Synchronization of estrus and ovulation: a practitioner's perspective

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Abstract

Artificial insemination as a management tool can be facilitated by synchronization of estrus or ovulation. Although estrus detection will remain an important part of successful breeding programs, timed artificial insemination is increasingly used in cattle herds. The practicing theriogenologist must utilize his or her knowledge of the physiology of the bovine estrous cycle and reproductive pharmacology, consider constraints imposed by availability of labor and facilities, and choose among the available protocols the one which is most likely to be successfully implemented. This article reviews the physiology of the bovine estrous cycle, and some of the protocols currently in use.

Keywords: Bovine estrous cycle, estrus synchronization, artificial insemination

Introduction

Artificial insemination as a management tool has been extensively utilized by dairy producers for over 50 years, and use in beef cattle has become increasingly common over the last 2 decades. Heat detection and insemination based on estrus signs and behavior remain necessary components for most successful artificial insemination programs, but the veterinary practitioner is increasingly called upon to suggest and monitor protocols aimed at increasing efficiency or convenience of artificial insemination programs by synchronizing estrus or ovulation. Knowledge of the physiology of the

estrous cycle, reproductive pharmacology, and management of cattle and people are all necessary components of successful programs.

Estrous cycle control has developed in distinct phases, as the events of the estrous cycle have been understood and pharmacologic agents have become available. Today, the most successful protocols combine control of the length of the luteal phase with control of the emergence of follicular waves, often with control of the time of ovulation and timed artificial insemination. An understanding of the physiology of the estrous cycle, including emergence of follicular waves, development of the dominant follicle, follicular atresia, ovulation, development of the corpus luteum (CL), and luteolysis is a necessary prerequisite to evaluate and implement synchronization strategies.

Integration of the physiology of the estrous cycle with reproductive pharmacology, the age, breed and lactation status of the targeted group, the animal handling skills and facilities present on the farm or ranch, the availability of sufficient skilled personnel at critical times, effective communication and record keeping are all important to success of protocols. The practicing theriogenologist must take all of these into consideration as he or she consults with their clients.

A brief outline of critical points is presented below, and discussion of the some of the more common protocols which have evolved follows.

The bovine estrous cycle

Cattle are reproductively non-seasonal, polyestrous, monovulatory, with an interestrus interval ranging from 18-23 days. Observable estrus behavior is detectable even in the absence of the male.

Heat, or estrus, is best demonstrated by standing to be mounted, whether by a bull or by other females in the herd. Typical estrus behavior is triggered by rising estradiol in an environment where progesterone concentrations are falling.

The rising concentrations of circulating estradiol accompanying estrus induce the leutinizing hormone (LH) surge, and the LH surge induces ovulation. Ovulation follows the onset standing heat by 24 to 32 hours.

Ovulation is followed by development of the CL, a transient endocrine organ that arises on the ovary at the site of ovulation. The CL produces a number of endocrine products, the most important for this discussion being progesterone, which is necessary for maintenance of pregnancy following insemination. Should pregnancy not occur, the CL will regress and another ovulatory follicle will develop. This follicle will produce sufficient estradiol to again induce psychic estrus and the LH surge, and ovulation will follow. The cycle will repeat indefinitely in the absence of pregnancy. This very basic outline of the events of the estrous cycle has proven to be sufficient for insemination programs based on heat detection. As we move to more precisely control the estrous cycle, an understanding of other, less obvious events becomes necessary.

The cycle is sometimes conveniently divided into the luteal and follicular phases, based on the dominant ovarian structure present. The follicular phase begins with the regression of the CL and encompasses about 20% of the cycle, the period from the onset of luteolysis until ovulation. Estradiol from the developing dominant or preovulatory follicle is the predominant sex steroid during the follicular phase. The luteal phase begins at ovulation, and continues until regression of the CL. During this time, the sex steroid dominance shifts to progesterone produced by the CL. The luteal phase includes

both metestrus, the period of CL development following ovulation, and diestrus, the period spanned by the life of the mature CL. Follicles continue to emerge and grow during the luteal phase, and although they produce estradiol, luteal progesterone inhibits LH sufficiently to prevent development to preovulatory size

The endocrine signal that results in regression of the CL in the absence of pregnancy has been known to be prostaglandin F2alpha (PGF2ά) for several decades. More recently, the signal responsible for maternal recognition of pregnancy, which effectively blocks luteolysis, has been shown to be interferon tau produced by the fetal trophoblast.[1] The signals that result in emergence, growth, and regression of ovarian follicles became evident following the advent of ultrasound as a research tool coupled with endocrine assays in the early 1980's.[2] As mechanisms regulating the ovarian cycle were discovered and pharmacologic agents became available, opportunities to develop protocols to control the events of the estrous cycle developed.

Follicular dynamics

During the 1980's, ultrasound technology allowed basic research that described waves of follicular growth and regression. These revelations were coupled with endocrine assays to explain the dynamic nature of ovarian follicles. Understanding of the processes that initiated follicular emergence, selection, atresia, dominance and ultimately ovulation, coupled with strategic administration of the limited number of available pharmacologic agents have become the basis for programs to manipulate the estrous cycle and in many instances precisely control the time of ovulation. The basic research on follicular dynamics has been reviewed extensively in recent publications,[3,4] and the reader is

referred to these resources for a more detailed description. A brief summary, applicable to clinical practice follows.

Estrus is characterized by the presence of a large ovarian follicle, producing sufficient estrogen to induce the psychic changes associated with estrus behavior and to induce a surge of LH, which eventually results in ovulation of the follicle and release of the oocyte. Following ovulation, the granulosa and thecal cells surrounding the follicular antrum transform into luteal cells (luteinization) and the CL develops. Steroid production shifts from estrogen to progesterone and this period of progesterone dominance initiates the luteal phase.

At the time of the LH surge which initiates the events of ovulation, both LH and follicle-stimulating hormone (FSH) are released from the pituitary. This release of FSH is followed closely by a second surge of FSH, and these peri-ovulatory surges of FSH are associated with the emergence of a cohort of small follicles from the ovarian follicular pool shortly after ovulation. Emergence, defined as the last day the potential dominant follicle was less than 4 mm, is perhaps more easily understood as the time that follicles are easily detectable with commonly used ultrasound equipment. Emergence of follicles is generally coincident with the peak of the FSH surge. As growth of these follicles is stimulated by FSH, follicular hormonal activity increases and the antral follicles produce a number of endocrine products, including the protein hormone inhibin, and later estradiol.[5] Estradiol and inhibin exert negative influence on the anterior pituitary to decrease or inhibit release of FSH from the anterior pituitary, so that following follicular emergence, FSH concentrations decline. FSH has a long half life relative to LH, but concentrations of FSH remain sufficient to support follicular growth only to a diameter of

8-9 mm,[6] although exogenous FSH administration will allow follicular development to continue past this stage.[7]

Within the cohort of emerged follicles, a single follicle either has or gains a slight developmental advantage as FSH concentrations approach their nadir. As this developmentally advantaged follicle grows, it acquires additional LH receptors in the granulosa cells surrounding the follicular antrum and oocyte.[8] The acquisition of LH receptors is critical for continued development of the follicle, and LH support allows this follicle to continue progressive development as the growth rate of the other follicles in the cohort declines. The time at which the growth rate of the future dominant follicle exceeds the growth rate of the largest dominant follicle is referred to as follicular deviation and the largest follicle is referred to as the dominant follicle. As this selected follicle continues to grow in response to pulsatile release of LH it continues to produce estradiol and inhibin, and FSH support for the remaining follicles is lost. These subordinate follicles, lacking LH receptors in a low FSH environment, begin the process of atresia as the dominant follicle continues to grow. As the dominant follicle grows and produces estradiol, a positive feed back loop with the hypothalamus increases the frequency of gonadotropin-releasing hormone (GnRH) pulses, which in the face of continued inhibition of FSH release, results in increased pulsatile LH, but not FSH, release from the pituitary.

Coincident with the development of this first post ovulatory follicular wave, and eventual development of a single dominant follicle, is the development of the CL and the associated increase in circulating progesterone concentrations. As noted above, the dominant follicle grows and increasingly produces estrogen which feeds back on the

hypothalamus to increase GnRH and elicit LH pulses from the pituitary. However, in the high progesterone environment of the luteal phase, GnRH release is limited, and the resultant LH pulsatility is insufficient to support continued development of the dominant follicle to pre-ovulatory size. The dominant follicle, deprived of sufficient LH for continued development, eventually joins the other members of the cohort, and undergoes atresia. Atresia of the dominant follicle removes the source of estradiol and inhibin, and shortly after its demise, another surge of FSH from the anterior pituitary stimulates emergence of a second follicular wave.[9] The second follicular wave develops in a manner similar to the first, and a new dominant follicle develops. The dominant follicle of the second follicular wave may go on to become the ovulatory follicle, or may undergo atresia and be replaced by a dominant follicle from a third follicular wave which will become the ovulatory follicle.

In the non-pregnant animal, the endometrium releases PGF2ά by day 16 -17 post ovulation, and destruction of the CL (luteolysis) follows. As circulating progesterone concentrations decrease following luteolysis, the inhibitory effect of progesterone on GnRH release is removed. LH pulse frequency increases, and the dominant follicle responds with continued growth. This growth is accompanied by increasing estrogen production, and feedback on the hypothalamus further increasing pulsatile release of GnRH, which it turn acts on the pituitary to increase LH pulse frequency. Follicular development continues in this low progesterone environment with the production of estrogen eventually reaching the threshold necessary to trigger the LH surge.

Most cattle exhibit either 2 or 3 follicular waves during an estrous cycle. It is necessary to recognize that follicles which achieve dominance can only reach pre-

ovulatory status in the low progesterone environment that follows luteolysis, and that any of the dominant follicles produced in either 2 or 3 wave cycles can grow to ovulatory size if luteolysis is induced and the inhibitory effect of progesterone removed. Figure 1 is a schematic representation of ovarian follicular development during a typical three wave estrous cycle.

Pharmacologic control of the estrous cycle

Programs to control the time of estrus and ovulation developed as the events controlling various portions of the estrous cycle were recognized, and as products for pharmacologic manipulation of these events became available. Even though the number of pharmacologic agents available is limited, producers and veterinarians are offered an ever increasing number of protocols for control of the estrous cycle, with seemingly endless variations and refinements. Veterinarians actively practicing in the field as theriogenologists are asked and expected to evaluate estrus and ovulation synchronization programs, and make recommendations regarding selection and implementation on specific premises. Understanding the available hormones and their interactions with the events of the estrous cycle are essential in this task.

Pharmacologic agents to control the bovine estrous cycle

Opportunities for manipulation of the estrous cycle in cattle are limited to control the length of the luteal phase, initiation a new follicular wave, and control the time of ovulation. Many of the currently available programs rely on control of all three. Precise control of ovulation has increasingly led to adoption of timed artificial insemination (TAI) protocols.

Drug availability is influenced by legal constraints which prohibit the use of some drugs and classes of drugs. Products must be available from commercial sources, and the use of compounded drugs for estrous cycle control is prohibited by the Animal Medical Drug Use Clarification Act (AMDUCA). Enforcement of restrictions seems likely to increase, driven by food safety and consumer demands.

Available products meeting these criteria and commonly utilized in clinical settings are limited to progesterone and progestins, prostaglandin F2ά (PGF2ά) and its analogs, and GnRH agonists. Other potentially useful products such as FSH, LH, or human chorionic gonadotropin (hCG), are uncommonly utilized in synchronization protocols. Noticeably, and deliberately absent from this list are injectable estrogenic compounds, which although valuable and historically widely used, are not currently approved for estrus control in food animals, and not commercially available in the United States. As mentioned earlier, compounded products, including estrogen, must be avoided. Table 1 provides a list of some commercially available products.

Control of the length of the luteal phase

The length of the luteal phase may be extended by administration of exogenous progesterone or progestins, or truncated by the administration of luteolytic doses of prostaglandin.

Extending the luteal phase with progestins

Early attempts at synchronizing estrus focused on administration of progesterone or progestins, followed by acute withdrawal. In this manner, the period of progesterone dominance is extended beyond the normal lifespan of the corpus luteum. Exogenous progesterone inhibits release of LH, preventing development of ovarian follicles to

preovulatory size. Removal of the exogenous source or progesterone from groups of cattle results in a relatively synchronized estrus followed by ovulation. An additional benefit of progestin therapy is the ability to hasten the onset of estrous cyclicity in animals that may be anestrous at the beginning of the protocol.

Melengestrol acetate. Melengestrol acetate (MGA), an orally active progestational steroid, was developed in the 1960s and first marketed to both improve feed efficiency and rate of gain in feedlot heifers and suppress estrus behavior. Suppression of ovulation and estrus behavior occurs when consumption was approximately 0.5 mg per head per day.[10]

Early efforts at synchronization with MGA in cycling cattle relied on feeding 0.5 to 1 mg daily for 14 to 18 days, a period sufficient to allow spontaneous luteal regression in all animals within a group. Because the luteal phase is extended past the time of luteolyis, follicular development and ovulation is suppressed. Following withdrawal of MGA from the diet, follicular development resumes and a synchronized estrus follows. A majority of animals treated in this manner exhibit signs of estrus and ovulate 3 to 7 days following MGA withdrawal. Pregnancy rates to this synchronized estrus are variable and generally disappointing. The reduction in fertility, however, is not apparent in subsequent cycles. Because the reduction in fertility is confined to the first post treatment estrus, programs have developed to take advantage of the synchrony of the second post treatment cycle. These programs are plagued by decreasing synchrony of the second post treatment estrus due to the inherent variability of estrous cycle length. These protocols require that the synchronization be planned well in advance of the onset of the breeding season.

The reduced fertility of the first synchronized estrus following MGA withdrawal has been attributed to altered development of ovarian follicles. Dominant follicles which would either undergo atresia or ovulate during a normal ovarian cycle persist beyond their normal lifespan in the sub-luteal progesterone environment provided by the exogenous MGA. Following withdrawal of the progestin, these persistent follicles grow, produce sufficient estradiol to induce estrus and the LH surge, and ovulate, but the oocytes associated with these follicles are often developmentally compromised.

Administrating MGA in the feed for shorter intervals partially overcomes the negative effects of persistent follicles, but synchrony of estrus is decreased. Incorporating higher doses of MGA has not been effective in overcoming problems associated with development of persistent follicles.[11] Although some reports indicate acute administration of progesterone late in the artificially lengthened luteal phase will induce follicular turnover,[12] the lack of FDA approval for injectable progesterone products precludes use of this strategy.

Several variations of the initial MGA protocol utilizing PGF2ά have been developed to overcome problems associated with lack of synchrony of the second post treatment estrus, and will be discussed in a later section.

Intra-vaginal progesterone. Intra-vaginal delivery of progesterone as a method of extending or controlling the length of the luteal phase of the cycle has been available in the United States since 2002. Currently the only FDA approved device is the Eazi-Breed CIDR® (Pfizer Animal Health, New York, NY, USA). The Eazi-Breed CIDR® has been available for many years in other countries prior to introduction to the U.S. and a

large number of clinical and research trials have demonstrated the efficacy of progesterone delivery by this route.

Labeled protocols for Eazi-Breed CIDR® specify that the pessary be placed intra-vaginally for 7 days, with administration of PGF2ά either one day prior to pessary removal, or on the day of removal. The protocols yield similar results.[13] This combination approach allows precise control of the length of the luteal phase, and synchronizes estrus. Because of inherent variations in follicular wave emergence, estrus activity following this protocol is not synchronized sufficiently to permit appointment breeding. The range of estrus activity is often much more tightly controlled than in programs which use prostaglandins as the sole agent.

Progesterone-releasing pessaries are often incorporated into other synchronization programs to improve the degree of control of the luteal phase, and like other progestin based synchronization protocols, may hasten the onset of cyclicity in anovular cattle.[14,15] Truncating the luteal phase with prostaglandin F2ά

Almost 3 decades ago, PGF2 ά and its analogs were introduced as the first drugs approved to control the estrous cycle in cycling cows. Prostaglandin is released from the endometrium after mid-cycle in non-pregnant cattle, and result in regression of the CL, removing the progesterone mediated inhibition of LH pulsatility. Although the developing CL is resistant to the effects of prostaglandin, sensitivity increases as the CL matures, and by day 6 post-estrus, the luteal phase can be terminated in virtually all cows administered exogenous PGF2ά.

Administration of PGF2ά or its analogs after day 5-6 of the estrous cycle results in CL regression and loss of progesterone dominance. When administered to groups of

cycling cattle, the synchronized end of the luteal phase is followed by a synchronized estrus.

Following the commercial introduction, PGF2ά protocols rapidly became popular in estrus synchronization protocols both for artificial insemination and in synchronizing estrous cycles for recipients in embryo transfer programs. The two commercially available products, dinoprost (Lutalyse®; Pfizer Animal Health, New York, NY, USA) and cloprostenol (Estrumate®;Intervet/Schering–Plough animal Health, Summit, NJ, USA), are in similar in their action, and while cloprostenol has a longer duration of action, their effects are clinically comparable.[16]

Following a single injection of PGF2ά to randomly cycling groups of cows or heifers, 60 to 80% can be expected to be in estrus within a few days following luteolysis. Poor to no response is expected in cattle which have ovulated recently. Cattle which have undergone spontaneous luteolysis would typically exhibit estrus coincidentally with their "synchronized" herd mates. Single injection protocols can be tailored to a producer's needs is several ways.

Traditional estrus detection with artificial insemination for 6 to 7 days, followed by PGF2ά administration to those not previously inseminated increases the percent response by removing those with immature CLs from the pool. Animals injected are expected to exhibit estrus in 2 to 5 days. Drug costs are very low in this protocol, but the number of days during which animals need to be observed and bred is fairly high. These single injection programs are useful and widely practiced in many farm settings, but many management situations exist in which a shorter window of estrus synchronization is

desired. In these cases, group synchronization utilizing 2 injections of prostaglandin may offer advantages.[17]

In two injection schemes, animals which respond to the first injection and animals in the group which have undergone spontaneous luteolysis just prior to administration of the drug are expected to be in heat in from 2 to 5 days. Those animals with an immature CL, (~ day 1-5 of the cycle) are not be expected to respond to the injection of PGF2ά, and luteal development will continue. At the time of the second injection, all animals in the group should be an appropriate stage of the luteal phase and expected to exhibit a synchronized estrus.

Although label indications for dinoprost suggest a two injection scheme with the injections separated by 10 to 12 days, 14 day intervals are more commonly used and 14 day programs are considered equally effective if not superior to the shorter intervals specified on the product label.

Strategies aimed at identifying cows at the appropriate stage of the cycle to respond to PGF2ά injections eliminate injections in animals that will not respond to exogenous prostaglandin. Transrectal palpation for the presence of a CL, determination of milk progesterone concentrations,[18] or identification of the CL with ultrasound have been investigated as methods to identify cattle with functional luteal tissue. Although these techniques can be effective, routine administration of PGF2ά to non-inseminated animals at random stages of the estrous cycle has remained the most common, and perhaps most economically justifiable, procedure.

Estrus synchronization protocols which use PGF2ά as the sole agent are characterized by an inherent variability in the time of the onset of estrus, typically

exhibiting a bell shaped curve with estrus activity beginning 2 days post injection, peaking in 3 to 4 days, and declining rapidly after day 5. While this synchronization of estrus is useful and advantageous compared to observing and handling cattle over an entire estrous cycle, synchrony of ovulation is too variable to allow timed artificial insemination protocols.

MGA plus prostaglandin F2ά

The availability of PGF2ά products led to estrus synchronization protocols which combined feeding MGA for various lengths of time with injections of prostaglandin. Feeding MGA for 14 to 18 days, as described above, results in synchrony of estrus, but poor fertility is associated with the first estrus following prolonged feeding of the progestational agent. Because estrous cycles in cattle fed MGA are synchronized following withdrawal of the progestin, there is an opportunity to administer PGF2ά to a group of animals with a synchronized luteal phase following MGA withdrawal. Treatment with prostaglandin late in the luteal phase reduces the variability in the interval from PGF2ά injection to estrus. Following treatment with PGF2ά, synchrony of the second estrus following MGA withdrawal is better than in MGA protocols which do not control the luteal phase length prior to the second synchronized estrus, and fertility to the synchronized estrus is not compromised. Initial protocols injected prostaglandins at 17 days after withdrawal of MGA, and more recently protocols in which prostaglandin injection has been delayed until 19 days after MGA withdrawal have shown tighter estrus synchrony.[19] Detection of estrus and breeding based on the signs of estrus are recommended. These combination programs are quite effective and economical in

situations where feed intake is adequately controlled. The length of treatment in these MGA protocols remains a disadvantage, requiring a long lead time prior to insemination.

Shorter MGA programs combined with prostaglandin injections at the time of MGA withdrawal either did not overcome the infertility associated with longer feeding periods,[20] or did not show improvement over PGF2ά alone.[21]

Synchronization of the emergence of the follicular wave

Following atresia of the dominant follicle, a new cohort of follicles emerges in response to FSH, and the transition from the dominance of one follicle to emergence of a new cohort of developing ovarian follicles is termed follicular turnover. Treatments aimed at ending the period of follicular dominance will induce follicular turnover, allowing more precise control of the events and timing of the estrous cycle.

At least three methods to induce synchronous emergence of a new follicular wave are practiced, but only one lends itself to mass synchronization schemes.[22] Aspiration or ablation a mid-cycle dominant follicle removes the inhibitory effects of estradiol and inhibin on pituitary FSH release, and is followed by wave emergence in 1 to 2 days. Administration of an acute dose of exogenous estradiol or a combination of estradiol and progesterone results in emergence of a new follicular wave in approximately 3-4 days. Administration of GnRH can induce ovulation or luteinization of a dominant follicle, removing the source of inhibin and estradiol, followed by emergence of a new follicular wave in 1.5-2 days.[22,23]

Follicular ablation is not practical in on farm settings involving synchronization of the estrous cycle of groups of cattle. As previously noted, the lack of commercial sources of injectable estrogens and the legal atmosphere surrounding the use of

compounded pharmaceuticals in food animals effectively removes administration of estrogen or estrogen/progesterone injections as an option for synchronizing follicular wave emergence.

Although not specifically approved by the Food and Drug Administration for this purpose, GnRH is available in the United States and licensed for use in cattle. Administration of GnRH is currently the basis for control of follicular wave emergence and ovulation in many synchronization programs.

Gonadotropin-releasing hormone

GnRH, a decapeptide hormone produced in the hypothalamus and transported to the anterior pituitary, influences the secretion of the hormones FSH and LH. Commercial formulations have been available in the United States and worldwide for nearly 3 decades, and are approved for treatment of cystic ovarian disease. Currently, no meat or milk withdrawal periods are required for use of this product in cattle.

Endogenous GnRH stimulates release of LH and FSH, with a varying magnitude of response and ratio depending on the ovarian structures and resultant hormonal mix present. During the luteal phase of the cycle, high progesterone concentrations limit the release of GnRH, and subsequently LH pulse frequency. Conversely, during the follicular phase, removal of the negative influence of progesterone is followed by continued growth of the dominant follicle, and increasing production of estradiol by the follicle triggers increasing pulsatile release of GnRH from the hypothalamus. This feedback loop culminates in the pre-ovulatory LH surge, which induces ovulation and initiates the onset of another luteal phase.

Exogenous GnRH administered in the luteal phase during the period of follicular dominance results in ovulation or luteinization of dominant follicles, and is followed by emergence of a new follicular wave. Administration shortly after follicular wave emergence is less likely to result in initiation of a new follicular wave. Administration of exogenous GnRH following spontaneous or induced luteolysis can be used to induce an LH surge and control the time of ovulation. The actions of GnRH have been incorporated into synchronization protocols which control the time of follicular wave emergence and ovulation.

Ovsynch and related protocols

In 1995, a protocol for TAI using sequential administration of GnRH, PGF2ά, and GnRH to synchronize follicular wave emergence, luteolysis, and ovulation was introduced.[24] This protocol, widely known as Ovsynch, was the first allowing appointment breeding to gain widespread acceptance. The basic program with numerous modifications is the basis for many of the successful ovulation synchronization protocols used today.

Figure 2a illustrates a timeline schematic for the Ovsynch protocol. Figure 2b outlines an injection scheduling calendar for Ovsynch. On day 0, all eligible cattle receive GnRH, followed 7 days later by PGF2ά. Forty eight hours later, a second injection of GnRH is administered, and TAI, without regard to heat detection, is carried out 16 to 20 hours following the second GnRH. The initial injection of GnRH is intended to synchronize emergence of a new follicular wave following induction of ovulation of a dominant follicle. The corpus luteum formed following this diestrus ovulation will not become susceptible to the luteolytic effects of prostaglandin for several days, and will

provide a source of progesterone should the CL which developed following the previous ovulation be destroyed. The prostaglandin injection 7 days after initiation of the protocol will initiate luteolysis and allow continued development of the dominant follicle which arose from the induced follicular wave to pre-ovulatory size. The second injection of GnRH will induce an LH surge, with ovulation following in approximately 28 hours.[24]

Not all randomly cycling cattle have equivalent ovarian structures. Synchrony of ovulation is improved if a dominant follicle is present at the time of the first GnRH injection compared to initiation prior to the time of follicular deviation. The day of the cycle at which Ovsynch is initiated influences pregnancy rates to timed artificial insemination. Cows in which Ovsynch was initiated near mid-cycle had greater rates of synchronous ovulation following the first injection of GnRH, and programs beginning on day 5 to 12 of the estrous cycle resulted in greater pregnancy rates.[25,26]

Many modifications to the initial Ovsynch protocol have been developed with the goal of increasing rate of synchronous ovulation and pregnancy. The most common have utilized 2 prostaglandin injections, approximately 2 weeks apart, with the last injection 12 or 14 days prior to the first GnRH injection of Ovsynch. These programs increase the percentage of animals at the optimum stage of the cycle when the first injection of GnRH is administered, and are collectively referred to as Pre-Synch Ovsynch programs, or Prostaglandin Pre-Synch programs. Pregnancy rates to TAI are improved compared to Ovsynch alone.[27] A sample schedule for a Pre-Synch Ovsynch protocol is shown in Figure 3. The long lead time (initiation of hormone injections 36-38 days prior to insemination) present an obstacle to use of Pre-Synch, which is minimized in dairy herds

by initiating the protocol during the voluntary waiting period during which breeding is commonly withheld for 45 to 70 days following parturition.

More recently, protocols utilizing both GnRH and prostaglandin to increase the percentage of cows that ovulate following the first GnRH injection have been developed with improvement in pregnancy rate following timed artificial insemination similar to or better than that seen with Pre-Synch Ovsynch programs.[28,29] The injection schedule for the G6G protocol described by Bello is illustrated in Figure 4. The injection of PGF2ά which initiates the protocol induces luteolysis of mid- and late-cycle CL's. Two days later, an injection of GnRH synchronizes ovulation following luteolysis, and initiates a new follicular wave in animals which have a dominant follicle. An Ovsynch protocol is initiated six days later. The goal is to optimize the number of animals with a dominant follicle at the onset of Ovsynch, and ultimately synchronization of ovulation at the completion of Ovsynch.[28] The G6G protocol has a shorter lead time than Pre-Synch, but has scheduling disadvantages. Increased pregnancy rates to timed artificial insemination compared to Ovsynch alone are reported.

MGA plus PGF2ά plus GnRH protocols

Estrus synchronization protocols incorporating oral administration of MGA, followed with administration of PGF2ά have been modified by incorporating GnRH near the time of the first post MGA estrus to synchronize the first follicular wave and ovulation.[30-32] These programs generally allow for shorter duration of MGA feeding, and offer the potential advantage of hastening the onset of cyclicity in the late pre-pubertal period or post partum period. Adaptations allowing fixed-time artificial insemination[33]

or heat detection are utilized. Figure 5 illustrates a treatment schedule for the MGA 7-11 Synch modification with fixed time insemination.

Synchronization of ovulation and timing of insemination

GnRH administration in the low progesterone environment following luteolysis results in an LH surge followed by ovulation in 24 to 32 hours. Protocols which used estrogen to induce ovulation enjoyed some popularity,[34] but no approved product is available for veterinarians at this time. Agents with LH activity, such as hCG, could be a suitable substitute for GnRH, but protocols utilizing this strategy are not common.

Timing of insemination should include ample opportunity for sperm capacitiation prior to ovulation, and insemination at 16 to 20 hours following the ovulation-inducing dose of GnRH is the most common recommendation.[35] Earlier insemination, including at the time of administration of GnRH (Co-Synch protocol), may result in acceptable pregnancy rates, while insemination later than 24 hours after GnRH leads to less favorable outcomes.

In the original Ovsynch protocol, the second GnRH injection, administered to synchronize ovulation, was given 48 hours following induction of luteolysis with PGF2ά. This timing was convenient for dairies, and resulted in grouping of chores associated with the protocol around times when cows were handled or locked up. Recent studies suggest that delaying the administration of the second GnRH injection until 56 hours after induced luteolysis will improve pregnancy rates.[36] Modification of any protocol should take into consideration farm schedules to insure compliance, particularly if handling or restraint occurs outside the normal daily or weekly routine.

Choosing and implementing the right protocol

Many estrus synchronization and TAI programs are available, and when implemented properly, most are effective. Selecting a protocol for a particular herd will depend on the ability of herd management to comply with the time lines inherent in each protocol. A lack of commitment or cavalier attitude concerning the timing of injections and breeding will negatively impact the ultimate success any program.

An old saying in the dairy business is "When heat detection is everybody's job, it usually means it is nobody's job". This wisdom is applicable to scheduling and administration of protocols. Unless a job is a priority, compliance will suffer.

Accurate record keeping and proper administration of all hormones are essential. Injection schedules can become very complicated when dealing with large herds and when multiple protocols used within a herd. Both PCDART and DAIRYCOMP 305 have routines which will generate action lists to facilitate scheduling of injections.

Timed artificial insemination programs may overcome some management problems associated with estrus detection, but management problems with nutrition, housing, semen handling, and concurrent disease are not eliminated.

Drug cost, availability of trained technicians, class of livestock and the facilities available for repeatedly handling cattle will influence the choice of protocols for individual farms. Selection of the appropriate protocol for a particular herd or management system means selection of the protocol herd management will be most likely to efficiently and consistently implement, rather than the newest or most popular protocol available.

Discussion

Today, theriogenologists have an increasing number of options to synchronize estrus and ovulation. These systems developed in concert with an increased understanding of reproductive endocrinology and the proper selection and implementation of any of the available protocols requires an understanding of follicular dynamics, luteolysis and ovulation. Programs which allow for the successful utilization of TAI require much more precise control of the events of the estrous cycle than those which incorporate estrus detection. Although the legal and regulatory environment restricts or forbids access to certain potentially useful pharmacologic agents, successful strategies utilizing available agents have been devised, and offer adequate control of the cycle.

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Figure 1.

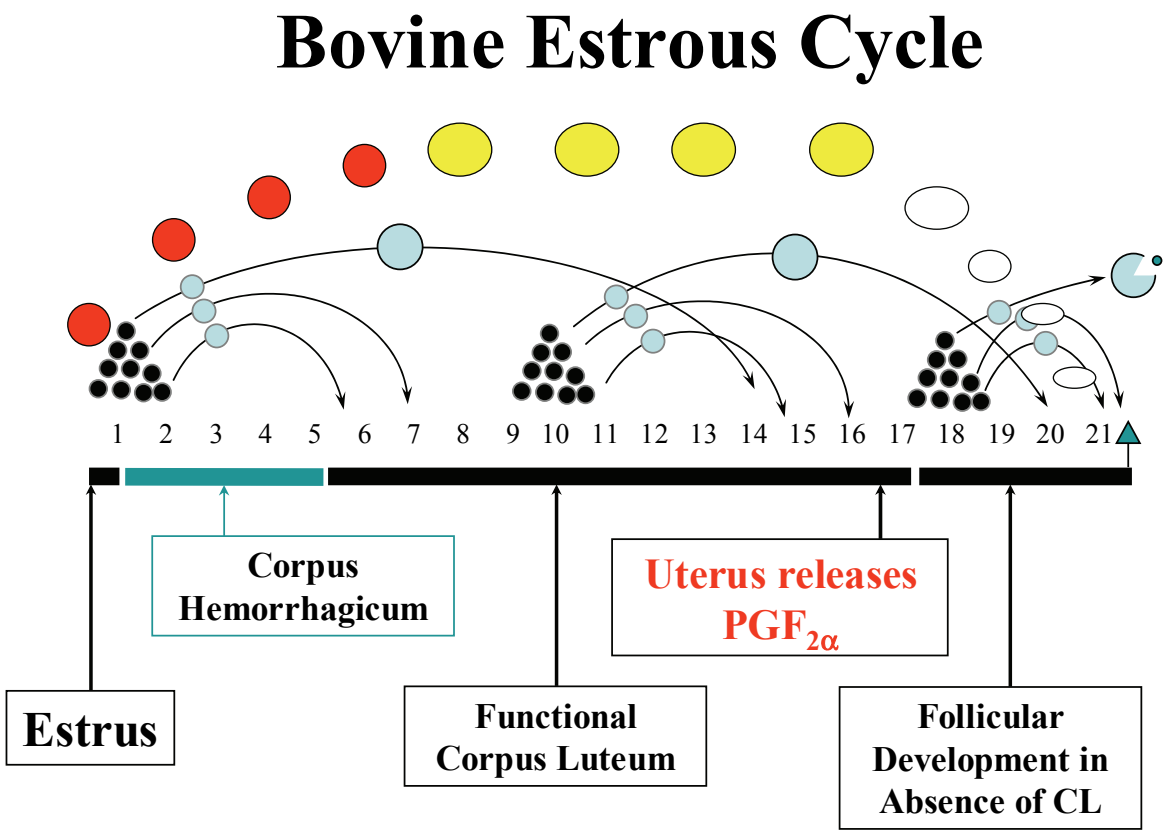

Figure 1: Development of successive waves of ovarian follicles during the estrous cycle. Shortly after ovulation, a cohort of follicles emerges; a single follicle gains an advantage that allows it to become the dominant follicle, only to undergo atresia in the high progesterone environment of the luteal phase. A second follicular wave emerges around day 8-9 of the cycle, and the dominant follicle that follows meets the same fate. A third wave emerges on day 16-17 following demise of the second wave dominant follicle. Prostaglandin-induced luteolysis destroys the source of progesterone, the inhibition of LH pulses is removed, and this third wave dominant follicle develops to preovulatory size. Estradiol produced by the dominant follicle increases past the threshold necessary

to induce the LH surge, and ovulation follows. Image courtesy of Dr. M. Daniel Givens, Auburn University

Figure 2a.

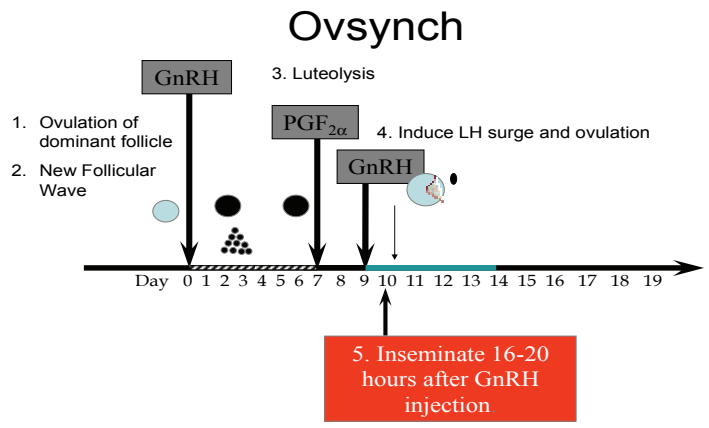

Figure 2a: OVSYNCH PROTOCOL. An injection of GnRH on day 0 induces ovulation or luteinization of a dominant follicle, and a new follicular wave emerges 2 days later. Injection of prostaglandin F2alpha on day 7 induces luteolysis, and injection GnRH on day 9 induces an LH surge, with ovulation 28 hours later. Insemination at 16-20 hours after the second GnRH injection (~8 hours ahead of ovulation) allows time for sperm capacitation prior to ovulation.

Figure 2b.

	SUNDAY	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SAT
Week 1		GnRH					
Week 2		PGF2ά		GnRH	TAI (16-20 hours post GnRH)		

Figure 2b: Injection calendar for a Pre-Synch Ovsynch protocol that avoids weekend chores.

PGF2ά = prostaglandin F2alpha, GnRH = gonadotropin-releasing hormone, TAI = timed artificial insemination.

Figure 3.

	SUNDAY	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SAT
Week 1		PGF2ά					
Week 2							
Week 3		PGF2ά					
Week 4							
Week 5		GnRH					
Week 6		PGF2ά		GnRH	TAI (16-20 hours post GnRH)		

Figure 3: Injection calendar for a Pre-Synch Ovsynch protocol which utilizes 2 prostaglandin injections, 14 days apart, with the second injection given 14 days prior to the start of Ovsynch. This protocol minimizes the number of days per week on which injections are given, avoids weekend chores, and places all prostaglandin injection on the same day of the week. A modification in which the first two prostaglandin injections are given on Wednesday of week 1 and 3 could be utilized and may have advantages. PGF2ά = prostaglandin F2alpha, GnRH = gonadotropin-releasing hormone, TAI = Ttmed artificial insemination.

Figure 4.

	SUNDAY	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SAT
Week 1		PGF2ά		GnRH			
Week 2			GnRH				
Week 3			PGF2ά		GnRH	TAI (16-20 hours post GnRH)	

Figure 4: Injection calendar for a modified G6G protocol. G6G shortens the lead time prior to insemination compared to Pre-Synch Ovsynch, but has the disadvantage of having assigned chores related to the protocol on more days of the week. Scheduling to avoid weekend chores may be more complicated with this protocol.

PGF2ά = prostaglandin F2alpha, GnRH = gonadotropin-releasing hormone, TAI = timed artificial insemination.

Figure 5.

	SUNDAY	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SAT
Week 1						MGA (first day)	MGA
Week 2	MGA	MGA	MGA	MGA	MGA (last day) PGF2ά		
Week 3		GnRH					
Week 4		PGF2ά		GnRH and TAI (60 hours after PGF2ά)			
Week 5							
Week 6							

Figure 5: Treatment calendar for MGA 7-11 Synch with TAI. MGA feeding begins on day 0, and is discontinued on day 7. Prostaglandin injections are given on day 7, and followed on day 11 with GnRH injections. On day 18, prostaglandin is injected to end the luteal phase, and GnRH is injected and cows are inseminated at 60 hours following the prostaglandin injection. Variations eliminating the last GnRH injection utilize heat detection and breeding based on the signs of estrus.

MGA = melengestrol acetate incorporated into feed, PGF2ά = prostaglandin F2alpha, GnRH = gonadotropin-releasing hormone, TAI = timed artificial insemination.

Table 1.

Class	Drug	Trade Name	Route of Adminstration	Manufacturer
Progestatational agents	Melengestrol acetate	MGA®	Oral	Pfizer
	Progesterone	Eazi-Breed CIDR®	Intravaginal pessary	Pfizer
Prostaglandin F2ά and analogs	Dinoprost tromethamine	Lutalyse®	Intramuscular injection	Pfizer
	Cloprostonol	Estrumate ®	Intramuscular injection	
GnRH agonists	Gonadorelin diacetate tetrhydrate	Cystorelin®	Intramuscular injection	Merial
		Ovacyst®	Intramuscular injection	

	Gonadorelin hydrochloride	Factrel®	Intramuscular injection	Fort Dodge Animal Health

Table 1: Commercially available products used to manipulate the estrous cycle of cattle.