

ESTABLISHMENT OF PREGNANCY IN BEEF CATTLE: APPLICATION OF BASIC PRINCIPLES

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Introduction

Reproductive efficiency is the most important factor impacting the economics of a cow calf operation. The economic value of reproduction for commercial beef producers was reported to be five times greater than calf growth (Trenkle and Willham, 1977). Maximizing reproductive efficiency depends upon the successful completion of the following events: 1) a heifer must reach puberty before the start of the breeding season, 2) conceive early in the breeding season, 3) calve unassisted, 4) raise the calf to the time it is marketed, and 5) the heifer/cow must conceive in time to calve early during the subsequent calving season. Any interruption in the preceding cycle will constitute reproductive loss, which is estimated to cost the US beef industry around \$500 million annually (Bellows et al., 2002). Therefore, minimizing reproductive loss needs to be a high priority.

Recent years have witnessed the rapid development of technologies utilized to increase reproductive efficiency and (or) improve the genetic merit of a herd. Some of these technologies include: estrous synchronization, artificial insemination, gender-selected semen, in vitro embryo production, embryo transfer, ultrasonography, transgenics, and cloning. Of the preceding reproductive technologies, estrous synchronization and artificial insemination are among the most powerful and applicable technologies for genetic improvement of beef herds (Seidel, 1995). The development of new and improved methods of synchronizing estrus and ovulation depends on our understanding of the physiological and hormonal mechanisms controlling the estrous cycle and the initiation of estrous cyclicity in prepubertal heifers and postpartum cows. Although estrous synchronization products and protocols have changed over time, the basic physiological principles underlying how these products work have not. An understanding of the bovine estrous cycle and how estrous synchronization products work will facilitate the application of these technologies in groups of cycling and anestrous females. This article reviews the endocrine regulation of the estrous cycle with specific emphasis on the regulation of growth of a dominant follicle and the lifespan of the corpus luteum. In addition, emphasis will be given to estrous synchronization products that are commercially available, and the physiologic mechanisms by which these products synchronize estrus and(or) ovulation in cattle.

Principles of the Bovine Estrous Cycle

Characteristics of the estrous cycle. In cattle, the estrous cycle normally varies from 17 to 24 days and the duration of estrus is generally 10 to 18 hrs; however, considerable variation exists among individual animals (range < 8 to > 30 hr; O'Connor and Senger, 1997). The primary sign of estrus in cattle is standing to be mounted and secondary signs of estrus include frequent mounting, watery mucus from the vulva, and restlessness.

There are commercially available estrous detection aids that can be used in conjunction with visual observation to increase estrous detection efficiency in beef herds. The HeatWatch Estrus Detection System is probably the only tool that can replace visual observation, since this system provides precise data on the onset, intensity, and duration of estrus. Some of the more common estrous detection aids include tail chalk/paint, pressure mount detectors, gomer (spotter) bulls (teaser bulls; rendered sterile by vasectomy, epididectomy, and (or) penile deviation), and androgenized cows. Table 1 provides a list of common estrous detection aids, a description of how they work, some potential concerns, and relative cost.

Table 1. A list of estrus detection aids in beef cattle, a description of how they work, potential concerns, and relative cost.

Heat Detection Aid	How it Works	Potential Concerns	Relative Cost
Tail Chalk	Chalk is applied to tailhead. When animal is mounted the color will be rubbed off and hair will be ruffled.	Removal by trees, water, fences, or licking by other animals	\$
Heat Mount Detectors	Detectors are applied to tailhead and turn a different color when mounted.	Partial activation or loss of detector requires interpretation, false activation (e.g. trees, fences, other animals)	\$\$
Heat Watch	Transmitters are attached to tailhead region. When transmitter is depressed a signal is sent to receiver.	Expensive to replace lost sensors, data interpretation, appropriate facilities/terrain	\$\$\$
Gomer Bulls	Vasectomized, epididymectomized, and (or) penile-deviated animals are used as teaser animals and will mount females in estrus.	Feeding and maintenance expense, potential loss of desire to mate, and disease transmission by non penile-deviated animals	\$\$\$
Chin Ball Marking Harness	Detector animal is fitted with harness leaving an inkmark on the back and neck of females that have been mounted.	Maintenance of equipment, feeding and maintenance of animal, ill-defined markings	\$\$
Androgenized cows	Testosterone injections before and during the breeding season or androgen implant causes cow to mount other females in heat.	Cost and labor of administering drug, variable response to hormone	\$\$

Rorie et al., (2002) utilized the HeatWatch system with 500 Angus cows to evaluate the effect of the intensity of estrus on pregnancy rate. Estrus was synchronized with the Select Synch protocol (Gonadotropin releasing hormone [GnRH] followed seven days later with an injection of prostaglandin F_{2α}). Length of estrus ranged from 0.5 to 24 hr and there was no effect of length of estrus on pregnancy status. However, cows that became pregnant were mounted more times per estrus than cows that did not conceive. These data are similar to another study with Angus cows in which cows that became pregnant were mounted more times per estrus than cows that did not become pregnant (Kuhlman et al., 1998).

A seasonal effect on estrous behavior has been reported in Angus x Hereford cows located in Oklahoma (White et al., 2002). In the preceding study, the length of estrus was greater in summer compared to winter or spring; however, cows were mounted more frequently per estrus

in winter compared to summer or spring. Therefore, estrous detection may need to occur more frequently in winter compared to spring or summer; whereas, in summer estrous detection may need to occur for a longer duration at each check. In this study, there was no effect of season on the interval from the onset of estrus to ovulation (Mean = 31 hr). In Florida, an increase in the temperature-humidity index (THI) decreased the number of mounts per estrus (Landaeta-Hernandez et al., 2002).

The number of mounts per estrus increases as the number of females in estrus increases (Helmer and Britt, 1985; Landaeta-Hernandez et al., 2002). This is likely due to the formation of sexually active groups of cattle which is known to increase the number of mounts per female (Hurnick et al., 1975; Galina et al., 1994). In nonsynchronized cattle there will be fewer sexually active groups (or fewer animals per group) and less mounting activity. Therefore, improved estrous detection efficiency is an advantage of an estrous synchronization program. However, it is also true that frequent animal handling and restraint are stressors (Dobson and Kamonpatana, 1986) and that increased handling and restraint of heifers during a synchronized estrus decreased the number of mounts per estrus (Lemaster et al., 1999). Depending upon the estrous synchronization protocol, a fixed-time insemination protocol should reduce the amount of animal handling associated with sorting estrual heifers at the time of insemination.

In contrast to other livestock species, cattle ovulate following the end of estrus (approximately 28 to 32 hr after the onset of estrus or 12 to 20 hr following the end of estrus). Although characteristics of the estrous cycle are similar among most beef breeds, important differences have been reported between *Bos Taurus* and *Bos Indicus* breeds (Galina et al., 1987; Inskip et al., 1982). In general, it is more difficult to detect estrus in *Bos Indicus* females compared to *Bos Taurus* females. This is likely because *Bos Indicus* females are reported to have a shorter duration of behavioral estrus compared to *Bos Taurus* females (Brewester and Cole, 1941; Plasse et al., 1970). In addition, *Bos Indicus* females had a decreased interval from onset of estrus to ovulation (Randel, 1976), decreased magnitude of the preovulatory luteinizing hormone surge (Randel, 1976), smaller corpora lutea (Irvin et al., 1978), and lower luteal phase concentrations of progesterone (Adeyemo and Heath, 1980) than *Bos Taurus* females.

Hormonal Patterns During the Estrous Cycle

The estrous cycle is divided into three stages (follicular phase, estrus, and luteal phase) and is regulated by hormones secreted by the hypothalamus (GnRH), anterior pituitary gland (follicle stimulating hormone [FSH] and luteinizing hormone [LH]), ovary (estradiol and progesterone), and uterus (prostaglandin $F_{2\alpha}$ [$PGF_{2\alpha}$]). The preceding hormones serve as chemical messengers that travel in the blood to specific target tissues which contain receptors that are hormone specific and regulate the phases of the estrous cycle. The combination of hormone secretion and metabolism (liver, kidneys, and lungs) maintain the correct hormonal balance during the follicular phase, estrus, and luteal phase of the cycle. For a list of hormones, their biological functions, their role in estrous synchronization, and product names see Table 2.

A preovulatory follicle and the subsequently formed corpus luteum are the two primary ovarian structures that regulate the estrous cycle through secretion of estradiol and progesterone, respectively. Changes in a preovulatory follicle and corpus luteum, patterns of secretion of LH, estradiol and progesterone, and changes in ovarian blood flow during the ruminant estrous cycle are depicted in Figure 1.

Table 2. Reproductive hormones, their functions during the estrous cycle, roles in estrous synchronization, product name, dosages, and route of administration.

Hormone	Endocrine Gland	Function of Hormone	Biological Action in Estrous Sync.	Product Name	Dosage	Route of Administration
Progesterone	Corpus luteum	Inhibit estrus	Inhibit estrus	Melengestrol Acetate (MGA®)	0.5 mg/hd/day	Feed
		Inhibit ovulation	Inhibit ovulation			
		Prepares animal for pregnancy	Induce cyclicity	EAZI-BREED CIDR®	1 CIDR per animal (1.38 g prog)	Vaginal insert
		Maintenance of pregnancy	Dominant follicle turnover			
Prostaglandin F _{2α}	Uterus	Induce luteal regression	Induce premature luteal regression	Lutalyse®	5 ml	im inject
			ProstaMate®	5 ml	im inject	
			In Synch®	5 ml	im inject	
			Estrumate®	2 ml	im inject	
			estroPLAN®	2 ml	im inject	
Gonadotropin releasing hormone (GnRH)	Hypothalamus	Controls secretion of LH Induces gonadotropin surge	Synchronize follicle wave	Cystorelin®	2 ml	im inject
				Factryl®	2 ml	im inject
				Fertagyl®	2 ml	im inject
				OvaCyst®	2 ml	im inject
Follicle Stimulating Hormone (FSH)	Anterior Pituitary Gland	Initiation of a follicular wave	Superovulation	Follitropin®	Depends on application	im inject
Luteinizing Hormone (LH)	Anterior Pituitary Gland	Stimulated by GnRH	Synchronize follicular wave	N/A	N/A	N/A
		Induction of ovulation	Induction of ovulation			
		Oocyte maturation				
		Luteal tissue formation				
Estradiol	Ovarian follicle	Estrous behavior	Dominant follicle turnover	N/A	N/A	N/A
		Induction of gonadotropin surge	Estrous behavior			
		Sperm transport				

GnRH = gonadotropin releasing hormone; prog = progesterone; N/A = not applicable

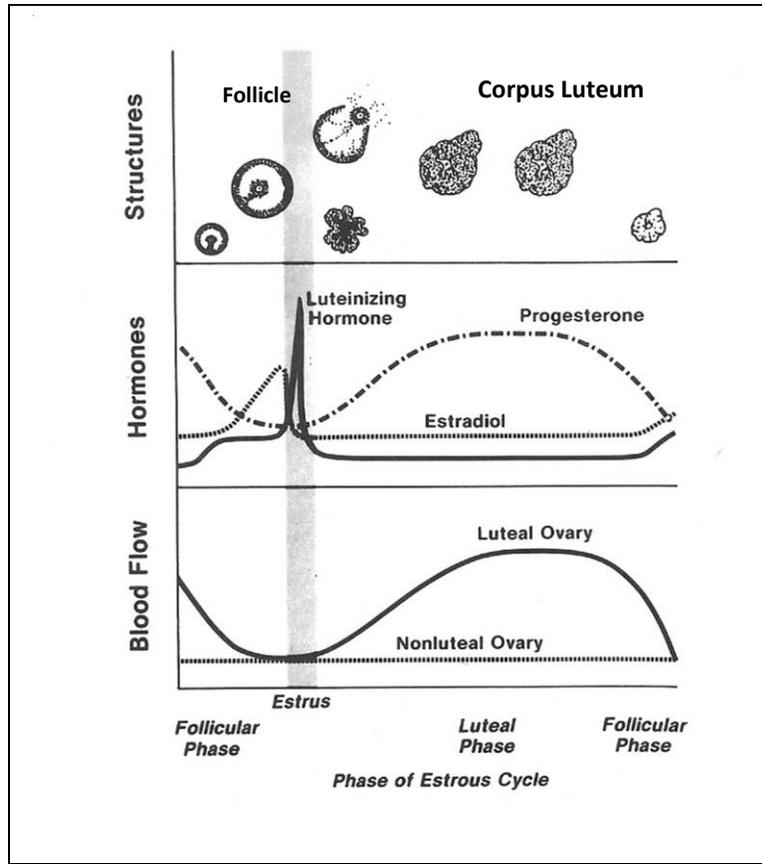


Figure 1. Changes in ovarian structures (preovulatory follicle and corpus luteum), hormones (luteinizing hormone, estradiol, and progesterone) and ovarian blood flow (ovary containing [luteal ovary] or not containing [nonluteal ovary] a corpus luteum) during the three phases of the estrous cycle (follicular, estrus, and luteal phase; Modified from Garverick and Smith, 1993).

Follicular phase. The follicular phase (proestrus) begins with the initiation of corpus luteum regression (luteolysis) and ends with the onset of estrus. Luteolysis is accompanied by a rapid decrease in progesterone resulting in a decrease in the negative feedback on pituitary LH secretion. As circulating concentrations of progesterone decrease, LH pulse frequency increases followed by a rapid increase in follicular estradiol secretion. The production of follicular estradiol results from the coordinated actions of LH and FSH on theca and granulosa cells, respectively (Fortune, 1986; Fortune and Quirk, 1988). The follicle wall consists of two distinct cell layers (granulosa and theca cells) that are separated by a basement membrane. Granulosa cells are located in the compartment with the oocyte; whereas, theca cells surround the granulosa cells and are in close association with a wreath of capillaries. Theca cells have membrane receptors that bind LH resulting in synthesis of androgens that subsequently diffuse through the basement membrane into granulosa cells. Following FSH binding to membrane receptors on granulosa cells there is an increase in aromatase activity, which converts androgens to estradiol. Increased circulating concentrations of estradiol initiate estrous behavior and induce the preovulatory gonadotropin surge, which is essential for ovulation. In addition, estradiol can act within granulosa cells to increase LH receptor concentration and thereby prepare the preovulatory follicle to respond to the gonadotropin surge (Richards, 1980).

Regulation of follicular waves. Two general patterns of antral follicular development are present in mammals. In cattle, sheep, and horses, ovulatory sized follicles develop in sequential waves during both the follicular and luteal phases of the cycle (Figure 2). In primates, pigs, and rodents, however, ovulatory follicles only develop during the follicular phase of the cycle (Fortune, 1994). The bovine estrous cycle usually consists of two to three follicular waves and each wave begins with the recruitment of a cohort of antral follicles from a pool of growing small follicles. One follicle is subsequently selected from this cohort for continued growth and becomes dominant. The remaining follicles in the cohort become atretic. During a nonovulatory follicular wave, the dominant follicle eventually becomes atretic and a new follicular wave is initiated. A viable dominant follicle present at luteolysis will generally become the ovulatory follicle (Adams, 1999). The estrous cycle length of cows that have three follicular waves is generally longer (20-24 days) compared to cows with two follicular waves (18-20 days).

In cattle, follicular waves can be detected during most reproductive states including the prepubertal period, estrous cycle, gestation, and postpartum anestrous period (Adams, 1999). The only exception to the continuous growth and development of follicular waves in cattle is during the last 21 days of gestation. During this time follicles greater than 6 mm in diameter have not been detected (Ginther et al., 1996a). Following parturition, follicular waves resumed following a rise in circulating concentrations of FSH (Schallenberger and Prokopp, 1985), and the first dominant follicle appeared between days 7 and 15 postpartum in both beef and dairy cows (Murphy et al., 1990; Crowe et al., 1993).

Follicular waves have been studied most extensively in cattle and consist of the following three stages: recruitment, selection, and dominance.

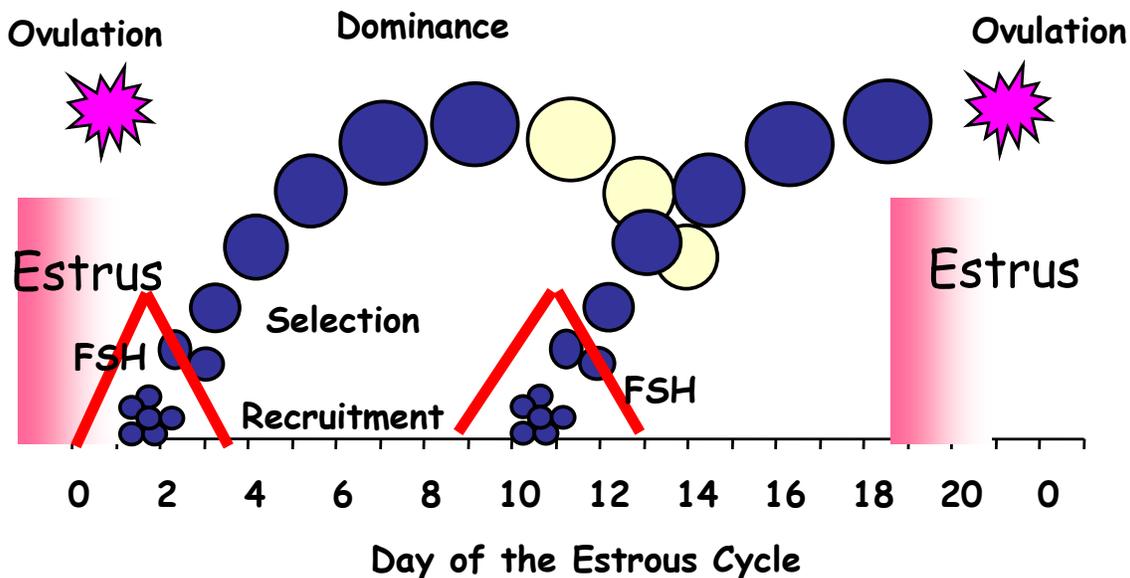


Figure 2. Relationship between circulating concentrations of follicle stimulating hormone (FSH) and stages of a bovine follicular wave (recruitment, selection, and dominance). A transient increase in FSH (solid line) initiates recruitment of a cohort of follicles, from which a single follicle is normally selected to become the dominant follicle. If the corpus luteum regresses in the presence of a viable dominant follicle ovulation will occur (second follicular wave). However, in the absence of luteal regression, the dominant follicle becomes atretic (regresses; light circles; Modified from Kojima and Patterson, 2003).

Recruitment. Recruitment of a cohort of follicles, around 3 mm in diameter, is stimulated on each ovary by a transient rise in FSH (Figure 2). Inhibition of both FSH and LH arrested follicular growth at 2 to 4 mm, however, when physiological levels of FSH were infused for 48 hr follicular growth from 5 to 8 mm was stimulated (Gong et al., 1996). The peak concentration of FSH occurred when the future dominant follicle attained a mean diameter of approximately 4 mm, after which concentrations of FSH declined (Figure 2; Ginther et al., 1996b), and were at basal concentrations by the time follicular selection occurred (Ginther et al., 2000a). The mechanism responsible for the initial decline in FSH concentration is unknown; however, estradiol and inhibin are follicular products that probably play a major role in the decline of FSH (Adams, 1999).

Selection. Follicular selection is the process by which a single follicle from the recruited cohort is selected to continue to grow and become dominant, while the remaining follicles of the cohort undergo atresia. With the decline in circulating FSH concentrations, small follicles are presumably unable to continue growth and the selected follicle (dominant follicle) may shift its dependency from FSH to LH (Beg and Ginther, 2006; Lucy, 2007). The decreased circulating concentrations of FSH at the time of selection are likely important for the selection of a single dominant follicle (Figure 2). The decline in circulating concentrations of FSH is presumably driven by increasing concentrations of estradiol (and perhaps inhibin) produced by the cohort of recruited follicles (Ginther et al., 2000b; Beg and Ginther, 2006). Increased concentrations of estradiol and inhibin may feedback on the hypothalamic-pituitary axis to selectively suppress FSH secretion (Martin et al., 1988). At follicular deviation, the selected follicle continues to grow while the subordinate follicles enter atresia (Ginther et al., 1996b). In cattle, deviation usually occurs when the largest follicle reaches a diameter of approximately 8 mm, approximately 2.7 days after the initiation of a follicular wave (Ginther et al., 1997; Ginther et al., 1999) or 61 hr after the LH surge (Kulick et al., 1999).

Dominance. The dominance phase of the follicular wave occurs when a follicle has been selected and continues to grow at a faster rate than the largest subordinate follicle, and inhibits the emergence of a new follicular wave (Ginther et al., 1996b; Lucy, 2007). Following selection and establishment of a dominant follicle, follicular recruitment is inhibited until dominance is lost or ovulation occurs. Inhibition of follicular recruitment may be mediated by inhibiting the transient rise in circulating concentrations of FSH (Adams, 1999). An alternative hypothesis is that the dominant follicle directly inhibits growth of small follicles through the secretion of a factor(s) that acts directly on other follicles in the ovary. Regardless of the mechanism, destruction or ovulation of a dominant follicle results in a transient rise in circulating concentrations of FSH and subsequent initiation of a new follicular wave (Adams et al., 1992).

Estrous phase. Increasing circulating concentrations of estradiol following luteolysis initiate estrous behavior, increase uterine contractions (facilitate sperm transport), and induce the preovulatory gonadotropin surge. The preovulatory gonadotropin surge coordinates the following events that are critical to the establishment of pregnancy: resumption of meiosis within the oocyte, follicular rupture, and luteinization of follicular cells. LH is generally considered to be the primary gonadotropin that controls the preceding events; however, FSH also has been shown to cause ovulation and luteal tissue formation (Galway et al., 1990). The end of the estrous phase of the cycle is marked by follicular rupture, which is the culmination of a complex cascade of events leading to the activation of proteolytic enzymes that digest the follicular wall

and allows the egg (oocyte) to be released for fertilization. This process is similar to mechanisms associated with inflammation. Injection of GnRH will induce a surge of LH within 2 to 4 hr and ovulation of a dominant follicle will occur 24 to 36 hr after injection (Figure 3).

Estrus and ovulation are not always linked and frequently occur as independent events. The incidence of anovulatory estrus in peripuberal heifers was 22% and 13% for years 1 and 2, respectively and this phenomenon has been called nonpuberal estrus (Nelsen et al., 1985; Rutter and Randel, 1986). The incidence of nonpuberal estrus may be affected by age, breed, and photoperiod or season of the year (Nelsen et al., 1985). Formation of a cystic follicle can also result in estrous behavior without ovulation; however, the incidence of cystic follicles is low in beef cattle. Cystic follicles are normally treated by injecting GnRH, to luteinize the follicular tissue followed by an injection of PGF_{2α} 7 days later to regress the luteal tissue.

Alternatively, ovulation without estrus is not uncommon in beef cattle. The first ovulatory estrus in heifers and postpartum cows is preceded by a transient increase in progesterone (short luteal phase; Gonzalez-Padilla et al., 1975). This is presumably due to ovulation without estrus. Increased concentrations of progesterone may be involved in preparation of the uterus for the possibility of pregnancy or in the establishment of patterns of gonadotropin secretion characteristic of cycling females. Short-term exposure of prepuberal heifers or anestrous postpartum beef cows to a progestin (Melengestrol Acetate [MGA] or Controlled Internal Drug Release [CIDR]) has been used extensively in estrous synchronization protocols to mimic this short period of progesterone exposure and will be discussed in more detail later.

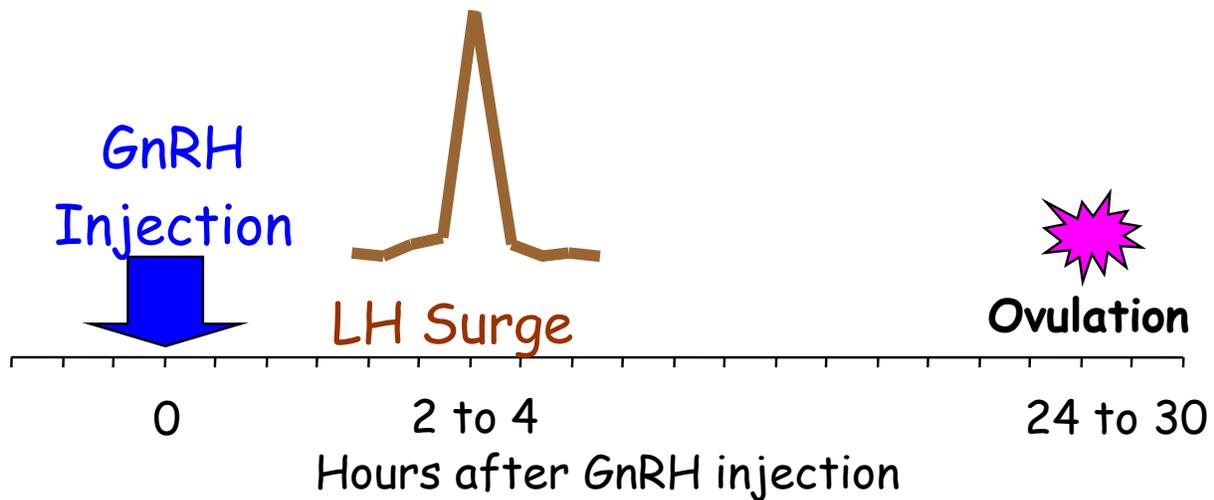


Figure 3. Injection (im) of GnRH will induce a surge of LH within 2 to 4 hr and ovulation of a viable dominant follicle (≥ 10 mm) will occur within 24 to 36 hr (Modified from Kojima and Patterson, 2003).

Luteal phase. The luteal phase spans the time of corpus luteum formation and maintenance which begins with ovulation and ends with luteolysis (Figure 4). Progesterone is the primary secretory product of the corpus luteum and is regulated by secretions of the anterior pituitary, uterus, ovary, and embryo (Niswender et al., 1976). The regulation of progesterone secretion is likely controlled by a balance of luteotropic (stimulate progesterone) and luteolytic (inhibit progesterone) stimuli, given that both types of stimuli are secreted concurrently during the estrous cycle. In ruminants, LH is considered to be the primary luteotropic hormone and

concentration of luteal LH receptors is positively correlated with changes in progesterone and luteal growth (Niswender et al., 2000). Corpora lutea receive the majority of the ovarian blood flow (Figure 2) and blood flow to the luteal ovary and progesterone secretion are highly correlated (Niswender et al., 1976). Progesterone has a central role in the regulation of the estrous cycle as it determines estrous cycle length and is required for the maintenance of pregnancy.

In cattle, $\text{PGF}_{2\alpha}$ is the uterine luteolysin and is commonly used to synchronize estrus in cattle. In the absence of an embryo, the uterine concentrations of $\text{PGF}_{2\alpha}$ increase during the late luteal phase and $\text{PGF}_{2\alpha}$ is secreted as pulses into the uterine veins on days 17 to 20 following estrus (Figure 4; day 0 = estrus; Inskeep and Murdoch, 1980). $\text{PGF}_{2\alpha}$ is transported from the utero-ovarian vein into the ovarian artery via a counter-current transfer mechanism (Hixon and Hansel, 1974; McCracken et al., 1972) and is transported to the corpus luteum. $\text{PGF}_{2\alpha}$ may have both a direct and an indirect effect on a ruminant corpus luteum to cause luteolysis. In the presence of an embryo, pulsatile secretion of $\text{PGF}_{2\alpha}$ is reduced and the corpus luteum does not regress. Maintenance of high circulating concentrations of progesterone in pregnant animals prevents the expression of estrus and ovulation.

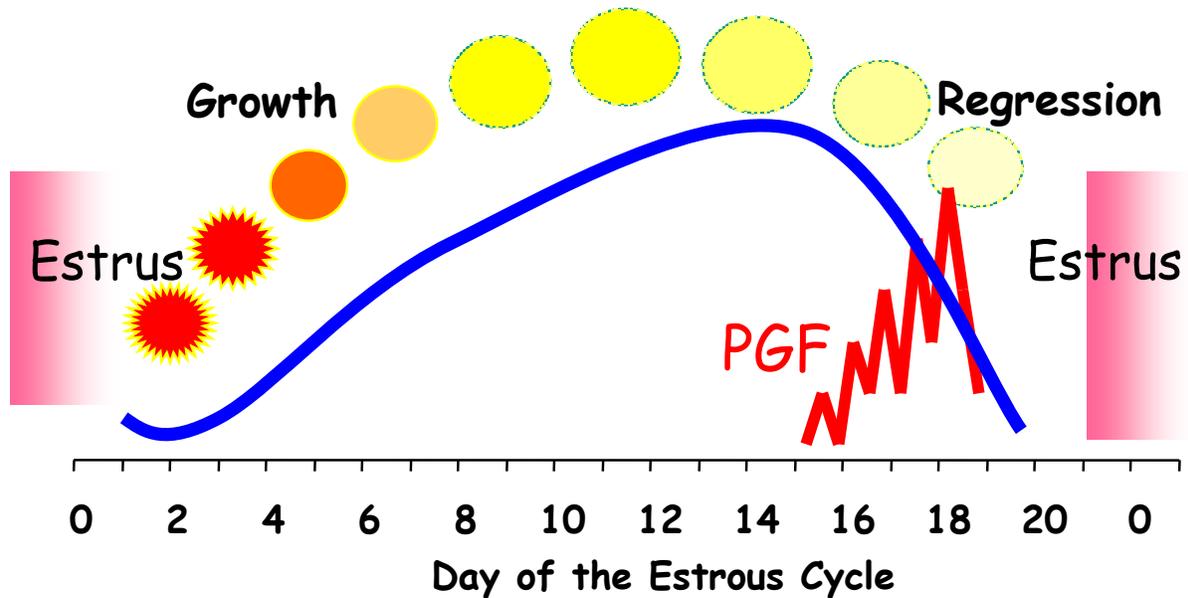


Figure 4. Changes in corpus luteum development, circulating concentrations of progesterone, and circulating concentrations of prostaglandin $\text{F}_{2\alpha}$ (PGF) during the luteal phase of the bovine estrous cycle are depicted above. Luteal secretion of progesterone inhibits the expression of estrus, inhibits ovulation, and is essential for the maintenance of pregnancy. In the absence of an embryo, $\text{PGF}_{2\alpha}$ is secreted as pulses that cause a precipitous decrease in progesterone and regression of the corpus luteum. Products that mimic the action of progesterone (progestins) are commonly used in estrous synchronization. Progestin administration in cows that have experienced corpus luteum regression will delay the expression of estrus and ovulation until after progestin withdrawal (Modified from Kojima and Patterson, 2003).

Follicular determinants of corpus luteum function. Corpora lutea are a continuation of follicular maturation; consequently, changes in the hormonal stimulation of a preovulatory follicle may have a subsequent effect on luteal progesterone secretion. The endocrine microenvironment of a preovulatory follicle is unique relative to surrounding nonovulatory

follicles and is important for preparation of follicular cells for luteinization and secretion of progesterone (McNatty et al., 1975). McNatty et al. (1979) suggested that development of a normal corpus luteum may depend upon a preovulatory follicle meeting the following criteria: 1) an adequate number of granulosa cells, 2) an adequate number of LH receptors on granulosa and theca cells, and 3) granulosa cells capable of synthesizing adequate amounts of progesterone following luteinization. Furthermore, the ability of luteinized human granulosa cells to secrete progesterone increased when the cells were collected from follicles having increased follicular fluid concentrations of estradiol compared to granulosa cells collected from follicles that had lower concentrations of estradiol (McNatty et al., 1979). Premature induction of ovulation in ewes was associated with luteal insufficiency (Murdoch et al., 1983). These data are relevant to fixed-time insemination protocols in which physiologically immature dominant follicles are induced to ovulate at AI and the subsequent circulating concentrations of progesterone are lower than in cows in which a larger dominant follicle is induced to ovulate with GnRH (Perry et al., 2005). Inadequate luteal function following induced ovulation may be due to a reduced number of follicular cells and(or) inadequate preparation of follicular cells for luteinization and secretion of progesterone.

Estrous Synchronization Products and Mechanism of Action

Effective estrous synchronization protocols are designed to synchronize follicular maturation with the onset of corpus luteum regression. In general, development of estrous synchronization protocols in cycling animals has involved the following three approaches: 1) Inhibit ovulation following spontaneous corpus luteum regression (long-term progestin treatment), 2) Induction of corpus luteum regression (PGF_{2α} treatment), and 3) a combination of 1 and 2. Most of the protocols utilized today can be categorized under the third approach. The first approach requires long-term progestin treatment (14 days) and is effective at synchronizing estrus; however, fertility at the synchronized estrus is frequently reduced due to the presence of persistent follicles (see section below). The second approach results in good fertility; however, animals that are in the first 5 to 6 days of their cycle will not respond to the PGF_{2α} injection, resulting in a reduced synchronization response. The third approach allows effective synchronization of estrus, regardless of stage of the cycle, without compromising fertility. This is particularly true when an injection of GnRH is administered at the beginning of progestin treatment to ovulate a dominant follicle and synchronize a new follicular wave. The following section is focused on specific hormonal changes that are required for the establishment of pregnancy and a description of how specific estrous synchronization products mimic these changes (Figure 5). Subsequent papers in the proceedings will provide detailed information on specific estrous synchronization protocols.

Hormonal Changes That Optimize Pregnancy Success. The following physiological changes, before and after fertilization, are required to maximize pregnancy rate: 1) High circulating concentrations of progesterone for several days prior to the onset of corpus luteum regression, 2) Rapid and complete regression of the corpus luteum resulting in basal concentrations of progesterone by the time of AI, 3) Increased secretion of estradiol by a physiologically mature follicle before and at the time of AI, and 4) Ovulation followed by an increase in adequate amounts of progesterone in circulation by the newly formed corpus luteum. Estrous synchronization protocols that result in synchronized pregnancy rates that are similar to pregnancy rates following spontaneous expression of estrus have been designed to mimic the

preceding physiological changes. The specific physiological contributions of the basic components of most fixed-time synchronization protocols are described below.

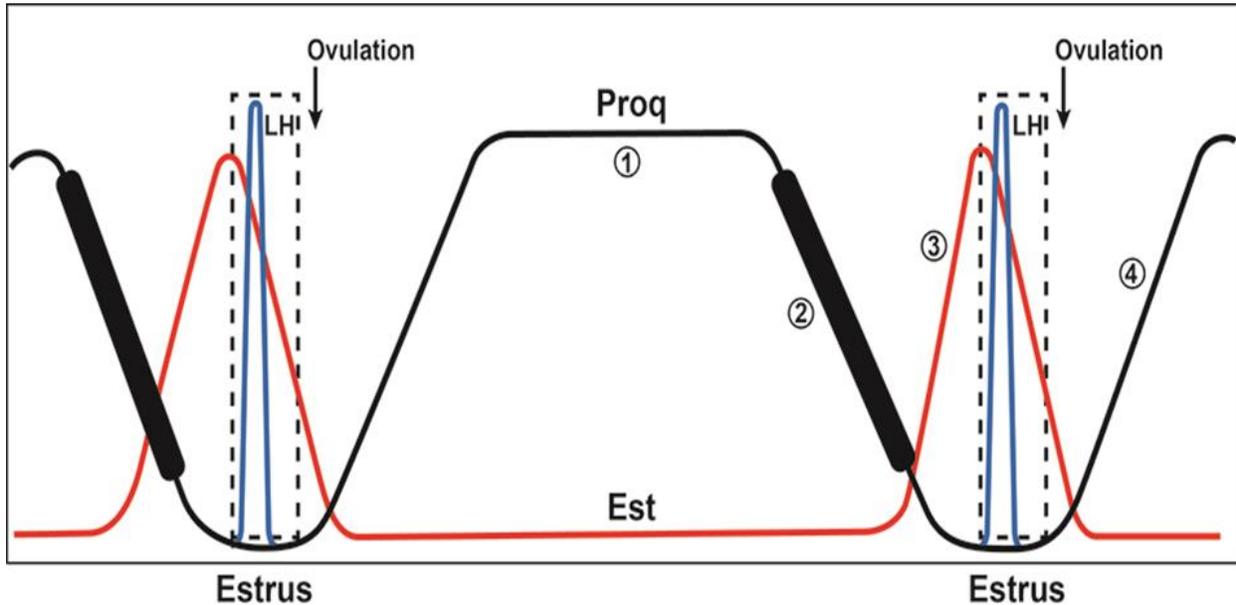


Figure 5. Hormonal changes during a bovine estrous cycle that are required for the establishment of pregnancy: 1) High circulating concentrations of progesterone (Prog) for several days prior to the onset of corpus luteum regression are important for management of follicular waves and oocyte quality in cycling animals. In prepuberal heifers and anestrus cows an increase in progesterone is important for inducing normal estrous cycles. The primary progestin products available for accomplishing this include the CIDR and MGA, 2) Rapid and complete regression of the corpus luteum resulting in basal concentrations of progesterone by the time of AI is required for fertility. There are numerous reports of decreased pregnancy rates to fixed-time AI when progesterone concentrations are elevated at the time of AI. Induction of luteal regression is accomplished by administration of PGF_{2α} products, 3) Increased secretion of estradiol (Est) by a physiologically mature follicle before and at the time of AI. Estradiol is the follicular hormone that coordinates several processes that are important for fertilization and the establishment of pregnancy such as estrous expression and sperm transport. Increased circulating concentrations of estradiol are normally achieved by inseminating at the recommended time following the induction of corpus luteum regression. Injection of a GnRH product will induce a surge of luteinizing hormone (LH), which is the signal for ovulation. Initiation of the ovulatory process will decrease the production of estradiol and increase production of progesterone. Therefore, injecting GnRH before the recommended time may decrease circulating concentrations of estradiol, and 4) Ovulation followed by an increase in adequate amounts of progesterone in circulation by the newly formed corpus luteum. Progesterone is essential for the maintenance of pregnancy and a rapid increase in progesterone after ovulation can increase the rate of embryonic development.

High circulating concentrations of progesterone prior to the onset of corpus luteum regression. Elevated serum concentrations of progesterone during the cycle before conception have been associated with increased pregnancy rates. Although it is not known how circulating concentrations of progesterone during one cycle affects pregnancy rates following the subsequent estrus, it is clear that low concentrations of progesterone during the luteal phase can adversely affect the quality of the oocyte that is ovulated. Increased circulating concentrations of progesterone before AI have been shown to be important for management of the follicular wave and improved oocyte quality. In an estrous synchronization protocol, elevated concentrations of progesterone are accomplished by feeding Melengestrol Acetate (MGA; an orally active progestin) or insertion of an intravaginal insert containing progesterone (CIDR; Controlled

Internal Drug Release). However, if circulating concentrations of progesterone are low due to the absence of a corpus luteum and the presence of a CIDR or MGA, this can result in prolonged growth of a dominant follicle which will decrease oocyte quality. This is due to the formation of a persistent follicle as described in the section below. In addition, in prepubertal heifers or anestrus cows, exposure to progesterone before fixed-time AI can initiate cyclicity. A short luteal phase usually occurs in prepubertal heifers and postpartum beef cows following the first ovulation (Perry et al., 1991; Werth et al., 1996). This short exposure to progesterone is believed to be necessary for reprogramming the reproductive axis to resume normal estrous cycling. The short lived corpus luteum can be mimicked by feeding MGA or inserting a CIDR. Consequently, increased circulating concentrations of progesterone before induction of luteal regression can induce normal cycles in a proportion of anestrus females and increase oocyte quality.

Rapid and complete regression of the corpus luteum. There are reports that pregnancy rates to fixed-time AI are reduced when corpus luteum regression is not complete. This is because incomplete luteal regression results in elevated circulating concentrations of progesterone at AI. Progesterone is secreted by the corpus luteum and has a number of actions that are opposite to the actions of estradiol. For example, progesterone can block estrus expression, inhibit the ability of estradiol to induce an LH surge (signal for ovulation), and inhibit the transport of sperm and the oocyte within the reproductive tract. In addition, elevated concentrations of progesterone around the time of AI can decrease circulating concentrations of estradiol, which is known to have an important role in the establishment of pregnancy. Therefore, ensuring that the corpus luteum regresses in response to an injection of PGF_{2α} and that the dominant follicle has adequate time to secrete estradiol before induction of ovulation is essential. It is important to remember that the corpus luteum is not responsive to PGF_{2α} during the first five days of the estrous cycle, which is why PGF_{2α} is administered at CIDR removal instead of CIDR insertion. The reason that two injections of PGF_{2α} are recommended with the 5 day CO-Synch CIDR + PG protocol is because PGF_{2α} is being given at a time when the corpus luteum that formed in response to GnRH is just becoming responsive to PGF_{2α}.

Increased secretion of estradiol by a physiologically mature follicle at the time of AI. The oocyte resides within a follicle and there is bidirectional communication, between the oocyte and surrounding follicular cells that are essential for proper development of both the follicle and oocyte. Estradiol is the follicular hormone that initiates estrus expression and cows that express estrus around the time of fixed-time AI have higher pregnancy rates than cows that do not express estrus. Estradiol has a number of important functions that increase the probability that a cow will conceive including estrus expression, contractions of the uterus and oviduct that facilitate sperm transport, and preparation of the uterus for pregnancy. From our own studies we know that increased circulating concentrations of estradiol around the time of fixed-time AI increased fertilization rate and pregnancy rate (Atkins et al 2013, Jinks et al 2013). The purpose of the GnRH injection at the start of a fixed-time AI protocol is to synchronize a follicular wave which will result in a more physiologically mature follicle that can secrete adequate amounts of estradiol by the time of AI.

Ovulation followed by an increase in adequate amounts of progesterone. It is well known that progesterone is required for the establishment and maintenance of pregnancy in numerous species, including cattle. More specifically, progesterone has a critical role in the production of

uterine secretions that sustain the embryo before placental development. A more rapid increase in circulating concentrations of progesterone following AI resulted in a larger embryo that was able to produce more of the signal for maternal recognition of pregnancy (interferon-tau). As previously mentioned, the corpus luteum is a continuation of the maturation of a dominant follicle. Physiologically mature dominant follicles that secrete adequate amounts of estradiol subsequently secrete adequate amounts of progesterone after a corpus luteum forms. These data are relevant to fixed-time insemination protocols in which physiologically immature dominant follicles may be induced to ovulate at AI and the subsequent circulating concentrations of progesterone are lower than in cows in which a larger dominant follicle is induced to ovulate with GnRH (Perry et al., 2005). Inadequate luteal function following induced ovulation may result in lower pregnancy rates due to decreased secretion of progesterone.

Hormonal management of the luteal phase for synchronization of estrus. Successful estrous synchronization protocols require control of the timing of both dominant follicle development and luteal regression. During the estrous cycle when a corpus luteum is present and circulating concentrations of progesterone are high, standing estrus and ovulation are inhibited; however, when the corpus luteum regresses and progesterone concentrations decrease, circulating concentrations of estradiol increase and the animal returns to standing estrus. Progestins mimic the actions of progesterone produced by the corpus luteum and inhibit estrus/ovulation which can delay the interval to estrus when luteal tissue is not present. Following the removal of the progestin, progesterone concentrations will be low and standing estrus and ovulation will occur.

Progestins. Two progestin products that are commercially available for estrous synchronization include Melengestrol Acetate (MGA) and the CIDR (Controlled Internal Drug Release). In cycling cows and heifers, administration of MGA or CIDRs does not affect the time of corpus luteum regression. However, once corpus luteum regression has occurred, progestin administration can prevent a cow or heifer from showing estrus and ovulating. Consequently, progestin administration in cows that have experienced corpus luteum regression will delay the expression of estrus and ovulation until after progestin withdrawal.

Role of progestins in anestrus. At the start of a breeding season, most herds consist of a mixture of cycling and anestrous females. An effective estrous synchronization protocol must be able to induce a fertile estrus or ovulation in both anestrous and cycling heifers and cows. As previously mentioned, a short luteal phase usually occurs in prepuberal heifers and postpartum beef cows following the first ovulation (Perry et al., 1991; Werth et al., 1996). This short exposure to progesterone is believed to be necessary for reprogramming the reproductive axis to resume normal estrous cycling. Therefore, in herds that have a large proportion of prepuberal heifers or anestrous cows, progestin pretreatment before induction of ovulation can initiate estrous cycling status and eliminate or at least reduce the occurrence of short estrous cycles.

Administration of low levels of a progestin (i.e. MGA), in the absence of a corpus luteum, can result in the formation of a persistent follicle (see below). However, the effect of progestin treatment on persistent follicle formation differs between cycling and anestrous animals. Administration of low concentrations of progestins did not induce persistent follicle formation in early postpartum anestrous dairy heifers (Rhodes et al., 1997) or anestrous postpartum beef cows (Perry et al., 2002). It is not clear why persistent follicles did not form in anestrous cows.

Progestin administration and formation of persistent follicles. Persistent follicles are characterized by an extended dominant follicle life span and increased estradiol production (Zimbelman and Smith, 1966b; Sirois and Fortune, 1990; see review by Fortune and Rivera, 1999). Treatment of cycling heifers or cows with low levels of a progestin, following luteolysis, resulted in the formation of persistent follicles that had a large diameter, extended lifespan, and increased production of estradiol (Zimbelman and Smith, 1966a; Sirois and Fortune, 1990; Fortune et al., 2001). Administration of low (subluteal) concentrations of progestins to cattle, in the absence of luteal tissue, increased LH pulse frequency (Savio et al., 1993; Kojima et al., 1995; Kinder et al., 1996); however, treatment with midluteal phase concentrations of progesterone decreased LH pulse frequency and persistent follicles did not form (Sirois and Fortune, 1990; Savio et al., 1993). Thus, the formation of persistent follicles has been associated with increased LH pulse frequency, and infusion of exogenous LH induced persistent follicle formation (Duffy et al., 2000).

Insemination immediately following long-term progestin treatment and ovulation of a persistent follicle has been associated with decreased fertility (Mihm et al., 1994). No difference was reported in fertilization rate following ovulation of persistent follicles, but fewer zygotes developed into embryos containing 16 or more cells compared to ovulation of oocytes from control follicles (Ahmad et al., 1995). Decreased fertility following formation and ovulation of persistent follicles may result from alterations in the uterine environment due to increased estradiol secretion (Butcher and Pope, 1979) and/or premature resumption of meiosis due to prolonged exposure to increased LH pulse frequency (Mattheij et al., 1994).

Progestin administration-management tips. Melengestrol acetate is an orally-active progestin and each animal must receive the appropriate daily dose of MGA throughout the treatment period. The effect of MGA treatment (14 days) on cows in different stages of the estrous cycle is illustrated in Figure 6. If you detect an animal in standing estrus while feeding MGA then it is likely the animal did not receive the appropriate dose of MGA. Melengestrol acetate should be fed at a dose of 0.5 mg/hd/day in 2 to 5 lb of a highly palatable carrier. The MGA should not be top-dressed on a large amount of feed such as silage. If cattle are on a lush pasture it can be helpful to remove salt from the pasture and include the salt (0.5 oz/cow/day) in the MGA carrier. In addition, it is a good idea to feed carrier alone for several days before administering the MGA so that the cattle become accustomed to coming to the bunk. There should be a minimum of 18 in. of bunk space for heifers and 24 in. for cows. Remember to not inseminate cattle at the estrus immediately following long-term (14 days) MGA treatment since fertility will be reduced due to the ovulation of persistent follicles (see previous section).

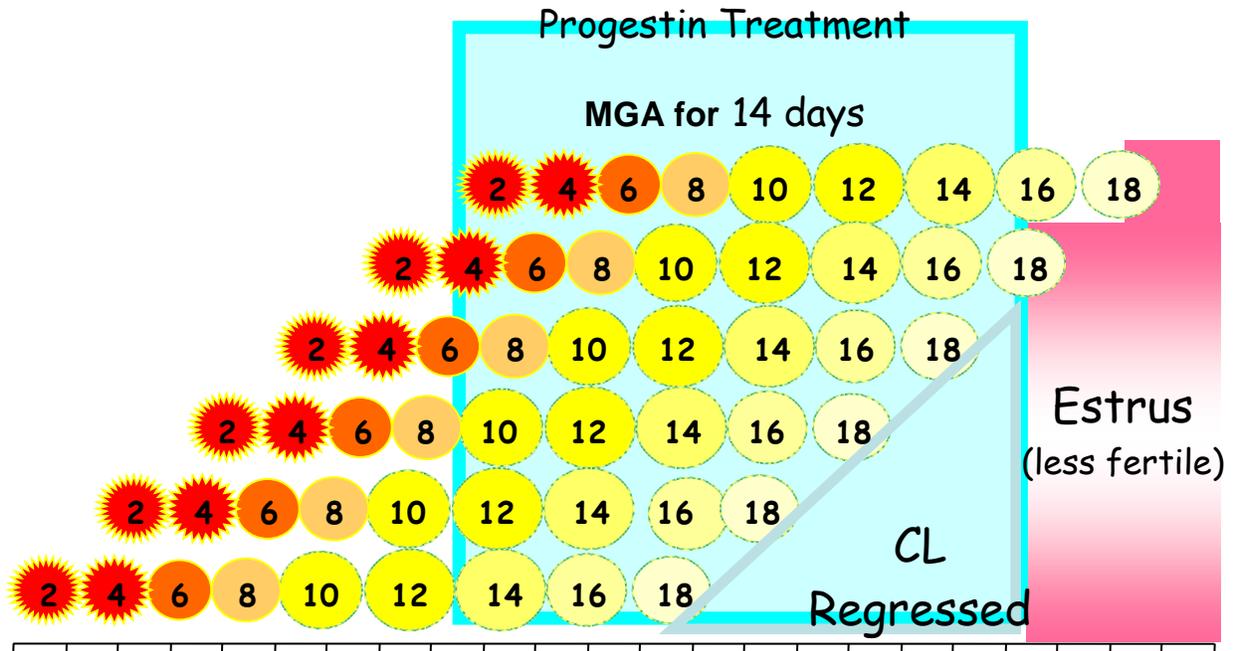


Figure 6. Effect of 14 days of melengestrol acetate (MGA) feeding on estrous synchronization of cows in different stages of the estrous cycle. Each row of circles represent development and regression of corpora lutea (CL) for an individual heifer. Numbers inside each circle represent days of the cycle. In this diagram, spontaneous luteal regression occurs around day 17 to 18 of the cycle. Note that at the end of progestin treatment all corpora lutea have regressed or are in the process of regressing (Modified from Kojima and Patterson, 2003).

In the absence of a corpus luteum, a CIDR functions as an artificial corpus luteum by releasing progesterone and thereby suppressing estrus and ovulation for seven or more days. CIDR's consist of a "T" shaped nylon backbone that is coated with a silicone layer containing 10% progesterone by weight. The CIDR's are inserted into the vagina with a lubricated applicator following disinfection of the applicator and vulva. CIDR's are easily removed by pulling the flexible nylon tail. Although a small amount of vaginitis is a common observation at CIDR removal, fertility is not compromised. The retention rate of CIDR's is approximately 95%. If the retention rate is considerably less than 95% the device may have been inserted incorrectly or other animals may be pulling the CIDR's out by biting on the nylon tails. In the latter case, the problem can be remedied by trimming the nylon tails.

Prostaglandin $F_{2\alpha}$. Prostaglandins are naturally occurring compounds that are produced by most cells in the body and have a variety of biological actions. Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) is a naturally occurring luteolytic hormone that has also been utilized to synchronize estrus and induce abortion in cattle through induction of corpus luteum regression. In the absence of an embryo, uterine concentrations of $PGF_{2\alpha}$ increase during the late luteal phase. $PGF_{2\alpha}$ is secreted in pulses and transported to the corpus luteum via a counter-current mechanism. The mechanisms associated with $PGF_{2\alpha}$ -induced luteolysis are not completely understood; however, $PGF_{2\alpha}$ probably has both a direct and indirect (decreased blood flow) action. Luteal cells are known to have $PGF_{2\alpha}$ receptors on the plasma membrane and direct inhibitory effects of $PGF_{2\alpha}$ on luteal progesterone secretion have been demonstrated (Niswender et al., 2000). In addition,

PGF_{2α} is known to reduce luteal blood flow due to vasoconstrictor activity (Niswender and Nett, 1988).

Administration of PGF_{2α} to domestic ruminants does not induce luteolysis during the early luteal phase (Figure 7). For purposes of estrous synchronization, injection of PGF_{2α} is only effective in cycling heifers and cows (approximately day 6 to 16 following estrus; day 0 = estrus). Although functional PGF_{2α} receptors and signal transduction mechanisms are present in developing ovine corpora lutea (Tsai et al., 1997; Tsai and Wiltbank, 1998), the acquisition of luteolytic capacity is not established until after day 4 postestrus (Tsai and Wiltbank, 1998).

Injection of PGF_{2α} into prepubertal heifers or anestrous cows is not effective due to the absence of luteal tissue. Furthermore, PGF_{2α} treatment will not induce cycling activity in noncycling cattle. Therefore, when using PGF_{2α} alone to synchronize estrus it is important to assess the proportion of cycling animals before initiating the treatment. In herds containing both cycling and noncycling females, the most effective estrous synchronization protocols combine treatment with a progestin and an injection of PGF_{2α}. In pregnant feedlot heifers, PGF_{2α} is highly effective at inducing abortion before 100 days of gestation.

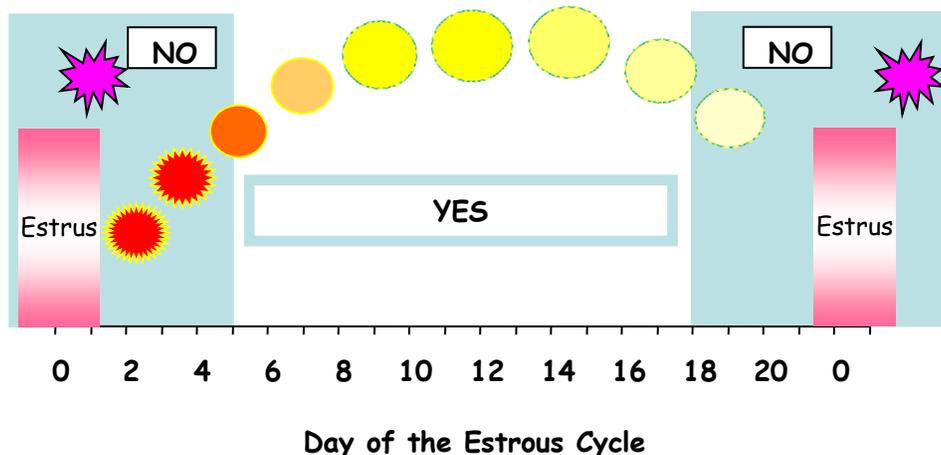


Figure 7. Effect of stage of the bovine estrous cycle on luteal responsiveness to PGF_{2α}. Bovine corpora lutea will not respond to an injection of PGF_{2α} during the first five days of the cycle. Therefore, PGF_{2α} should not be injected at the beginning of progestin treatment (Modified from Kojima and Patterson, 2003).

Hormonal management of follicular waves for synchronization of estrus. The development of effective protocols for fixed-time insemination is dependent upon the precise synchronization of follicular waves culminating in a fertile ovulation at a predetermined time. Two approaches that have been used to synchronize bovine follicular waves include: 1) ovulating/destroying the dominant follicle and thereby initiating a new follicular wave, and 2) prolonging the lifespan of a dominant follicle (persistent follicle).

Initiation of a new follicular wave occurs following ovulation or turnover (atresia) of the dominant follicle. Administration of exogenous progesterone, estradiol, or GnRH has been utilized to turnover (progesterone and estradiol) or ovulate (GnRH) dominant follicles and to synchronize follicular waves in heifers and cows (see reviews by Bo et al., 1995; Diskin et al., 2002). Follicular turnover (atresia) of persistent follicles can be accomplished through the administration of progesterone. Progesterone as a single injection (Anderson and Day, 1994) or

administered over a 24 hr period (McDowell et al., 1998) effectively regressed persistent follicles and initiated new follicular waves. Reduction of LH pulse frequency and amplitude following the administration of exogenous progesterone may be the mechanism by which persistent follicles are induced to undergo atresia (McDowell et al., 1998).

Estradiol benzoate has also been used to induce atresia of dominant follicles and to initiate a new follicular wave approximately 4.5 days after injection (Burke et al., 2000). When treatment with progesterone and estradiol were combined, the dominant follicle stopped growing within 24 hr and became atretic resulting in the initiation of a new follicular wave 4 to 5 days after treatment (Burke et al., 1999). A single injection of a GnRH agonist is capable of ovulating dominant (\geq 10 mm) but not subordinate follicles (Figure 8; Ryan et al., 1998). Following GnRH administration, a new follicular wave was initiated approximately 1.6 days later (Roche et al., 1999) and selection occurred 3 to 4 days later (Twagiramungu et al., 1995). However, the ability of a single injection of GnRH to induce ovulation and initiate a new follicular wave is dependent on the stage of follicular development (Geary et al., 2000; Atkins et al., 2005).

Management Considerations for Selection of Heifers and Cows for Synchronization of Estrus

The success of an estrous synchronization program is largely based on understanding the bovine estrous cycle, the biological actions of estrous synchronization products (progestins, PGF_{2 α} , and GnRH), and the selection of heifers and cows that have a high likelihood of responding appropriately to the preceding products. Below are listed a few management tips for identifying heifers and cows that will be good candidates for an estrous synchronization program and likely respond appropriately.

Heifers. Heifers need to reach puberty prior to estrous synchronization to increase the likelihood of responding to a synchronization program. Furthermore, a 21% increase in fertility is experienced at a heifer's third estrus compared to her pubertal estrus (Byerley et al., 1987). Age at puberty is affected by a variety of factors, including genotype, body weight, nutrition, social environment, and season. Reproductive tract scores (RTS) provide an estimate of reproductive maturity in heifers and help predict their response to an estrous synchronization protocol. Heifers are assigned a RTS score ranging from one (immature) to four and five (cycling) based on rectal palpation or ultrasound of the uterus and ovaries. Qualified personnel should assess the RTS for heifers two weeks prior to synchronization or six to eight weeks prior to breeding. Heifers should have a minimum RTS score of two to be considered for breeding and at least 50% of the heifers should score a four or five in order to achieve a high response to synchronization.

Furthermore, replacement heifers should not receive growth promoting implants since implants administered within 30 days of birth may impair normal development of reproductive organs in growing heifers. At weaning, older heifers should be selected as potential replacement females and each heifer should attain 65% of their mature body weight before breeding and 85% prior to first calving. Feeding heifers separately from cows will assist heifers in attaining a targeted rate of gain.

Postpartum cows. In postpartum cows, the response to an estrous synchronization program is primarily dependent upon cow body condition and days postpartum. Body condition score (BCS) is a subjective measurement of an animal's fat reserves and ranges from extremely thin

(1) to obese (9). Cows should have a body condition score of 5 or greater at calving to be considered for an AI and estrous synchronization program. Cows that are too thin at calving are likely to have poor reproductive performance and are not good candidates for estrus synchronization/AI. In general, it takes 80 to 100 lbs to increase one BCS (i.e. 4 to 5). If possible, feed thin cows separately from well conditioned cows in order to promote a steady pattern of feed intake to attain the desired BCS.

The average number of days postpartum for cows at the start of an estrous synchronization program should be > 40 days. Increased energy requirements associated with lactation can result in a delay in the interval from calving to first estrus. A longer recovery period between calving and the beginning of the breeding season corresponds to a larger proportion of cows cycling at the start of the breeding season.

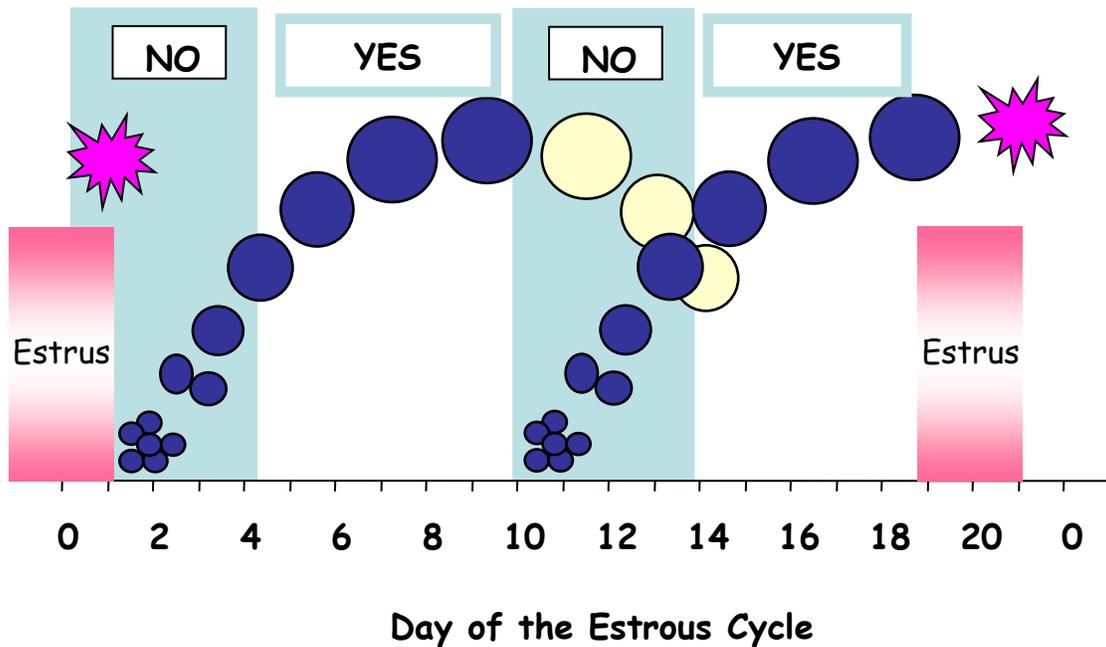


Figure 8. Injection of GnRH will induce ovulation of a dominant follicle (≥ 10 mm in diameter). Circles represent follicle development and atresia (light circles) during a wave. The above figure represents a “two-wave cow” and the shaded areas indicate when during a follicular wave follicles will ovulate (Yes) or not ovulate (No) in response to a single injection of GnRH (Modified from Kojima and Patterson, 2003).

Management Considerations for an Artificial Insemination Program

A successful AI program is dependent upon the following factors: optimization of the number of healthy cycling females at the beginning of the breeding season, careful attention to detection of estrus, purchase of high quality semen, and proper semen handling and insemination technique. To increase the number of animals cycling at the beginning of the breeding season, cows and heifers should be well-nourished, disease-free, mature enough to achieve puberty, or allowed an adequate period of recovery from calving to the subsequent breeding season. Animal health, semen quality/ handling, AI techniques, and the timing of insemination influence conception rates. Inadequacy in any of these management practices will decrease pregnancy rates.

Planning ahead will minimize the chance of making costly mistakes in estrous synchronization and AI programs. Estrous synchronization protocols should be followed precisely. A good practice is to write each of the days of treatment and insemination on a calendar to reduce the likelihood of making a mistake. Feeding MGA mandates adequate bunk space to ensure uniform consumption (cows – 24 inches per animal; heifers – 18 inches per animal). CIDR insertion should be performed as cleanly as possible in order to reduce the risk of spreading disease. Intramuscular injections should be administered using an eighteen-gauge, 1.5 inch needle.

Stress can suppress the expression of estrus and decrease conception rates. Working facilities should be designed to minimize stressing animals during handling. A well-designed facility will include sorting pens, a crowding tub, and an operable head gate or breeding box for animal restraint. The facility requirement will vary depending on the number and type of animals that will be bred as well as the estrous synchronization protocol being used. With a fixed-time AI program, facilities should be sufficient to handle the insemination of all animals within 2 to 3 hrs. Many AI companies or county extension offices have portable breeding chutes available to producers if needed.

Clear individual animal identification and accurate records allow producers to manage animals on an individual basis. When handling animals for synchronization, double check their ear tags for legibility and clip hair from the ears to facilitate reading the tags. Records should include detailed calving, breeding, and pregnancy information. At insemination, document the animal ID, date, time, AI technician, and sire. These records will allow producers to track the reproductive efficiency of individual animals, as well as the skill of the technician.

Sire selection will directly affect the genetic merit of the calf crop resulting from AI. Use sires with high accuracy EPDs collected from a certified semen services (CSS) facility and avoid unproven bulls. When breeding heifers, special attention should be paid to selection of bulls with EPDs for low birth weight or high calving ease. The choice of other sire traits will depend on the management goals of the producer. Seek advice from individuals in the AI industry to help make this important management decision.

It is essential to pay attention to details throughout an estrous synchronization and AI program. The success of these systems hinges on many factors (See a list of tips for a successful AI program in Figure 9). A fault in one area cannot be made up by success in a second area. Should a mistake occur in hormone administration or the treatment timeline, seek advice immediately from a veterinarian, an extension agent specializing in reproduction, or a representative from an AI company.

How do I determine what may have gone wrong during a FTAI program?

Occasionally the pregnancy rate following FTAI is much lower than expected. Trying to identify the root cause of a decreased pregnancy rate can be a daunting task due to the countless factors that can impact pregnancy rate following AI. When trying to trouble shoot what went wrong you should systematically work through the possibilities and not assume anything was done correctly – evaluate all the possibilities! A list of questions that may provide a systematic approach to identifying the problem is provided in Figure 10.

One of the most common problems accounting for reduced pregnancy rates following FTAI is that the heifers or cows do not meet the criteria for being good candidates for an estrus synchronization and AI program (see previous section). The second problem is poor choice of an estrus synchronization protocol and(or) protocol compliance. If you have a mixture of cycling and anestrus animals at the beginning of estrus synchronization treatment, you need to use a protocol that includes a progestin (e.g. CIDR or MGA).

Tips to Running a Successful Estrous Synchronization and AI Program

- Animal identification should be clear and easily readable.
- Keep accurate calving, breeding, and pregnancy records.
- Ensure herd health and disease prevention with a well-designed vaccination protocol prior to the breeding season.
- Vaccinate a minimum of 30 days before the breeding season begins.
- At least 50% of heifers should have a reproductive tract score (RTS) ≥ 3 by 2 weeks prior to the start of synchronization or 6-8 weeks prior to the breeding season.
- Heifers should weigh 65% of their mature body weight by the start of the breeding season.
- Synchronize and inseminate only cows with BCS ≥ 5.0 (1.0 = emaciated; 9.0 = obese).
- Cows should average ≥ 40 days postpartum by the start of estrous synchronization.
- Plan ahead and meticulously follow estrous synchronization protocols.
- If detecting estrus, spend as much time observing animals as possible.
- Use a minimum of one person to detect estrus per 100 head of synchronized cattle.
- Use estrous detection aides to facilitate detection.
- Use a properly trained AI technician.
- Purchase semen from a Certified Semen Services (CSS) collection facility.
- Select proven AI sires with high accuracy EPDs that match performance goals.
- Pregnancy check by 75 days after AI via ultrasound or 80-90 days after AI via rectal palpation to distinguish AI from bull bred pregnancies.
- PAY ATTENTION TO DETAILS!

Figure 9. Check list of tips to facilitate a successful estrous synchronization and artificial insemination (AI) program.

What are the most common mistakes when implementing an estrus synchronization and AI program?

One of the most common problems accounting for reduced pregnancy rates following FTAI is that the heifers or cows do not meet the criteria for being good candidates for an estrus synchronization and AI program (see previous section). The second problem is poor choice of an estrus synchronization protocol and(or) protocol compliance. If you have a mixture of cycling and anestrus animals at the beginning of estrus synchronization treatment, you need to use a protocol that includes a progestin (e.g. CIDR or MGA).

Progestin treatment will increase the proportion of prepuberal heifers and anestrus cows that will respond to the protocol. Furthermore, it is essential that each heifer or cow receives the correct estrus synchronization product, at the correct dose, and on the appropriate day. A third problem is that the facilities don't allow the cattle to be inseminated properly within a 2 to 3 hr time period and(or) cause undue stress on the cattle. With a FTAI protocol you have to carefully consider how many animals you can inseminate properly within the designated time period (e.g. 66 ± 2 hr for CO-Synch + CIDR protocol) with a minimum of stress. As previously mentioned, renting a breeding barn or contracting with an AI company that has breeding barns available can alleviate the problems associated with marginal facilities.

<p>Figure 10. Questions to ask when the pregnancy rate to FTAI is lower than expected.</p> <ul style="list-style-type: none"> • What was the pregnancy rate following estrus synchronization and fixed-time AI (FTAI)? • Was the pregnancy rate low or do you have unrealistic expectations (see Table 1)? <p>Consider asking the following questions to an AI company representative, your veterinarian, or a beef reproduction specialist to identify potential causes of the reduced pregnancy rate.</p>
<p>1) What was the pregnancy rate in your heifers or cows after 60 to 80 days over the past few years? If less than 85% there may be other issues that should be addressed before initiating an estrus synchronization and AI program.</p>
<p>2) What was the nutrition (protein, energy, phytoestrogens, etc) and mineral program before and after FTAI?</p>
<p>3) Did the animals meet the criteria for being good candidates for an estrus synchronization protocol (see earlier section)?</p>
<p>4) Did you use fixed-time AI or did you breed following detection of estrus? If you inseminated following detection of estrus, how frequently did you detect estrus (when did you begin and when did you end), what criteria did you use for detecting estrus, and when did you inseminate relative to detecting estrus?</p>
<p>5) What bull did you use and is there evidence that semen from this sire has resulted in acceptable pregnancy rates when using fixed-time AI or AI following estrous detection?</p>
<p>6) What protocol did you use and exactly when did you administer each of the products? You will need to confirm that the correct products were administered at the correct dosages and at the correct times. It is helpful to record on a calendar which product was administered on a particular day so you can check back to see if a mistake was made.</p>
<p>7) Was the biological activity of the various products compromised? You will need to verify that the products were not out of date and were stored and administered properly.</p>
<p>8) If using fixed-time AI, when did you inseminate the heifers or cows? Did you record who inseminated each animal? This will be helpful in identifying if there is a technician problem.</p>
<p>9) Where did you obtain the semen, how was it stored, and was the semen thawed correctly?</p>

Biological activity of the estrus synchronization products. It is not uncommon to hear someone blame a particular estrus synchronization product or the protocol for poor results. The commercially available products are effective when properly stored and administered. Furthermore, the protocols have been shown to consistently work in a variety of environments. The estrus synchronization protocols listed in the AI catalogs published by Select Sires, ABS Global, Genex, and Accelerated Genetics have been thoroughly tested in the field in a number of herds by numerous investigators in many states.

Rarely does one find that the biological activity of a particular product has been compromised provided the product has been stored properly, administered at the appropriate dose on the correct day of the protocol, and that the expiration date has not been exceeded. It is not uncommon for PGF or GnRH products to be administered at the wrong dose or to be injected subcutaneously instead of in the muscle. Intramuscular injections should be administered using an eighteen-gauge, 1.5 inch needle to minimize the possibility of back flow.

Potential problems associated with feeding melengestrol acetate (MGA). Occasionally there can be problems with feeding melengestrol acetate (MGA) if you don't pay attention to a few simple guidelines. The most common problem is that a heifer does not receive the correct dose (0.5 mg/hd/day). If a heifer does not receive enough MGA she may express estrus during the period of MGA feeding. Therefore, it is a good idea to watch the heifers for estrous activity as they come to the bunk. Alternatively, if a heifer receives more than the appropriate dose, expression of estrus may be delayed following the end of MGA feeding. To ensure that each heifer has an opportunity to receive the correct dose, MGA should be fed once daily in 3 to 5 pounds of carrier and each heifer should have 18 to 24 inches of bunk space. To be confident there is adequate bunk space and to train the heifers to come to the bunk it is a good idea to feed the carrier without MGA for a few days before the start of MGA treatment. At the end of 14 days of MGA feeding, heifers will express estrus within 2 to 5 days; however, heifers should not be inseminated at this estrus since pregnancy rates will be reduced. Be sure to inseminate the heifers at the designated time specified in the protocol.

Potential problems associated with CIDRs. Controlled Internal Drug Release (CIDR) is an intravaginal device that contains progesterone and acts like an artificial corpus luteum. Normally there are few problems associated with CIDR treatment. CIDRs should not be inserted in cows that are less than 21 days postpartum because the probability of inducing cyclicity is low. CIDR insertion should be performed as cleanly as possible in order to reduce the risk of spreading disease. When removing CIDRs it is not uncommon to detect a whitish discharge which is due to vaginal irritation from the wings of the CIDR and does not necessarily mean the animal has a vaginal infection. A difference in conception rate or pregnancy rate has not been detected between CIDR-treated animals that do or do not have a discharge.

Summary

There are significant benefits to genetic improvement and reproductive management that can be gained from the implementation of an estrus synchronization and AI program in heifers and postpartum beef cows. Artificial insemination in beef cattle is more practical than ever due to advances in FTAI, identification of sires with highly accurate EPDs, and a market structure that can identify and reward producers for the quality of their cattle. Above all, a successful estrus synchronization and AI program is dependent upon being proactive, well organized, and attention to detail. The success of these systems hinges on many factors.

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