Handbook of Estrous Synchronization: 2nd Edition

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Abstract

Over the past 25 years, many systems to synchronize estrus in beef cattle have been developed and/or optimized. This progress has been driven by scientific discoveries that have arisen from fundamental research into the reproductive physiology and endocrinology of cattle. This fundamental research has identified specific reproductive endpoints that must be achieved for estrous to be effectively synchronized and pregnancy to be established. These advances have led to the development of a wide variety of systems of varying complexity that effectively synchronize estrus and/or ovulation when used on the appropriate classification of female cattle. An applied understanding of the estrous cycle in cattle, the challenges presented by anestrous cows and prepubertal heifers, endocrine deficiencies that limit fertility, the commercial pharmaceutical products available, and the impact of these products on the reproductive system of cattle is necessary to choose the appropriate system of estrus synchronization. An awareness of the production, management, and physiological requirements of estrous control and environmental and biological factors that limit effectiveness of synchronization is essential. Finally, the details of the treatments given within each system and a working knowledge of the purpose of each step within a protocol are necessary to choose a suitable method of estrous synchronization and correctly apply this system. The information presented is based primarily on research performed in *Bos taurus* beef cattle, and limitations exist for some of the protocols in beef cattle with a large percentage of *Bos indicus* breeding. Likewise, while many of the programs described are effective in lactating dairy cows, the intent is that the information presented be applied to female beef cattle of predominantly *Bos taurus* parentage.

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Foreword

Members of Multi-State Regional Project W-2112 (Reproductive Performance in Domestic Ruminants) have worked as a group for more than 40 years to obtain a better understanding of the basic physiological mechanisms controlling the estrous cycle of beef cattle. Information obtained by this group and others has led to the development of several effective systems of estrous synchronization for beef cattle. This publication provides a source of applied information that will permit readers of varying educational backgrounds to understand the process of estrous synchronization in beef cattle of predominantly *Bos taurus* parentage and enable them to apply the existing technology in an appropriate manner.

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Acknowledgements

The authors would like to acknowledge the founders of W-112 as we have come to know it and the contributions they made in laying the foundation to establish all of the collaborative efforts that were fostered. Identifying our founders has not been an easy task, so we hope that we don't miss anyone along the lines. Rumors of the start of W-112 include some of the earlier members meeting in a bar in Denver and deciding to start a regional project and arm wrestling, perhaps about who was going to be Chair or Member at Large. But W-112 was really hatched from a previous W-49 project that included members like Jim Wiltbank, Perry Cupp, Gordon Niswender, and Colin Kaltenbach. In the Fall of 1969, Gordon, Colin, Rue Jensen, and others met in the basement of and old hotel in Salt Lake City to hammer out the renewal of W-49 which was approved and renamed W-112. Thus, we acknowledge the roles of early W-112 members like: Colin Kaltenbach, Jerry Reeves, Gordon Niswender, Jim Wiltbank, Tom Dunn, Guy Kiracofe, Terry Nett, Ron Randel, Fred Stormshak, Dennis Hallford, Gary Moss, Charlie Weems, Jon Wheaton, and others, in our many successes.

Synchronization of the Estrous Cycle

Cattle are in heat (estrus) approximately every 21 days with the "normal range" being 17-24 days. This periodic pattern of sexual receptivity is the result of an organized and rather complex series of changes that occur in the reproductive system of cattle. This 21-day sequence of changes is collectively referred to as the "estrous cycle". The systems of estrous control that are used to synchronize estrus and/or ovulation are designed to manipulate various components or functions of the estrous cycle. Thus, it is necessary to understand the estrous cycle in order to regulate it.

The primary glands, organs, or tissues that control the estrous cycle are the hypothalamus, pituitary, ovary and uterus. Each of these components of the reproductive system secretes chemical compounds called hormones, which regulate their own function, or the function of other components. Many hormones are involved in control of the estrous cycle and their release into the bloodstream can be measured experimentally. The major hormones, which are most commonly manipulated or administered to animals to synchronize estrus, are outlined in Table 1.

Table 1: Site of production and commercially available hormones used for estrous control.

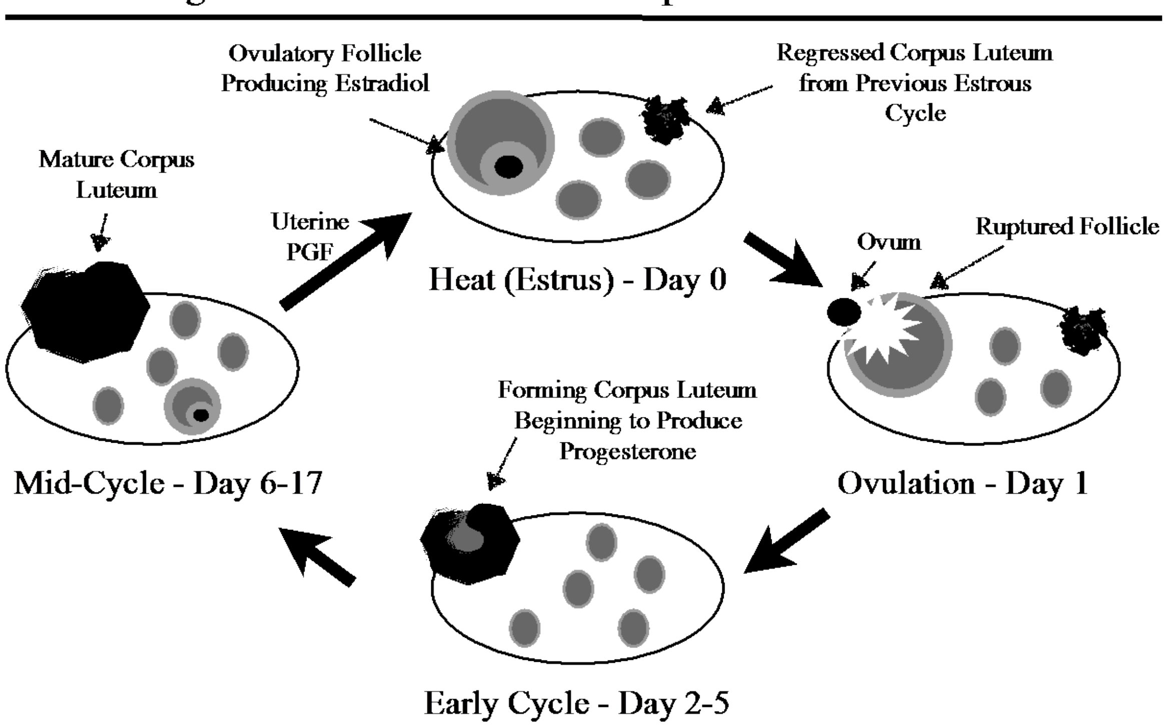
Gland	Hormone (Abbreviation)	Commercial Products[1]
Hypothalamus	Gonadotropin Releasing Hormone (GnRH)	Cystorelin®, Factrel®, Fertagyl®, OvaCyst®, GONAbreed®
Pituitary	Luteinizing Hormone (LH)	Not used in current systems
	Follicle Stimulating Hormone (FSH)	Folltropin-V®
Ovary		
-Follicles	Estradiol	Not used in current systems
-Corpus luteum	Progesterone	Melengestrol acetate (MGA®), Intravaginal Progesterone Releasing Insert (CIDR®)
Uterus	Prostaglandin $F_2\alpha$ (PGF)	Lutalyse®, Estrumate®, ProstaMate®, In Sync®

[1]The commercial products often do not have the same chemical composition as the hormone produced by the animal's body. These compounds do have similar effects on the reproductive system of cattle. Compounds that have actions similar to progesterone are called progestins.

The hormones listed in Table 1 influence the secretion of other hormones and the function of the components of the reproductive system to cause and control estrus, ovulation, formation and regression of the corpus luteum and the growth of follicles in the ovaries. The overall aim of estrous synchronization systems are to make some, or all of these physiological changes occur in <u>all animals at the same time</u>, in order to synchronize the timing estrus and/or ovulation.

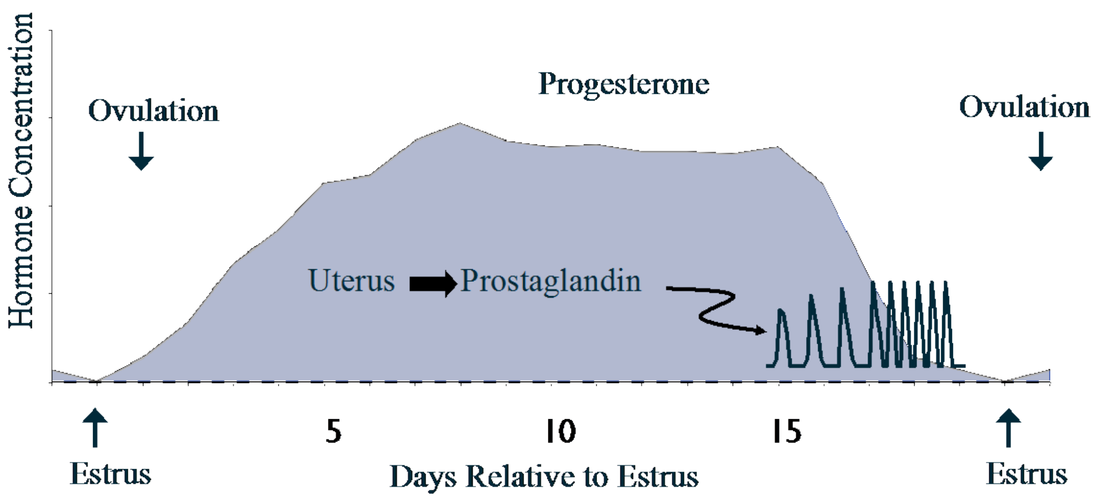
The 21 -day interval between heats in cattle is dictated by the lifespan of a structure on the ovary called the corpus luteum (CL). Figure 1 illustrates the process of formation, development and regression of the CL. The CL originates from cells of an ovulatory follicle that is present on the day the animal is in heat (Day 0). The ovulatory follicle is a fluid filled structure on the ovary, which contains the egg to be fertilized (ovum) and layers of cells around the wall of the structure. This follicle produces the hormone estradiol, which causes the animal to show heat, and is responsible for inducing hormonal changes that will cause this follicle to ovulate. Estradiol is also important for development of the ovum and preparation of the uterus for the impending pregnancy. On Day 1, the ovulatory follicle ruptures or "ovulates" and releases the ovum into the oviduct (a funnel and tube organ connected to the uterus). On days 2 to 5, important changes occur in the cells that line the ovulated follicle. These cells are transformed, or "luteinized", into the structure called the corpus luteum or CL. A primary function of the CL is to produce progesterone; the "pro – gestational" hormone. As the name implies, this hormone supports pregnancy. If the egg released on Day 1 is fertilized, the CL will be maintained throughout the pregnancy and helps support this pregnancy. **In terms of estrous synchronization, a very important function of progesterone is that if it is present in the bloodstream of female cattle, they will not show heat.** We can use this to our advantage in controlling the estrous cycles of cattle. If the animal is not pregnant, this structure will be maintained on the ovary until approximately Day 17. At this time, the cows reproductive system has recognized that she is not pregnant. The uterus then secretes the hormone prostaglandin $F_2\alpha$ (PGF). This hormone destroys the CL, causing it to regress, and stop producing progesterone. The decline in progesterone in the cow's bloodstream allows formation of a new ovulatory follicle, increased estradiol production, estrus, and another opportunity for the female to conceive.

Figure 1. Formation of the Corpus Luteum in Cattle



The formation, life and demise of the CL results in a distinctive pattern of progesterone in the blood stream of cattle during the 21-day estrous cycle. This pattern, and that of PGF, is shown over an estrous cycle in Figure 2.

Figure 2. Pattern of Progesterone and PGF During the Estrous Cycle



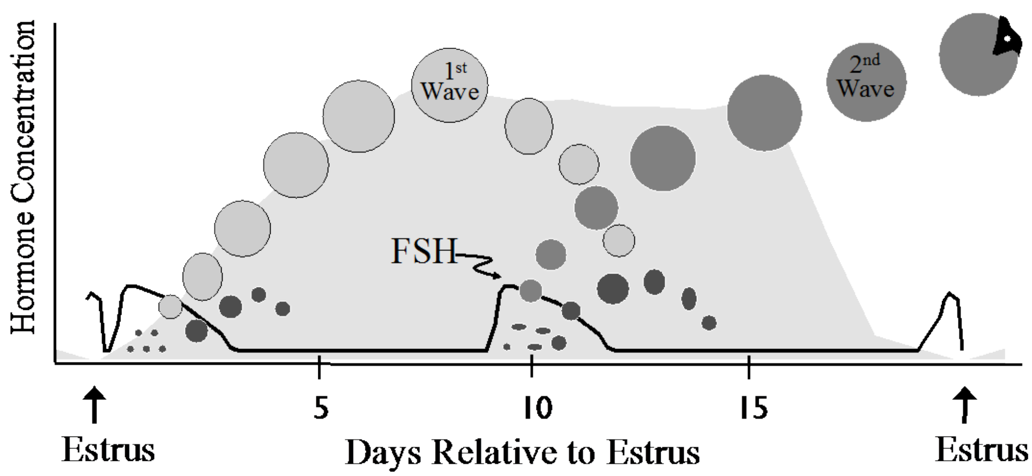
If pregnancy is not established, the CL regresses causing a decrease in progesterone, allowing the female another heat and opportunity to conceive. In non-pregnant cattle, uterine secretion of PGF is responsible for controlling the lifespan of the CL. **This action by PGF is a critical component of estrous control programs.** The ability to administer PGF to regress the CL and remove progesterone from the bloodstream, will cause cattle to show heat 2 – 5 days later. Synchronized induction of CL regression in all animals is a fundamental part of all estrous synchronization programs. Management of progesterone and PGF for estrous synchronization are outlined below. Some of the key functions and/or management will be discussed later in the bulletin.

Table 2. Key functions and management of progesterone and prostaglandin $F_2\alpha$.

Hormone	Key Functions	Necessary Management
Progesterone	Prevents estrus and ovulation, even at low concentrations Modifies growth of follicles Exposure to progesterone is necessary before conception in anestrous females	Synchronize decline in progesterone in all females by: - Regression of CL - Removal of progestin from vagina or feed
$PGF_2\alpha$ (PGF)	Induce regression of the CL and stops progesterone production	Administer at appropriate stage of the estrous cycle to regress the CL

In addition to the hormones progesterone and PGF, another important aspect of the estrous cycle in cattle is the continued growth and atresia (death) of follicles on the ovary. Ovarian follicles grow in a wave-like pattern (Figure 3) on the ovaries and discovery of this pattern of growth has greatly heightened our ability to control the estrous cycle for synchronization purposes. **The capacity to control this wave-like pattern of follicles is required for the success of timed insemination programs and beneficial to the precision of the timing of estrus when inseminating based upon detection of heat.**

Figure 3. Waves of Follicular Development and Pattern of FSH



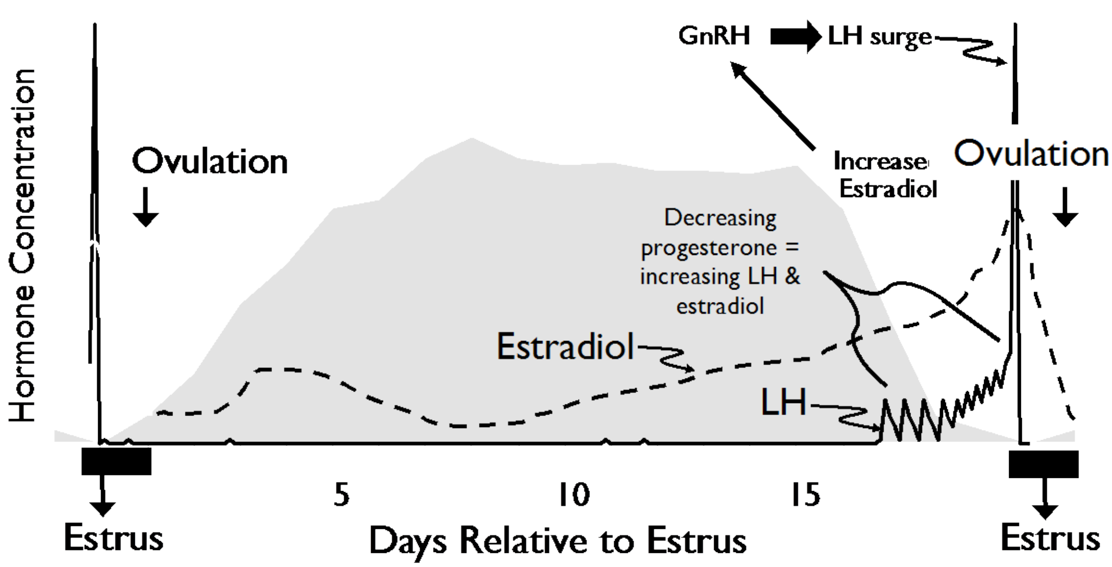
Ovarian follicles grow in what are normally referred to as "waves" during the estrous cycle. Most cattle have either 2 or 3 waves of follicle growth during each estrous cycle. The first wave begins immediately after ovulation. The second wave starts on approximately day 10. In some cows, a third wave will start on day 16 to 18 (not shown in Figure 3). A wave consists of a group of small follicles that all begin to grow at the same time. The growth of each wave is initiated by a rise in FSH in the bloodstream. As this group of follicles continues to grow, one of them becomes dominant over the others and suppresses the secretion of FSH. After dominance is obtained, LH controls continued growth and maturation of the dominant follicle. The dominant follicle continues to grow, while the others in the group undergo follicle death, or atresia. The dominant follicle of the first wave (in females with two waves) and the dominant follicle of both the first and second wave (in females with three waves) are destined to undergo atresia, and be replaced by a new group of small follicles. The reason that these dominant follicles undergo atresia is that elevated concentrations of progesterone prevent them from progressing to ovulation. Once the dominant follicle undergoes atresia, its suppressive effects on other small follicles and FSH are removed to allow a new follicular wave to begin. In the 2-wave example in Figure 3, the decline in progesterone on day 18, due to regression of the CL by PGF, permits this dominant follicle to grow to a stage that allows increased estradiol production and eventual ovulation. **A fundamental component of all timed insemination protocols is to synchronize the waves of follicle development in all females, so that when ovulation is induced, all females have a dominant follicle that is of the same age and size.** Administration of GnRH is used to induce ovulation in timed insemination approaches. The key functions, and necessary management of follicle waves for estrous synchronization are outlined below (Table 3). Some of the functions and/or management will be discussed later in the bulletin.

Table 3. Key functions and management of follicle waves and FSH.

Hormone	Key Functions	Necessary Management
Follicle Waves	Delivers ovum for fertilization	Synchronize time of initiation of follicle waves to permit:
	Provides a follicle to produce estradiol to cause estrus	- Timed AI - Synchronized ovulation
	Size at CL regression and growth rate influences time interval to estrus	Synchronize follicle waves to prevent ovulation of: - Aged (persistent) or immature follicles
FSH	Stimulates start of each wave Early growth of follicles Inhibited by the factors from the dominant follicle	Induce atresia of dominant follicles to permit a new wave at the appropriate time in response to FSH

In addition to the ovum, another major product of the follicles is the hormone estradiol (Figure 4). Generally, the pattern of estradiol secretion follows the waves of follicle growth. Estradiol reaches its highest concentrations following the decrease in progesterone. The small pulses in LH that are released due to decreased progesterone cause the preovulatory follicle to increase in size and produce greater amounts of estradiol. The elevated estradiol acts on behavioral centers in the brain of cattle and causes estrus (heat). An equally important function of the high concentrations of estradiol is to induce a surge release of GnRH from the hypothalamus, which in turn causes a surge of LH from the pituitary gland. The preovulatory surge of LH is directly responsible for initiating ovulation.

Figure 4. Pattern of Estradiol and LH During the Estrous Cycle



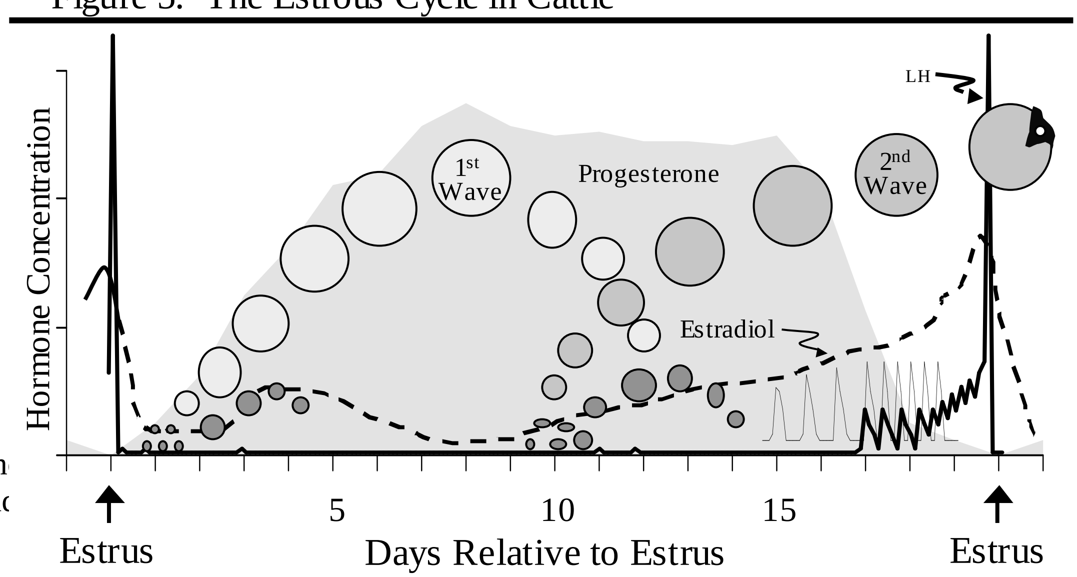
For estrous synchronization programs in which females are inseminated following detection of estrus, the sequence of increased LH pulses, increased estradiol, estrus and the LH surge are allowed to progress spontaneously without intervention. In most timed insemination programs, an injection of GnRH is given to cause the LH surge, and thus ovulation, at a set time. In these programs, an interval of 48 to 72 hours, after the decrease in progesterone is used to allow the earlier changes in LH and estradiol to occur. Limiting the period between CL regression and GnRH administration, termed proestrus, results in reduced estradiol concentrations prior to ovulation and can reduce fertility in cattle. **Controlling the timing of the LH surge with an injection of GnRH is a fundamental part of most timed insemination programs.** The key functions, and necessary management of estradiol and LH are outlined below in Table 4 and some will be discussed later in the bulletin.

Table 4. Key functions and management of estradiol and LH.

Hormone	Key Functions	Necessary Management
Estradiol	Signal for the onset of estrus Signal for the surge of LH Preparation of uterus for pregnancy	Managed through control of follicular waves Maximize prior to induced ovulation
LH	Small pulses for final growth of preovulatory follicles Surge causes ovulation Inhibited by progesterone Stimulated by GnRH	Allow small peaks to grow follicles for 48+ hours after the decrease in progesterone Induce an LH surge with GnRH to cause ovulation -for timed AI -to initiate a new follicle wave

Combining all these key components and hormones of the estrous cycle presents a complex picture of the process resulting in estrus and ovulation (Figure 5).

Figure 5. The Estrous Cycle in Cattle



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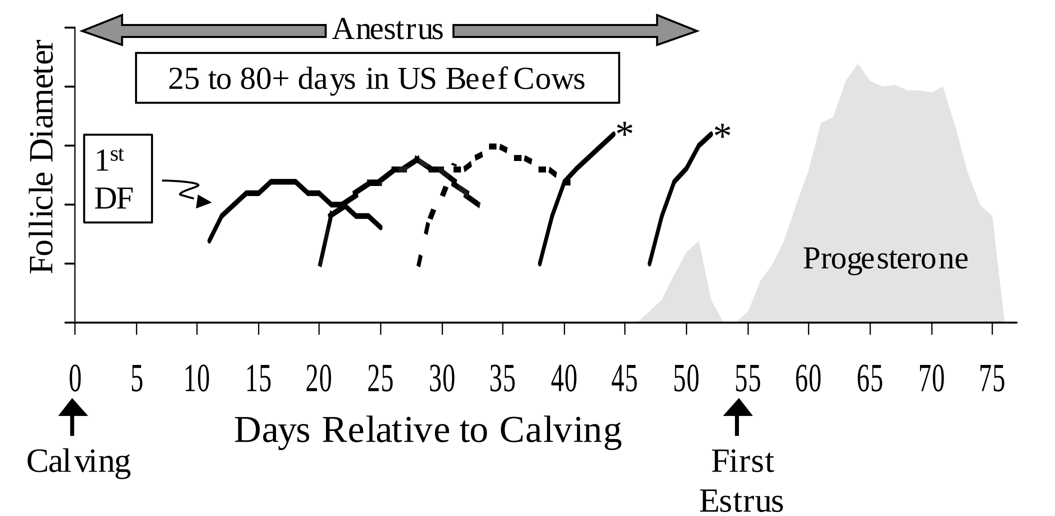
task of the estrous synchronization program is to modify hormones and follicular waves so that regardless of the day of the estrous cycle the female is in when the protocol is initiated, every female will be in estrus or even ovulate at the same time. With the complexity of the estrous cycle, achieving this endpoint is one hurdle. However, the challenges for cattle producers that wish to synchronize estrus do not end here.

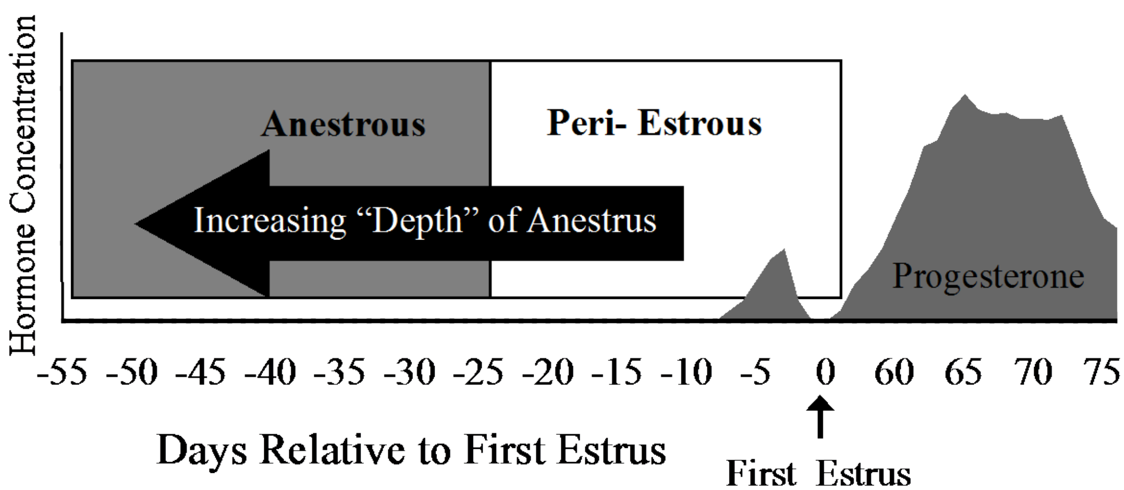
The second major obstacle to successful estrous control is that within all groups of postpartum cows and yearling heifers, there is a proportion of females that are either in postpartum anestrus (cows) or prepubertal anestrus (heifers). Anestrus is the lack of estrous cycles and expression of heat. Anestrus is a normal occurrence, and prevents females from conceiving before they have reached sexual maturity (heifers) or at a time after calving that will substantially alter their annual calving interval (postpartum cows). The proportion of females that are anestrus at the start of their annual breeding season can vary from 20 – 70% between herds, with this proportion dependant on many management factors that are discussed later. Heifers reach puberty at 12 to 15 months of age, and the duration of postpartum anestrus ranges from 25 to >80 days in postpartum cows. Figure 6 illustrates postpartum anestrus in terms of follicular development and progesterone concentrations in postpartum cows.

Figure 6. Postpartum Anestrus in Beef Cows



Notice in this example of a cow with an anestrous period of 55 days, from calving until about day 45, there is no progesterone in the animal's system. During this time, the female is not ovulating or forming a CL. She is not having estrous cycles. There are, however, waves of follicular development occurring during this time. The line labeled "1st DF" in Figure 6 indicates the growth and atresia of the first dominant follicle of the first wave after calving. Waves of follicle growth begin 7-14 days after calving and continue through pregnancy. The first ovulation after calving in this example occurred on day 45 and is indicated by the asterisk at the end of the 4th DF. Notice, however, that the increase in progesterone after this ovulation, and the interval to the next ovulation (2nd asterisk) is not 21, but only about 10 days. This "short – cycle" is also a normal occurrence in greater than 80% of postpartum cows at their first ovulation after calving and at the first ovulation in prepubertal heifers. Some cows (20-30%) will show heat at the beginning of the short cycle, however, cows that are inseminated or mate with a bull at this heat will not become pregnant. The purpose of the short cycle is to provide a period of progesterone exposure before the first "normal" ovulation on day 55. The short period of progesterone exposure is necessary for the next cycle to be a normal 21-day estrous cycle. **In terms of estrous synchronization systems designed to induce postpartum anestrous cows to ovulate, it is essential that the cow be provided with this short period of progesterone exposure before the estrus/ovulation to avoid pregnancy failure following AI due to short-cycles.** Pregnancy success of cows that do not have this period of progesterone exposure is thought to be very low.

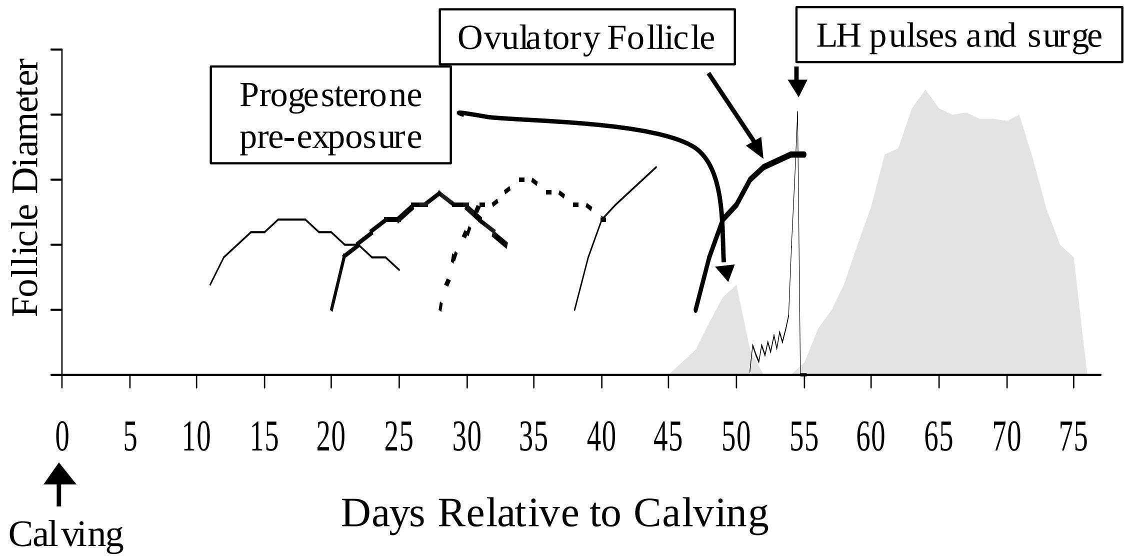
Figure 7. "Depth" of Postpartum Anestrus in Beef Cows



The capacity to initiate estrous cycles in anestrous cows is further complicated by the knowledge that all anestrous females are not equally responsive to treatments designed to induce heat. This variation among anestrous females is described by the concept of "depth" of anestrus (Figure 7). Cows that are near the re-initiation of a normal estrous cycle (less than 2-3 weeks; "Peri-estrous") at the start of a synchrony program are believed to be very responsive to many of our current estrous control programs. Those females that are "deeper" in anestrus (greater than 2-3 weeks before spontaneous resumption of estrous cycles) are less responsive, and require the more powerful of the estrous control systems to induce the onset of estrous cycles. Within a herd, the proportion of anestrous cows that fall into each of these categories can vary widely, depending upon calving distribution, age, genetics, nutritional program, and other management factors. Prediction of which cows are peri–estrus and which are deeper in anestrus is difficult or impossible.

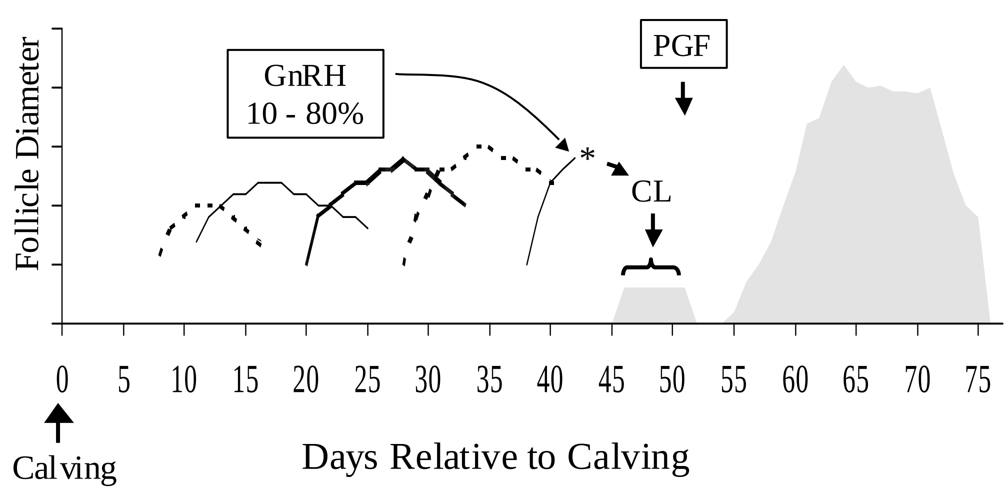
Since the number of females in anestrus can represent a major proportion of a cow or heifer herd, and this proportion varies between years, it is critical that estrous control systems are able to induce anestrous females to begin to cycle. The stimuli necessary to induce a majority of anestrous cows to cycle are outlined in Figure 8. In the natural progression of events during the postpartum anestrous period, cows will begin progressively growing follicular waves soon after calving. As the postpartum period progresses, LH concentrations increase, allowing for sequentially larger dominant follicles. Eventually, the endocrine conditions will align and ovulation will occur. This subsequently results in a brief period of progesterone, the short-cycle, that then establishes the progesterone exposure required for continued estrous cycles of normal duration. Often when synchronizing estrous, we provide external stimulus to expedite these normal sequential events. These stimuli include exogenous progesterone and the induced ovulation of dominant follicles by administration of GnRH.

Figure 8. Requirements to Induce Anestrous Cows to Cycle



The requirements to induce anestrous cows to cycle and provide an opportunity for these females to become pregnant during a synchronization period are outlined above. It has been established that pre – exposure to a short period of progesterone is required for 2 reasons. First, as mentioned earlier, pre-exposure is necessary to ensure that the subsequent synchronized heat or ovulation results in a fertile estrous cycle of normal length. Second, it has been shown that following treatment with progesterone (or a progestin) for 5 to 14+ days, a substantial proportion of cows will start to cycle on their own. Withdrawal of the progesterone will initiate the pulses of LH, followed by the surge of LH in some cows. This period of progestin exposure can currently be provided to the animal through two approaches. In one approach, the progestin is provided by giving an injection of GnRH to induce ovulation of a follicle and formation of a short – lived CL (Figure 9).

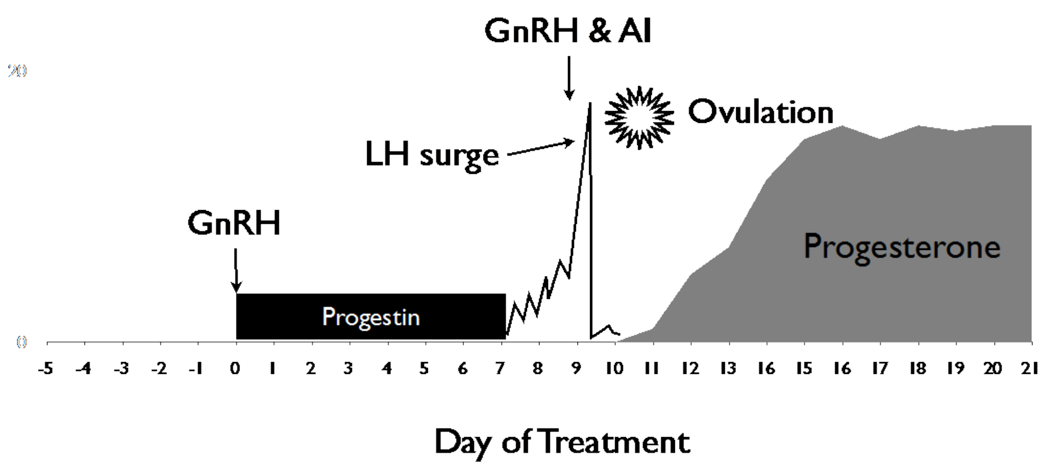
Figure 9. Progesterone Pre – exposure Using GnRH



With this approach cows are given an injection of GnRH to cause the existing dominant follicle to ovulate. The success of this method is dependent upon a dominant follicle being present on each cow's ovaries that is responsive to GnRH. Due to the dynamics of the follicular waves in cattle, there is not always a dominant follicle that is able to respond to an LH surge. If the dominant follicle is of adequate size to ovulate in response to GnRH, the resulting CL is short – lived, however this is irrelevant since the entire purpose of this is to provide a short period of progestin exposure. The limitation of this approach is that within groups of anestrous cows, the response in terms of ovulation can vary from 10 to 80%. It is presumed that in herds with many anestrous cows in deep anestrus, the response to GnRH is low, and in herds with most anestrous cows in the peri-estrous category, the response is high. The alternative approach to using GnRH to provide progesterone pre-exposure is to administer a progestin directly either through the feed (MGA; heifers only) or with the intravaginal insert (CIDR). This is a more predictable method, as delivery of progesterone is 100% in all females.

The number of cows that ovulate following the withdrawal of the progestin can be increased substantially by providing a stimulus for the surge of LH. This is achieved in some current systems with an injection of GnRH 2 to 3 days after withdrawal of the progestin (Figure 10).

Figure 10. Use of Progestins and GnRH to Induce Ovulation



The sequence of progestin pre-exposure (with either GnRH, a CIDR or MGA), followed 2 to 3 days after progestin removal with administration of GnRH (to induce an LH surge) is the most powerful tool available to US producers to induce anestrous cows to resume estrous cycles or heifers to reach puberty. With this sequence, females can be inseminated at the induced ovulation on day 9 or 10 (Figure 10) with a reasonable expectation for pregnancy.

The challenges of estrous synchronization should be relatively obvious as one considers the preceding pages. On a given day in the spring (or fall), the objective is to start an estrous synchronization program on a group of females that includes cyclic females in all stages of the estrous cycle and anestrous females in varying depths of anestrus. In most herds, it is difficult to identify into which of these categories individual females fit. Therefore, if synchronization of the entire herd is the goal, a single sequence of treatments is required to cause all of these animals to show estrus and/or ovulate on a single day for timed AI, or over a 3 – 5 day period for insemination based upon estrous detection. Fortunately, the pace of discoveries in reproductive physiology and the development of new technologies have made this seemingly insurmountable task achievable. In recent years, increased understanding of the endocrine and physiological parameters that influence reproductive competence in cattle has been obtained and with this knowledge, new approaches of synchronization within the limitations of the pharmaceutical products available have been developed.

Production and Physiological Requirements for Estrous Control

Widespread adoption of estrous synchronization and AI is dependent upon a variety of factors, but perhaps most importantly is the efficacy and consistency of the protocols available. Effectiveness can be defined in several ways because each producer has distinct goals to achieve when implementing protocols. For example, conducting timed insemination rather than mating based on estrous detection is of paramount importance to some producers but less critical for others. As a second example, some producers may feel it is essential to include the entire herd in a synchrony program, while others may choose only to include a sub-group of females (e.g. cows that calved in the first 30 days of the calving season). For systems in which the entire herd will be included in the synchronization program, the following endpoints represent viable production goals.

1. The system of synchrony should enhance the reproductive performance of the cow herd as measured by average day of conception during the breeding season, rather than simply concentrating the times that AI will take place.

2. The system should be effective in <u>all</u> cows and heifers within the herd. Estrus and/or ovulation should be induced in a majority of anestrous females and fertility of the synchronized estrus/ovulation, especially in cyclic females, must be equal to or greater than that achieved with mating based upon a spontaneous estrus.

3. Greater than 50% of the herd should become pregnant to AI over a maximum of 3 days or to a timed insemination protocol.

4. The system should minimize cattle handling, use treatments that are easy to administer and be of reasonable cost while attaining the above goals.

5. A hidden requirement *essential* for achieving the above goals is that the herd must be adequately managed from both a reproductive and nutritional standpoint. Cows must be maintained in an adequate body condition, a defined breeding season is imperative, the first-calf heifers should calve before the cowherd or early in the main calving season, and appropriate replacement heifer selection and management is necessary.

In order to achieve the production endpoints outlined above, there are 4 key physiological requirements that must be met by the estrous control system.

1. A synchronous decline in progesterone (or exogenous progestin) must occur in all animals as a result of luteal regression or withdrawal of a progestin source.

2. Adequate stimulus must be provided to the reproductive system of anestrous females to induce ovulation in a majority of females in this reproductive classification.

3. Ovarian follicle growth must be managed to: a) ensure that follicles which ovulate yield oocytes of normal fertility and b) that stage of follicular growth is approximately equal at the time of the progestin decline to permit precise timing of estrus and/or ovulation (timed AI).

4. Elevated concentrations of estradiol are achieved prior to induced ovulation (for timed AI protocols) and elevated concentrations of progesterone are achieved in the subsequent estrous cycle.

In recent years, a considerable amount of research has focused on identifying endocrine and physiological factors that influence the probability of pregnancy establishment in cattle. Understanding of function of the reproductive system in cattle and the impact of various treatments on this system, have made possible the development of synchronization systems that meet these production and physiological requirements. Various characteristics of the ovulatory follicle have been investigated to identify what components of follicle maturation correspond to increased reproductive competence. Numerous studies have investigated the relationship between ovulatory follicle diameter at time of ovulation and subsequent fertility. Collective consideration of these studies suggests that optimal fertility is obtained in a finite window of ovulatory follicle diameter. Induction of ovulation of follicles that are exceedingly small or large reduces the probability of pregnancy. In itself, however, ovulatory follicle diameter appears to be only an indirect measurement of follicle maturity, serving as a potential indicator of the capacity of the ovulatory follicle to produce estradiol and potentially related to the duration of follicle dominance prior to induced ovulation. Females with greater concentrations of estradiol prior to induced ovulation have greater pregnancy success than those with lesser concentrations. Elevated concentrations of estradiol produced by the ovulatory follicle affect the development and competence of the oocyte within the follicle as well as program uterine function for the preparation of pregnancy. The relationship between ovulatory follicle size and fertility is likely in large part due to the inability of small follicles to produce adequate concentrations of estradiol prior to ovulation; with the reduction in fertility in females induced to ovulate an exceedingly large follicle due to an extended duration of follicular dominance and ovulation on an aged oocyte. Therefore, new approaches to timed-AI have been designed to maximize preovulatory estradiol concentrations by attempting to increase the proestrus period and/or minimize the proportion of exceedingly small or large follicles being induced to ovulate at the conclusion of the protocol.

State of follicular maturity at induced ovulation can also influence the quality of CL developed after ovulation and concentrations of progesterone in the subsequent estrous cycle. Following ovulation, a rapid increase in progesterone is required for optimal fertility. These increasing concentrations of progesterone are requisite for proper uterine function, conceptus growth, and establishment and maintenance of pregnancy. Therefore, current approaches to estrous synchronization attempt to ensure that CL function after ovulation, especially in scenarios where ovulation is induced with GnRH, is not compromised. Many studies in beef cattle have investigated the potential to increase pregnancy success by increasing progesterone concentrations during early gestation via progesterone supplementation (CIDR insertion) or pharmaceutical interventions to increase CL function or number of CL present. The improvement in fertility when these systems were employed were variable, with the potential improvement in fertility dictated by the inherit level of fertility of the herd in which treatment was implemented.

In addition to the physiological and endocrine parameters that impact fertility, other managerial factors influence the probability of pregnancy. One of the most critical is nutrition. Nutritional status of the female can influence age at puberty, duration of postpartum anestrus, follicular development, and general fertility. In cows, nutritional status is often assessed by evaluation of body condition through a subjective body condition scoring system. In the US, body condition scores are on a scale of 1 to 9 with 1 being emaciated and 9 being obese. At calving, females should be in a body condition score of 5 or 6. Inadequate body condition results in an extended period of postpartum anestrus and reduced pregnancy success to estrous synchronization. In heifers, inadequate nutrition can delay puberty and recent findings indicate that inadequate nutritional inputs during early gestation may reduce pregnancy success.

To maximize pregnancy success to estrous synchronization and AI, animal stress must be minimized. Therefore, it is critical to employ low-stress handling procedures when implementing an estrous synchronization protocol. In addition, transport of females can also induce a large stress response and if done at an inappropriate time relative to insemination, result in embryonic losses. If females must be transported following AI, this should occur within the first few days following AI, if possible. If this cannot be accomplished, then the producer should wait until 40 days following AI to transport females.

Mathematical “Laws” of Estrous Synchronization

As one considers the implementation of estrous control programs, and decides to compare and contrast various systems, there are a few simple “laws” or “facts” that are often overlooked.

Law #1 – There are 365 days in a year, gestation length in cattle is approximately 285 days, and cows are expected to produce a calf on an annual basis.

From this statement, it can be calculated that in order to calve a herd at the same time each year, there will always be approximately 80 days from the start of the calving season to the start of the next breeding season (365 – 285 = 80). Thus, the first cows to calve in a herd will be about 80 days postpartum when the next breeding season starts. The later a cow calves, the fewer days postpartum she will be at the start of the breeding season, and the greater the likelihood that she will be anestrus. As an example, if the breeding season for the previous year was 60 days, cows conceiving on the last day of that season will calve approximately 20 days before the start of their next breeding season. If the previous breeding season was 90 days in length, cows may still be calving when the breeding season for the current year is started. This relationship cannot be changed, unless one decides to calve later in each successive year. This law stresses the importance of controlled breeding seasons of 60 days or less, especially if a goal is to synchronize the entire herd at the start of each breeding season. The second point that should be derived from this is that the synchrony system should be as short as possible. In most herds, there will not be 20+ days from the end of calving to the start of breeding; thereby limiting the desirable length of a synchronization system.

Law #2 – The pregnancy rate of a herd to an AI program is determined by 2 factors: Pregnancy Rate = Submission Rate x Conception Rate.

Again, this seems simple; however, it is quite easy to get distracted by what the true endpoint should be for an AI program. In most cases, the desire is to get as many cows pregnant by AI at the start of the breeding season. Hence, maximizing submission rate and conception rate are equally important. Focusing on each variable independently can lead to less than ideal responses.

Table 5 illustrates the relationship amongst submission rate, conception rate, and the ultimate impact on pregnancy rate. Improving either separately or submission rate and conception rate collectively equates to increased pregnancy success. As will be discussed, using timed AI results in a 100% submission rate because all females are inseminated irrespectively of estrous expression. Therefore, the major driver of pregnancy response in timed AI protocols is conception rate, which can be influenced by many of the factors previously discussed.

Table 5. Influence of submission rate and conception rate on pregnancy rate

	Submission rate, %							
	40		60		80		100	
Conception Rate, %	50	70	50	70	50	70	50	70
Pregnancy Rate, %	**20**	**28**	**30**	**42**	**40**	**56**	**50**	**70**

If the factor of conception rate is considered independently, the easiest way to maximize this factor within a herd is to apply a synchronization system to all animals, and inseminate only those that show estrus. If cows have the capacity to grow a follicle to the level that it will cause the cow to come into heat, the conception rate will be determined primarily by the skill of the individuals that are detecting estrus, the AI technician, semen quality, and the inherent fertility of the cowherd. In this case you are not "making" cows show heat and/or ovulate; instead, you are just grouping their expression of heats into a similar time. It is critical to realize that not all females will show heat with any of the systems available in the USA. The losses in terms of animals not "submitted" for breeding can be attributed to three primary categories: 1) cows that show heat but are not observed, 2) cows that ovulate but do not show outward signs of heat, and 3) cows that are anestrus, or are ineffectively synchronized, that do not ovulate or show heat during the synchrony period. If the approach were taken to only inseminate cows that show estrus in a whole-herd synchrony program, it would not be unusual for submission rate to range from 30 to 80%. If we do the math for a herd with a 50% estrus response and a 70% conception rate, there will be 35% of the herd pregnant to AI during the synchronization period. This is unacceptable, or marginally acceptable to most producers. As another example, if only 60% of the herd is enrolled into a program (e.g. exclude cows that calve in the 2nd half of the season, two year – old cows, etc), submission rate for this sub-group will likely be as high as 80+ %. However, the math still shows that submission rate = 48% (80% detected of 60% of the herd). Therefore, with a conception rate of 70% and a submission rate of 48%, pregnancy rate for the herd is 34%.

In terms of submission rate, it is very easy to make this equal 100% if timed AI is used. With this approach, all females are inseminated, regardless of whether they show estrus. If appropriate control of the timing of ovulation is in place, and mainly animals from categories "1 and 2" in the above paragraph are added to the insemination group, conception rate would not be expected to decline substantially, and whole – herd pregnancy rate would increase. However if there were a large number of cyclic females that were not synchronized, or anestrous females that did not ovulate (category "3", above), these animals will not conceive to a timed insemination and conception rate could decline considerably. The two examples outlined in the table below (Table 6) are for a herd in which 50% would have been detected in heat without timed AI.

Considering Example 1, this illustrates how timed AI can be beneficial to pregnancy rate if it increases submission rates of cows that are potentially fertile, but not detected or showing heat. Example 2, however, demonstrates that females must be successfully synchronized and induced to ovulate (in the case of anestrous cows), if this approach to increasing submission rate is to be profitable. In herds with a sizeable proportion of anestrous females, synchrony systems that induce these females to initiate cycles are imperative for success.

Another tactic that may be valuable, especially in heifers, is a 'hybrid' approach to estrous synchronization. These 'hybrid' protocols, also referred to as 'Select-Synch & timed-AI', involve a period of estrous detection and AI followed by timed insemination of all females not observed in heat. This type of an approach offers two major advantages. First, inseminating off of an observed estrus maximizes conception rates. Second, including a timed-AI allows all females to be inseminated, regardless of estrous expression, thus allowing females that may be synchronized but not exhibiting estrus the opportunity to conceive to AI.

Table 6. Influence of herd response category on pregnancy rate to timed AI

	Example 1			**Example 2**		
Sub – group of herd	# AI	Conceive %	# Preg.	# AI	Conceive %	# Preg.
Would have been detected in heat	50	65%	33/50	50	65%	33/50
Ovulating correctly but not in heat	40	60%	24/40	10	60%	6/10
Not synchronized and/or ovulating	10	0%	0/10	40	0%	0/40
Total	100	57%	57/100	100	39%	39/100

Law #3 – The overall conception rate to a synchronized estrus will decrease as the proportion of the herd that is inseminated increases.

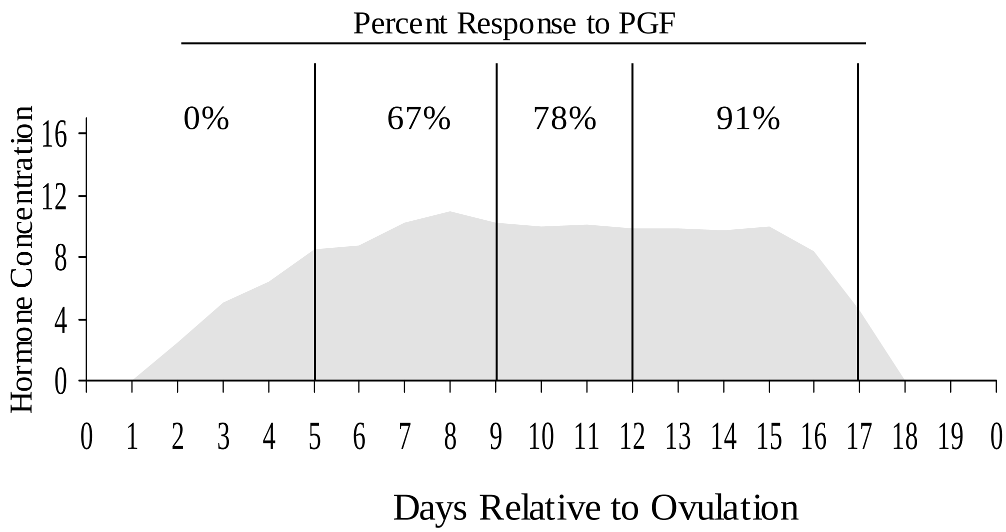
This statement is somewhat redundant with Law #2, but deserves mention in itself. There are some synchronization programs that are quite powerful, and effective in inducing a potentially fertile ovulation in a majority of anestrous females. With these systems, cows that normally would have shown estrus 1 to 5+ weeks after the start of the breeding season are induced to ovulate on the first day of the breeding season and inseminated. This provides them an opportunity to conceive early, but typically their conception rates are lower. This should not be surprising as we are forcing their reproductive system to function before it is fully prepared to function spontaneously. In a herd with 50% of the females in anestrus, an aggressive synchrony program that allows one to inseminate these females, at a 40 to 50% conception rate would be considered a success in many cases. The alternative is no synchronized pregnancies in 50% of the cowherd. Conception rates can vary considerably and are dependent upon technician proficiency and fertility of both the semen and the cowherd. It is important to have realistic expectations. Excellent conception rates are typically about 75% in females observed in estrus.

It is important to keep in mind that one can make any synchronization system available appear to be tremendously effective by avoiding the “high – risk” animals within a herd. Often excellent results are reported for select groups of females (usually mature cows that calved in the first 3 to 5 weeks of the calving season). Essentially any synchronization system available can make a high proportion of these animals show heat and result in or approach excellent conception rates; because they are all cyclic or peri – estrous. See Law #2 to evaluate the true impact of this approach on reproductive efficiency of the cowherd.

Systems of Synchronization using Prostaglandin $F_2\alpha$

Introduction: The most widespread approach to estrous synchronization in the USA has been through the use of prostaglandin $F_2\alpha$ (PGF). Prostaglandin $F_2\alpha$ regulates a female’s estrous cycle by causing regression of the CL. An injection of a synthetic PGF (see Table 1) will mimic natural PGF release to cause CL regression. Synchronized regression of the CL will cause a decline in progesterone and result in the final growth of the dominant follicle, allowing it to produce estradiol and stimulate behavioral heat. Females with a mature CL on their ovaries when they receive an injection of PGF will usually exhibit heat 2 to 5 days later. Thus, in order for PGF to be effective, females must be cyclic, and in a responsive phase of the estrus cycle. The responsiveness of cows to PGF at various times in the cycle is diagrammed in Figure 11. The most important aspect of Figure 11 to consider is the fact that PGF will not regress an immature CL (days 1 to 5). Thus administration of PGF prior to day 5 after ovulation is not effective. Prostaglandin $F_2\alpha$ also has no effect after the CL has started to regress (day 17+), but cows between days 17-20 of their estrous cycle are coming into heat over the next 1 - 4 days anyway, and appear to respond to the injection. Anestrous cows and prepubertal heifers will not respond to an injection of PGF since no CL exists. If they represent a major portion of the herd, response rates could be quite low.

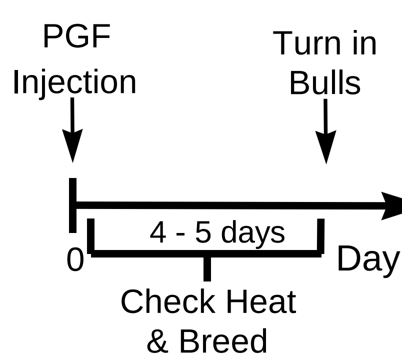
Figure 11. Stage of the Estrous Cycle and CL Regression with PGF



If PGF is to be used exclusively to synchronize estrus, it is necessary to ensure that most cyclic females have a mature CL that is susceptible to regression by PGF. For optimal responses, it is helpful if only cows that are likely to be cyclic are enrolled in the synchronization program. With PGF systems, control of follicular waves is not provided, and cows must be inseminated following detection of estrus; not to a timed insemination. Thus, proper estrous detection is required when using these synchronization approaches. There are four PGF programs being used to synchronize estrus in cattle. Two of these programs require two injections of PGF and two require a single injection. Fertility of cows at an estrus resulting from an injection of PGF is similar to that of females that show heat spontaneously.

One Injection of PGF with Insemination after PGF: Inject all cows, check heat and inseminate all cows 12 hours after detection of standing heat (Figure 12). With a single injection of prostaglandin, ~70% of the cyclic cows would be expected to display heat (those on day 6 or greater of the cycle at injection) during the next 4 - 5 days. The percentage of the animals injected that are anestrus can have a major influence on response rates. For example, if 100 females are injected, and only 50 are cyclic, the estrus response would be 35% (70% of 50 cyclic females/100 females injected).

Figure 12. One Injection PGF Protocol

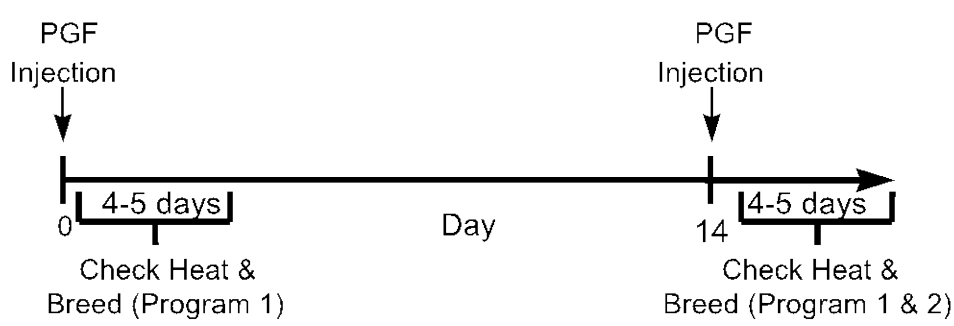


Two Injection PGF Programs: The two injection programs for synchronization with PGF are designed to increase the proportion of females with a CL that is responsive to regression with PGF. Administering PGF 11 to 14 days apart ensures that cyclic females will regress their CL in response to at least one of the PGF doses. With the first PGF injection, ~70% of the cyclic cows would be expected to display heat (those on day 6 or greater of the cycle at injection) during the next 4 - 5 days. Those animals that were not responsive to the first injection (i.e. on days 1-5 of the cycle at the first injection), would respond to the second injection. Also, cows in heat after the first injection would be on day 6 or greater of their next estrous cycle, and would be expected to show heat a second time; following the second PGF injection. With all systems that use PGF, another PGF injection could be added to any protocol (11-14 days later) in cows not inseminated to provide another opportunity for AI. Remember though, if cows did not initially respond because they were anestrus, an additional PGF injection will be of little benefit.

Program 1: Initially, an injection of PGF is given to all cows. Within 5 days after the first injection, ~ 70 % of the cyclic females should be in heat. Females detected in heat are inseminated 12 hours later (Figure 13). With this program, animals <u>not</u> inseminated after the first PGF receive a second PGF injection 11-14 days later, and are inseminated 12 hours after detection of heat. Do not give the 2^{nd} PGF to cows that have been inseminated after the first PGF; this will terminate that pregnancy.

Program 2: This approach is similar to Program 1, except that no inseminations are performed after the first PGF injection and all females receive the 2^{nd} PGF. This sequence should, theoretically, synchronize estrus in essentially 100% of cyclic cows within 1 to 5 days after the second PGF. In application, some cows that display heat after the first injection fail to do so after the second PGF, and synchronization responses of 75 – 85% after the 2^{nd} PGF are common with this protocol. With either program, the number of anestrous cows that are treated will influence estrus responses in a manner similar to that described for the single injection system.

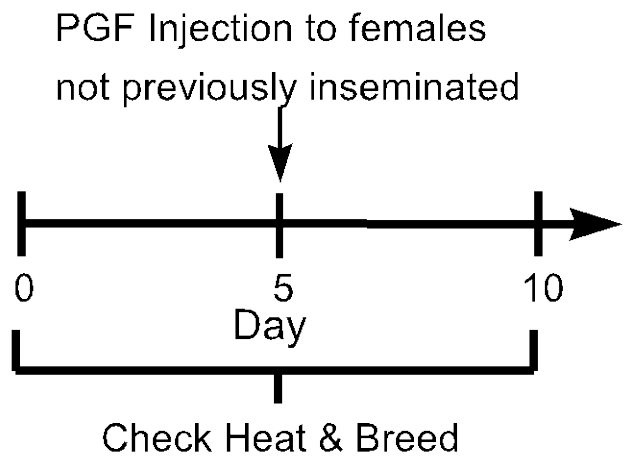
Figure 13. Two Injection, PGF Protocols With Breeding After Both (Program 1) or Only After the Second Injection (Program 2).



One Injection With Inseminations Before <u>and</u> After PGF: Check heat and inseminate all cows detected in estrus for the first five days of the breeding season. Inject all females not previously inseminated with PGF on day 5 and inseminate 12 hours after detection of heat (Figure 14). By inseminating cows in heat for the 5 days before PGF, no cows that receive PGF will be on day 1-5 of the estrous cycle. Thus, all cows that are cyclic should show estrus within 5 days after the

PGF injection. This is the most popular protocol that uses only PGF to synchronize estrus, and can result in greater than 90% of cyclic cows being bred during the first 10 days of the season.

Figure 14. One PGF Injection With 10 Days of Heat Detection



Systems of Synchronization using Prostaglandin $F_2\alpha$ and a Progestin

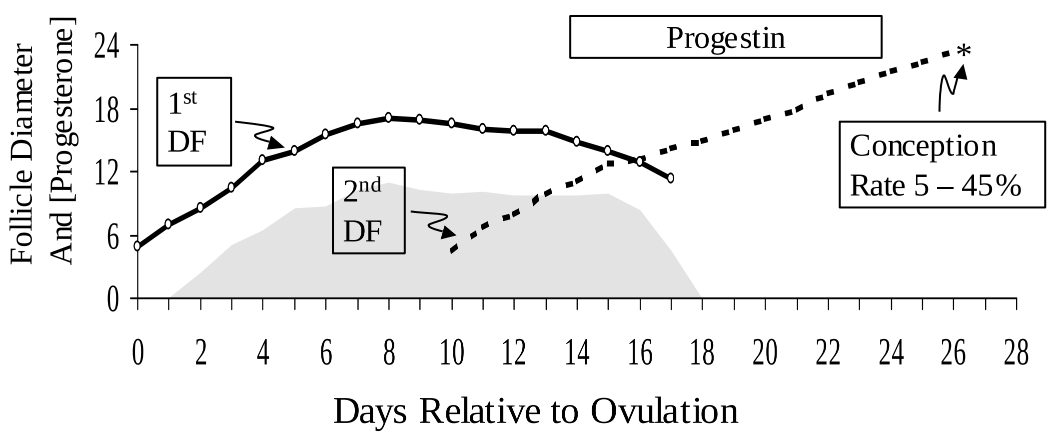
Introduction: While the use of PGF is included in essentially every program cited, some programs have been developed that incorporate the use of a progestin with PGF. The addition of a progestin addresses two aspects of estrous control. Most importantly, providing a progestin will induce some prepubertal heifers and anestrous cows to begin cycling and to have a normal - length estrous cycle following the first ovulation. Through this action, a proportion of anestrous cows within a herd have an opportunity to be inseminated at the start of the breeding season. A second advantage is that since progestins prevent animals that are cyclic from showing heat, including a progestin for at least 5 days can increase the number of animals with a CL that are responsive to PGF at the time this hormone is injected.

There are two types of progestins available in the USA. The first is MGA, an orally active progestin that is added into feed. Feeding MGA to lactating cows is prohibited and thus MGA should only be used in non-lactating replacement heifers. The second progestin source available is the CIDR; inserted into the vagina to deliver a constant amount of ‘natural’ progesterone. Unlike MGA, CIDR inserts can be used in both lactating cows and heifers. Although both progestin sources can be used to stimulate the initiation of estrous cycles in anestrous females; it has been demonstrated that CIDR inserts are more effective at inducing estrous cycles than MGA in anestrous postpartum cows.

Progestins are an extremely valuable tool for estrous synchronization; however, they must be used appropriately to avoid negative effects on fertility that are inherent to their use. The progestins that are available for use in the USA effectively prevent estrus and induce anestrous cows to cycle, but can cause inappropriate follicular growth and reduced fertility if used indiscriminately. Abnormal follicular growth occurs when estrus is inhibited by progestins in the absence of a functioning CL, producing progesterone. In this situation, the dominant follicle does not undergo atresia and persists on the ovary (persistent follicle) for an extended period. When the progestin is withdrawn, the persistent follicle ovulates and the ovum released is of lowered fertility (Figure 15). The reduction in fertility is most evident in cattle that start a progestin treatment late in the estrous cycle, and the longer we give a progestin to a group of cyclic females, the greater the reduction in fertility.

In Figure 15, the dominant follicle of the 2nd wave would be expected to ovulate on day 21 with no intervention. The progestin prevents this, and the follicle grows to an abnormal diameter and age. After withdrawal of the progestin on day 24 in this example, the persistent follicle ovulates, and fertility is low. In systems that use progestins, 3 approaches are taken to avoid this reduction in fertility. The first approach is to avoid the problem, and following progestin removal, wait until the next heat period to inseminate. The second option is to use a short period of progestin exposure to minimize the reduction in fertility.

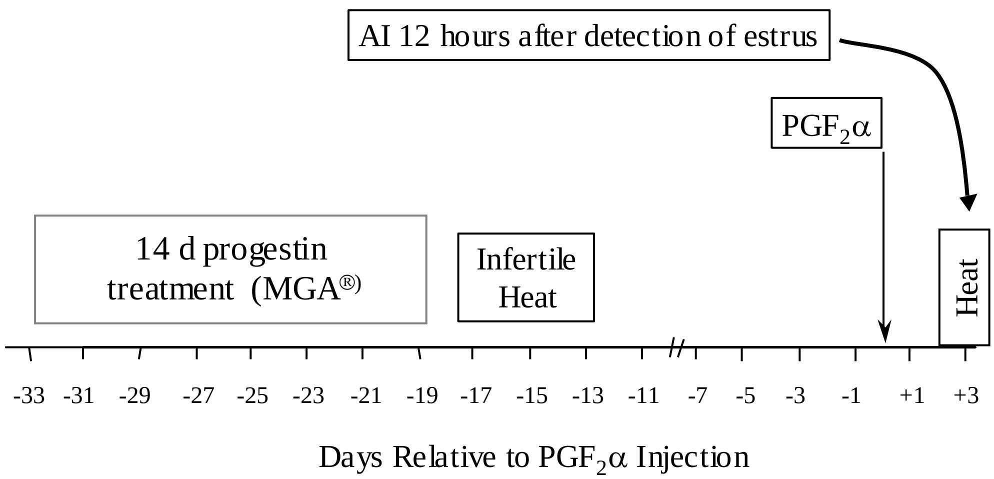
Figure 15. Persistent Follicle Formation with Progestins



The third method is to administer a treatment at the start of the synchronization treatment that will reset follicular growth, and prevent the development of persistent follicles. Each of these options is incorporated into systems described below.

Melengestrol Acetate (MGA) and PGF: One system of estrous synchronization in heifers uses the progestin, MGA, and PGF (Figure 16). this system, MGA is fed at .5 mg/head/day for 14 days. Feeding MGA for 14 days prevents cyclic females from showing heat even if their CL regresses, until the MGA is removed from their feed and is cleared from circulation. Essentially all cyclic females, and some anestrous females, will exhibit estrus within a week after withdrawal of the MGA. This is an **infertile** heat, with many females ovulating a persistent follicle. Heifers should not be inseminated at this estrus. A single injection of PGF, administered 17 - 19 days after the MGA has been withdrawn, causes the CL that developed following the infertile heat to regress. Most females will show estrus 48 to 72 hours after PGF and can be inseminated 12 hours after detection of estrus. The most common approach is to check heat for 5-7 days and inseminate upon detection. Alternatively, timed AI of all heifers, or just those that have not yet displayed heat by 72 hours after PGF, can often result in acceptable pregnancy rates.

Figure 16. MGA- PGF for Heifer Synchronization

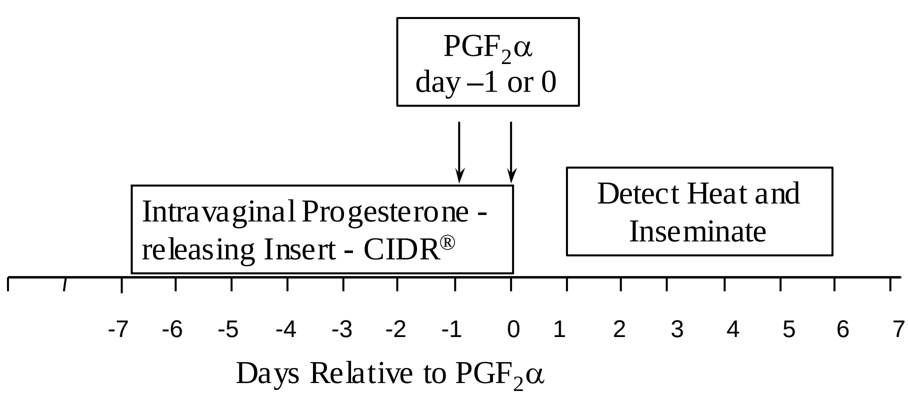


There are two major reasons that this is an effective system in heifers. First, the 14 - day feeding period of MGA will group the cyclic females, and induce onset of cycles in some prepubertal heifers. Variable proportions of the anestrous heifers will be induced to ovulate; probably dependent upon their distribution between peri – estrous and anestrous status (see Figure 7). Secondly, the 17-19 day waiting period was carefully chosen, in order to ensure that most females would be in the latter stages of the estrous cycle (after day 12) when PGF was given. The effectiveness of PGF to cause regression of the CL is highest at this time (refer to Figure 11). It is not uncommon for estrus response rates to exceed 80%, and conception rates to be 70% or greater when using this system on heifers. The 19 - day interval between the last day of feeding MGA and PGF yields a more precise estrous response.

An important consideration of this system is to ensure that all heifers consume MGA on a daily basis. If consumption is inadequate, heifers will show estrus during the MGA feeding period, and the initial synchronizing effect of the MGA will be lost. A disadvantage of this system is the length of time between initiation of feeding MGA and the start of the breeding season (31 – 33 days). With yearling heifers, however, this can be accommodated with careful planning.

Intravaginal progesterone – releasing insert (CIDR) and PGF: A second progestin – PGF system involves the use of progesterone, delivered via a CIDR, and PGF. The sequence of treatment for this system is described in Figure 17.

Figure 17. CIDR® and PGF Synchronization System



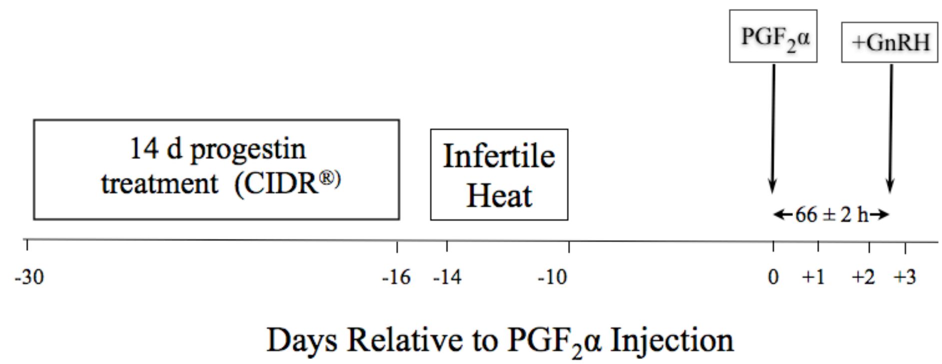
With this system, the CIDR is inserted into the vagina of the female for 7 days. An injection of PGF is given either 1 day before or on the day of CIDR withdrawal. Females are inseminated based upon detection of estrus during the 3-5 day period following treatment. This system will effectively synchronize estrus in a large proportion of cyclic females; since, treatment for 7 days with the CIDR prevents animals from being in the early stage of the cycle and unresponsive to regression of the CL by PGF. Variable proportions of the anestrous females will be induced to ovulate <u>and</u> show heat during the synchronization period. This variation is probably the result of the distribution of animals in the peri-estrous and anestrous categories (see Figure 7). Some data suggests that a greater proportion will ovulate than will show heat and thus some of the anestrous females will display their first heat approximately 21 days later.

A putative disadvantage of this system is that the potential exists for persistent follicles to develop in cyclic females that are in the latter stages of their estrous cycle when the treatment is initiated. However, data to this time indicates fertility is normal at the synchronized estrus in yearling heifers or postpartum cows receiving this synchrony system. Lastly, since no attempt is made to synchronize follicular development in this protocol, it is not an effective method for timed-AI.

14-day CIDR and PGF: This approach to estrous synchronization follows similar rationale to those employed in the MGA and PGF protocol (Figure 16). The 14-day CIDR and PGF protocol (Figure 18) involves the insertion of a CIDR for 14 days. This long-term progestin treatment serves to pre-synchronize estrous cycles prior to PGF delivery given 16 days after CIDR insert removal. This protocol does not directly synchronize follicular waves like other protocols using GnRH, rather estrous cycle synchronization is accomplished after removal of the long-term CIDR. Studies have been conducted which include GnRH administration 7 days prior to PGF, however, pregnancy rates were not improved. Because follicular waves are not synchronized with GnRH in this program, some variation in follicle wave synchrony may exist due to differences in timing of estrus after CIDR removal and follicular wave pattern between individual females. The shorter duration between CIDR removal and PGF administration with the 14-day CIDR and PGF protocol and MGA and PGF protocol is due to clearance of progesterone in circulation once the progestin source is removed. Upon removal of a CIDR, progesterone in circulation is cleared within a few hours. However, MGA is a synthetic progestin that is stored in fat. Once MGA is removed from the diet, progesterone in circulation is not cleared for 2-3 days. Following PGF administration, a tight synchrony of estrus has been reported in the 14-day CIDR and PGF protocol. Females can be either inseminated 12 hours after being observed in heat or administered GnRH 66 hours after PGF administration and time inseminated. Alternatively, a period of heat detection and AI, followed by timed AI (and GnRH administration) at 70-74 hours in females not observed in heat can be implemented.

The 14-day CIDR and PGF protocol has been demonstrated to deliver acceptable AI pregnancy rates in heifers and cows. The CIDR eliminates the need for daily feeding and concerns about consistent feed consumption. The long duration of the protocol (33 days), however, often limits its utilization in postpartum cows. In postpartum cows with an annual calving interval, it is much more difficult to implement this type of system as there are not typically 33 days available between calving and the start of the next breeding system (see Law #1).

Figure 18. 14-day CIDR® - PGF Synchronization System



Systems of Synchronization Using GnRH

Introduction: None of the systems described up to this point have included treatments that address the "physiological requirement" of managing follicle growth to ensure that follicles will ovulate ova of normal fertility and to standardize stage of follicular growth to cause the timing of estrus to be more precise and/or synchronizing ovulation for timed AI (refer to page 11). Each of the preceding systems permits the females to show heat based upon spontaneous follicle growth and are inseminated accordingly. As an example, the expected response for 3 animals injected with PGF on different days of the estrous cycle is diagrammed in Figure 19.

Figure 19. Estrus and Ovulation After PGF by Stage of the Cycle

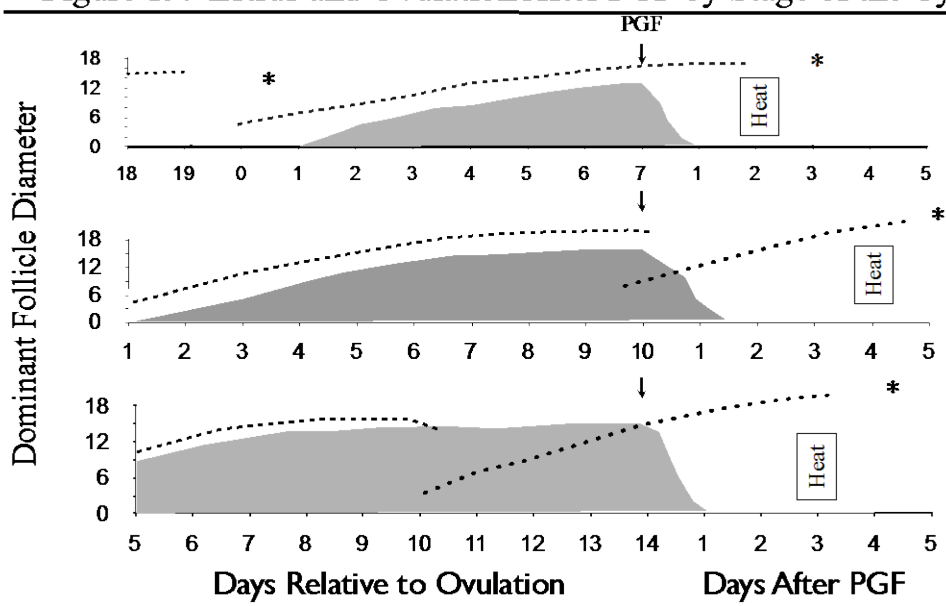
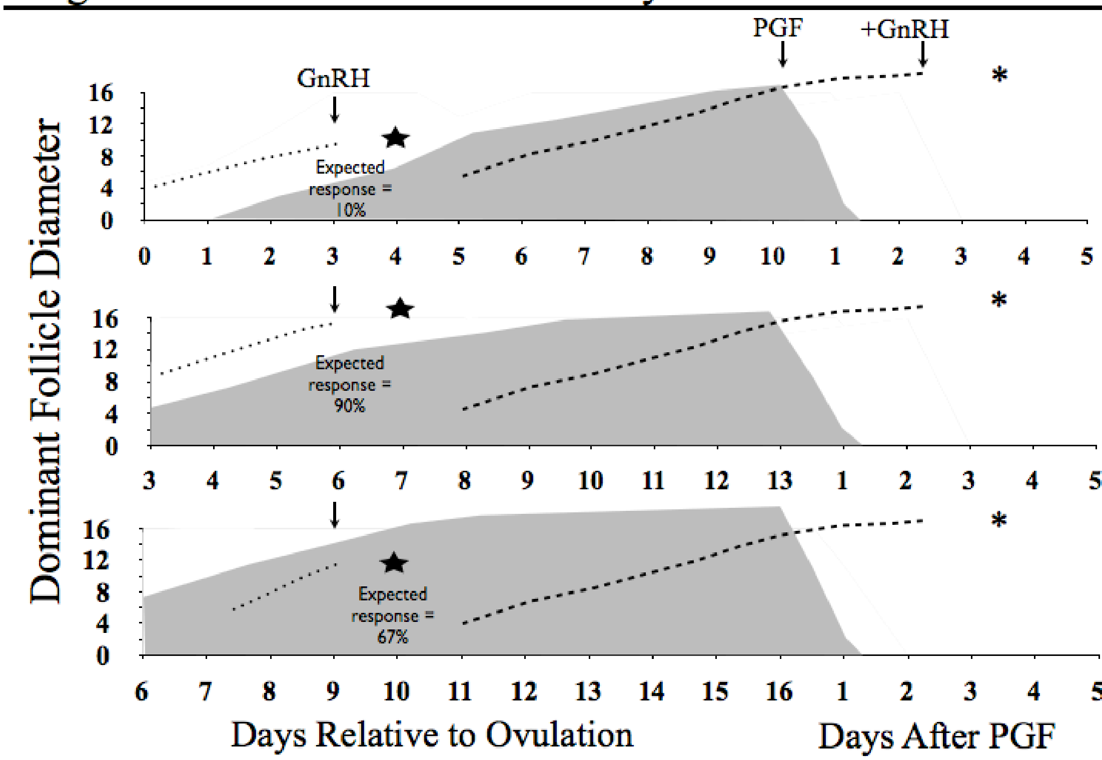


Figure 19 describes a typical response in terms of follicle growth, estrus and ovulation following injection of PGF on days 7, 10 and 14 of the estrous cycle. In each case, progesterone (shaded area) declines within 24 hours. For the example of PGF on day 7, the dominant follicle of the first wave (solid line) is at near maximal size at the time of PGF. This follicle will rapidly produce adequate estradiol concentrations to cause heat 2 days after PGF and ovulation the following day. In contrast, with injection of PGF on day 10, the first wave follicle has undergone atresia, and the dominant follicle of the second wave is immature. The interval of time it takes for the immature follicle to grow large enough to produce sufficient estradiol and cause estrus is 4 days. With injection of PGF on day 14, the dominant follicle of the second wave is intermediate in size, and estrus occurs 3 days after PGF. This variation is normal and is the result of the normal pattern in which follicles grow. This does not present a problem for synchrony systems based entirely upon heat detection to determine the time of insemination. However, GnRH provides a means to standardize the pattern of follicular growth in a majority of animals. This is advantageous to avoid development of persistent follicles and for estrous control systems in which an injection is used to synchronize the precise time of ovulation for timed AI.

When GnRH is used for this purpose, it is typically given the first day of a synchronization system to program emergence and growth of the subsequent wave of follicles through inducing luteinization and/or ovulation of dominant follicles in the ovary. Through removal of the existing dominant follicle, the emergence of a new wave of follicles approximately 2 days later is achieved in most females (Figure 20).

Figure 20. Influence of GnRH to Synchronize Dominant Follicles



In Figure 20, examples of the influence of GnRH on follicle growth when given on days 3, 6 or 9 of the estrous cycle are shown. On day 3 and 6, the largest follicle of the first wave is dominant. On day 9, the second wave of follicles has emerged in this example. Regardless of stage at the time of GnRH, the key effect of the GnRH is to stimulate emergence of a new wave of follicles 2 days later in all females. As a result of this action, the dominant follicle present on the day of PGF is of similar age and diameter in all cows. If females are allowed to show heat spontaneously after PGF, the timing of estrus will be less variable than with the PGF example in Figure 19. The +GnRH treatment that is shown (Figure 20) is a second GnRH that can be used to synchronize ovulation for timed AI. Since all females have follicles of similar diameter, the appropriate time to make these follicles ovulate in all cows can be chosen. It would not be possible to pick a single time to induce ovulation in the PGF example in Figure 19.

Diameter of the dominant follicle at GnRH administration dictates effectiveness of GnRH at inducing ovulation. Due to follicular wave patterns in cattle, when a synchronization system that includes GnRH administration at the onset of the protocol is initiated in a group of females that are all on a random day of the estrous cycle, a dominant follicle responsive to GnRH is not on the ovary of all animals. For example, in Figure 20, GnRH would be effective in nearly 100% of cows on day 6 of the cycle, when a large dominant follicle is present. On days 3 and 9 of the cycle, a proportion of cows would not have a follicle that would ovulate in response to GnRH because they would lack a large enough follicle to be affected by GnRH. This variation in response is most detrimental in cows that do not have a dominant follicle on days 15-17 of the estrous cycle. This sub-group of cows will be in heat 5 – 7 days after GnRH following the impending regression of the CL. Fertility is normal, but these females will not be synchronized with the rest of the cows. Some of the systems described below account for these “early heats” by either conducting estrous detection and AI prior to PGF administration or by administration of a progestin between GnRH and PGF to prevent estrous expression.

The inability of GnRH administration to induce ovulation of all follicles at a start of a synchronization system means that in a subpopulation of females, follicle waves are not controlled. This loss of control can result in alterations in the maturity and fertility of the follicle being induced to ovulate and timed AI. Females induced to ovulate follicles of lesser or greater diameter than normal have reduced pregnancy success. The reduction in fertility in these females is not due to follicle size per se but rather reduced estradiol concentrations prior to ovulation and subsequent impediments with uterine function and potentially ovum quality. In dairy herds, the deficiency of GnRH at resetting follicular growth in all randomly cycling females has been addressed by incorporating pre-synchronization systems in an attempt to ensure that all cows are at a stage of follicular growth to respond to GnRH administration. In beef cattle, pre-synchronization approaches are not practical, thus alternative approaches have been attempted, which will be discussed below, to overcome the deficiency of GnRH at resetting follicular waves and initiation of a synchronization protocol.

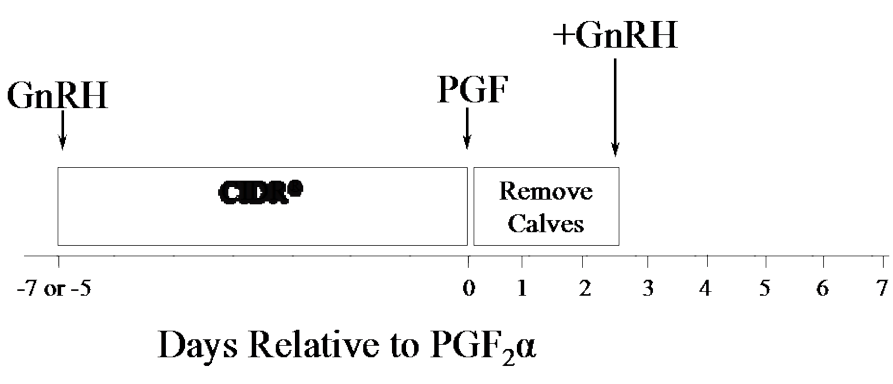
When using GnRH in whole herd synchrony, the other important action of this hormone is to induce ovulation in some anestrous cows, thereby providing a source of progestin pretreatment. The value of this effect is described in an earlier section (Figures 9 and 10).

A series of GnRH – based systems of varying complexities have been developed. The simplest programs are designed for use primarily in cyclic females with insemination based on detection of estrus. Others have been developed in order to support the use of timed AI. Often, these GnRH-based systems also incorporate the use of a progestin treatment to assist in inducing a varying proportion of anestrous females to ovulate and prevent premature expression of estrus for improved synchrony in timed AI approaches.

Most GnRH-based synchronization approaches follow a similar pattern of administration (Figure 21). At protocol initiation, GnRH is administered to program follicle growth and to induce ovulation in anestrous females to provide a period of progestin exposure via the developed CL. Five to seven days after GnRH administration, depending upon the synchronization approach, PGF is given to induce the regression of the CL that are present and cause a decline in progesterone. A second dose of GnRH is given 2 to 3 days after PGF to induce ovulation of the dominant follicle that has been pre-programmed by the first GnRH treatment. Often, inclusion of a CIDR is incorporated between the initial GnRH and PGF to prevent the occurrence of the "early" heats in cyclic cows, and provide progestin pre-exposure to the anestrous females. **The use of MGA between GnRH and PGF administration in these short-term protocols is not recommended.** In addition, calves can be removed from their dams during the period between PGF and the second GnRH. This withdrawal of the suckling stimulus provided by the calf will accelerate follicle development; however, removal of the calf for greater than 48 h can result in reduced calf performance.

Most of the original GnRH-based estrous synchronization protocols were designed to include a 7-day period between GnRH and PGF administration. The variants in the 7-day protocol are addressed below. Recently, new protocols have been devised that have reduced the interval from the initial GnRH to PGF from 7 to 5 days (Figure 21). These protocols will be described later in this review.

Figure 21. GnRH-based Synchronization Systems



7-day GnRH-PGF Protocols:

***GnRH – PGF System*:** This combination represents the simplest GnRH – based system and involves the GnRH treatment followed 7 days later by the PGF treatment (Figure 21). A common name that is often used for the GnRH – PGF system is "Select Synch". Some cows (~8 %) will exhibit estrus up to 36 hours before PGF. The "early" heats are fertile and cows can be inseminated 12 hours after detection. The PGF treatment is not necessary in "early" cows that have already exhibited estrus, but will not compromise the pregnancy if given. The peak estrous response will occur 2-3 days after PGF with a range of days 1 – 5. With this system, a minimum of 5 days of estrous detection after PGF and 2 days preceding PGF is required to detect most heats. A large proportion of cyclic females will be in estrus during this 7 - day period. This protocol will initiate estrous cycles in some anestrous cows although results can be unpredictable (described by Figure 9). The GnRH – PGF system is most effective if used on cyclic cows, or cows that would be expected to spontaneously resume estrous cycles within the first couple weeks of the breeding season.

GnRH – PGF + GnRH Systems: This approach is the GnRH – PGF system, with the addition of a second GnRH injection (+ GnRH) given to all, or some cows, between 48 and 72 hours after PGF (day 2-3), with timed AI on all or a portion of the herd. These systems are most effective if used on cyclic cows, or anestrous cows that would be expected to spontaneously resume estrous cycles within the first couple weeks of the breeding season. Within this sequence, several variations are available.

a. GnRH – PGF +GnRH with Timed AI of <u>All</u> Females: This system involves giving the GnRH treatment on day –7, PGF on day 0, +GnRH on day 2.5, and inseminating all cows at the time of the + GnRH injection (Figure 21). No heat detection is performed. In beef cows, it has been reported that the ideal interval from PGF to +GnRH and timed AI in this protocol to be 60-66 hours after PGF; in heifers the ideal interval to perform timed AI and +GnRH is 52-56 hours after PGF. A common name for this system is "CO – Synch". In beef cows, the CO-Synch protocol often delivers acceptable timed AI pregnancy rates, but may be better adapted to operations with a history of short breeding / calving seasons and/or those operations that have a history of successful AI use. However, in beef heifers considerable variability exists when using this protocol. A major setback to this strictly timed AI approach is that cows which exhibited estrus before PGF or very early post-PGF will have 0% pregnancy rate. A minor modification to the system is based upon findings that pregnancy rates to this system may be increased by 2-8% if cows are inseminated 8 – 16 hours after the +GnRH treatment. This is an approach commonly used in dairy herds, with a common name of "Ovsynch". This increase in pregnancy rate must be balanced against the labor costs, stress and inconvenient time interval for moving the cows back through the chute 8 – 16 hours after giving the +GnRH treatment.

b. GnRH – PGF +GnRH with Heat Detection Until Hour 72, Timed AI at 72 Hours: This approach is actually a hybrid between the *GnRH – PGF* and the *GnRH – PGF +GnRH* systems. Thus, one common name for this system is "Hybrid Synch" and another is "Select Synch & Timed-AI". This approach is based upon the knowledge that in most herds, a majority of cows that are going to be in estrus will display heat by 60 – 72 hours (Day 2.5 to 3) after PGF. With 5 days of heat detection (Day – 2 to Day 3) cows that will display estrus are detected and inseminated 12 hours after detection of heat. This time interval "permits" cows that will show estrus in a timely manner to do so, and optimizes conception rates for these animals. The remaining animals (not detected in estrus) receive the +GnRH treatment on Day 3 (72 hours) and are mass inseminated at this time. The conception rates for the animals that are timed inseminated can vary widely, depending upon if the lack of observed heat was due to failure to response to synchronization, anestrous, failure to exhibit heat but properly synchronized, or failure to accurately observe heat.

GnRH – Based Systems + Progestins: As indicated in Figure 21, an option is to add a CIDR to GnRH based programs. As indicated earlier, it is not advisable to use MGA in these short-term protocols, rather the use of a CIDR is recommended. When a exogenous progestin is used in a synchronization system it is common lingo to add the name of the progestin to the common name of the synchronization system identified above. For example, if a CIDR were added to a CO-Synch program, this would be called a "CO-Synch + CIDR" program. When using a CIDR, it is normally inserted at the time of the initial GnRH injection, and withdrawn at the time of the PGF injection

Inclusion of a CIDR in GnRH – based programs guarantees that females will be exposed to a progestin during the period between GnRH and PGF. One reason the progesterone exposure is beneficial is that it ensures that most ovulations occurring in previously anestrous cows, either spontaneously or in response to the +GnRH treatment during the synchrony period, will result in normal (~21 day) rather than short (~10 day) cycles. Furthermore, since the withdrawal of a progestin has been demonstrated to induce onset of cycles in some anestrous females, the likelihood of an ovulation (either spontaneous or in response to +GnRH) is enhanced. Since the initial response to GnRH can be variable, the inclusion of a progestin removes the requirement that ovulation be induced by GnRH on day –7. A second benefit to inclusion of a progestin in GnRH – based programs is that the early heats (days – 2 to day 1) that are inherent to these systems are prevented. The progestin prevents estrus and ovulation between days –7 and 1. This increased control of the time of ovulation is particularly important in timed AI systems in which no heat detection is performed.

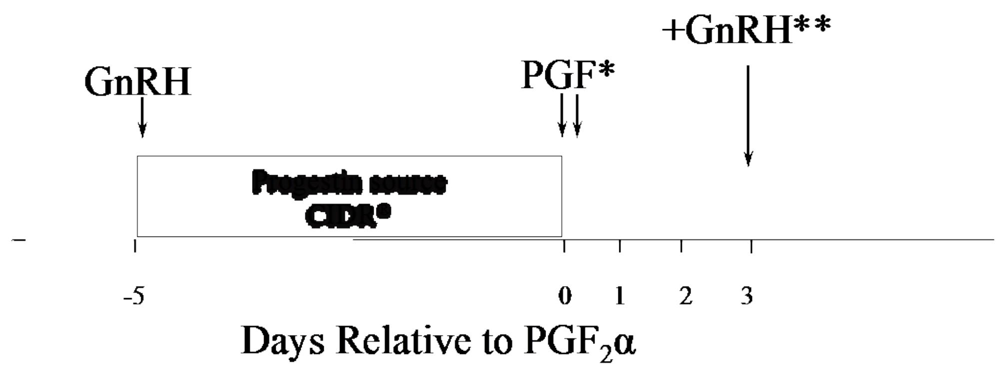
5-day CO-Synch, Select Synch, and Select Synch & Timed AI Protocols:

Although the 7-day approaches to estrous synchronization often yield acceptable pregnancy rates in beef cows and heifers, variability in response and new insights into physiology of reproduction led to the development of a GnRH-based protocol that includes only a 5 day interval from GnRH to PGF. In addition, this protocol has been almost exclusively investigated with incorporation of a CIDR between GnRH and PGF (Figure 22). The rationale for development of this 5-day approach to synchronization was two fold. First, numerous research studies demonstrated that increasing estradiol concentrations prior to +GnRH and timed AI resulted in improve pregnancy rates to timed AI. Within the 5-day approach, the shorter interval from the initial GnRH to PGF allows the interval from PGF to +GnRH to be extended, from 60-66 hours in cows (7 day) to 72 hours, and from 52 hours (7 day) in heifers, to 60 hours. This allows for increased estradiol production prior to ovulation, which has been demonstrated to improve pregnancy success. Second, in females that fail to ovulate in response to the initial GnRH administration, the follicle wave pattern is not as perturbed and there is less variation in maturity of the ovulatory follicle at +GnRH, thus the incidence of inducing the ovulation of a follicle that is abnormally large or small should be diminished.

In recent years, the 5-day protocol (Figure 22) has been the most rigorously tested estrous synchronization approach in beef cows and heifers as well as dairy cattle. In most studies in beef cows and heifers, the 5-day GnRH-based approach to estrous synchronization has been demonstrated to yield greater timed AI pregnancy rates than the 7-day programs. In addition, the 5-day approach to synchronization can be used exclusively with estrous detection and AI, timed

AI, or a hybrid approach with a period of estrous detection and AI followed by mass timed insemination of all females not yet observed in heat.

Figure 22. 5-Day CIDR Synchronization Systems



*Two full doses of PGF required. See text for recommendations on appropriate interval between the 2 doses of PGF
** See text for recommendations on appropriate interval from CIDR removal to timed AI for cows and heifers

Figure 22 illustrates the 5-day approach to estrous synchronization. On day -5, females receive GnRH in an attempt to program follicular wave patterns and are administered a CIDR. Five days later, on day 0 the CIDR is removed and PGF is administered. Delivery of PGF is unique in the 5-day system. Because the interval from GnRH to PGF is only 5 days, cows that ovulated to the initial GnRH will have a CL that is just becoming responsive to the luteolytic actions of PGF. Therefore, in the 5-day system, additional doses of PGF are required. The original recommendation was to administer one dose of PGF at CIDR removal with a second dose 8 to 12 hours later. Subsequently, it was demonstrated that the interval between doses could be reduced to 2 hours. Most recently, however, a large multi-state study in beef cows observed that delivering both doses of PGF simultaneously at CIDR removal was as effective as administering two doses at an 8-hour interval. This alteration negates the need to handle the animals an additional time. Within beef heifers, research varies on whether there is a necessity to delivery a single or two doses of PGF at CIDR removal. In some herds, a single dose of PGF is adequate, however, in others, timed-AI pregnancy rates are improved if two doses are administered. Therefore, administering two doses of PGF at the time of CIDR removal may be the best approach.

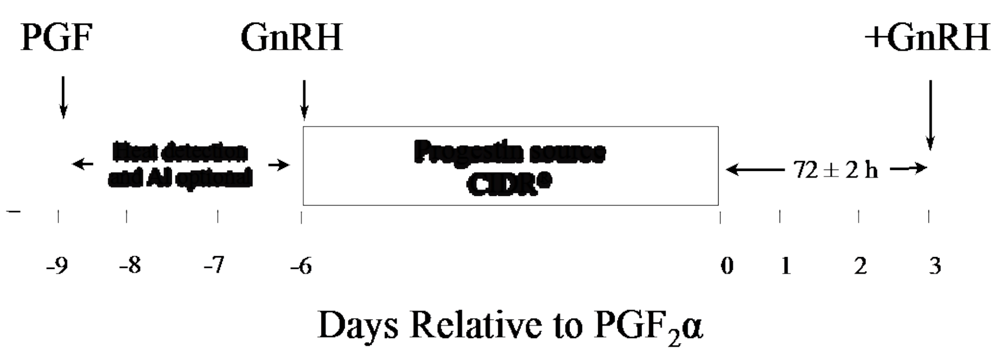
Following PGF administration in the 5-day approach, females can be observed for heat and inseminated via the AM/PM rule. Although effective, if only estrous detection is used, there has been no demonstrated improvement in pregnancy success with the 5-day over the 7-day programs. A period of heat detection followed by timed AI of females not observed in estrus at 72 h after CIDR removal is also an effective approach with the 5-day system (referred to as 5-day Select Synch + CIDR & Timed AI). This approach has proven very effective in heifers by optimizing conception rates in those heifers that exhibit estrous 24 to 48 hours after CIDR removal. Moreover, research has demonstrated that the hybrid approach with the 5-day CIDR program yields improved overall AI pregnancy rates than the hybrid approach with the 7-day

CIDR program. Lastly, the 5-day system can be used for strict timed AI. The ideal interval from CIDR removal to +GnRH and timed AI varies between cows and heifers. Cows should be timed inseminated, concurrent with +GnRH, from 70-74 hours after CIDR removal. In beef heifers, this interval is reduced to 56-66 hours, with +GnRH at timed-AI.

Variants of GnRH-Based Systems:

PGF 6-day CIDR protocol: The deficiency of GnRH to reset follicular patterns in all females has resulted in the development of a new approach to synchronization. The PGF 6-day CIDR program (Figure 23) was devised as a method to increase the proportion of cows that ovulate to GnRH and reduce the proportion of females in which follicular patterns are not controlled. This program begins with administration of PGF to all females. In theory, PGF administration here eliminates the CL in a proportion of females (cyclic females on days 5-17 of estrous cycle), removing progesterone from circulation, thus allowing increased follicle growth. It is hypothesized that this increased follicle growth results in a follicle that is more responsive to GnRH when it is administered 3 days after PGF. At GnRH delivery 3 days after PGF a CIDR is also inserted. Six days after CIDR insertion, CIDRs are removed and another dose of PGF is given. Females can then be inseminated based on heat expression, receive timed AI 66 to 72 hours after CIDR removal with +GnRH administration, or a combination of heat detection and timed AI can be conducted. A potential added benefit of this program is the flexibility to conduct estrous detection and AI after the initial PGF administration at protocol initiation. After the initial PGF administration, females can be observed for estrus and inseminated, with only females that fail to exhibit estrous continuing in the program. This allows some flexibility in the program and if females are inseminated after the initial PGF, reduces the cost of synchronization as fewer females receive the additional pharmaceutical products. Data suggests that 50 to 60% of cyclic females may be in heat within 3 d of PGF.

Figure 23. PGF 6-Day CIDR Synchronization System



Summary

A wide variety of synchronization programs are available for use in cattle. These systems range from programs that are effective only in cyclic cattle, to complex programs that regulate follicular development, provide multiple stimuli designed to induce anestrous females to cycle and treatments to synchronize ovulation for the purposes of timed AI. There is not a single program that can be identified as "ideal" for all situations. The management capabilities for a given group of females and the mathematical laws of estrous synchronization discussed above have a major impact upon the system chosen. For example, if maximal conception rate is the primary goal, then systems that use estrous detection and AI are a probable choice. In situations when heat detection is not an option, a timed AI program is the primary option. If the cows to be synchronized calve in a short season, or at a time earlier than planned for the subsequent year, longer-term programs may be a logical choice, but an advantage has not been demonstrated. Management considerations, such as a desire to minimize the number of times animals are handled, or the inability to deliver treatments such as MGA in the feed are also key determinants of the program chosen. In general, as estrous synchronization requirements are compared for cyclic vs. anestrous females, the complexity of the program necessary to effectively control estrous cycles increases for anestrus. Likewise, in comparison of programs in which AI is performed based upon detection of spontaneous heat vs. timed AI, more complex programs are necessary for timed AI. All programs described have been demonstrated to provide acceptable results when used in the appropriate animal classes. To maximize effectiveness of any protocol, strict compliance with the protocol, regardless of protocol implemented, is required. In addition, proper intramuscular drug delivery of the proper dosage and handling of pharmaceutical products is necessary.

Producers of pure Bos indicus cattle (Brahman) must be aware that small differences exist between their animals and the Bos taurus (European and British) or Bos taurus x Bos indicus cattle utilized to develop the estrous synchronization systems described in this bulletin. Timed insemination protocols and protocols to control follicular waves have not been as effective in Bos indicus as in Bos taurus cattle. Caution should be used in selection of an estrous synchronization protocol that has not been fully tested for Bos indicus cattle. Of the programs described, the PGF – based systems have been shown to be equally effective in Bos indicus and Bos taurus cattle, although with the two injection scheme, an interval of 14 or more days between PGF injections has been demonstrated to be most effective. Additionally, Bos indicus cattle respond well to the PGF and progestin systems described when cattle are inseminated 12 hours following onset of estrus. Lastly, a new approach for timed AI has been designed exclusively for Bos indicus cattle. Simplistically put, it is the 5-day Co-Synch + CIDR protocol with administration of an additional dose of PGF at CIDR insertion. To date, this protocol appears to be the most effective at facilitating timed AI in cattle of Bos indicus breeding.