Proceedings, Applied Reproductive Strategies in Beef Cattle
September 11 and 12, 2007, Billings, Montana

# PHYSIOLOGICAL PRINCIPLES UNDERLYING SYNCHRONIZATION OF ESTRUS

M.F. Smith, G.A. Perry, J. B. Hall, J.A. Atkins, D.C. Busch, and D.J. Patterson
Division of Animal Sciences, University of Missouri, Columbia
Department of Animal and Range Sciences, South Dakota State University
Department of Animal and Poultry Science, VPI & SU

## 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 U.S. 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 commercially available, and the physiologic mechanisms by which these products synchronize estrus and/or ovulation in cattle.

## Principles of the Bovine Estrous Cycle

### Characteristics of the Estrous Cycle

In cattle, the estrous cycle normally varies from 17 to 24 days and the duration of estrus is generally 10 to 18 hrs; however, considerable variation exists among individual animals (range < 8 to > 30 hr; O'Connor and Senger, 1997). The primary sign of estrus in cattle is standing to be mounted and secondary signs of estrus include frequent mounting, watery mucus from the vulva, and restlessness. There are a number of estrous detection aids available to assist producers including pressure mount detectors, tail chalk/paint, androgenized cows, and teaser bulls (rendered sterile by vasectomy, epididectomy, and(or) penile deviation). However, the HeatWatch electronic estrous detection system is the most effective estrous detection aid and provides precise information on the onset, intensity, and duration of estrus. Rorie et al., (2002) utilized the HeatWatch system with 500 Angus cows to evaluate the effect of the intensity of estrus on pregnancy rate. Estrus was synchronized with the Select Synch protocol (Gonadotropin releasing hormone [GnRH] followed seven days later with an injection of prostaglandin $F_{2\alpha}$). Length of estrus ranged from 0.5 to 24 hr and there was no effect of length of estrus on pregnancy status. However, cows that became pregnant were mounted more times per estrus than cows that did not conceive. These data are similar to another study with Angus cows in which cows that became pregnant were mounted more times per estrus than cows that did not become pregnant (Kuhlman et al., 1998).

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

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

In contrast to other livestock species, cattle ovulate following the end of estrus (approximately 28 to 32 hr after the onset of estrus or 12 to 20 hr following the end of estrus). Although characteristics of the estrous cycle are similar among most beef breeds, important differences have been reported between bos taurus and bos indicus breeds

(Galina et al., 1987; 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 (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.

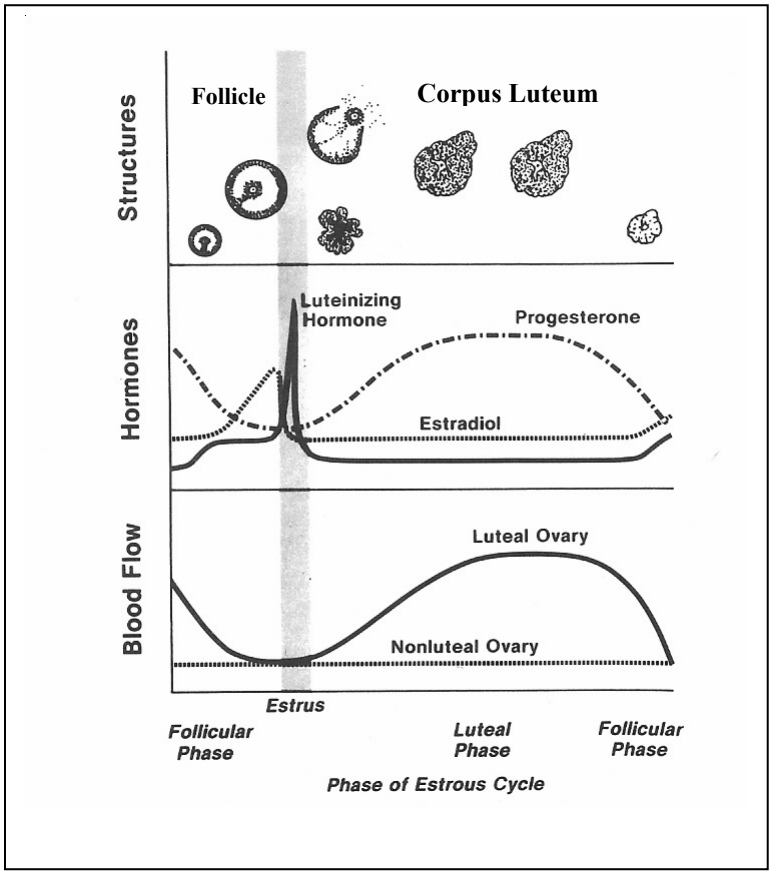


**Figure 1.** Changes in ovarian structures (preovulatory follicle and corpus luteum), hormones (luteinizing hormone, estradiol, and progesterone) and ovarian blood flow (ovary containing [luteal ovary] or not containing [nonluteal ovary] a corpus luteum) during the three phases of the estrous cycle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

**Follicular Phase**.

The follicular phase (proestrus) begins with the initiation of corpus luteum regression (luteolysis) and ends with the onset of estrus. Luteolysis is accompanied by a rapid decrease in progesterone resulting in a decrease in the negative feedback on pituitary LH secretion. As circulating concentrations of progesterone decrease, LH pulse frequency increases followed by a rapid increase in follicular estradiol secretion. The production of follicular estradiol results from the coordinated actions of LH and FSH on theca and granulosa cells, respectively (Fortune, 1986; Fortune and Quirk, 1988). The follicle wall consists of two distinct cell layers (granulosa and thecal cells) separated by a basement membrane. Granulosa cells are located in the compartment with the oocyte; whereas, theca cells surround the granulosa cells and are in close association with a wreath of capillaries. Theca cells have membrane receptors that bind LH resulting in 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).

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

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

**Table 1.** 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<br>Inhibit ovulation<br>Prepares animal for pregnancy<br>Maintenance of pregnancy | Inhibit estrus<br>Inhibit ovulation<br>Induce cyclicity<br>Dominant follicle turnover | Melengestrol Acetate (MGA®)<br>EAZI-BREED CIDR® | 0.5 mg/hd/day<br>1 CIDR per animal (1.38 g prog) | Feed<br>Vaginal insert |
| Prostaglandin $F_{2\alpha}$ | Uterus | Induce luteal regression | Induce premature luteal regression | Lutalyse®<br>ProstaMate®<br>In Synch®<br>Estrumate®<br>estroPLAN® | 5 ml<br>5 ml<br>5 ml<br>2 ml<br>2 ml | im inject<br>im inject<br>im inject<br>im inject<br>im inject |
| GnRH | Hypothalamus | Controls secretion of LH<br>Induces gonadotropin surge | Synchronize follicle wave<br>Induce ovulation | Cystorelin®<br>Factryl®<br>Fertagyl®<br>OvaCyst® | 2 ml<br>2 ml<br>2 ml<br>2 ml | im inject<br>im inject<br>im inject<br>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<br>Induction of ovulation<br>Oocyte maturation<br>Luteal tissue formation | Synchronize follicular wave<br>Induction of ovulation | N/A | N/A | N/A |
| Estradiol | Ovarian follicle | Estrous behavior<br>Induction of gonadotropin surge<br>Sperm transport | Dominant follicle turnover<br>Estrous behavior | N/A | N/A | N/A |

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

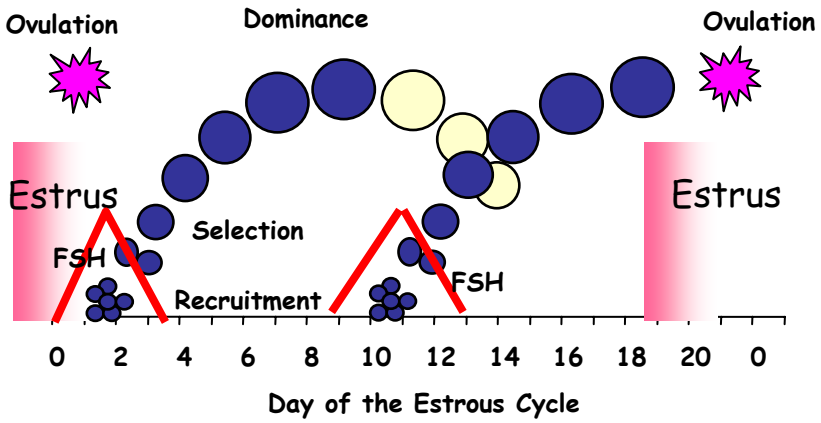


**Figure 2.** Relationship between circulating concentrations of follicle stimulating hormone (FSH) and stages of a bovine follicular wave (recruitment, selection, and dominance). A transient increase in FSH initiates recruitment of a cohort of follicles, from which a single follicle is normally selected 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).

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

*Selection.* Follicular selection is the process by which a single follicle from the recruited cohort is selected to continue to grow and become dominant, while the remaining follicles of the cohort undergo atresia. With the decline in circulating FSH concentrations, small follicles are presumably unable to continue growth and the selected follicle (dominant follicle) may shift its dependency from FSH to LH (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 hr after the LH surge (Kulick et al., 1999).

*Dominance.* The dominance phase of the follicular wave occurs when a follicle has been selected and continues to grow at a faster rate than the largest subordinate follicle, and inhibits the emergence of a new follicular wave (Ginther et al., 1996b). 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 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).

**Estrous Phase**

Increasing circulating concentrations of estradiol following luteolysis initiate estrous behavior, increase uterine contractions (facilitate sperm transport), and induce the preovulatory gonadotropin surge. The preovulatory gonadotropin surge coordinates the following events 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 hr and ovulation of a dominant follicle will occur 24 to 36 hr after injection (Figure 3).

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

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

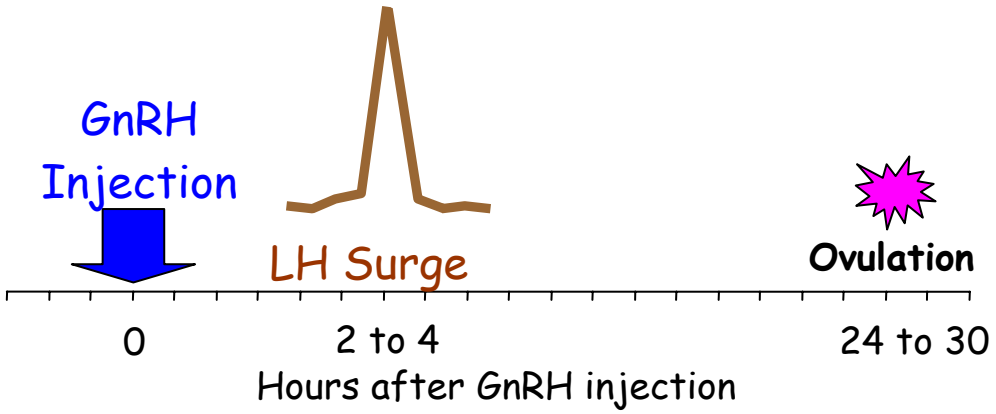


**Figure 3.** Injection (i.m.) of GnRH will induce a surge of LH within 2 to 4 hr and ovulation of a viable dominant follicle (≥ 10 mm) will occur within 24 to 36 hr (Modified from Kojima and Patterson, 2003).

**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.

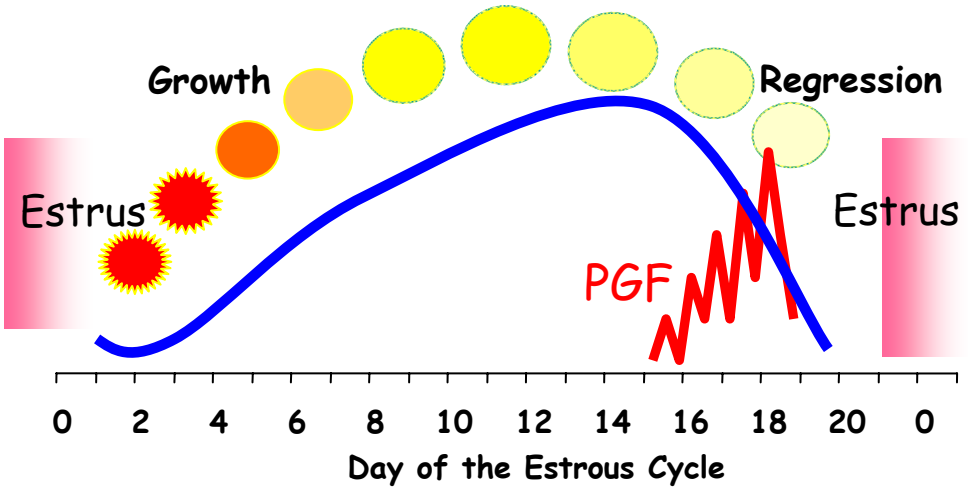


**Figure 4.** Changes in corpus luteum development, circulating concentrations of progesterone, and circulating concentrations of prostaglandin $F_{2\alpha}$ (PGF) during the luteal phase of the bovine estrous cycle are depicted above. Luteal secretion of progesterone inhibits the expression of estrus, 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 development of a normal corpus luteum may depend upon a preovulatory follicle meeting the following criteria: 1) an adequate number of granulosa cells, 2) an adequate number of LH receptors on granulosa and theca cells, and 3) granulosa cells capable of synthesizing adequate amounts of progesterone following luteinization. Furthermore, the ability of luteinized human granulosa cells to secrete progesterone increased when the cells were collected from follicles having increased follicular fluid concentrations of estradiol compared to granulosa cells collected from follicles that had lower concentrations of estradiol (McNatty et al., 1979). Premature induction of ovulation in ewes was associated with luteal insufficiency (Murdoch et al., 1983). These data are relevant to fixed-time insemination protocols in which physiologically immature dominant follicles are induced to ovulate at AI and the subsequent circulating concentrations of progesterone are lower than in cows in which a larger dominant follicle is induced to ovulate with GnRH (Perry et al., 2005). Inadequate luteal function following induced ovulation may be due to a reduced number of follicular cells and/or inadequate preparation of follicular cells for luteinization and secretion of progesterone.

## Estrous Synchronization Products and Mechanism of Action

Effective estrous synchronization protocols are designed to synchronize follicular maturation with the onset of corpus luteum regression. In general, development of estrous synchronization protocols in cycling animals has involved the following three approaches: 1) Inhibit ovulation following spontaneous corpus luteum regression (long-term progestin treatment), 2) Induction of corpus luteum regression ($PGF_{2\alpha}$ treatment), and 3) a combination of approaches 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 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 which can delay the interval to estrus when luteal tissue is not present. Following the removal of the progestin, progesterone concentrations will be low and standing estrus and ovulation will occur.

### Progestins

Two progestin products 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 with 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 (MGA) 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 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).

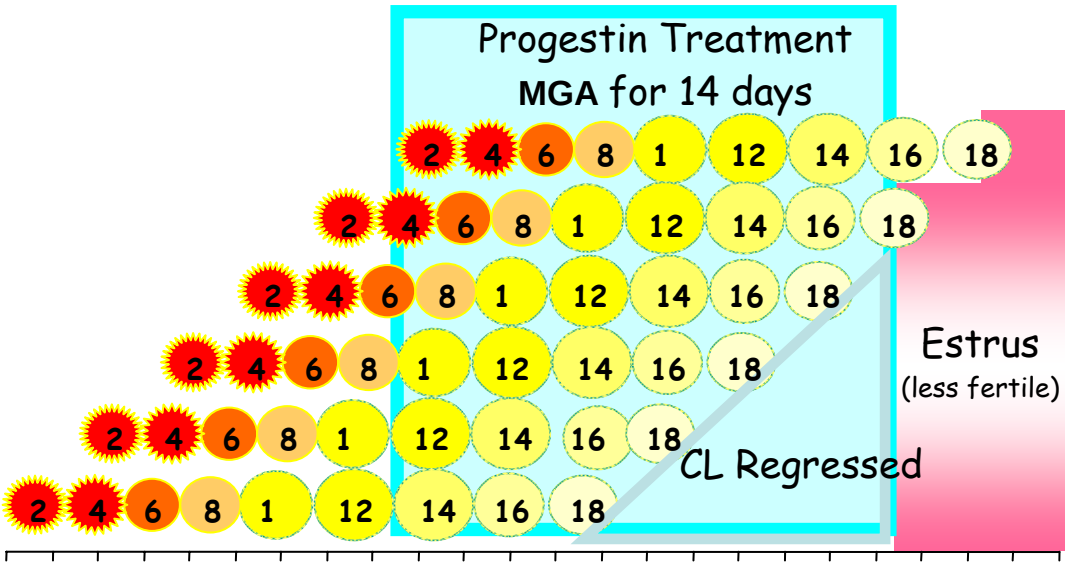


**Figure 5.** Effect of 14 days of melengestrol acetate (MGA) feeding on estrous synchronization of cows in different stages of the estrous cycle. Circles represent development and regression of corpora lutea (CL). Numbers inside each circle represent days of the cycle. In this diagram, spontaneous luteal regression occurs around day 17 to 18 of the cycle. Note 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 coated with a silicone layer containing 10% progesterone by weight. The CIDR's are inserted into the vagina with a lubricated applicator following disinfection of the applicator and vulva. CIDR's are easily removed by pulling the flexible nylon tail. Although a small amount of vaginitis is a common observation at CIDR removal, fertility is not compromised. The retention rate of CIDR's is approximately 95%. If the retention rate is considerably less than 95% the device may have been inserted incorrectly or other animals may be pulling the CIDR's out by biting on the nylon tails. In the latter case, the problem can be remedied by trimming the nylon tails.

**Prostaglandin $F_{2\alpha}$**

Prostaglandins are naturally occurring compounds produced by most cells in the body and have a variety of biological actions. $PGF_{2\alpha}$ is a naturally occurring luteolytic hormone also 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 d 6 to 16 following estrus; d 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).

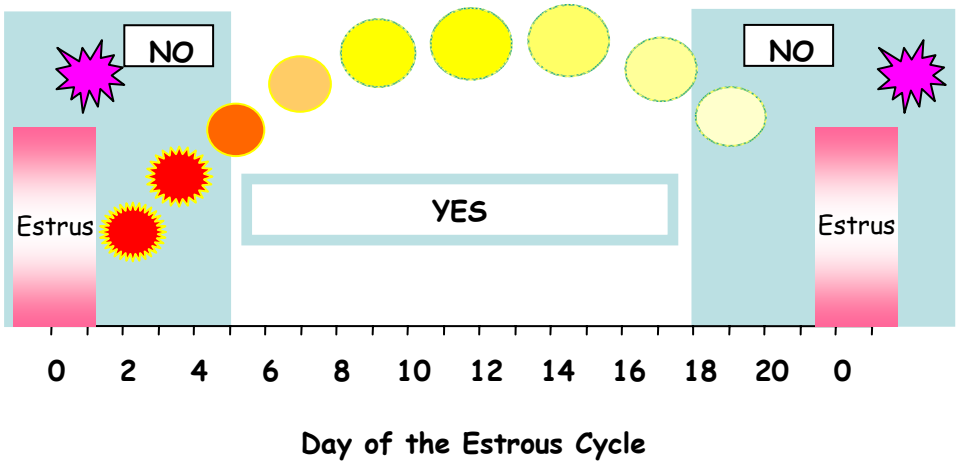


**Figure 6.** Effect of the bovine estrous cycle stageon luteal responsiveness to $PGF_{2\alpha}$. Bovine corpora lutea will not respond to an injection of $PGF_{2\alpha}$ during the first five days of the cycle. Therefore, $PGF_{2\alpha}$ should not be injected at the beginning of progestin treatment (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 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 hr period (McDowell et al., 1998) effectively regressed persistent follicles and initiated new follicular waves. Reduction of LH pulse frequency and amplitude following the administration of exogenous progesterone may be the mechanism by which persistent follicles are induced to undergo atresia (McDowell et al., 1998).

Estradiol benzoate has also been used to induce atresia of dominant follicles and to initiate a new follicular wave approximately 4.5 days after injection (Burke et al., 2000). When treatment with progesterone and estradiol were combined the dominant follicle stopped growing within 24 hr and became atretic resulting in the initiation of a new follicular wave 4 to 5 days after treatment (Burke et al., 1999). A single injection of a GnRH agonist is capable of ovulating dominant (≥ 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).

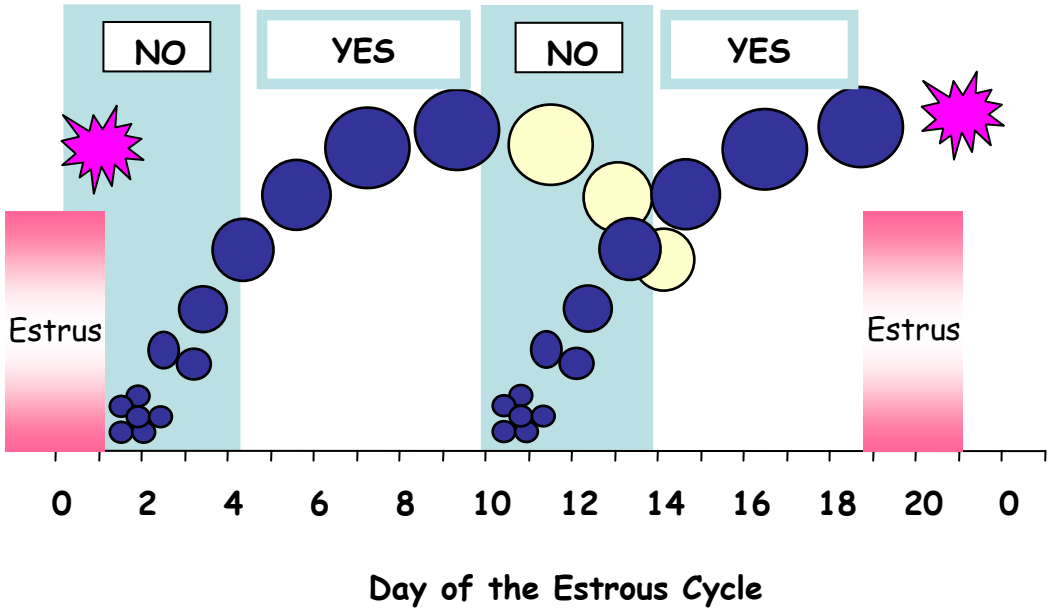


**Figure 7.** Injection of GnRH will induce ovulation of a dominant follicle (≥ 10 mm in diameter). Circles represent follicle development and atresia (light circles) during a wave. The above figure represents a "two-wave cow" and the shaded areas indicate when during a follicular wave follicles will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

## Mechanisms of Puberty and Postpartum Anestrous

Anestrous or a lack of estrous cycles is a normal occurrence before puberty and immediately postpartum. Many factors including age, nutrition, genetics, and photoperiod can influence the timing of the onset of puberty or the resumption of cycles after calving. In addition, weaning strategies can impact length of the postpartum interval as well as timing of onset of puberty. Management strategies to reduce age at puberty or the length of the postpartum interval will be presented later in this symposium. However, knowledge of the physiological control of these two periods of anestrous is important to understanding the potential success or failure of an estrous synchronization program.

### Puberty

Although puberty in heifers usually occurs between 10 and 18 months, the reproductive axis of the heifer is able to respond to LH, FSH, estradiol, and progesterone at young ages. By 5 to 6 months of age, heifers are able to develop follicles when supplied with LH and FSH, produce an LH surge in response to estradiol, and ovulate (Seidel et al., 1971; Schillo et al., 1983; McLeod et al., 1985). As early as the 1940s, it was proven heifers as young as 1 to 2 months could be superovulated (Cassida et al., 1943). However, despite the ability of young animals to respond to hormonal treatments, young heifers do not form a CL with a normal lifespan nor do they continue to cycle.

Prepuberal anestrous is primarily the result of a lack of a normal pattern of LH release from the pituitary. Release of LH is controlled by GnRH from the hypothalamus which causes LH to be released in pulses except during the ovulatory LH surge. The frequency and amplitude of these LH pulses dictate whether follicular growth continues or declines. In the cycling heifer, LH (and FSH) release is affected by estradiol, progesterone, and perhaps inhibin concentrations in the circulation. In prepuberal heifers, estradiol is the primary hormone that inhibits GnRH release thereby suppressing release of LH and FSH (Kiser et al., 1981; Day et al., 1984).

Prepuberal heifers have waves of follicular growth similar to cycling females (Adams et al., 1994). Follicles in these anovulatory waves are relatively small and produce limited amounts of estradiol. However, the hypothalamus of the developing heifer is extremely sensitive to the suppressive effects (or negative feedback) of estradiol on GnRH release. As the heifer matures, the sensitivity to this negative feedback diminishes due to a decrease in the number of estrogen receptors in the hypothalamus, and sufficient LH and FSH are released to initiate cycles (Day et al., 1987). The majority of the increase in LH release occurs in the last thirty days before puberty.

The first ovulatory estrus in heifers is often preceded by a transient increase in progesterone called a short luteal phase (Gonzalez-Padilla et al., 1975). Increased concentrations of progesterone before the first normal ovulation and luteal phase may aid in preparation of the uterus for pregnancy or establish patterns of gonadotropin secretion characteristic of cycling females. This short luteal phase may be the result of an ovulation without estrus or luteinization of a follicle without ovulation. Mimicking the short luteal phase by exposure of heifers to 7 to 10 days of a progestin (MGA or CIDR) can induce cycles in prepubertal heifers (Anderson et al., 1996; Imwalle et al., 1998). Induction of cycles with progestins is more effective the closer a heifer is to naturally attaining puberty (Short et al., 1976; Tanaka et al., 1995; Hall et al., 1997).

Fertility in heifers increases from the puberal estrus to third estrus following puberty (Byerley et al., 1987). It appears maternal recognition of pregnancy, uterine environment, and/or synchronization of estrus and ovulation is improved with an increased number of cycles. Management of heifers to increase the number of cycles attained before the breeding season will improve fertility in heifers.

Age at puberty in heifers is influenced by breed, nutrition, biostimulation, and photoperiod (Schillo et al., 1992). Overall, age at puberty is dictated by genetics as breeds mature at different rates. However, within the same breeds there are lines that may be later or earlier maturing. Crossbred females reach puberty earlier than their purebred counterparts. Undernutrition delays onset of puberty. In contrast, increased energy intake and dietary protein content may dramatically affect age at puberty depending on when the exposure to increased nutrition occurs. Many studies indicate increased energy intake post-weaning can reduce age at puberty by 30-45 days (McShane et al., 1989; Hall et al., 1995; Schillo et al., 2003). The effect of high energy intake between 90 and 200 days of age increases precocious puberty in heifers (Gasser et al, 2006). More recently, it appears prenatal nutrition may also affect age at puberty (Martin et al, 2007).

Estrus can occur without an associated ovulation. In past research, 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). Unfortunately, nonpuberal estrus may cause producers to overestimate the percentage of cycling heifers in the herd.

In contrast, precocious puberty can create difficulties with heifers becoming pregnant while still nursing their dams. Precocious puberty is usually defined as estrus and ovulation before 300 days of age. Incidence of precocious puberty in most herds is estimated at 2-5% (Wehrman et al., 1996). However, early weaning combined with high concentrate diets may result in the incidence of precocious puberty as high as 50% (Gasser et al., 2006).

**Postpartum Anestrus**

The mechanism of resumption of cycles after calving is similar to initiation of cycles at puberty. Essentially, activity of the GnRH pulse generator is reduced after prolonged exposure to progesterone during gestation. Anovulatory follicular waves continue during gestation until the last 21 days of gestation (Ginther et al., 1996a). Like the puberal transition, early postpartum anestrus appears to be a result of hypersensitivity to estradiol negative feedback. Soon after calving, pituitary stores of LH return to normal and the GnRH pulse generator is capable of "restarting" as indicated by the rapid return to estrus in cows that lose calves at or near calving.

Sensitivity to estradiol negative feedback appears to diminish by 20 to 40 days postpartum. Therefore cows have the "endocrine potential" to cycle early after calving. Similar to heifers, many postpartum cows exhibit a short luteal phase immediately before the first ovulatory estrus. Therefore, including progestins in an estrous synchronization program may induce cyclicity in some anestrous females.

The GnRH pulse generator is also suppressed by a variety of factors including nutrition, energy reserves, and suckling (Short et al., 1990). The strongest suppressors of

the GnRH pulse generator are suckling and presence of the calf (Williams, 1990) and nutritional status (Short et al., 1990). Several experiments clearly indicate the presence of the calf may be a stronger factor in suppression of cycles than mammary stimulation caused by nursing or milking (McMillian, 1983; Lamb et al., 1999). In addition to presence of the calf, the nutritional demands on the dam may also suppress resumption of cycles, but perhaps through different mechanism(s) than the calf. Luteinizing hormone levels increase after only 48 hours of calf removal (Whisnant et al., 1985). Therefore, temporary calf removal may be a management option.

Energy intake or reserves are also major factors influencing return to estrus. Cows calving in BCS 5 (1 = emaciated to 9 = obese) or better have shorter postpartum intervals than cows calving in thin condition (Selk et al., 1988; Wettemann et al., 2003). Cows gaining weight postpartum have decreased postpartum intervals compared to cows that lose weight (Wettemann et al., 2003). Primiparous cows are more sensitive to the effects of undernutrition on postpartum interval (Ciccioli et al., 2003). It appears the concomitant nutritional demands of lactation and growth create additional nutritional stress for these young cows.

Other factors such as biostimulation, photoperiod, milking ability, and breed can also affect the length of postpartum anestrus. Management strategies to reduce postpartum interval are examined in several other presentations in this symposium. It appears many of the factors affecting postpartum anestrus act independently through different mechanisms. Therefore, several factors may have an additive effect on postpartum interval.

**Depth of Anestrus**

Whether management practices can alter timing of puberty onset or resumption of cycles postpartum depends on the "depth" of anestrus. As previously indicated, many factors influence age at puberty and length of the postpartum interval. How "deep" an animal is in anestrus may be dictated by the number of inhibitory factors impinging on the reproductive axis.

It is the interplay of inhibitory (i.e. estradiol negative feedback, undernutrition, suckling) and positive (i.e. age, days postpartum, body condition, biostimulation) factors that dictate whether the cycles begins. Most importantly females must be released from estradiol negative feedback. Usually, this means heifers are old enough (12-14 months) and postpartum cows are thirty or more days post-calving. Once animals are released from negative feedback, then other factors such as undernutrition become the limiting factor.

Energy availability from body fat stores and dietary intake are major affecters of depth of anestrus. Cows calving in poor body condition or undernourished heifers will have prolonged anestrous periods. To reduce the length of anestrus in these animals, dietary energy intake must be increased and nutritional demands must be reduced (i.e. early weaning). In contrast, well nourished cows have a shorter anestrous period and respond well to management strategies to reduce the length of the anestrous period such as biostimulation.

For example, 10-month old heifers weighing only 400 lbs are in "deeper" anestrus than 14-month old heifers weighing 600 lbs. The younger heifers are very sensitive to estradiol negative feedback and undernutrition is also a negative factor. Therefore, these

heifers are in deep anestrus. It is doubtful these 10-month old heifers will become puberal in response to a variety of management techniques. It will take a combination of time and nutrition to release these heifers from anestrus. In contrast, undernutrition is probably the major limiting factor for the older heifers. Providing a high energy diet in combination with a progestin may trigger cycles in these "lighter" anestrous heifers.

The amount of animals in deep anestrus should be considered when planning an estrous synchronization program. Although many protocols may induce cycles in females close to resuming cycles, these protocols will not affect females in deep anestrous. Failure to consider the cyclic status of the herd may result in an unsuccessful attempt to synchronize animals incapable of responding to estrous synchronization drugs.

## 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 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.

## Literature Cited

Adams, G.P., R.L. Matteri, J.P. Kastelic, J.C. Ko, and O.J. Ginther. 1992. Association between surges of follicle-stimulating hormone and the emergence of follicular waves in heifers. J. Reprod. Fertil. 94:177-188.

Adams, G.P., A.C. Evans, and N.C. Rawlings. 1994. Follicular waves and circulating gonadotrophins in 8-month-old prepubertal heifers. J. Repro. Fert. 100: 27-33.

Adams, G.P. 1999. Comparative patterns of follicle development and selection in ruminants. J. Reprod. Fertil. Suppl. 54:17-32.

Adeyemo, O., and E. Heath. 1980. Plasma progesterone concentration in *Bos Taurus* and *Bos Indicus* heifers. Theriogenology 14:411.

Ahmad, N., F.N. Schrick, R.L. Butcher, and E.K. Inskeep. 1995. Effect of persistent follicles on early embryonic losses in beef cows. Biol. Reprod. 52:1129-1135.

Anderson, L.H. and M.L. Day. 1994. Acute progesterone administration regresses persistent dominant follicles and improves fertility of cattle in which estrus was synchronized with melengestrol acetate. J. Anim. Sci. 72:2955-2961.

Atkins, J.A., D.C. Busch, J.F. Bader, D.J. Schafer, M.C. Lucy, D.J. Patterson, and M.F. Smith. 2005. GnRH-induced ovulation in heifers: Effects of stage of follicular wave. Biol. Reprod (Special Issue) p231.

Bellows, D.S., S.L. Ott, and R.A. Bellows. 2002. Review: Cost of reproductive diseases and conditions in cattle. The Professional Animal Scientist 18:26-32.
Bo, G.A., G.P. Adams, R.A. Pierson, and R.J. Mapletoft. 1995. Exogenous control of follicular wave emergence in cattle. Theriogenology 43:31-40.
Brewester J. and C.L. Cole. 1941. The time of ovulation in cattle. J. Dairy Sci. 24:111.
Burke, C.R., M.P. Boland, and K.L. Macmillan. 1999. Ovarian responses to progesterone and oestradiol benzoate administered intravaginally during dioestrus in cattle. Anim. Reprod. Sci. 55:23-33.
Burke, C.R., M.L. Day, C.R. Bunt, and K.L. Macmillan. 2000. Use of a small dose of estradiol benzoate during diestrus to synchronize development of the ovulatory follicle in cattle. J. Anim. Sci. 78:145-151.
Butcher, R.L., and R.S. Pope. 1979. Role of estrogen during prolonged estrous cycles of the rat on subsequent embryonic death or development. Biol. Reprod. 21:491-495.
Byerley, D.J., R.B. Staigmiller, J.G. Beradinelli, and R.E. Short. 1987. Pregnancy rates of beef heifers bred on puberal or third estrus. J. Anim. Sci. 65:645-650.
Cassida, L.E., R.K. Meyer, W.H. McShan, and W.Wisnicky. 1943. Effects of pituitary gonadotropins on the ovaries and induction of superfecundity in cattle. Amer. J. Vet. Res. 4:76.
Ciccioli, N.H., R.P. Wettemann, L.J. Spicer, C.A. Lents, F.J. White, and D.H. Keisler. 2003. Influence of body condition at calving and postpartum nutrition on endocrine function and reproductive performance of primiparous beef cows. J. Anim. Sci. 81:3107-3120.
Crowe, M.A., D. Goulding, A. Baguisi, M.P. Boland, and J.F. Roche. 1993. Induced ovulation of the first postpartum dominant follicle in beef suckler cows using a GnRH analogue. J. Reprod. Fertil. 99:551-555.
Day, M.L., K. Imakawa, P.L. Wolf, R.J. Kittock, and J.E. Kinder. 1984. Endocrine mechanisms of puberty in heifers: Estradiol negative feedback regulation of luteinizing hormone secretion. Biol. Reprod. 31:332-341.
Day, M.L., K. Imakawa, M. Garcia-Winder, D.D. Zalesky, B.D. Schanbacher, R.J. Kittock, and J.E. Kinder. 1987. Endocrine mechanisms of puberty in heifers: Role of hypothalamo-pituitary estradiol receptors in negative feedback of estradiol on luteinizing hormone secretion. Biol. Reprod. 37:1054-1065.
Diskin, M.G., E.J. Austin, and J.F. Roche. 2002. Exogenous hormonal manipulation of ovarian activity in cattle. Domest. Anim. Endocrinol. 23:211-228.
Dobson, H., and M. Kamonpatana. 1986. A review of female cattle reproduction with special reference to a comparison between buffaloes, cows, and zebu. J. Reprod. Fertil. 77:1-36.
Duffy, P., M.A. Crowe, M.P. Boland, and J.F. Roche. 2000. Effect of exogenous LH pulses on the fate of the first dominant follicle in postpartum beef cows nursing calves. J. Reprod. Fertil. 118:9-17.
Fortune J.E. 1986. Bovine theca and granulose cells interact to promote androgen production. Biol. Reprod. 35:292.
Fortune, J.E., and S.M. Quirk. 1988. Regulation of steroidogenesis in bovine preovulatory follicles. J. Anim. Sci. 66:1.
Fortune, J.E. 1994. Ovarian follicular growth and development in mammals. Biol. Reprod. 50:225-232.

Fortune, J.E., and G.M. Rivera. 1999. Persistent dominant follicles in cattle: basic and applied aspects. Arq. Fac. Vet. 27:24-36.
Fortune, J.E., G.M. Rivera, A.C. Evans, and A.M. Turzillo. 2001. Differentiation of dominant versus subordinate follicles in cattle. Biol. Reprod. 65:648-654.
Galina, C.S., A. Orihuela, A. and Duchateau. 1987. Reproductive physiology in Zebu cattle. Vet. Clin. North Am. Food. Anim. Pract. 3:619.
Galina, C.S., A. Orihuela, and I. Rubio. 1994. Behavioral characteristics of zebu cattle with emphasis on reproductive efficiency. In M.J. Fields and R.S. Sands, editors. Factors affecting calf crop. Boca Raton: CRC Press p345-361.
Galway, A.B., P.S. Lapolt, A. Tsafriri, C.M. Dargan, I. Boime, and A.J.W. Hsueh. 1990. recombinant follicle stimulating hormone induces ovulation and tissue plasminogen activator expression in hypophysectomized rats. Endocrinology 127:3023.
Garverick, H.A., and M.F. Smith. 1993. Female reproductive physiology and endocrinology of cattle. In. The Veterinary Clinics of North America. Eds W.F. Braun and R.S. Youngquist. W.B. Saunders Co. Philadelphia, p223-247.
Gasser, C.L., C.R. Burke, M.L. Mussard, E.J. Behlke, D.E. Grum, J.E. Kinder, and M.L. Day. 2006. Induction of precocious puberty in heifers II: Advanced ovarian follicular development. J. Anim. Sci. 84:2042–2049.
Geary, T.W., E.R. Downing, J.E. Bruemmer, and J.C. Whittier. 2000. Ovarian and Estrous Response of suckled beef cows to the select synch estrous synchronization protocol. Prof. Anim. Sci. 16:1-5.
Ginther, O.J., K. Kot, L.J. Kulick, S. Martin, and M.C. Wiltbank. 1996a. Relationships between FSH and ovarian follicular waves during the last six months of pregnancy in cattle. J. Reprod. Fertil. 108:271-279.
Ginther, O.J., M.C. Wiltbank, P.M. Fricke, J.R. Gibbons, and K. Kot. 1996b. Selection of the dominant follicle in cattle. Biol. Reprod. 55:1187-1194.
Ginther, O.J., K. Kot, L.J. Kulick, and M.C. Wiltbank. 1997. Emergence and deviation of follicles during the development of follicular waves in cattle. Theriogenology 48:75-87.
Ginther, O.J., D.R. Bergfelt, L.J. Kulick, and K. Kot. 1999. Selection of the dominant follicle in cattle: establishment of follicle deviation in less than 8 hours through depression of FSH concentrations. Theriogenology 52:1079-1093.
Ginther, O.J., D.R. Bergfelt, L.J. Kulick, and K. Kot. 2000a. Selection of the dominant follicle in cattle: role of two-way functional coupling between follicle-stimulating hormone and the follicles. Biol. Reprod. 62:920-927.
Ginther, O.J., D.R. Bergfelt, L.J. Kulick, and K. Kot. 2000b. Selection of the dominant follicle in cattle: role of estradiol. Biol. Reprod. 63:383-389.
Gong, J.G., B.K. Campbell, T.A. Bramley, C.G. Gutierrez, A.R. Peters, and R. Webb. 1996. Suppression in the secretion of follicle-stimulating hormone and luteinizing hormone, and ovarian follicle development in heifers continuously infused with a gonadotropin-releasing hormone agonist. Biol. Reprod. 55:68-74.
Gonzalez-Padilla E., J.N. Wiltbank, and G.D. Niswender. 1975. Puberty in beef heifers I. The interrelation between pituitary, hypothalamic and ovarian hormones. J. Anim. Sci. 40:1091.

Hall, J.B., R.B. Staigmiller, R.A. Bellows, R.E. Short, W.M. Moseley, and S.E. Bellows. 1995. Body composition and metabolic profiles associated with puberty in beef heifers. J. Anim. Sci. 73:3409-3420.

Hall, J.B., R.B. Staigmiller, R.E. Short, R.A. Bellows, M.D. MacNeil, and S.E. Bellows. 1997. Effect of age and pattern of gain on induction of puberty with a progestin in beef heifers. J. Anim. Sci. 75:1606-1611.

Helmer, S.D and J.H. Britt. 1985. Mounting activity as affected by stage of estrous cycle in Holstein heifers. J. Dairy Sci. 68:1290-1296.

Hixon, J.E., W. Hansel. 1974. Evidence for preferential transfer of prostaglandin $F_{2\alpha}$ to the ovarian artery following intrauterine administration in cattle. Biol. Reprod. 11:543.

Hurnick, J.F., G.J. King, and H.A. Robertson. 1975. Estrous and related behavior in postpartum Holstein cows. Applied Animal Ethology 2:55-68.

Imwalle, D.B., D.J. Patterson, and K.K. Schillo. 1998. Effects of melengestrol acetate on onset of puberty, follicular growth, and patterns of luteinizing hormone secretion in beef heifers. Biol. Reprod 58:1432-1436.

Inskeep, E.K. and W.J. Murdoch. 1980. Relation of ovarian functions to uterine and ovarian secretion of prostaglandins during the estrous cycle and early pregnancy in the ewe and cow. *In* Greep, R.O. (ed): Reproductive Physiology III, International Review of Physiology, Vol. 22. Baltimore, University Park Press, 325.

Inskeep, E.K., R.A. Dailey, and R.C. Rhodes. 1982. Some considerations on the value of hormonal assays and a knowledge of hormonal profiles to reproduction of red meat animals. S. Afr. J. Anim. Sci. 12:85.

Irvin, H.J., R.D. Randel, and W.E. Haensley. 1978. Reproductive studies of Brahman cattle. III. Comparison of weight, progesterone content, histological characteristics, and 3β-hydroxysteroid dehydrogenase activity in corpora lutea of Brahman, Hereford and Brahman X Hereford heifers. Theriogenology 10:417.

Kinder, J.E., F.N. Kojima, E.G. Bergfeld, M.E. Wehrman, and K.E. Fike. 1996. Progestin and estrogen regulation of pulsatile LH release and development of persistent ovarian follicles in cattle. J. Anim. Sci. 74:1424-1440.

Kiser, T.E., R.R. Krealing, G.B. Rampacek, B.J. Landmeier, A.B. Caudel, and J.D. Chapman. 1981. J. Anim. Sci. 53:1545-1550.

Kojima, F.N., J.R. Chenault, M.E. Wehrman, E.G. Bergfeld, A.S. Cupp, L.A. Werth, V. Mariscal, T. Sanchez, R.J. Kittok, and J.E. Kinder. 1995. Melengestrol acetate at greater doses than typically used for estrous synchrony in bovine females does not mimic endogenous progesterone in regulation of secretion of luteinizing hormone and 17 beta-estradiol. Biol. Reprod. 52:455-463.

Kojima N.F. and D.J. Patterson 2003. Guide to estrous synchronization of beef cattle. University of Missouri-Columbia Extension Publications #MM101.

Kuhlmann K.K., D.R. Shelby, C.B. Scott, B.J. May, and G.R. Engdahl. 1998. The use of an electronic estrous detection system to monitor estrous behavior in Angus females of various ages. J. Anim. Sci. 1998:81 (Suppl 1):271. Abstr.

Kulick, L.J., K. Kot, M.C. Wiltbank, and O.J. Ginther. 1999. Follicular and hormonal dynamics during the first follicular wave in heifers. Theriogenology 52:913-921.

Lamb, G.C., B.L. Miller, J.M. Lynch, K.E. Thompson, J.S. Heldt, C.A. Loest, D.M. Grieger, and J.S. Stevenson. 1999. Twice daily suckling but not milking with calf presence prolongs postpartum anovulation. J. Anim. Sci. 77:2207–2218.

Landaeta-Hernandez, A.J., J.V. Yelich, J.W. Lemaster, M.J. Fields, T. Tran, C.C. Chase Jr., D.O. Rae, and P.J. and Chenoweth. 2002. Environmental, genetic, and social factors affecting the expression of estrus in beef cows. Theriogenology 57:1357-1370.

Lemaster, J.W., J.V. Telich, J.R. Kempfer, and F.N. Schrick. 1999. Ovulation and estrous characteristics in crossbred Brahman heifers treated with an intravaginal progesterone-releasing insert in combination with prostaglandin $F_{2\alpha}$ and estradiol benzoate. J. Anim. Sci. 77:1860-1868.

Martin, G.B., C.A. Price, J.C. Thiery, and R. Webb. 1988. Interactions between inhibin, oestradiol and progesterone in the control of gonadotrophin secretion in the ewe. J. Reprod. Fertil. 82:319-328.

Martin, J.L., K.A. Vonnahme, D.C. Adams, G.P. Lardy, and R.N. Funston. 2007. Effects of dam nutrition on growth and reproductive performance of heifer calves. J. Anim. Sci. 85: 841-847.

Mattheij, J.A., J.J. Swarts, H.M. Hurks, and K. Mulder. 1994. Advancement of meiotic resumption in graafian follicles by LH in relation to preovulatory ageing of rat oocytes. J. Reprod. Fertil. 100:65-70.

McCracken, J.A., J.C. Carlson, M.E. Glew, J.R. Goding, D.T. Baird, K. Green, and B. Samuelson. 1972. Prostaglandin $F_{2\alpha}$ identified as a luteolytic hormone in sheep. Nature 238:129.

McDowell, C.M., L.H. Anderson, J.E. Kinder, and M.L. Day. 1998. Duration of treatment with progesterone and regression of persistent ovarian follicles in cattle. J. Anim. Sci. 76:850-855.

McLeod, B.J., A.R. Peters, W. Haresign, and G.E. Lamming. 1985. Plasma LH and FSH responses and ovarian activity in prepubertal heifers treated with repeated injections of low doses of GnRH for 72 h. J. Reprod. Fert. 74:589-596.

McMillan, K. L. 1983. Postpartum interval to oestrus in monozygotic twin cows and possible effects of maternal bonding. N.Z. J. Agric. Res. 26:451-454.

McNatty, K.P., W.M. Hunter, A.S. MacNeilly, and R.S. Sawers. 1975. Changes in the concentration of pituitary and steroid hormones in the follicular fluid of human graafian follicles throughout the menstrual cycle. J. Endocrinol. 64:555-571.

McNatty, K.P., D.M. Smith, A. Makris, R. Osathanondh, and K.J. Ryan. 1979. The microenvironment of the human antral follicle: interrelationships among the steroid levels in antral fluid, the population of granulosa cells, and the status of the oocyte in vivo and in vitro. J. Clin. Endocrinol. Metab. 49:851-860.

McShane, T.M., K.K. Schillo, J.A. Boling, N.W. Bradley, and J.B. Hall. 1989. Effects of Recombinant DNA-Derived Somatotropin and Dietary Energy Intake on Development of Beef Heifers: I. Growth and Puberty. J. Anim. Sci. 67: 2230-2236.

Mihm, M., A. Baguisi, M.P. Boland, and J.F. Roche. 1994. Association between the duration of dominance of the ovulatory follicle and pregnancy rate in beef heifers. J. Reprod. Fertil. 102:123-130.

Murdoch, W.J., M. De Silva, and T.G. Dunn. 1983. Luteal phase insufficiency in the ewe as a consequence of premature induction of ovulation by intrafollicular injection of gonadotropins. J. Anim. Sci. 57:1507-1511.

Murphy, M.G., M.P. Boland, and J.F. Roche. 1990. Pattern of follicular growth and resumption of ovarian activity in post- partum beef suckler cows. J. Reprod. Fertil. 90:523-533.

Nelsen, T.C., R.E. Short, D.A. Phelps, and R.B. Staigmiller. 1985. Nonpuberal estrus and mature cow influences on growth and puberty in heifers. J. Anim. Sci. 61:470.

Niswender, G.D., T.J. Riemers, M.A. Diekman, and T.M. Nett. 1976. Blood flow: a mediator of ovarian function. Biol. Reprod. 14:64-81.

Niswender, G.D. and T.M. Nett. 1988. The corpus luteum and its control. *In* Knobil E, Neill J.D., Ewing LL, et al (eds): The Physiology of Reproduction, Vol. 1. New York, Ravel Press. p489.

Niswender, G.D., J.L. Juengel, P.J. Silva, M.K. Rollyson, and E.W. McIntush. 2000. Mechanisms controlling the function and life span of the corpus luteum. Physiological Reviews 80:1-29.

O'Connor, M.L. and P.L. Senger. 1997. Estrus Detection. In Current Therapy in Large Animal Theriogenology. Ed. R.S. Youngquist. W.B. Saunders Co. Philadelphia, pp276-285.

Perry, R.C., L.R. Corah, G.H. Kiracofe, J.S. Stevenson, and W.E. Beal. 1991. Endocrine changes and ultrasonography of ovaries in suckled beef cows during resumption of postpartum estrous cycles. J. Anim. Sci. 69:2548-2555.

Perry, G.A., F.N. Kojima, B.E. Salfen, J.F. Bader, D.J. Patterson, and M.F. Smith. 2002. Effect of an orally active progestin on follicular dynamics in cycling and anestrous postpartum beef cows. J. Anim. Sci. 80:1932-1938.

Perry, G.A., M.F. Smith, M.C. Lucy, J.A. Green, T.E. Parks, M.D. MacNeil, A.J. Roberts, and T.W. Geary. 2005. Relationship between follicle size at insemination and pregnancy success. PNAS 102:5268-5273.

Plasse, D, A.C. Warnick, and M. Koger. 1970. Reproductive behavior of *Bos Indicus* in a subtropical environment. IV. Length of oestrous cycle, duration of oestrus, time of ovulation, fertilization, and embryo survival in grade Brahman heifers. J. Anim. Sci. 30:63.

Randel, R.D. 1976. LH and ovulation in Brahman X Hereford and Hereford heifers (abstract). J. Anim. Sci. 43:300.

Rhodes, F.M., B.A. Clark, M.L. Day, and K.L. Macmillan. 1997. Can persistent ovarian follicles be induced in young postpartum dairy cows? In: Australian Society of Reproductive Biology, Canberra, Australia. p103.

Richards, J.S. 1980. Maturation of ovarian follicles: actions and interactions of pituitary and ovarian hormones on follicular cell differentiation. Physiol. Rev. 60:51.

Roche, J.F., E.J. Austin, M. Ryan, M. O'Rourke, M. Mihm, and M.G. Diskin. 1999. Regulation of follicle waves to maximize fertility in cattle. J. Reprod. Fertil. Suppl. 54:61-71.

Rorie, R.W., T.R. Bilby, and T.D. Lester. 2002. Application of electronic estrus detection technologies to reproductive management of cattle. Theriogenology 137-148.

Rutter, L.M. and R.D. Randel. 1986. Nonpuberal estrus in beef heifers. J. Anim. Sci. 63:1049.

Ryan, M., M. Mihm, and J.F. Roche. 1998. Effect of GnRH given before or after dominance on gonadotrophin response and fate of that follicle wave in postpartum dairy cows. J. Reprod. Fertil. 21:61 (abstract).
Savio, J.D., W.W. Thatcher, G.R. Morris, K. Entwistle, M. Drost, and M.R. Mattiacci. 1993. Effects of induction of low plasma progesterone concentrations with a progesterone-releasing intravaginal device on follicular turnover and fertility in cattle. J. Reprod. Fertil. 98:77-84.
Schallenberger, E., and S. Prokopp. 1985. Gonadotrophins and ovarian steroids in cattle. IV. Re-establishment of the stimulatory feedback action of oestradiol-17 beta on LH and FSH. Acta Endocrinol. (Copenh.) 109:44-49.
Schillo, K.K., D.J. Dierschke, and E.R. Hauser. 1983. Estrogen-induced release of luteininzing hormone in prepubertal and postpubertal heifers. Theriogenology 19:727-738.
Schillo, K.K., J.B. Hall and S.M. Hileman. Effects of nutrition and season on the onset of puberty in the beef heifer. 1992. J. Anim. Sci. 70:3994-4005.
Seidel, G.E., L.L. Larson, and R.H. Foote. 1971. Effects of age and gonadotropin treatment on superovulation in the calf. J. Anim. Sci. 33:617-622.
Seidel G.E. 1995. Reproductive biotechnologies for profitable beef production. In Proc. Beef Improvement Federation. p28. Sheridan, WY.
Selk, G.E., R.P. Wettemann, K.S. Lusby, J.W. Oltjen, S.L. Mobley, R.J. Rasby, and J.C. Garmendia. 1988. Relationships among Weight Change, Body Condition and Reproductive Performance of Range Beef Cows. J. Anim. Sci. 66:3153-3159.
Short, R.E., R.A. Bellows, J.B. Carr, R.B. Staigmiller, and R.D. Randel. 1976. Induced or synchronized puberty in heifers. J. Anim. Sci. 43:1254.
Short, R. E., R. A. Bellows, R. B. Staigmiller, J. G. Berardinelli, and E. E. Custer. Physiological mechanisms controlling anestrus and infertility in postpartum beef cattle**.** J. Anim. Sci. 68:799-816.
Sirois, J. and J.E. Fortune. 1990. Lengthening the bovine estrous cycle with low levels of exogenous progesterone: a model for studying ovarian follicular dominance. Endocrinology 127:916-925.
Tanaka, Y., D.L. Vincent, K.S. Ledgerwood, and C.W. Weems. 1995. Variable progesterone response and estradiol secretion in prepubertal beef heifers following treatment with Norgestomet implants. Theriogenology 43:1077.
Trenkle, A. and R.L. Willham. 1977. Beef production efficiency: The efficiency of beef production can be improved by applying knowledge of nutrition and breeding. Science 198:1009-1015.
Tsai, S.J., and M.C. Wiltbank. 1998. Prostaglandin $F_{2\alpha}$ regulates distinct physiological changes in early and mid-cycle bovine corpora lutea. Biol. Reprod. 58:346-352.
Tsai, S.J., J.L. Juengel, and M.C. Wiltbank. 1997. Hormonal regulation of monocyte chemoattractant protein-1 messenger ribonucleic acid expression on corpora lutea. Endocrinology 138:4517-4520.
Twagiramungu, H., L.A. Guilbault, and J.J. Dufour. 1995. Synchronization of ovarian follicular waves with a gonadotropin- releasing hormone agonist to increase the precision of estrus in cattle: a review. J. Anim. Sci. 73:3141-3151.

Wehrman, M. E., F. N. Kojima, T. Sanchez, D. V. Mariscal, and J. E. Kinder. 1996. Incidence of precocious puberty in developing beef heifers. J. Anim. Sci. 74:2462-2467.

Werth, L.A., J.C. Whittier, S.M. Azzam, G.H. Deutscher, and J.E. Kinder. 1996. Relationship between circulating progesterone and conception at the first postpartum estrus in young primiparous beef cows. J. Anim. Sci. 74:616-619.

Wettemann, R.P., C.A. Lents, N.H. Ciccioli, F.J. White, and I. Rubio. 2003. Nutritional and suckling-mediated anovulation in beef cows. J. Anim. Sci. 81: E48-59E.

Whisnant, C. S**.,** T. E. Kiser and F. N. Thompson. 1985. Effect of calf removal on serum luteinizing hormone and cortisol concentrations in postpartum cows. Theriogenology 24 119.

White, F.J., R.P. Wettemann, M.L. Looper, T.M. Prado, and G.L. Morgan. 2002. Seasonal effects on estrous behavior and time of ovulation in nonlactating beef cows. J. Anim. Sci. 80:3053-3059.

Williams, G. L. 1990. Suckling as a regulator of postpartum rebreeding in cattle: a review. J. Anim. Sci. 68:831-852

Zimbelman, R.G., and L.W. Smith. 1966a. Control of ovulation in cattle with melengestrol acetate. II. Effects on follicular size and activity. J. Reprod. Fertil. 11:193-201.

Zimbelman, R.G., and L.W. Smith. 1966b. Control of ovulation in cattle with melengestrol acetate. I. Effect of dosage and route of administration. J. Reprod. Fertil. 11:185-191.

**NOTES**