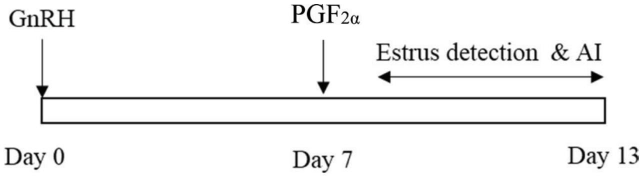

Figure 5. Schematic presentation of Select Synch estrus synchronization program

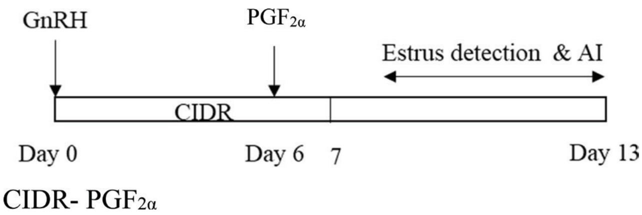

Figure 6A. Schematic presentation of Select-Synch + CIDR estrus synchronization protocol with $PGF_{2\alpha}$ treatment the day before CIDR removal

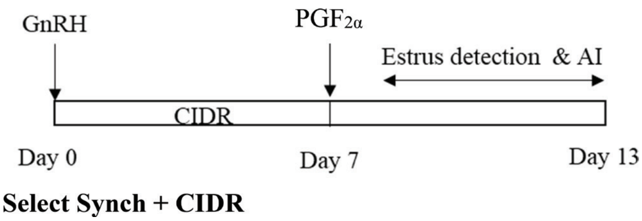

Figure 6B. Schematic presentation of Select-Synch + CIDR estrus synchronization protocol

CIDR + $PGF_{2\alpha}$ or Select Synch + CIDR

Cows receive a CIDR (1.3 g progesterone) insert for 7 days and an injection of $PGF_{2\alpha}$ the day before (day 6) (Figure 6A) or at CIDR removal (day 7) (Figure 6B), with improved estrus synchrony and conception rates. When GnRH was given 6 or 7 days prior to $PGF_{2\alpha}$, 70-83% of cows were in estrus within 4 days.[20] It is advantageous to include the CIDR when more cows are anestrus and/or when estrus detection before $PGF_{2\alpha}$ treatment is not feasible. With Select Synch, 5-20% of cattle may exhibit estrus 2 days before $PGF_{2\alpha}$ treatment. The best strategy is to apply both protocols to the same group of cows, placing CIDRs in young, thin, and/or late-calving cows. In heifers, progesterone supplentation (oral or intravaginal) promotes cyclicity.

MGA

MGA is an orally active progestin; fed at 0.5 mg/day per animal, estrus is suppressed and ovulation is prevented.[18,21] Feeding MGA is specifically approved for estrus suppression only in heifers. Level of feeding and consumption of MGA are critical to success. In this program, beef heifers are fed MGA for 14 days, followed by a $PGF_{2\alpha}$ injection 19 days later on day 33.[18,21] This will have heifers in the late luteal stage of the estrous cycle at $PGF_{2\alpha}$ injection, and will maximize conception rate (Figure 7A). Alternately, if there is a concern about consistent delivery of MGA, producers/clinicians can use a CIDR insert in place of MGA (Figure 7B). Both MGA and CIDR control the estrous cycle.[22-24] Furthermore, a 7-11 modified MGA synchroinzation protocol reduces the protocol from 37 to 26 days (Figure 8). The 7-11 program[25,26] utilizes a 7-day progestin supplementation (MGA; fed from days 0 to 7) with $PGF_{2\alpha}$ given at the conclusion of the MGA feeding (day 7). GnRH is

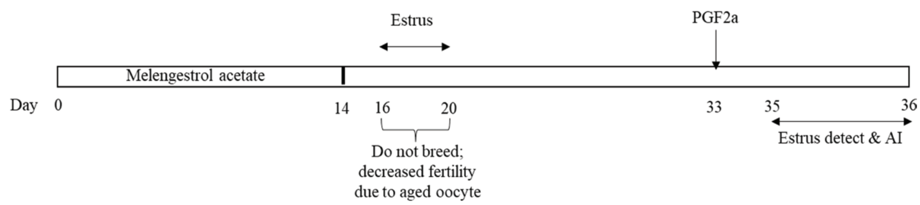

Figure 7A. Schematic presentation of 14 d MGA + $PGF_{2\alpha}$ estrous synchronization protocol in beef heifers

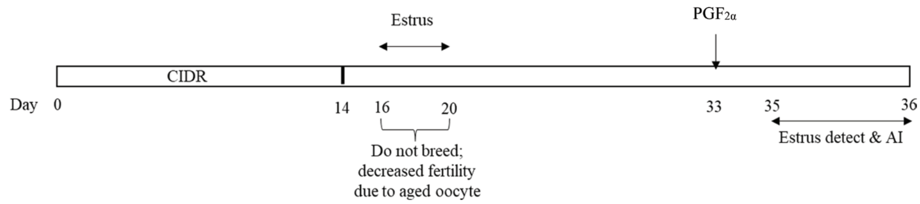

Figure 7B. Schematic presentation of 14 d CIDR + $PGF_{2\alpha}$ estrous synchronization protocol in beef heifers

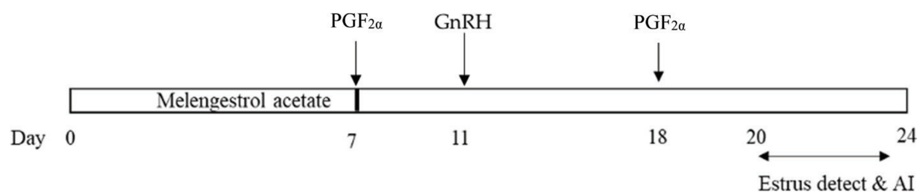

Figure 8. Schematic presentation of 7-11 Synch program

given on day 11, $PGF_{2\alpha}$ on day 18, estrus detected from days 20-24, with AI using AM-PM rule.

MGA programs are used for NS and AI. Field trials involving heifers where MGA was used in conjunction with NS, or with $PGF_{2\alpha}$ prior to AI at oberved estrus.[27] The pregnancy rate for synchronized estrus was 9 percentage points greater for NS over AI, substantiating the flexibility in implementing precise synchronization protocols with this particular management system.[27] Further, the MGA + Select protocol involves an injection of GnRH on day 26 in a 14-day MGA + $PGF_{2\alpha}$ program.[28,29] Although pregnancy rate to synchronized estrus in 2-4 year old cows were similar to both MGA-Select and MGA + $PGF_{2\alpha}$ protocols (62 versus 69%, respectively), the MGA + Select program is advantageous, as more cows < 5 years of age became pregnant compared to an MGA +$PGF_{2\alpha}$ program (71 versus 46%).[28,29]

Substituting CIDR inserts for MGA in the MGA + Select protocol in beef heifers were evluated. Although pregnancy per AI was greater in CIDR versus MGA-treated heifers (63 versus 47%; $p < 0.01$), final pregnancy rate did not differ ($p > 0.10$) between treatments.[25] Pregnancy rates to AI were similar following implementation of 14-day MGA + $PGF_{2\alpha}$ and 7-11 synch protocols.[30] AI success with various protocols is illustreated (Table 4).

Ovulation synchronization

Ovulation synchronization is aimed at synchronizing ovulation and allowing TAI rather than relying on estrus detection to determine when to breed. Ovulation synchronization is based on forcing 'turnover' of a dominant follicle as it is the main limiting factor to synchronize emergence of a new follicular wave in all eligible beef females at random stages of the estrous cycle at protocol initiation. In addition, the ovulation synchronization program promotes oocyte viability. As illustrated (Figure 9), 3 principles are involved and should be achieved in a timely manner to promote success of ovulation synchronization programs. Intiation of a new follicular wave and synchronized follcular wave growth can be achieved by GnRH, estrogen (licensed use varies with country), or follicle ablation.

GnRH

GnRH induces ovulation/luteinization of follicles > 10 mm in 85% of cows and 55% of heifers when injected at random times of the estrous cycle. Further, treatment of GnRH at random stages of the estrous cycle inititates new follicular wave emergence in ~ 3-4 days.[20,41]

Estrogen

Estrogen causes regression of FSH-dependant follicles and luteinization/ovulation of LH dependent/estrogenic follicles via both negative and positive feedback. New follicular wave emergence occurs ~ 4 days later, depending on the type of estradiol used. Follicular wave emergence occurs 3.6, 4.1, and 4.4 days after estadiol 17β, estradiol benzoate (EB), and estradiol cypionate, respectively.[42-44] It should be noted that when

Table 4. Conception per AI following implementation of estrous synchronization protocols in beef heifers and cows

Protocol	CR/AI% (Total female)	Age group
Select Synch + CIDR*	53.8 (323/600)	Cows[31]
Select Synch + CIDR* (SS)	65.0 (160/246)	Cows[32]
Select Synch + CIDR* (CS)	66.7 (164/267)	
Select Synch + NS	56.9 (249/438)	Heifers[33]
Select Synch + CIDR*	46.9 (160/341)	Cows[34]
14-day CIDR + $PGF_{2\alpha}$	61.7 (267/433)	Heifers[35]
5-day Select Synch + CIDR	64.8 (287/443)	
Select Synch + CIDR*	55.5 (394/710)	Cows[36]
2 $PGF_{2\alpha}$ (14 days)	52.3 (376/723)	Heifers[37]
Select Synch	60.2 (100/166)	
CIDR + $PGF_{2\alpha}$	59.1 (528/894)	
5 day Select Synch + CIDR	72 (33/46)	Cows[38]
7 day Select Synch + CIDR	72 (36/50)	
CIDR+ $PGF_{2\alpha}$	61 (197/325)	Cows[39]
Select Synch	70 (217/309)	
Select Synch + CIDR	67 (230/345)	
MGA/Select Synch	46.0 (185/402)	Heifers[30]
MGA/ $PGF_{2\alpha}$	47.0 (185/394)	
Select Synch	47.0 (21/45)	Cows[25]
7-11 Synch	68.0 (30/44)	
Select Synch	65.7 (115/175)	Cows[40]
Select Synch + P_4	59.1 (123/208)	
2 $PGF_{2\alpha}$ (14 days)	60.6 (86/142)	

Citation: Clinical Theriogenology 2025, 17, 11650, http://dx.doi.org/10.58292/CT.v17.11650

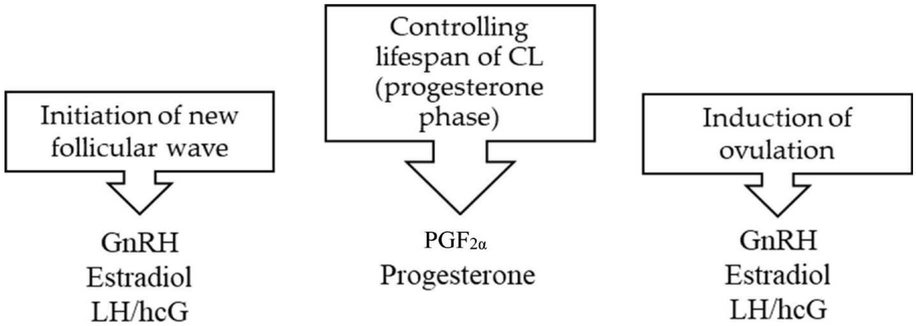

Figure 9. Illustration of 3 principles that are involved in the success of ovulation synchronization programs

using estrogen for follicular suppression, treatment of estrogen and progesterone is advisable (addition of progesterone is to suppress estrogen induced LH surge), whereas estrogen alone is sufficient in combination with a CIDR.

Limitations of synchronizing follicular waves

The simplistic explanation is the average length of estrous cycle is 21 days, with a range from 16 to 24 days. A typical bovine estrous cycle has 2 or 3 waves of follicle development, and the dominant follicle of the last wave ovulates. The follicular wave consists of 3 phases (growing, static, and regression) with 3 points (recruitment, selection, and dominance).

It should be noted that an 18-21 day range is associated in a 2 wave cycle and a 21-24 day range is associated with a 3 wave cycle.[45] Two-wave cycles are common in cattle and cycles with 1 or 4 waves cycle occur incidentally. Wave length in prepubertal heifers are 8 days. The interovulatory intervals of 3 wave cycles differed from 2 wave cycle in: 1. earlier emergence of the dominant follicles; 2. longer duration; and 3. shorter interval from emergence to ovulation. The reason for 2 or 3 wave cycles is unclear. Poor nutrition and stress (3 wave), lifespan of CL (shorter in 3 wave) and slowly growing dominant follicles (2 wave) are associated with the difference.[45]

Differences in number of follicular waves and range of estrous cycle and follicular wave lengths are illustrated (Table 5). It depicts variations in follicular dynamics when implementing estrus or ovulation synchronization protocols in a group of eligible beef females at random stages of the estrous cycle. Follicular wave length could vary 6-12 days.

While implementing synchronization, not only these variations, but prepubertal and peripubertal status in heifers and postpartum anestrus in cows and ovarian disorders such as cystic ovarian degenration and uterine disorders such as clinical and subclinical endometritis should also be taken in to account.

Ovulation synchronization treatment regimens with combinations of GnRH and $PGF_{2\alpha}$

Figure 10 shows the basic ovulation synchronization protocols that utilize the combination of GnRH and $PGF_{2\alpha}$.

Both Ovsynch and Presynch-Ovsynch are commonly used in dairy operations. Ovsynch includes a first dose of GnRH given on a random day of the estrous cycle (day 0). Seven days later, $PGF_{2\alpha}$ (day 7) is given and 48 hours later, a second dose of GnRH is given, with TAI 16 hours later,[46] requiring 4 handlings. Presynchronization, the initial portion of the protocol that precedes the Ovsynch portion, is achieved with 2 $PGF_{2\alpha}$ treatments, with a 2 week interval between the first and second $PGF_{2\alpha}$ treatment, in the weeks leading up to the initiation of Ovsynch. However, interval between the second $PGF_{2\alpha}$

Table 5. Differences in range of estrous cycle, number and length of follicular waves

Estrous cycle length	2 wave cycle	3 wave cycle
Short range – 18 day cycle	9 days per wave	**6 days per wave**
Long range – 24 day cycle	**12 days per wave**	8 days per wave
Average – 21 day cycle	10.5 days per wave	7 days per wave

The underline highlights the difference in the wave's length between a long- and short-range cycle and between a two- and three-wave cycle.

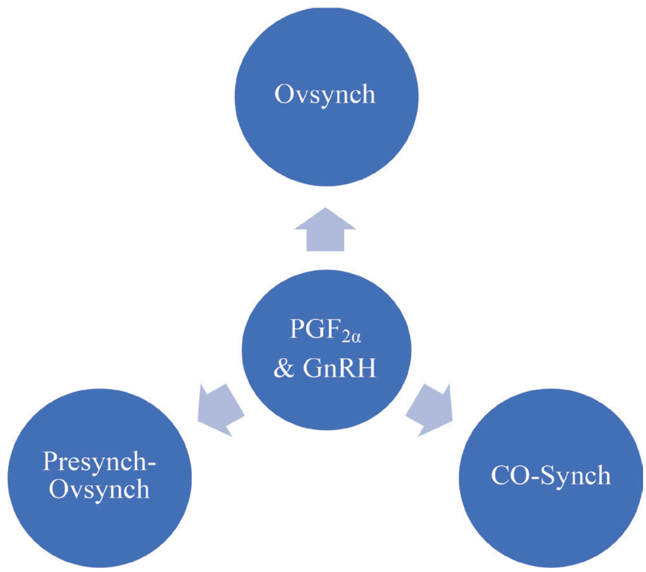

Figure 10. Ovulation synchronization protocols with the combination of GnRH and $PGF_{2\alpha}$

treatment and first GnRH of Ovsynch protocol is more variable. Usually, the selected interval is 10-14 days. The general objective of presynchronization is to increase the percentage of cows that are on days 5-8 of their estrous cycle, with intermediate circulating progesterone concentrations and a healthy, dominant follicle that can ovulate in response to first GnRH of Ovsynch protocol treatment.

CO-Synch

The CO-Synch protocol is one of the most commonly used protocols across the industry for TAI of beef females. The CO-Synch protocol, is similar to an Ovsynch protocol, except the second GnRH is given at TAI, with only 3 handlings. The CO-Synch protocol is implemented with or without CIDR. The CO-Synch + CIDR protocol was reasonably effective among pre/peri pubertal heifers and anestrous cows and it is a good option when a minimal number of animal handlings is desired.

CO-Synch without CIDR

Heifers are injected with GnRH on day 0 and $PGF_{2\alpha}$ is given on day 7. Heifers are inseminated 60 ± 4 hours after $PGF_{2\alpha}$ injection and a second dose of GnRH is given at TAI (Figure 11A).

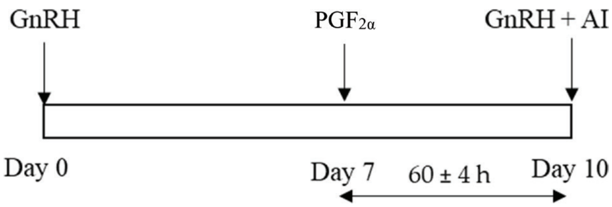

Figure 11A. Schematic presentation of CO-Synch ovulation synchroniazation protocol in heifers

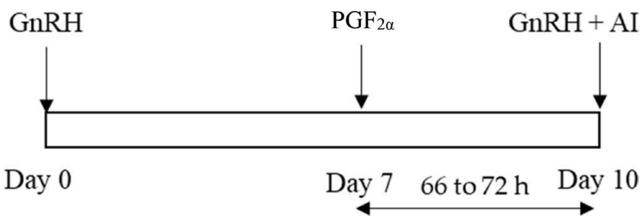

Figure 11B. Schematic presentation of CO-Synch ovulation synchroniazation protocol in cows

Cows are injected with GnRH on day 0, an injection of $PGF_{2\alpha}$ is given on day 7, with TAI (and a concommittant injection of GnRH) between 66 and 72 hours after $PGF_{2\alpha}$ injection.

CO-Synch + CIDR

The CO-Synch + CIDR program is similar to CO-Synch; in addition, a CIDR insert is placed and removed after 7 days (from GnRH to $PGF_{2\alpha}$ treatment). Heifers are inseminated at 60 ± 4 hours after $PGF_{2\alpha}$ injection and a second GnRH is given at TAI (Figure 12A). In cows, insemination is done between 66 and 72 hours after $PGF_{2\alpha}$, with a second GnRH at TAI (Figure 12B).

Pregnany per AI following utilization of various CO-Synch protocols, with or without CIDR, in cows and heifers that received insemination at a various fixed times following CIDR removal is given in Table 6.

5 day CO-Synch + CIDR

This protocol was developed based on the premise that reducing the length of CIDR treatment 7-5 days in the CO-Synch + CIDR protocol would increase secretion of estradiol by the preovulatory follicle, decrease incidence of induced ovulation of follicles with reduced estrogenic activity, and potentially improve TAI pregnancy rates, based on the assumption that day-4 dominant follicles have higher intrafollicular estradiol-17β.[49-51]

This protocol is similar to the 7 day CO-Synch + CIDR but involves a shorter interval (5 days) of CIDR treatment.[49,52] However, it requires giving 2 doses of $PGF_{2\alpha}$ approximately 6 to 8 hours apart (additional handling of cows and cost of second $PGF_{2\alpha}$). A larger field trial (n = 1817) had a small improvement in pregnancy rates to AI following a 5 day CO-Synch + CIDR protocol compared to the 7 day CO-Synch + CIDR

Table 6. Pregnancy per AI (P/AI) following implementation of estrous synchronization protocols, with or without progesterone supplementation in beef heifers and cows

Protocol	P/AI (total females)	Age group
CO-Synch + CIDR (72 hours)	65.0% (5470)	Heifers[47]
CO-Synch (72 hours)	55.3% (5099)	
CO-Synch + CIDR (60-66 hours)	54% (2868)	Cows[48]
CO-Synch (60-66 hours)	52% (871)	

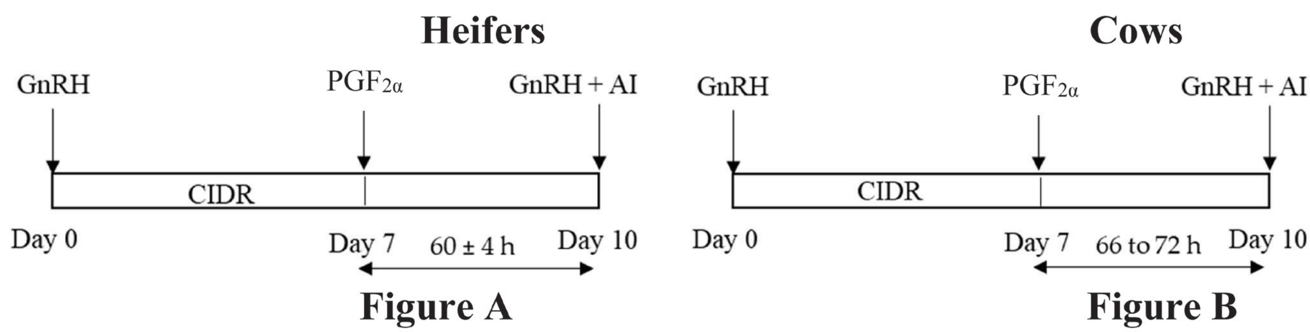

Figure 12. Schematic presentation of CO-Synch + CIDR ovulation synchroniazation protocol

protocol (58.1 versus 55.1%; p = 0.04) in cows,[53] whereas in heifers (n = 289), a 5 day CO-Synch + CIDR protocol tended to be greater (63.8 versus 53%; p = 0.07).[54]

Split-time AI

Pregnancy rates/AI can be optimized with a split-timed AI (STAI) approach. The rationale is that pregnancy percentages are greater in beef females that express estrus before insemination. The STAI involves not conducting AI in females that have not expressed estrus at the time of TAI. However, with heifers that have not expressed estrus at the time of TAI, there is an additional interval for behavioral estrus expression to occur before AI, with all heifers that have not expressed estrus by TAI being inseminated at the second TAI, with GnRH treatment either at first or at second TAI to heifers not detected in estrus.

In a large field trial in beef heifers (n = 3166), P/AI percentages were 58.9,63.4, 56.5, and 56.5% for 14 days/STAI, 5 days/STAI, 14 days/TAI and 5 days/TAI, respectively. The study concluded the 5 day CIDR regimen with 64 + 84 hours split-time AI combination acheived > P/AI.[35] In beef cows (n = 1062), P/AI for cows in the 65 + 85 hours treatment combination was > at 36 days than for cows in the 55 + 75 hours treatment combination (61.0 versus 51.4%), respectively.[55]

Another trial in beef cows (n = 695) was conducted to compare pregnancy percentages per embryo transfer (P/ET) following twice daily compared to split-time (64/84-hours) estrus detection in a CIDR + Select treatment regimen.[34] Percentage P/ET for cows in the split-time and twice daily estrus detection groups did not differ (49.2 [174/354] verus 46.9 [160/341]; p > 0.1). Further, percentage conception/ET for cows in the split-time and twice daily estrus detection groups were 60.0% (174/290) and 56.3% (160/284), respectively (p > 0.1), whereas conception rates for ET at 64 and 84 hours were 61.5% (150/244) and 52.2% (24/46).

Presynchronization in beef heifers

Exogenous progesterone hastened cyclicity in pre and peripubertal beef heifers and also increased pregnancy per AI. Progesterone (cyclic) status or progesterone supplementation at onset of synchronization protocols are critical for favorable pregnancy outcomes.

Presynchronization is synchronization of the estrus cycle prior to synchronization for TAI. There are various presynchronization methods, including 1 dose of $PGF_{2\alpha}$ (10 days before initiation of protocol) or 2 doses of $PGF_{2\alpha}$, 10-14 days apart, with the second dose 10-14 days before protocol initiation,[56] GnRH alone[57] or combined with $PGF_{2\alpha}$,[57,58] or a CIDR for 5, 7, 9, 14 or 18 days[53,59,60] before protocol initiation. Beef herds with a high percentage of prepubertal or peripubertal heifers at the start of the breeding season may benefit from presynchronization.

Treatments such as CIDR and/or GnRH before initiating a TAI program may hasten puberty.[38,47,61-63] Dominant follicles are present in prepubertal and peripubertal heifers and may be induced to ovulate with exogenous GnRH, depending on follicle size and maturity. However, smaller follicles (< 11 mm) induced to ovulate were less likely to result in pregnancy than ovulation of larger follicles (11-16 mm).[19,64-66] Using a CIDR promotes ovulation in prepubertal and peripubertal heifers[47] and anestrus postpartum cows. Further, ovarian responses in Angus-cross beef heifers presynchronized with CIDR-GnRH before a CO-Synch protocol had more heifers with a CL at $PGF_{2\alpha}$ and increased preovulatory follicle diameter at AI compared to a CIDR only before a CO-Synch protocol.[67]

Recently, 7 & 7 Synch protocol (CIDR insert and $PGF_{2\alpha}$ on day 0; GnRH on day 7, CIDR insert removal and $PGF_{2\alpha}$ on day 14 and GnRH + TAI, 66 hours after CIDR removal) was compared to 7 days CO-Synch + CIDR protocol in beef cows.[68,69] Improved pregnancy was observed following implementation of 7 & 7 Synch protocol compared to 7-days CO-Synch + CIDR protocol. In beef cattle, 5 days CO-Synch + CIDR protocol resulted in 10% greater pregnancy compared to 7 days CO-Synch + CIDR protocol in cows[49] and similar pregnancy rate compared to 14 days CIDR protocol in heifers.[24] Since 5 days CO-Synch + CIDR protocol resulted in greater pregnancy in beef heifers, it would be interesting to compare pregnancy rates following 7 & 5 and 5 & 5 treatment regimens in heifers. Preliminary and unpublished results from our trials in beef heifers had greater pregnancy for 7 & 5 protocol when $PGF_{2\alpha}$ (60.1%) was replaced with GnRH (52.8%) at CIDR insertion.

Utilization of sexed semen in a synchronization programs

AI with sexed semen should be used only on any cow or heifer that are observed in estrus[70-72] following synchronization with any protocol. To improve pregnancy success, it is recommended to use sexed semen on beef females that have exhibited estrus before insemination and use conventional semen on females that have not exhibited estrus, with concurrent GnRH treatment. For best results with sexed semen, AI is performed 16-28 hours after detecting estrus.[73,74]

Variations among bulls exist in pregnancy success following AI with sexed semen due to variation in sperm DNA longevity.[75] Field studies and reports could be used to identify bulls with sufficient number of inseminations and to identify bulls with true differences in fertility. Once identified, these bulls could be used in research to assess fertility in sex-sorted semen. Sperm DNA integrity is an important component of fertility not routinely evaluated by a standard semen analysis. Further, extent of sperm DNA fragmentation (SDF) correlated with siring capacity.[76] Reduced cleavage rate and developmental arrest for sexed sperm-derived embryos compared to conventional embryos has also been reported.[75] Increased amount of SDF in sex-sorted sperm was detected when samples were incubated for 48 hours.[75-78] Duration of DNA integrity in vitro of sexed sperm varied among bulls. Bulls with higher fertility following sexed semen AI had sperm DNA integrity for longer duration (up to 72 hours), whereas DNA longevity was shorter, ≤ 24 hours, for bulls with low fertility.[78] However, AI with sexed semen that occurred close to ovulation (28 hours after first standing estrus) eliminated variations in bull fertility.

Utilization of natural service sire in a synchronization programs

NS programs are dependent on bulls for success, and synchronization will result in a larger proportion of females in estrus for a shorter interval. Any bull used for breeding should have a bull breeding soundness examination done by a veterinarian prior to turnout. As well as evaluating semen quality and scrotal circumference, the veterinarian will also assess the bull's overall condition and physical structure. Individual bulls vary widely in their ability to cover cows. If using synchronization, it is advisable to be on the conservative side with respect to the bull-to-cow ratio. Most recommendations are to stock mature bulls at a rate of 1 bull to no more than 25 cows. Use of young, inexperienced sires after synchronization is discouraged due to the concentrated breeding window, and bull-to-cow ratios should be reduced if young bulls are used (e.g. 12 cows for a yearling bull and 18 cows for an 18-month-old bull). Single-sire breeding pastures also inherently involve more risk. Periodic observation of breeding groups is recommended to ensure mating occurs and females do not continue to return to estrus throughout the breeding season.

A trial[33] was conducted in beef heifers (n = 1,744) to compare estrous response and first service and breeding season pregnancy rates in Angus-cross beef heifers that received progesterone-based estrus-synchronization treatment regimens for timed artificial insemination (TAI), with or without short-term NS. Progesterone-based CO-Synch TAI with a short-term NS treatment regimen resulted in proportionately more pregnancies (60.3%) than without a short-term (54.2%) treatment regimen. In addition, 64/84 hour split-timed AI ([STI] 59.3%) or NS following Select-Synch (57.3%) treatment regimen could be implemented as an alternative, as these treatment regimens resulted in similar pregnancy rate as progesterone-based CO-Synch TAI with short-term NS (60.3%) treatment regimen.

Synchronization strategies in *Bos indicus* cattle

Physiological differences between *Bos indicus* and *Bos taurus* include a reduced capacity for LH secretion, greater sensitivity to exogenous gonadotrophins, an earlier LH surge and ovulation with shorter and less overt estrus expression (mainly at night), smaller CL, and lower blood progesterone concentrations in *Bos indicus* cattle.[79] This makes it difficult to implement AI programs using estrus syncronization protocols. In *Bos indicus* cattle, estrus response following $PGF_{2\alpha}$ program was ~ 30% less than percentages reported for *Bos taurus* cattle under the same conditions.[80,81] The combination of low and variable estrus response and the high incidence of anestrus in animals grazing tropical grasses result in wide variability in estrus response and pregnancy rates. Pregnancy rates following implementation of GnRH + $PGF_{2\alpha}$ based Ovysnch and CO-Synch protocols have often been lower than rates reported in *Bos taurus*, with low conception rates in anestrus cows.[82]

The most useful alternative to increase the number of females that are inseminated are protocols that enable AI without the need for estrus detection, usually called TAI; TAI protocols using progestin devices, estradiol and equine chorionic gonadotrophin (eCG) have resulted in consistent pregnancy rates in *Bos indicus* and *Bos indicus* crossbred cows. In additon, pregnancy in successive cycles and breeding season pregnancy rates are improved with progestin devices used at the beginning of the breeding season. Exogenous control of luteal and follicular development has facilitated application of assisted reproductive technologies in *Bos indicus*-influenced cattle, without necessity of estrus detection and should provide opportunities to improve reproductive performance of beef cattle in tropical climates.

Estradiol and progestin treatments have been increasingly used over the past several years in estrus synchronization programs in cattle. For example, giving 2 mg of intramuscular EB at insertion of the CIDR (day 0); on days 7 or 8 the device is removed and intramuscular $PGF_{2\alpha}$ is given, and 24 hours later, 1 mg of intramuscular EB is given,[81] with TAI between 52 and 56 hours after device removal. Results from 13,510 inseminations in *Bos taurus* and *Bos indicus* crossbred cattle, resulted in an average pregnancy rate of 52.7% (27.8-75.0%).[81] Factors that influenced pregnancy success were body condition score and cyclicity.

Treatment of 200-400 IU eCG before ovulation in *Bos indicus* cattle improved ovarian follicular development before ovulation and increased progesterone concentrations during early pregnancy.[83] This glycoprotein eCG has FSH and LH like activity in ruminants, with both hormones required for periovulatory follicle maturation. In addition, eCG half-life was estimated to be 45 hours in the bloodstream of cows,[84] providing sustained gonadotropin support before ovulation. This effect of eCG is especially important in postpartum anestrus cows where LH pulses are frequently deficient.[85] Addition of eCG to a progesterone and estradiol-based treatment for TAI improves ovulation rate and luteal function in anestrous cows. Consequently, eCG has been previously used in conventional progesterone-based treatments.[85,86] Cyclicity at the initiation of protocol did not affect the pregnancy between cows treated (56.3%) or not treated with eCG (56.5%); however, addition of eCG yielded pregnancy rates close to 50% in cows with a BCS of 2.[81] There were greater ($p < 0.05$) percentages of insemination and pregnancy in a 4 day breeding season in cows treated with CIDR + PGF + TW (temporary weaning) + eCG (50.9 and 29.4%) than in cows treated only with CIDR + PGF + TW (39.4 and 23.7%).[87] Clearly, progestin-releasing devices, estradiol and eCG advance resumption of cyclicity in anestrus cows and facilitate TAI in suckled *Bos indicus* cows.

Factors influncing success of synchronization

Compliance

Farm personnel should be working closely with veterinarians when they consider which synchronization protocol best fits their goals. Additionally, it is crucial to ensure protocols are followed. Implementation of a synchronization protocol includes: finding the right cow, using the correct reproductive hormone at the correct dose and route, giving each injection at the correct time on the correct day, and adhering to each step, from the first injection to AI. Clear and accurate animal identification, employee training, safe and efficient animal handling, maintaining a record-keeping system and updating it regularly, maintaining product labels, and providing staff with needles, syringes, gloves, etc, are all important in achieving protocol compliance. Tracking submission for AI and pregnancy rates are necessary to determine if compliance is being met. It should be noted that missing 5% of cows (or injections) results in 86% compliance across a 3 handling protocol.

Transportation of beef females after AI

Transporting cows/heifers after AI should be avoided. Embryos are vulnerable to stress-associated changes in circulating hormones and uterine environment during blastocyst formation, hatching, maternal recognition of pregnancy, and attachment to the uterus. It is essential to try to minimize stress and/or major changes after insemination to maximize

Table 7. Reproductive tract scoring system based on the size of uterus and ovarian structures

Tract score	Uterine horns	Ovarian structures
1	Immature, < 20 mm diameter, no tone	No palpable structures
2	20-25 mm diameter, no tone	8 mm follicles
3	20-25 mm diameter, slight tone	8-10 mm follicles
4	30 mm diameter, good tone	> 10 mm follicles, possibly CL
5	> 30 mm diameter	CL

pregnancy success. Transportation is recommended 1-4 days after AI or later (day 42) after AI.[88-90] Although reports suggest that transporting cows 5-42 days after AI can cause 10% decrease in pregnancy rates, in a small trial[91] ACTH treatment increased serum cortisol concentrations but did not increase serum concentrations of prostaglandin F metabolites or cause pregnancy loss during early pregnancy in cows.

Reproductive tract score in heifers

The Reproductive tract score (RTS) is a 5 point scoring system (1 anestrus/underdeveloped or infantile genitalia; 5 – cycling, mature) based on the size (development) of tubular reproductive tract and ovarian structures are given below (Table 7).[92] The RTS should be used before breeding as a replacement heifer selection criterion. This can be categorized as: prepubertal – score 1; peripubertal – scores 2 and 3; and pubertal – scores 4 & 5.

The RTS of 1 corresponds to the point in time at which the pattern of LH release is characterized by low-frequency pulses, as the hypothalamic-pituitary axis is highly responsive to estrogen negative feedback. Reproductive tract scores of 2 and 3 are associated with the peripubertal phase, at which responsiveness to estradiol negative feedback decreases, causing increases in LH pulse frequency, follicle growth, and estradiol secretion. The decline in estradiol negative feedback and increase in LH secretion promote ovarian follicular growth, and elevated concentrations of estradiol sufficient to induce estrus and the preovulatory LH surge. Reproductive tract scores of 4 and 5 are assigned to heifers that have reached puberty, but differ in stage of the estrous cycle at the prebreeding exam (follicular phase = 4; luteal phase = 5). Heifers with higher RTS achieve higher AI and breeding season pregnancy rates and become pregnant earlier in the breeding season compared to heifers with lower RTS.[93]

Temperament score in cows and heifers

Temperament is a reaction characteristic of cattle to human handling. Cattle that remain calm perform better than those that are excitable during handling. In general, excitable temperament has detrimental effects on growth, carcass quality and health of beef cattle. The hypothalamic-pituitary-adrenal axis is exquisitely sensitive to physiological and psychological insults. Secretion of glucocorticoids is the classic endocrine response to stress. However, broad endocrine changes occur in response to stress. Within seconds to minutes after the onset of stress were increases in catecholamines, cortisol releasing hormones and ACTH and decreases in GnRH, gonadotropins, prolactin and glucogan secretion.[94-96] In addition over hours to days, gonadal steroid hormones decline; these changes inhibit reproductive physiology and behavior, and decrease feeding and appetite. Various scoring systems (1-5 or 6 point, or 2 point) have been developed to measure temperament.[97-99] Current techniques include chute score, flight speed and exit score. Calm cows[100] and heifers[99] had higher AI and breeding season pregnancy rates and become pregnant earlier in the breeding season compared to cattle with excitable temperament. Cattle facility design influenced temperament and thus affected reproductive performance.[99] Cattle handling facility design by temperament group interactions significantly influenced progesterone, cortisol, prolactin and substance-P concentrations.[99] Inter and intra-rater agreements for 2 point temperament scoring were moderate and good. The predictive value for calm and pregnant to AI was 0.87, and excited and nonpregnant to AI was 0.76.[99]

Body condition score in beef cows and heifers

The body condition score (BCS) is a 1-9 point (1 – emaciate and 9 – obese) scoring system[101] based on visual observation of muscle and fat cover in the area of ribs/thorax, transverse processes, back and gluteal regions and tail head and perineal regions. The BCS is an indirect measure of nutitional status of cows. The BCS can be categorized as thin < 5, moderate – 5, good – 6 and 7, and obese > 7. It is preferrable to feed cattle to have a BCS of 5 at calving and maintained at BCS 5 until breeding.[102] It should be noted that BCS has better correlation in beef cows than beef heifers. Cows with thin BCS took longer to resume cyclicity after calving due to lower concentrations of IGF, estrogen and LH.[103,104] Adiponectin and leptin are also lower in thin cows.[105] Thin and obese cows had lesser estrus expression, and lower AI and breeding season pregnancy rates compared to cows with moderate to good BCS.[106] Cows that maintained or gained body condition after breeding, during first 2 months of pregnancy, had increased pregnancy rates compared to cows that lost body condition.[107]

Conclusion

Estrus/ovulation synchronization and an AI program are excellent tools that should be planned in collaboration with a veterinarian well-experienced in reproductive management and implemented by experienced personnel who can adhere to the protocol and breeders who can breed many cattle concurrently. The investment of time in selecting AI and NS bulls and carefully following protocols should reduce the calving interval and produce more uniform calves with genetic advantages, making them more marketable and improving profitability. However, sycnhronization will not solve all breeding or management problems. So, the key for success is consideration of all factors and options and making evidence-based decisions.

Acknowledgments

Authors acknowledge College of Veterinary Medicine, Washington State University, Pullman, WA, USA for the support and thank Dr. John Kastelic (University of Calgary) for reviewing the manuscript.

Conflict of interest

Authors declare that they have no conflict of interest.

References

1. NAHMS. Part III: reference of 1997 Beef Cow-Calf Production Management and Disease Control. 1998. p. 9-11. Available from: https://www.aphis.usda.gov/animal_health/nahms/beef-cowcalf/downloads/beef97/Beef97_dr_PartIII.pdf [cited 22 September 2023].
2. NAHMS. Part II: Reference of Beef Cow-Calf Management Practices in the United States, 2007–08. 2008. p. 5-31. Available from: https://www.aphis.usda.gov/animal_health/nahms/beef-cowcalf/downloads/beef0708/Beef0708_dr_PartII_1.pdf [cited 22 September 2023].
3. Johnson SK Fogleman SL, Jones R: Comparison of breeding system costs for estrus-synchronization protocols plus artificial insemination versus natural service. Kansas Agric Exp Station Res Rep 2003;0:105-116. doi: 10.4148/2378-5977.1661
4. Lamb GC: Impacts of estrous synchronization on cowherd performance. Range Beef Cow Symposium. 2015. p. 342. Available from: http://digitalcommons.unl.edu/rangebeefcowsymp/342 [cited 22 September 2023].
5. Hall JB: Capturing the Value of Artificial Insemination in Commercial Herds. Proceedings, The Range Beef Cow Symposium XXVI. Mitchell, NE, USA 2019. p 23-30. Available from: https://beef.unl.edu/documents/RBC-Symposium/2019/RBCS-2019-03-Capturing-the-Value-of-Artificial-Insemination-in-Commercial-Herds.pdf [cited 22 September 2023].
6. Rodgers JC, Bird SL, Larson JE, et al: An economic evaluation of estrous synchronization and timed artificial insemination in suckled beef cows. J Anim Sci 2012;90:4055-4062. doi: 10.2527/jas.2011-4836
7. Lauderdale JW: ASAS centennial paper: contributions in the Journal of Animal Science to the development of protocols for breeding management of cattle through synchronization of estrus and ovulation. J Anim Sci 2009;87:801-812. doi: 10.2527/jas.2008-1407
8. Cooper MJ: Control of oestrous cycles of heifers with a synthetic prostaglandin analogue. Vet Rec 1974;95:200-203. doi: 10.1136/vr.95.10.200
9. Macmillan KL, Day AM: Prostaglandin F(2alpha) – a fertility drug in dairy cattle? Theriogenology 1982;18:245-253. doi: 10.1016/0093-691x(82)90001-2
10. Rabiee AR, Lean IJ, Stevenson MA: Efficacy of Ovsynch program on reproductive performance in dairy cattle: a meta-analysis. J Dairy Sci 2005;88:2754-2770. doi: 10.3168/jds.S0022-0302(05)72955-6
11. Trimberger GW, Davis HP: Breeding efficiency in dairy cattle bred at various stages of estrus by artificial insemination. J Dairy Sci 1943;26:757-759.
12. Trimberger GW: Conception rate in dairy cattle by insemination at various intervals before and after ovulation. J Dairy Sci 1944;27:659-660. doi: 10.3168/jds.S0022-0302(45)95220-9
13. Dorsey BR, Kasimanickam R, Whittier WD, et al: Effect of time from estrus to AI on pregnancy rates in estrous synchronized beef heifers. Anim Reprod Sci 2011;127:1-6. doi: 10.1016/j.anireprosci.2011.07.014
14. Kristula M, Bartholomew R, Galligan D, et al: Effects of a prostaglandin F2 alpha synchronization program in lactating dairy cattle. J Dairy Sci 1992;75:2713-2718. doi: 10.3168/jds.S0022-0302(92)78033-3
15. Ferguson SD, Galligan DT: Reproductive programs in dairy herds. Proc Central Veterinary Conference 1993;1:161-178.
16. Pankowski JW, Galton DM, Erb HN, et al: Use of prostaglandin F2a as a postpartum reproductive management tool for lactating dairy cows. J Dairy Sci 1995;78:1477. doi: 10.3168/jds.S0022-0302(95)76770-4
17. Geary TW, Downing ER, Bruemmer JE, et al: Ovarian and estrous response of suckled beef cows to the Select Synch estrous synchronization protocol. Prof Anim Sci 2000;16:1-5. doi: 10.15232/S1080-7446(15)31653-3
18. Patterson DJ, Kiracofe GH, Stevenson JS, et al: Control of the bovine estrous cycle with melengestrol acetate (MGA): A review. J Anim Sci 1989;67:1895-1906. doi: 10.2527/jas1989.6781895x
19. Thomas JM, Locke JWC, Bishop BE, et al: Evaluation of the 14-d CIDR-PG and 9-d CIDR-PG protocols for synchronization of estrus in Bos indicus-influenced and Bos taurus beef heifers. Theriogenology 2017;92:190-6. doi: 10.1016/j.theriogenology.2017.01.020
20. Twagiramungu H, Guilbault LA, Dufour JJ: Synchronization of ovarian follicular waves with a gonadotropin-releasing hormone agonist to increase the precision of estrus in cattle: a review. J Anim Sci 1995;73:3141-3151. doi: 10.2527/1995.73103141x
21. Deutscher GH: Extending interval from seventeen to nineteen days in the melengestrol acetate-prostaglandin estrous synchronization program for heifers. Prof Anim Sci 2000;16:164-168. doi: 10.15232/S1080-7446(15)31687-9
22. Tauck SA, Wilkinson JR, Olsen JR, et al: Comparison of controlled internal drug release device and melengestrol acetate as progestin sources in an estrous synchronization protocol for beef heifers. Theriogenology 2007;68:162-167. doi: 10.1016/j.theriogenology.2007.03.027
23. Nash JM, Mallory DA, Ellersieck MR, et al: Comparison of long- versus short-term CIDR-based protocols to synchronize estrus prior to fixed-time AI in postpartum beef cows. Anim Reprod Sci 2012;132:11-16. doi: 10.1016/j.anireprosci.2012.03.013
24. Kasimanickam R, Schroeder S, Hall JB, et al: Fertility after implementation of long- and short-term progesterone-based ovulation synchronization protocols for fixed-time artificial insemination in beef heifers. Theriogenology 2015;83:1226-1232. doi: 10.1016/j.theriogenology.2015.01.004
25. Kojima FN, Salfen BE, Bader JF, et al: Development of an estrus synchronization protocol for beef cattle with short-term feeding of melengestrol acetate: 7-11 Synch. J Anim Sci 2000;78:2186-2191. doi: 10.2527/2000.7882186x
26. Bremer VR, Damiana SM, Ireland FA, et al: Optimizing the interval from PGF to timed AI in the CO-Synch+CIDR and 7-11 Synch estrus synchronization protocols for postpartum beef cows. J Anim Sci 2004;82(Suppl. 2):106.
27. Patterson DJ, Wood SL, Kojima FN, et al: Current and emerging methods to synchronize estrus with melengestrol acetate. In: 49th Annual Beef Cattle Short Course Proceedings "Biotechnologies of

Reproductive Biology". Gainesville, FL; Univesity of Florida: 2000. p. 45-66.
28. Wood SL, Lucy MC, Smith MF, et al: Improved synchrony of estrus and ovulation with addition of GnRH to a melengestrol acetate-prostaglandin F2α estrus synchronization treatment in beef heifers. J Anim Sci 2001;79:2210. doi: 10.2527/2001.7982210x
29. Funston RN, Ansotegui RP, Lipsey RJ, et al: Synchronization of estrus in beef heifers using either melengesterol acetate (MGA)/prostaglandin or MGA/Select Synch. Theriogenology 2002;57:1485-1491. doi: 10.1016/s0093-691x(02)00654-4
30. Bader JF, Kojima FN, Schafer DJ, et al: A comparison of progestin-based protocols to synchronize ovulation and facilitate fixed-time artificial insemination in postpartum beef cows. J Anim Sci 2005;83:136-143. doi: 10.2527/2005.831136x
31. Barnes M, Kasimanickam R, Kasimanickam V: Effect of subclinical endometritis and flunixin meglumine administration on pregnancy in embryo recipient beef cows. Theriogenology 2023;201:76-82. doi: 10.1016/j.theriogenology.2023.02.020
32. Kasimanickam R, Kasimanickam V, Ratzburg K: Pregnancy and offspring sex ratio following insemination with SexedULTRA and conventional semen in cows in a commercial beef operation. Reprod Domest Anim 2021;56:1435-1445. doi: 10.1111/rda.14008
33. Kasimanickam R, Kasimanickam V, Kappes A: Timed artificial insemination strategies with or without short-term natural service and pregnancy success in beef heifers. Theriogenology 2021;166:97-103. doi: 10.1016/j.theriogenology.2021.02.023
34. Kasimanickam R, Kasimanickam V, Kastelic J, et al: Effects of twice daily compared with split-time estrous detection on pregnancy percentage in recipient beef cows. Anim Reprod Sci 2020;219:106526. doi: 10.1016/j.anireprosci.2020.106526
35. Kasimanickam R, Jorgensen-Muga K, Beumeler J, et al: Estrous response and pregnancy percentages following use of a progesterone-based, split-time estrous synchronization treatment regimens in beef heifers. Anim Reprod Sci 2020;221:106544. doi: 10.1016/j.anireprosci.2020.106544
36. Kasimanickam RK, Hall JB, Estill CT, et al: Flunixin meglumine improves pregnancy rate in embryo recipient beef cows with an excitable temperament. Theriogenology 2018;107:70-77. doi: 10.1016/j.theriogenology.2017.10.043
37. Kasimanickam RK, Whittier WD, Hall JB, et al: Estrous synchronization strategies to optimize beef heifer reproductive performance after reproductive tract scoring. Theriogenology 2016;86:831-838. doi: 10.1016/j.theriogenology.2016.03.004
38. Wilson DJ, Mallory DA, Busch DC, et al: Comparison of short-term progestin-based protocols to synchronize estrus and ovulation in postpartum beef cows. J Anim Sci 2010;88:2045-2054. doi: 10.2527/jas.2009-2627
39. Larson JE, Lamb GC, Stevenson JS, et al: Synchronization of estrus in suckled beef cows for detected estrus and artificial insemination and timed artificial insemination using gonadotropin-releasing hormone, prostaglandin F2alpha, and progesterone. J Anim Sci 2006;84:332-342. doi: 10.2527/2006.842332x
40. Stevenson JS, Thompson KE, Forbes WL, et al: Synchronizing estrus and(or) ovulation in beef cows after combinations of GnRH, norgestomet, and prostaglandin F2alpha with or without timed insemination. J Anim Sci 2000;78:1747-1758. doi: 10.2527/2000.7871747x
41. Bó GA, Adams GP, Pierson RA, et al: Exogenous control of follicular wave emergence in cattle. Theriogenology 1995;43:31-40. doi: 10.1016/0093-691X(94)00010-R
42. Bó GA, Adams GP, Nasser LF, et al: Effect of estradiol valerate on ovarian follicle, emergence of follicular wave and circulating gonadotropins in heifers. Theriogenology 1993;40:225-239. doi: 10.1016/0093-691X(93)90261-3
43. Thundathil J, Kastelic JP, Mapletoft RJ: The effect of estradiol cypionate (ECP) on ovarian follicular development and ovulation in dairy cattle. Can J Vet Res 1997;61:314-316.
44. Martínez MF, Kastelic JP, Colazo MG, et al: Effects of estradiol on gonadotrophin release, estrus and ovulation in CIDR-treated beef cattle. Domest Anim Endocrinol 2007;33:77-90. doi: 10.1016/j.domaniend.2006.04.009
45. Adams GP, Jaiswal R, Singh J, et al: Progress in understanding ovarian follicular dynamics in cattle. Theriogenology 2008;69:72-80. doi: 10.1016/j.theriogenology.2007.09.026
46. Pursley JR, Stevenson JS, Minton JE: Ovarian follicular waves in dairy cows after administration of gonadotropin-releasing hormone at estrus. J Dairy Sci 1993;76:2548-2560. doi: 10.3168/jds.S0022-0302(93)77590-6
47. Kasimanickam RK, Kasimanickam VR, Oldham J, et al: Cyclicity, estrus expression and pregnancy rates in beef heifers with different reproductive tract scores following progesterone supplementation. Theriogenology 2020;145:39-47. doi: 10.1016/j.theriogenology.2020.01.028
48. Bridges A: Timed-artificial insemination in beef cows: what are the options? Purdue University Cooperative Extension Service, West Lafayette, IN, USA. 2008; AS-575-W. Available from: https://www.extension.purdue.edu/extmedia/as/as_575_w.pdf [cited 15 October 2023].
49. Bridges GA, Helser LA, Gru DE, et al: Decreasing the interval between GnRH and PGF2α from 7 to 5 days and lengthening proestrus increased timed-AI pregnancy rates in beef cows. Theriogenology 2008;69:843-851. doi: 10.1016/j.theriogenology.2007.12.011
50. Bridges GA, Lake SL, Kruse SG, et al: Comparison of three CIDR-based fixed-time AI protocols in beef heifers. J Anim Sci 2014;92:3127-3133. 94. doi: 10.2527/jas.2013-7404
51. Valdez KE, Cuneo SP, Turzillo AM: Regulation of apoptosis in the atresia of dominant bovine follicles of the first follicular wave following ovulation. Reproduction 2005;130:71-81. doi: 10.1530/rep.1.00430
52. Peterson C, Alkar A, Smith S, et al: Effects of one versus two doses of prostaglandin F2alpha on AI pregnancy rates in a 5-day, progesterone-based, CO-Synch protocol in crossbred beef heifers. Theriogenology 2011;75:1536-1542. doi: 10.1016/j.theriogenology.2010.12.017
53. Whittier WD, Currin JF, Schramm H, et al: Fertility in Angus cross beef cows following 5-day CO-Synch + CIDR or 7-day CO-Synch + CIDR estrus synchronization and timed artificial insemination. Theriogenology 2013;80:963-969. doi: 10.1016/j.theriogenology.2013.07.019
54. Ahmadzadeh A, Gunn D, Hall JB, et al: Evaluation of treatment with a 5-day versus 7-day controlled internal drug-release insert on reproductive outcomes of beef heifers using a modified

timed-artificial insemination protocol. Prof Anim Sci 2015;31:270-277. doi: 10.15232/pas.2014-01378
55. Stevenson JS, Hill SL, Grieger DM, et al: Two split-time artificial insemination programs in suckled beef cows. J Anim Sci 2017;95:5105-111. doi: 10.2527/jas2017.1805
56. Navanukraw C, Redmer DA, Reynolds LP, et al: A modified pre-synchronization protocol improves fertility to timed artificial insemination in lactating dairy cows. J Dairy Sci 2004;87:1551-1557. doi: 10.3168/jds.S0022-0302(04)73307-X
57. Macmillan KL, Thatcher WW: Effects of an agonist of gonadotropin-releasing hormone on ovarian follicles in cattle. Biol Reprod 1991;45:883-889. doi: 10.1095/biolreprod45.6.883
58. Stevenson JS, Sauls JA, Mendonça LGD, et al: Dose frequency of prostaglandin F2a administration to dairy cows exposed to presynchronization and either 5- or 7-day Ovsynch program durations: ovulatory and luteolytic risks. J Dairy Sci 2018;101:9575-9590. doi: 10.3168/jds.2018-14653
59. Knickmeyer ER, Thomas JM, Locke JWC, et al: Altering duration of the presynchronization period in a long-term progestin-based estrus synchronization protocol for timed artificial insemination of beef heifers. Theriogenology 2019;136:66-71. doi: 10.1016/j.theriogenology.2019.06.027
60. Small JA, Colazo MG, Kastelic JP, et al: Effects of progesterone presynchronization and eCG on pregnancy rates to GnRH-based, timed-AI in beef cattle. Theriogenology 2009;71:698-706. doi: 10.1016/j.theriogenology.2008.09.045
61. Monn RE, Mackey JC, Dudley HB, et al: Effect of a pre-synchronization protocol on beef heifer AI pregnancy rates. J Anim Sci 2016;95:56-57. doi: 10.2527/ssasas2017.0115
62. Colazo MG, Mapletoft RJ: A review of current timed-AI (TAI) programs for beef and dairy cattle. Can Vet J 2014;55:772-780.
63. Berardinelli JG, Dailey RA, Butcher RL, et al: Source of progesterone prior to puberty in beef heifers. J Anim Sci 1979;49:1276-1281. doi: 10.2527/jas1979.4951276x
64. Moriel P, Lancaster P, Lamb GC, et al: Effects of post-weaning growth rate and puberty induction protocol on reproductive performance of Bos indicus influenced beef heifers. J Anim Sci 2017;95:3523-3531. doi: 10.2527/jas.2017.1666
65. Bao B, Garverick HA: Expression of steroidogenic enzyme and Gonadotropin receptor genes in bovine follicles during ovarian follicular waves: a review. J Anim Sci 1998;76:1903-1921. doi: 10.2527/1998.7671903x
66. Sartori R, Fricke PM, Ferreira JC, et al: Follicular deviation and acquisition of ovulatory capacity in bovine follicles. Biol Reprod 2001;65:1403-1409. doi: 10.1095/biolreprod65.5.1403
67. Ratzburg K, Jorgensen-Muga K, Murugesan J, et al: Presynchronization with CIDR, with or without GnRH, prior to CO-Synch in beef heifers. Theriogenology 2020;146:80-87. doi: 10.1016/j.theriogenology.2020.02.005
68. Bonacker RC, Stoecklein KS, Locke JWC, et al: Treatment with prostaglandin F2α and an intravaginal progesterone insert promotes follicular maturity in advance of gonadotropin-releasing hormone among postpartum beef cows. Theriogenology 2020;157:350-359. doi: 10.1016/j.theriogenology.2020.08.018
69. Bonacker RC, Gray KR, Breiner CA, et al: Comparison of the 7 & 7 Synch protocol and the 7-day CO-Synch + CIDR protocol among recipient beef cows in an embryo transfer program. Theriogenology 2020;158:490-496. doi: 10.1016/j.theriogenology.2020.09.033
70. Seidel GE Jr: Update on sexed semen technology in cattle. Animal 2014;8 Suppl 1:160-164. doi: 10.1017/S1751731114000202
71. Maicas C, Hutchinson IA, Kenneally J, et al: Fertility of fresh and frozen sex-sorted semen in dairy cows and heifers in seasonal-calving pasture-based herds. J Dairy Sci 2019;102:10530-10542. doi: 10.3168/jds.2019-16740
72. Seidel GE, Jr, Schenk JL, Herickhoff LA, et al: Insemination of heifers with sexed sperm. Theriogenology 1999;52:1407-1420. doi: 10.1016/s0093-691x(99)00226-5
73. Nebel R: Time of insemination relative to onset of activity threshold of cow manager® is associated with pregnancy risk when using gender selected™ semen for Jersey cattle. Dairy Vet Sci J 2018;5:555653. doi: 10.19080/JDVS.2018.05.555653
74. Bombardelli GD, Soares HF, Chebel RC: Time of insemination relative to reaching activity threshold is associated with pregnancy risk when using sex-sorted semen for lactating Jersey cows. Theriogenology 2016;85:533-539. doi: 10.1016/j.theriogenology.2015.09.042
75. Gosálvez J, Ramirez MA, López-Fernández C, et al: Sex-sorted bovine spermatozoa and DNA damage: II. Dynamic features. Theriogenology 2011;75:206-211. doi: 10.1016/j.theriogenology.2010.09.011
76. Kasimanickam R, Nebel RL, Peeler ID, et al: Breed differences in competitive indices of Holstein and Jersey bulls and their association with sperm DNA fragmentation index and plasma membrane integrity. Theriogenology 2006;66:1307-1315. doi: 10.1016/j.theriogenology.2006.04.025
77. Steele H, Makri D, Maalouf WE, et al: Bovine sperm sexing alters sperm morphokinetics and subsequent early embryonic development. Sci Rep 2020;10:6255. doi: 10.1038/s41598-020-63077-6
78. Reese S, Pirez MC, Steele H, et al: The reproductive success of bovine sperm after sex-sorting: a meta-analysis. Sci Rep 2021;11:17366. doi: 10.1038/s41598-021-96834-2
79. Cavalieri J, Rubio I, Kinder JE, et al: Synchronization of estrus and ovulation and associated endocrine changes in Bos indicus cows. Theriogenology 1997;47:801-814. doi: 10.1016/S0093-691X(97)00036-8
80. Galina CS, Arthur GH: Review on cattle reproduction in the tropics. Part 4. Oestrus cycles. Anim Breed Abstracts 1990;58:697-707.
81. Bó GA, Cutaia L, Peres LC, et al: Technologies for fixed-time artificial insemination and their influence on reproductive performance of Bos indicus cattle. Soc Reprod Fertil Suppl 2007;64:223-236. doi: 10.5661/rdr-vi-223
82. Baruselli PS, Reis EL, Marques MO, et al: The use of hormonal treatments to improve reproductive performance of anestrous beef cattle in tropical climates. Anim Reprod Sci 2004;82-83:479-486. doi: 10.1016/j.anireprosci.2004.04.025
83. Pinheiro VG, Souza AF, Pegorer MF, et al: Effects of temporary calf removal and eCG on pregnancy rates to timed-insemination in progesterone-treated postpartum Nellore cows. Theriogenology 2009;71:519-524. doi: 10.1016/j.theriogenology.2008.08.018
84. Murphy BD, Martinuk SD: Equine chorionic gonadotropin. Endocr Rev 1991;12:27-44. doi: 10.1210/edrv-12-1-27

85. Roche JF, Crowe MA, Boland MP: Postpartum anoestrus in dairy and beef cows. Anim Reprod Sci 1992;28:371-378. doi: 10.1016/0378-4320(92)90123-U
86. McMillan KL, Peterson AJ: A new intravaginal progesterone releasing device for cattle (CIDR-B) for oestrous synchronisation, increasing pregnancy rates and the treatment of post-partum anoestrus. Anim Reprod Sci 1993;33:1-25. doi: 10.1016/0378-4320(93)90104-Y
87. Sá Filho OG, Dias CC, Lamb GC, et al: Progesterone-based estrous synchronization protocols in non-suckled and suckled primiparous Bos indicus beef cows. Anim Reprod Sci 2010;119:9-16. doi: 10.1016/j.anireprosci.2009.12.011
88. Harrington TE: Effect of Transportation Time on Pregnancy Rates of Synchronized Yearling Beef Heifers. Ph.D. Thesis, Colorado State University, Fort Collins, CO, USA, 1995.
89. Merrill ML, Ansotegui RP, Burns PD, et al: Effects of flunixin meglumine and transportation on establishment of pregnancy in beef cows. J Anim Sci 2007;85:1547-1554. doi: 10.2527/jas.2006-587
90. Salverson R: Effects of shipping and heat stress on embryonic mortality in cattle. South Dakota State University, Animal Science Department. South Dakota Board of Regents. 2020. Available from: https://extension.sdstate.edu/sites/default/files/2020-10/P-00189.pdf [cited 28 September 2023].
91. Geary TW: Effects of adrenocorticotropic hormone and flunixin meglumine on pregnancy retention in beef cows. J Anim Sci 2012;90:207-211. doi: 10.2527/jas.2010-3564
92. Anderson KJ, Lefever DG, Brinks JS, et al: The use of reproductive tract scoring in beef heifers. Agri Sci Pract 1991;12:123-128.
93. Gutierrez K, Kasimanickam R, Tibary A, et al: Effect of reproductive tract scoring on reproductive efficiency in beef heifers bred by timed insemination and natural service versus only natural service. Theriogenology 2014;81:918-924. doi: 10.1016/j.theriogenology.2014.01.008
94. Matteri RL, Carroll JA, Dyer CJ: Neuroendocrine responses to stress. In: Moberg GP, Mench JA: editors. The Biology of Animal Stress. Wallingford; CABI: 2000. p. 43-76.
95. Möstl E, Palme R: Hormones as indicators of stress. Domest Anim Endocrinol 2002;23:67-74. doi: 10.1016/s0739-7240(02)00146-7
96. Sapolsky RM, Romero LM, Munck AU: How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocr Rev 2000;21:55-89. doi: 10.1210/edrv.21.1.0389
97. Grandin T: Assessment of stress during handling and transport. J Anim Sci 1997;75:249-257. doi: 10.2527/1997.751249x
98. Voisinet BD, Grandin T, Tatum JD, et al: Feedlot cattle with calm temperaments have higher average daily gains than cattle with excitable temperaments. J Anim Sci 1997;75:892-896. doi: 10.2527/1997.754892x
99. Kasimanickam R, Schroeder S, Assay M, et al: Influence of temperament score and handling facility on stress, reproductive hormone concentrations, and fixed time AI pregnancy rates in beef heifers. Reprod Domest Anim 2014;49:775-782. doi: 10.1111/rda.12368
100. Kasimanickam R, Asay M, Schroeder S, et al: Calm temperament improves reproductive performance of beef cows. Reprod Domest Anim 2014;49:1063-1067. doi: 10.1111/rda.12436
101. Lake SL, Scholljegerdes EJ, Hallford DM, et al: Effects of body condition score at parturition and postpartum supplemental fat on metabolite and hormone concentrations of beef cows and their suckling calves J Anim Sci 2006;84:1038-1047. doi: 10.2527/2006.8441038x
102. Short RE, Bellows RA, Staigmiller RB, et al: Physiological mechanisms controlling anestrus and infertility in postpartum beef cattle. J Anim Sci 1990;68:799-816. doi: 10.2527/1990.683799x
103. Astessiano AL, Pérez-Clariget R, Quintans G, et al: Metabolic and endocrine profiles and hepatic gene expression in periparturient, grazing primiparous beef cows with different body reserves. Livest Sci 2014;170:63-71. doi: 10.1016/j.livsci.2014.10.008
104. Kasimanickam R, Firth P, Asay M, et al: Impact of body condition change post-breeding on reproductive performance of beef cows. Clinical Theriogenology 2012;4:469-476.
105. Kasimanickam R, Whittier WD, Currin JF, et al: Effect of body condition at initiation of synchronization on estrus expression, pregnancy rates to AI and breeding season in beef cows. Clinical Theriogenology 2011;3:29-41.
106. Whitman RH. Weight changes, body condition and beef-cow reproduction. PhD thesis. Colorado State University, Fort Collins, CO, USA, 1975.
107. Ciccioli NH, Wettemann RP, Spicer LJ, et al: Influence of body condition at calving and postpartum nutrition on endocrine function and reproductive performance of primiparous beef cows. J Anim Sci 2003;81:3107-3120. doi: 10.2527/2003.81123107x