

Estrus and ovulation synchronization strategies in beef cattle

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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

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 during 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

Table 1. AI and NS combinations in beef cattle

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

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 quiescence, release of prostaglandin F_{2α} (PGF_{2α}) from the endometrium, and lysis of the CL.

Control of follicular and luteal 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). Limiting 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_{2α}

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

Progesterone

Controlled Internal Drug Release

(CIDR) intravaginal 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 pharmaceuticals used for synchronization in cattle are given in Table 3.

Table 2. Principles, limiting 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 _{2α} – need a responsive/active CL Prolonging – induce CL and/or give intravaginal or oral progesterone

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.	Estrumate®
	CYSTORELIN® (gonadorelin)	NADA 098-379 Merial, Inc.	Estrumate®
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.	-

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

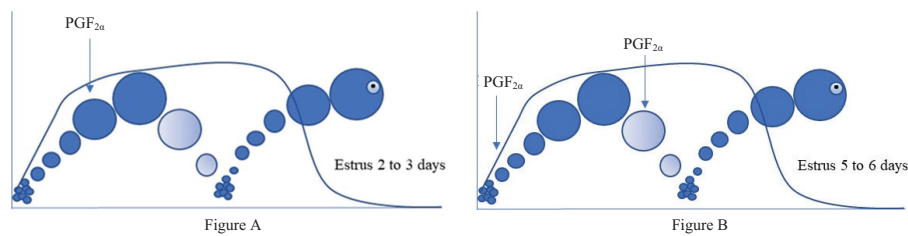


Figure 1. Schematic presentation of interval from PGF_{2α} treatment and estrus expression based on follicle size and phase of the follicular wave in cattle

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 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_{2α} 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_{2α} is given during growing and static phases, estrus occurs soon (Figure 1A), whereas estrus occurs later if PGF_{2α} is given during the regressing phase (Figure 1B) as it takes time for a new dominant follicle to emerge and reach preovulatory size. PGF_{2α} causes luteolysis, with subsequent surges in estrogen and LH, with ovulation occurring ~24 hours after the LH surge.

PGF_{2α} programs

PGF_{2α} 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_{2α} ~7 days

after ovulation. There are several $\text{PGF}_{2\alpha}$ protocols, according to management needs (Figures 2-4). However, implementation of a successful estrus detection program is essential to achieve good results with $\text{PGF}_{2\alpha}$ 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 $\text{PGF}_{2\alpha}$ program is generally similar to AI following spontaneous estrus.

Weekly Monday morning program

On Monday morning, all eligible cows receive an injection of $\text{PGF}_{2\alpha}$, 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 $\text{PGF}_{2\alpha}$ 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 $\text{PGF}_{2\alpha}$ program

On Monday morning, all eligible cows receive an injection of $\text{PGF}_{2\alpha}$ with⁶ or without AI for cows exhibiting estrus after $\text{PGF}_{2\alpha}$ treatment. Two weeks later (Monday), cows that did not

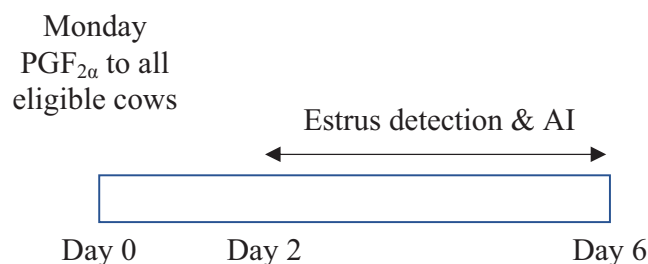


Figure 2. Schematic presentation of weekly Monday morning program in cattle

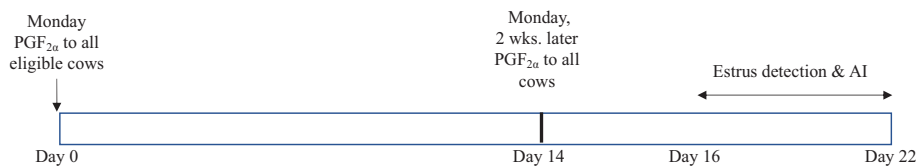


Figure 3. Schematic presentation of biweekly Monday morning program: in cattle

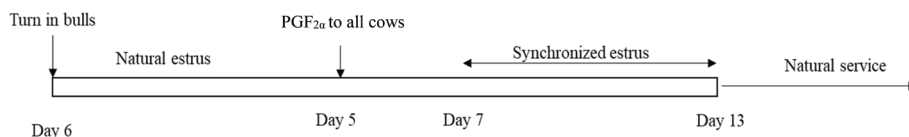


Figure 4. $\text{PGF}_{2\alpha}$ program before/after exposing beef females to bulls

have AI¹⁵ or all cows, receive another dose of $\text{PGF}_{2\alpha}$, 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.

There are several $\text{PGF}_{2\alpha}$ programs, including $\text{PGF}_{2\alpha}$ treatment for nonpregnant cows with an active CL at pregnancy diagnosis or repeated biweekly $\text{PGF}_{2\alpha}$ treatment as a postpartum reproductive management tool¹⁶ at 25-32, 39-46, and 53-60 days, with first insemination following the last injection. Cows not inseminated after $\text{PGF}_{2\alpha}$ injection between 53-60 days are given $\text{PGF}_{2\alpha}$ 14 days later.

$\text{PGF}_{2\alpha}$ programs are popular in dairy operations. However, some beef operations implement $\text{PGF}_{2\alpha}$ program before/after exposing cows to bulls (Figure 4). This approach generally resulted in more pregnancies and more calves compared to no $\text{PGF}_{2\alpha}$ 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 concomitant luteolytic dose of $\text{PGF}_{2\alpha}$ 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 + $\text{PGF}_{2\alpha}$ CIDR + $\text{PGF}_{2\alpha}$ and MGA + $\text{PGF}_{2\alpha}$.¹⁷⁻¹⁹

GnRH and $\text{PGF}_{2\alpha}$

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

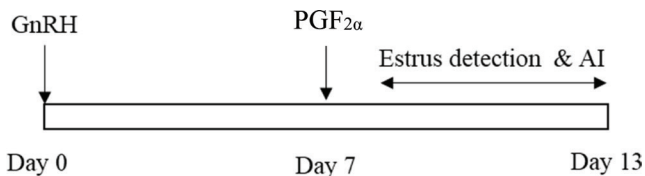
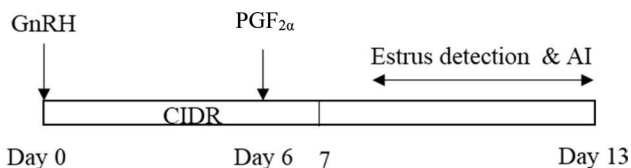
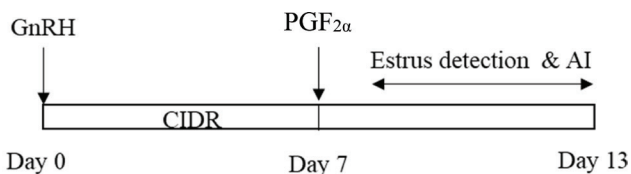


Figure 5. Schematic presentation of Select Synch estrus synchronization program



CIDR- PGF_{2α}

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



Select Synch + CIDR

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

CIDR + PGF_{2α} or Select Synch + CIDR

Cows receive a CIDR (1.3 g progesterone) insert for 7 days and an injection of PGF_{2α} 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_{2α}, 70-83% of cows were in estrus within 4 days.²⁰ It is advantageous to include the CIDR when more cows are anestrous and/or when estrus detection before PGF_{2α} treatment is not feasible. With Select Synch, 5-20% of cattle may exhibit estrus 2 days before PGF_{2α} 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 supplementation (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_{2α} injection 19 days later on day 33.^{18,21} This will have heifers in the late luteal stage of the estrous cycle at PGF_{2α} 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 synchronization 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_{2α} given at the conclusion of the MGA feeding (day 7). GnRH is

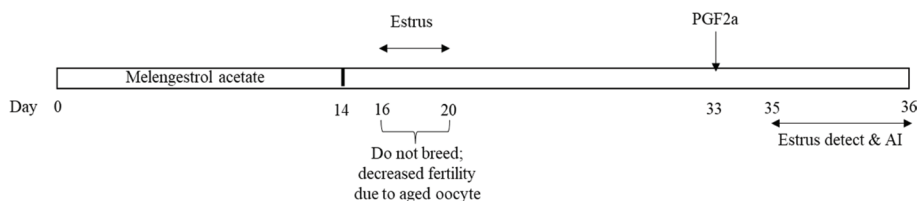


Figure 7A. Schematic presentation of 14 d MGA + PGF_{2α} estrous synchronization protocol in beef heifers

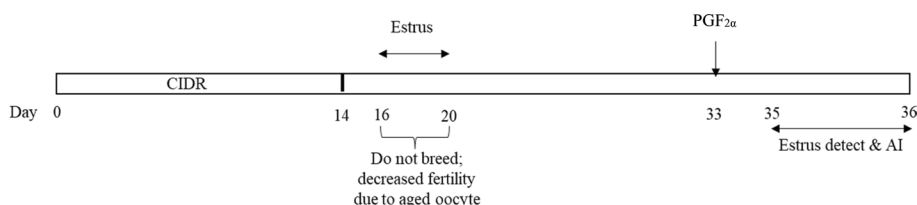


Figure 7B. Schematic presentation of 14 d CIDR + PGF_{2α} estrous synchronization protocol in beef heifers

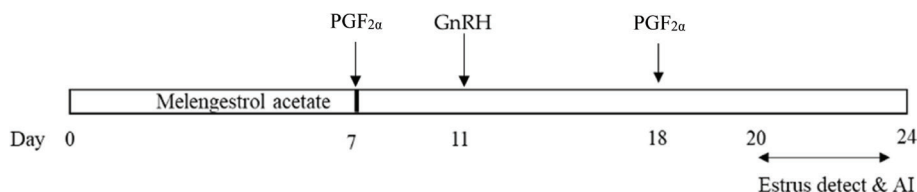


Figure 8. Schematic presentation of 7-11 Synch program

given on day 11, PGF_{2α} 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_{2α} prior to AI at observed 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_{2α} program.^{28,29} Although pregnancy rate to synchronized estrus in 2-4 year old cows were similar to both MGA-Select and MGA + PGF_{2α} 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_{2α} program (71 versus 46%).^{28,29}

Substituting CIDR inserts for MGA in the MGA + Select protocol in beef heifers were evaluated. 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_{2α} and 7-11 synch protocols.³⁰ AI success with various protocols is illustrated (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. Initiation of a new follicular wave and synchronized follicular 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 initiates 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 estradiol 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 _{2α}	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 _{2α} (14 days)	52.3 (376/723)	Heifers ³⁷
Select Synch	60.2 (100/166)	
CIDR + PGF _{2α}	59.1 (528/894)	
5 day Select Synch + CIDR	72 (33/46)	Cows ³⁸
7 day Select Synch + CIDR	72 (36/50)	
CIDR+ PGF _{2α}	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 _{2α}	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 _{2α} (14 days)	60.6 (86/142)	

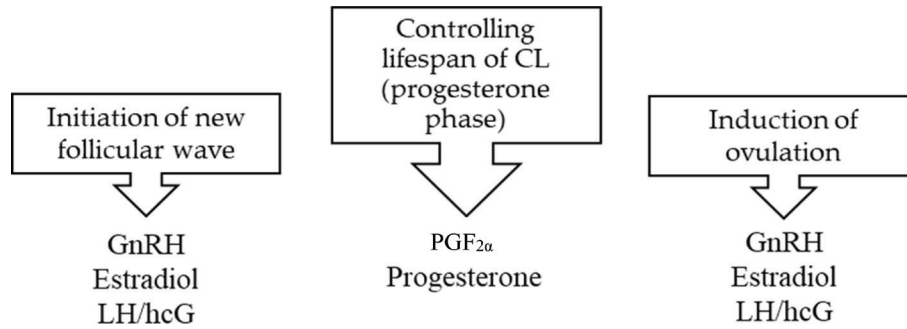


Figure 9. Illustration of 3 principles that are involved in the success of ovulation synchronization programs

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 degeneration 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_{2α}

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

Both Ovsynch and Presynch-Ovsynch are commonly used in dairy operations. Ovsynch includes a first dose of GnRH given

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	<u>6 days per wave</u>
Long range – 24 day cycle	<u>12 days per wave</u>	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.

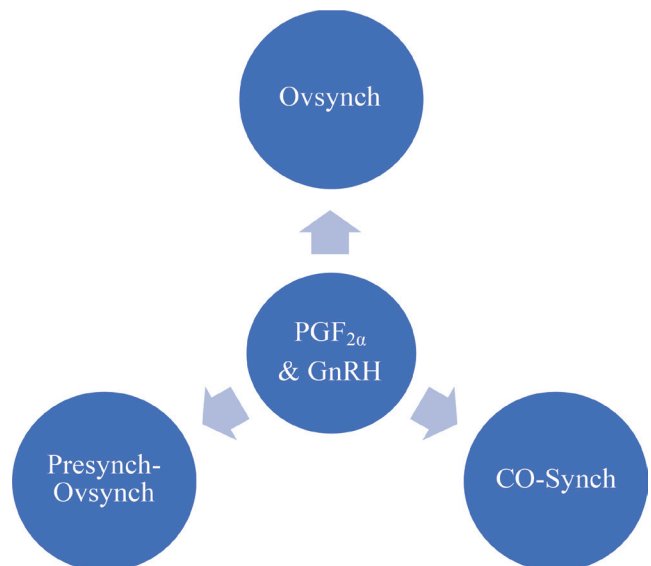


Figure 10. Ovulation synchronization protocols with the combination of GnRH and PGF_{2α}

on a random day of the estrous cycle (day 0). Seven days later, PGF_{2α} (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_{2α} treatments, with a 2 week interval between the first and second PGF_{2α} treatment, in the weeks leading up to the initiation of Ovsynch. However, interval between the second PGF_{2α}

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 anestrus 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_{2α} is given on day 7. Heifers are inseminated 60 ± 4 hours after PGF_{2α} injection and a second dose of GnRH is given at TAI (Figure 11A).

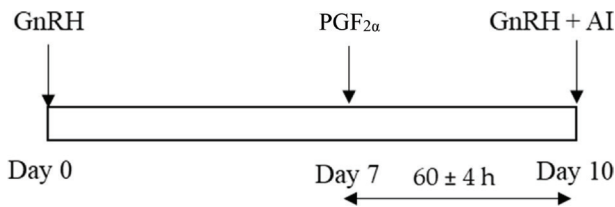


Figure 11A. Schematic presentation of CO-Synch ovulation synchronization protocol in heifers

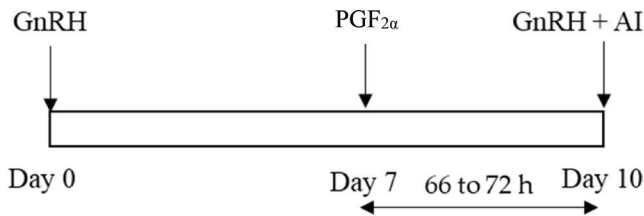


Figure 11B. Schematic presentation of CO-Synch ovulation synchronization protocol in cows

Cows are injected with GnRH on day 0, an injection of PGF_{2α} is given on day 7, with TAI (and a concomitant injection of GnRH) between 66 and 72 hours after PGF_{2α} 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_{2α} treatment). Heifers are inseminated at 60 ± 4 hours after PGF_{2α} injection and a second GnRH is given at TAI (Figure 12A). In cows, insemination is done between 66 and 72 hours after PGF_{2α} with a second GnRH at TAI (Figure 12B).

Pregnancy 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_{2α} approximately 6 to 8 hours apart (additional handling of cows and cost of second PGF_{2α}). 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)	

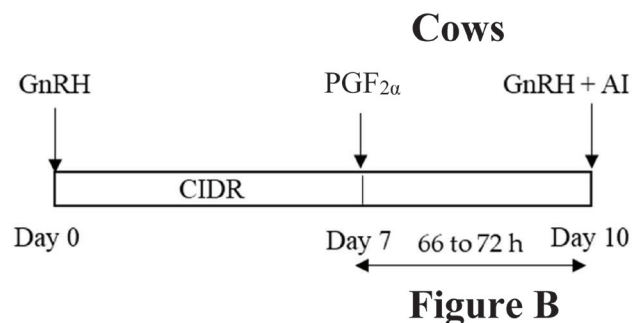
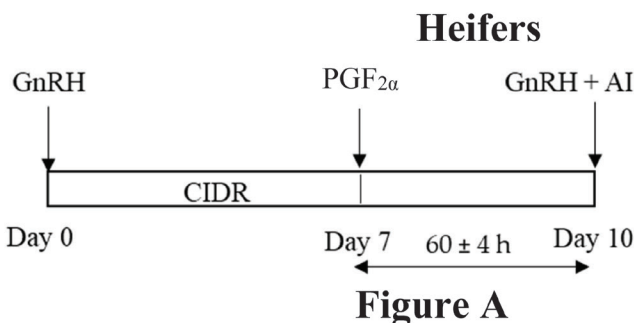


Figure 12. Schematic presentation of CO-Synch + CIDR ovulation synchronization protocol

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 achieved > P/AI.⁵⁵ 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] versus 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 $\text{PGF}_{2\alpha}$ (10 days before initiation of protocol) or 2 doses of $\text{PGF}_{2\alpha}$, 10-14 days apart, with the second dose 10-14 days before protocol initiation,⁵⁶ GnRH alone⁵⁷ or combined with $\text{PGF}_{2\alpha}$,^{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 heifers⁴⁷ 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 $\text{PGF}_{2\alpha}$ 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 $\text{PGF}_{2\alpha}$ on day 0; GnRH on day 7, CIDR insert removal and $\text{PGF}_{2\alpha}$ 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 cows⁴⁹ 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 $\text{PGF}_{2\alpha}$ (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 estrus⁷⁰⁻⁷² 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 trial³³ 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 synchronization protocols. In *Bos indicus* cattle, estrus response following PGF_{2α} 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_{2α} based Ovsynch 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 addition, 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_{2α} 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 anestrus 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 influencing 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 trial⁹¹ 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 glucocan 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 cows¹⁰⁰ and heifers⁹⁹ 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 intrarater 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 system¹⁰¹ 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 nutritional status of cows. The BCS can be categorized as thin < 5, moderate – 5, good – 6 and 7, and obese > 7. It is preferable 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, synchronization 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.

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Conflict of interest

Authors declare that they have no conflict of interest.

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