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PHYSIOLOGICAL PRINCIPLES UNDERLYING SYNCHRONIZATION OF ESTRUS

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Introduction

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

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

Principles of the Bovine Estrous Cycle

Characteristics of the Estrous Cycle

In cattle, the estrous cycle normally varies from 17 to 24 days and the duration of estrus is generally 10 to 18 hrs; however, considerable variation exists among individual animals (range < 8 to > 30 hr; O'Connor and Senger, 1997). There are a number of estrous detection aids available to assist producers; however, the HeatWatch electronic estrous detection system provides information on the intensity of estrus. Rorie et al., (2002) utilized the HeatWatch system with 500 Angus cows to evaluate the effect of estrous intensity on fertility. Estrus was synchronized with the Select Synch protocol (GnRH followed seven days later with an injection of prostaglandin $F_{2\alpha}$). Length of estrus ranged from 0.5 to 24 hr and there was no effect of length of estrus on pregnancy status. However, pregnant cows were mounted more times per estrus than nonpregnant cows. These data are similar to another study with Angus cows in which pregnant cows were mounted more times per estrus than nonpregnant cows (Kuhlman et al., 1998).

A seasonal effect on estrous behavior has been reported in Angus x Hereford cows located in Oklahoma (White et al., 2002). In the preceding study, the length of estrus was greater in summer compared to winter or spring; however, cows were mounted more frequently per estrus in winter compared to summer or spring. Therefore, estrous detection may need to occur more frequently in winter compared to spring or summer; whereas, in summer estrous detection may need to occur for a longer duration at each check. In this study, there was no effect of season on the interval from the onset of estrus to ovulation (Mean = 31 hr).

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

Hormonal Patterns During the Estrous Cycle

The estrous cycle is divided into three stages (follicular phase, estrus, and luteal phase) and is regulated by hormones secreted by the hypothalamus (gonadotropin releasing hormone [GnRH]), anterior pituitary gland (follicle stimulating hormone [FSH] and luteinizing hormone [LH]), ovary (estradiol and progesterone), and uterus (prostaglandin $F_{2\alpha}$; $PGF_{2\alpha}$). The preceding hormones serve as chemical messengers that travel in the blood to specific target tissues which contain receptors that are hormone specific and regulate the phases of the estrous cycle. The combination of hormone secretion and metabolism (liver, kidneys, and lungs) maintain the correct hormonal balance during the follicular phase, estrus, and luteal phase of the cycle. For a list of hormones, their biological functions, their role in estrous synchronization, and product names see Table 1.

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

Table1. Reproductive hormones, their functions during the estrous cycle, roles in estrous synchronization, product name, dosages, and route of administration.

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

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

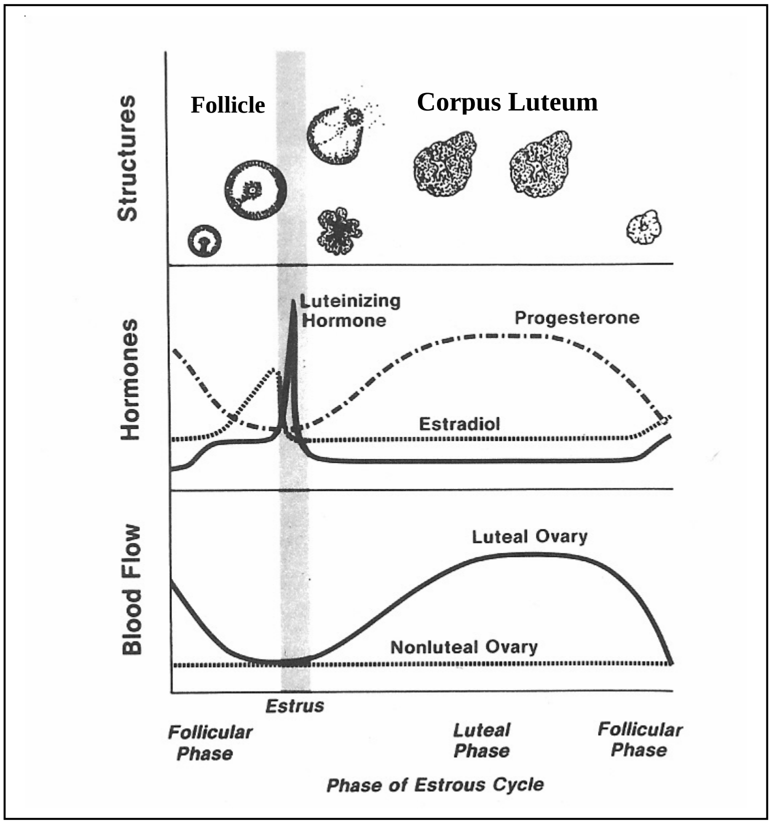

Figure 1. Changes in ovarian structures (preovulatory follicle and corpus luteum), hormones (luteinizing hormone, estradiol, and progesterone) and ovarian blood flow (ovary containing [luteal ovary] or not containing [nonluteal ovary] a corpus luteum) during the three phases of the estrous cycle (follicular,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

Follicular Phase

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

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

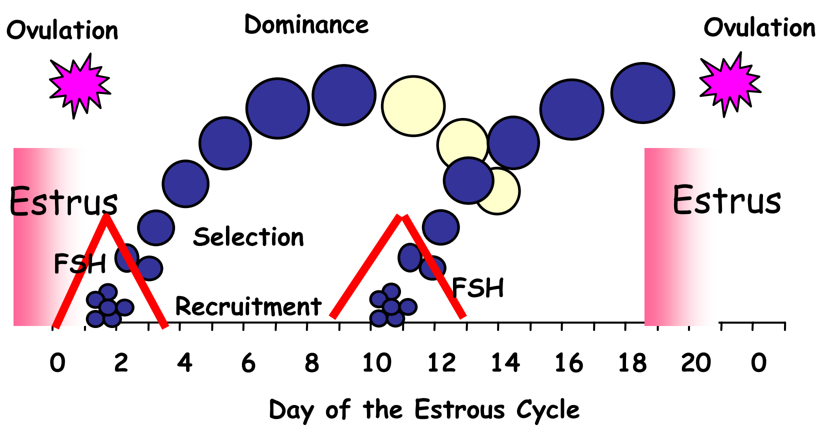

Figure 2. Relationship between circulating concentrations of follicle stimulating hormone (FSH) and stages of a bovine follicular wave (recruitment, selection, and dominance). A transient increase in FSH initiates recruitment of a cohort of follicles, from which a single follicle is normally selected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

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

Recruitment. Follicular waves have been studied most extensively in cattle and consist of the following three stages: recruitment, selection, and dominance. Recruitment of a cohort of follicles, around 3 mm in diameter, is stimulated on each ovary by a transient rise in FSH (Figure 2). Inhibition of both FSH and LH arrested follicular growth at two to four mm, however, when physiological levels of FSH were infused for 48 hours follicular growth from five to eight mm was stimulated (Gong et al., 1996). The peak concentration of FSH occurred when the future dominant follicle attained a mean diameter of approximately four mm, after which concentrations of FSH declined (Figure 2; Ginther et al., 1996b), and were at basal concentrations by the time follicular selection occurred (Ginther et al., 2000a). The mechanism responsible for the initial decline in FSH concentration is unknown, however, estradiol and inhibin are follicular products that probably play a major role in the decline of FSH (Adams, 1999).

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

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

Estrus Phase

Increasing circulating concentrations of estradiol following luteolysis initiate estrous behavior, increase uterine contractions (facilitate sperm transport), and induce the preovulatory gonadotropin surge. The preovulatory gonadotropin surge coordinates the following events that are critical to the establishment of pregnancy: resumption of meiosis within the oocyte, follicular rupture, and luteinization of follicular cells. LH is generally considered to be the primary gonadotropin that controls the preceding events; however, FSH also has been shown to cause ovulation and luteal tissue formation (Galway et al., 1990). The end of the estrus phase of the cycle is marked by follicular rupture, which is the culmination of a complex cascade of events leading to the activation of proteolytic enzymes that digest the follicular wall and allows the egg (oocyte) to be released for fertilization. This process is similar to mechanisms associated with inflammation. Injection of GnRH will induce a surge of LH within 2 to 4 hours and ovulation of a dominant follicle will occur 24 to 36 hr after injection (Figure 3).

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

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

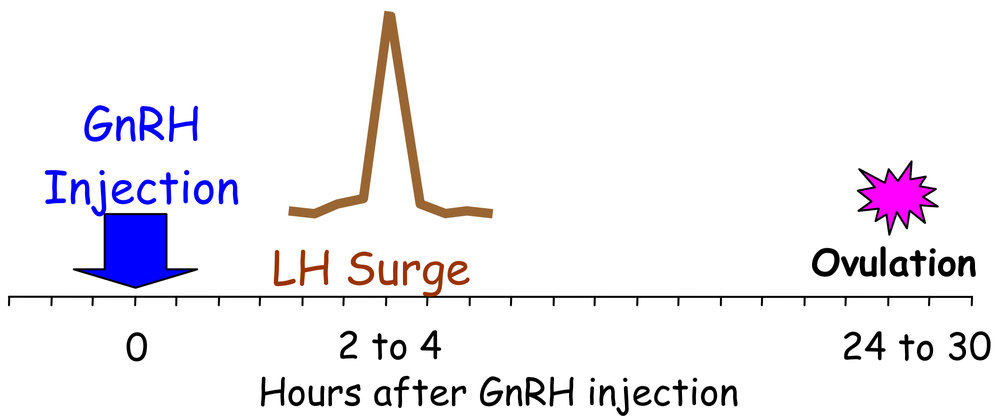

Figure 3. Injection (im) of GnRH will induce a surge of LH within 2 to 4 hr and ovulation of a viable dominant follicle (≥ 10 mm) will occur within 24 to 36 hr (Modified from Kojima and Patterson, 2003).

Luteal Phase

The luteal phase spans the time of corpus luteum formation and maintenance which begins with ovulation and ends with luteolysis (Figure 4). Progesterone is the primary secretory product of the corpus luteum and is regulated by secretions of the anterior pituitary, uterus, ovary, and embryo (Niswender et al., 1976). The regulation of progesterone secretion is likely controlled by a balance of luteotropic (stimulate progesterone) and luteolytic (inhibit progesterone) stimuli, given that both types of stimuli are secreted concurrently during the estrous cycle. In ruminants, LH is considered to be the primary luteotropic hormone and concentration of luteal LH receptors is positively correlated with changes in progesterone and luteal growth (Niswender et al., 2000). Corpora lutea receive the majority of the ovarian blood flow (Figure 2) and blood flow to the luteal ovary and progesterone secretion are highly correlated (Niswender et al., 1976). Progesterone has a central role in the regulation of the estrous cycle as it determines estrous cycle length and is required for the maintenance of pregnancy.

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

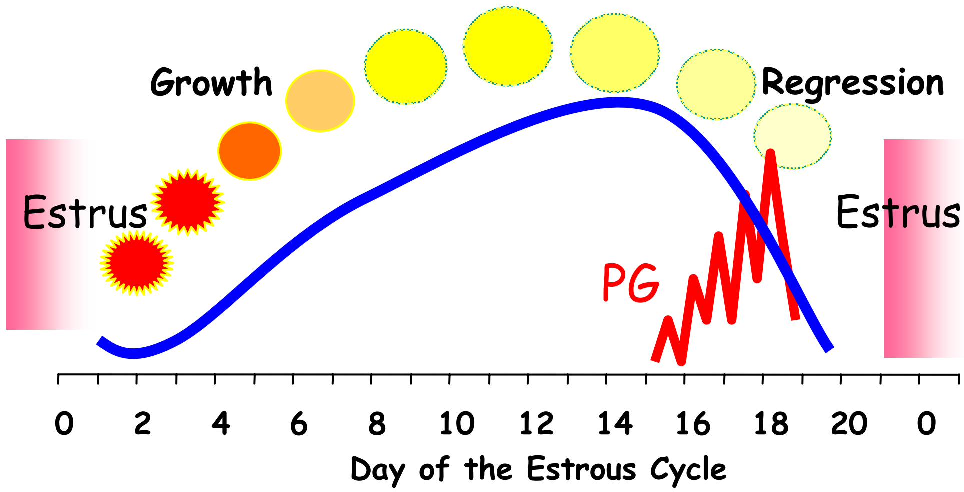

Figure 4. Changes in corpus luteum development, circulating concentrations of progesterone, and circulating concentrations of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) during the luteal phase of the bovine estrous cycle are depicted above. Luteal secretion of progesterone inhibits the expression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

Follicular Determinants of Corpus Luteum Function

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

Estrous Synchronization Products and Mechanism of Action.

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

Hormonal Management of the Luteal Phase for Synchronization of Estrus

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

Progestins

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

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

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

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

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

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

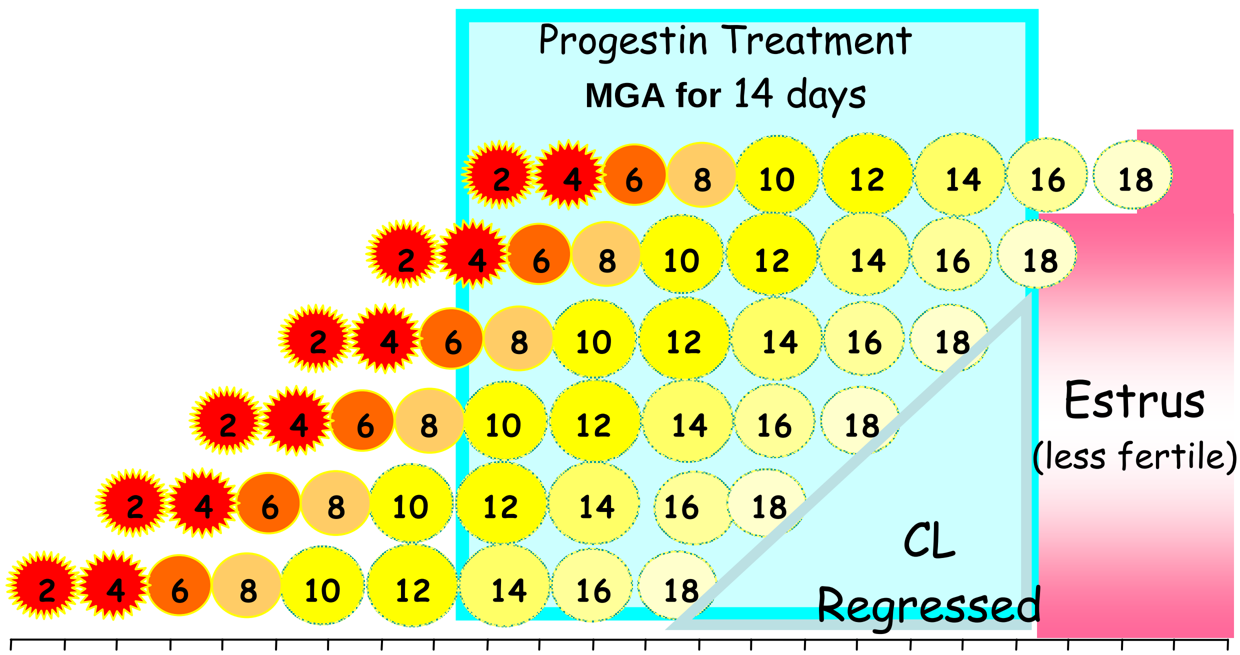

Figure 5. Effect of 14 days of MGA feeding on estrous synchronization of cows in different stages of the estrous cycle. Circles represent development and regression of corpora lutea. Numbers inside each circle represent days of the cycle. In this diagram, spontaneous luteal regression occurs around day 17 to 18 of the cycle. Note that at the end of progestin treatment all corpora lutea have regressed or are in the process of regressing (Modified from Kojima and Patterson, 2003).

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

Prostaglandin $F_{2\alpha}$

Prostaglandins are naturally occurring compounds that are produced by most cells in the body and have a variety of biological actions. Prostaglandin $F_{2\alpha}$ is a naturally occurring luteolytic hormone that has also been utilized to synchronize estrus and induce abortion in cattle through induction of corpus luteum regression. In the absence of an embryo, uterine concentrations of $PGF_{2\alpha}$ increase during the late luteal phase. $PGF_{2\alpha}$ is secreted in pulses and transported to the corpus luteum via a counter-current mechanism. The mechanisms associated with $PGF_{2\alpha}$ –induced luteolysis are not completely understood; however, $PGF_{2\alpha}$ probably has both a direct and indirect (decreased blood flow) action. Luteal cells are known to have $PGF_{2\alpha}$ receptors on the plasma membrane and direct inhibitory effects of $PGF_{2\alpha}$ on luteal progesterone secretion have been demonstrated (Niswender et al., 2000). In addition, $PGF_{2\alpha}$ is known to reduce luteal blood flow due to vasoconstrictor activity (Niswender and Nett, 1988).

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

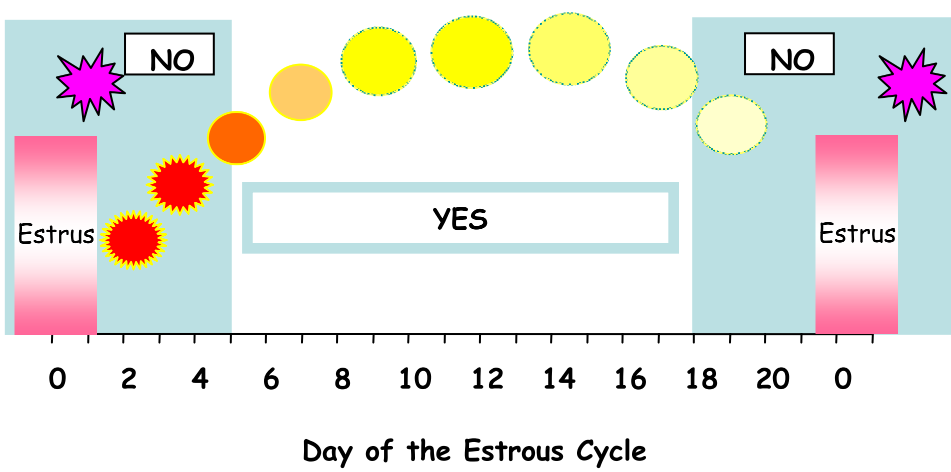

Figure 6. Effect of stage of the bovine estrous cycle on luteal responsiveness to $PGF_{2\alpha}$ Bovine corpora lutea will not respond to an injection of $PGF_{2\alpha}$ during the first five days

of the cycle. Therefore, $PGF_{2\alpha}$ should not be injected at the beginning of progestin treatment (Modified from Kojima and Patterson, 2003).

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

Hormonal Management of Follicular Waves for Synchronization of Estrus

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

Initiation of a new follicular wave occurs following ovulation or turnover (atresia) of the dominant follicle. Administration of exogenous progesterone, estradiol, or GnRH have been utilized to turnover (progesterone and estradiol) or ovulate (GnRH) dominant follicles and to synchronize follicular waves in heifers and cows (see reviews by Bo et al., 1995; Diskin et al., 2002). Follicular turnover (atresia) of persistent follicles can be accomplished through the administration of progesterone. Progesterone as a single injection (Anderson and Day, 1994) or administered over a 24-hour period (McDowell et al., 1998) effectively regressed persistent follicles and initiated new follicular waves. Reduction of LH pulse frequency and amplitude following the administration of exogenous progesterone may be the mechanism by which persistent follicles are induced to undergo atresia (McDowell et al., 1998).

Estradiol benzoate has also been used to induce atresia of dominant follicles and to initiate a new follicular wave approximately 4.5 days after injection (Burke et al., 2000). When treatment with progesterone and estradiol were combined the dominant follicle stopped growing within 24 hours and became atretic resulting in the initiation of a new follicular wave 4 to 5 days after treatment (Burke et al., 1999). A single injection of a GnRH agonist is capable of ovulating dominant ($\geq$ 10 mm) but not subordinate follicles (Figure 7; Ryan et al., 1998). Following GnRH administration, a new follicular wave was initiated approximately 1.6 days later (Roche et al., 1999) and selection occurred 3 to 4 days later (Twagiramungu et al., 1995). However, the ability of a single injection of GnRH to induce ovulation and initiate a new follicular wave is dependent on the stage of follicular development (Geary et al., 2000; Atkins et al., 2005).

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

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

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

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

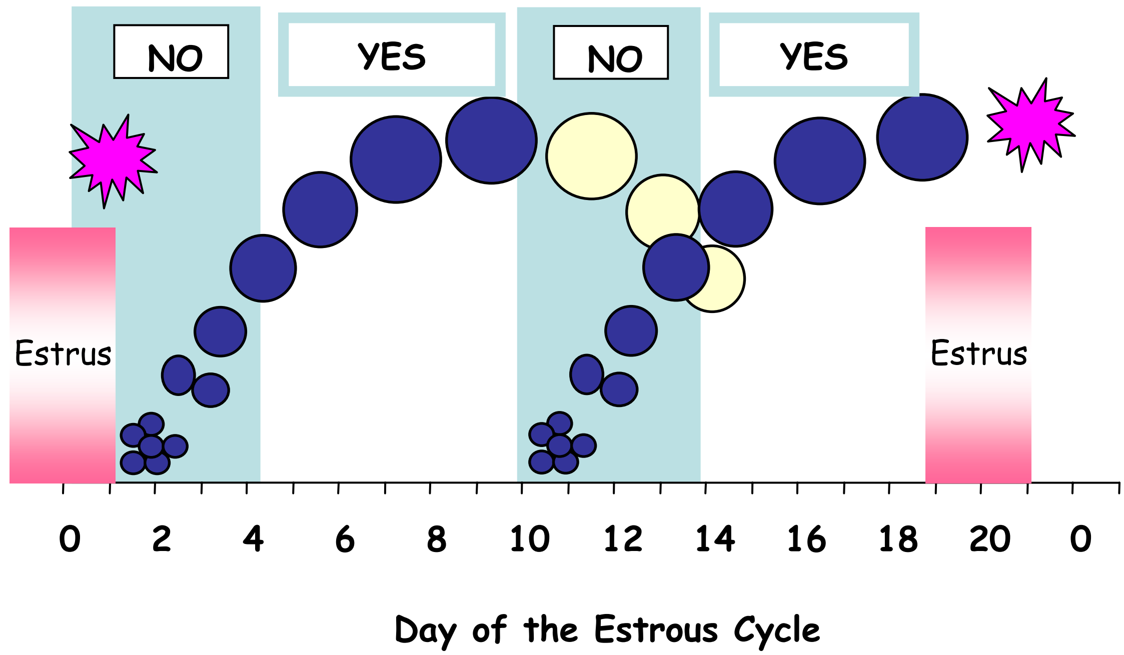

Figure 7 Injection of GnRH will induce ovulation of a dominant follicle (≥ 10 mm in diameter). Circles represent follicle development and atresia (light circles) during a wave. The above figure represents a "two-wave cow" and the shaded areas indicate when during a follicular wave follicles will ovulate (Yes) or not ovulate (No) in response to a single injection of GnRH (Modified from Kojima and Patterson, 2003).

Postpartum Cows: In postpartum cows, the response to an estrous synchronization program is primarily dependent upon cow body condition and days postpartum. Body condition score (BCS) is a subjective measurement of an animal's fat reserves and ranges from extremely thin (1) to obese (9). Cows should have a body condition score of 5 or greater at calving to be considered for an AI and estrous synchronization program. Cows that are too thin at calving are likely to have poor reproductive performance and are not good candidates for AI. In general, it takes 80 to 100 lbs to increase one BCS (i.e. 4 to 5). If possible, feed thin cows separately from well conditioned cows in order to promote a steady pattern of feed intake to attain the desired BCS.

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

Summary

Understanding the basic principles of the bovine estrous cycle and how estrous synchronization products affect the cycle is essential when choosing the best protocol for heifers or cows and for determining what went wrong when pregnancy rates following a synchronized estrus are less than expected. Three general approaches that have been used to develop estrous synchronization protocols include the following: 1) Inhibit ovulation following spontaneous corpus luteum regression (long-term progestin treatment), 2) Induction of corpus luteum regression ($PGF_{2\alpha}$ treatment), and 3) a combination of 1 and 2. Most of the protocols utilized today can be categorized under the third approach. The ability to synchronize bovine follicular waves through an injection of GnRH has added a new and important dimension to estrous synchronization and has made fixed-time AI in cows a viable option. Many of the current protocols are able to synchronize the growth of a dominant follicle in addition to the time of corpus luteum regression.

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