

**THE ESTROUS CYCLE IN CATTLE:
PHYSIOLOGY, ENDOCRINOLOGY, AND FOLLICULAR WAVES^{a,b}**

Freddie N. Kojima^c
Department of Animal Science
University of Missouri
Columbia, MO 65211

Introduction

Estrous synchronization is a valuable tool to enhance reproductive management in beef cattle. Procedures that 1) facilitate synchronization of estrus in cycling cattle and 2) induce ovulatory estrus in peripubertal heifers and postpartum anestrous cows, will increase reproductive rates and expedite genetic progress through use of artificial insemination (AI). Effective reproductive management requires the successful application of available knowledge to current estrous synchronization protocols. To that end, this review covers the basic physiology and endocrinology of the estrous cycle, and provides an overview of our current understanding of follicular waves.

Use of Estrous Synchronization and Artificial Insemination in Beef Industry. A review from the USDA National Animal Health Monitoring System in 1994 concluded that only 3.3 and 4.3 % of the beef cattle operations in the United States practice AI on their heifers and cows, respectively, and only 3 and 4.3 % of total operations use any form of estrous synchronization to facilitate their AI programs in their heifers and cows, respectively. Although hormonal treatment of heifers and cows to group estrous periods has been a commercial reality for years, producers have been slow to adapt this management practice. This is perhaps caused by past failures, which resulted when females that were placed on estrous synchronization treatments failed to express behavioral estrus or become pregnant by AI following treatment. Other reasons cited most in the NAHMS survey in 1998 for not using these practices are led by “lack of time and labor”, followed by “too complicated” or “costly”. Estrous synchronization and AI remain, however, the most important and widely applicable reproductive biotechnologies available (Seidel, 1995).

Low adoption rates of reproductive management practices leads one to question the future competitive position of the U.S. beef cattle industry, especially when one considers the rate of technology adoption that is occurring in other parts of the world. For instance, the United States and Brazil are world leaders in total number of beef cows in production. Growth in the use of AI

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^c To whom correspondence should be addressed: Department of Animal Science, S132 Animal Science Research Center, University of Missouri, Columbia, MO 65211 (phone: 573-884-4071; fax: 573-882-4798; e-mail: KojimaN@missouri.edu).

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in Brazil outpaced that in the United States by 93 % between 1993 and 1998 (ASBIA, 1998; NAAB, 1998). In Red Angus alone, domestic semen sales increased 17 % from 1997 to 2000, whereas semen sales to Brazil increased more than 100 % during the same time period (Hough, 2002). Total domestic sales of beef semen, however, have been relatively unchanged, approximately between 700,000 to 1,200,000 units, from 1980 to 2000 (Hough, 2002; Note: the Red Angus Association is the only breed organization that has publicly presented international semen sale data). Beef producers in Brazil are inseminating 3.5 times more cows annually than U.S. producers, based on the sale of imported and domestic beef semen. Furthermore, nearly one-half of the semen used in Brazil is imported, a large portion coming from the United States. Given this scenario, it is reasonable to assume that in the years ahead, elite seedstock herds in the U.S. will provide a sizable percentage of the genetics used worldwide. Unless commercial beef producers in the U.S. begin to aggressively approach reproductive and genetic improvement within their herds, one could argue that this country will lose its competitive advantage in the production of high-quality beef (reviewed by Patterson et al., 2000).

Facilitating the widespread use of estrous synchronization and AI in the U.S. necessitates development of effective educational materials for commercial beef producers (Kojima and Patterson, 2002). A better understanding of the physiology and endocrinology of the estrous cycle will improve reproductive management of beef cattle and facilitate the successful application of estrous synchronization protocols.

Discussion

Secretion of Gonadotropins and Gonadal Steroids During the Estrous Cycle

The reproductive cycle in the adult female consists of all processes leading to conception, gestation and birth of offspring. The average length of the estrous cycle of cattle is 21 d, and normally ranges from 17 to 24 d (Wishart, 1972; Salisbury et al., 1978). Estrus lasts 12 to 18 h (Roberts, 1956), and ovulation occurs 25 to 30 h after the onset of estrus (Christenson et al., 1975; Bernard et al., 1983). The estrous cycle generally is divided into luteal and follicular phases. During these two phases of the estrous cycle, gonadotropins influence gametogenesis, folliculogenesis, ovulation, corpus luteum function and steroidogenesis in the ovary. Regulation of gonadotropin secretion involves an exquisite balance between complex hormonal interactions in the hypothalamic-pituitary-gonadal axis which, in turn, regulates development and regression of ovarian follicles and corpora lutea.

Endocrine and neuroendocrine mechanisms regulate secretion of the gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), from the gonadotrophs of the anterior pituitary. Release of LH from the anterior pituitary occurs in response to gonadotropin-releasing hormone (GnRH) secreted by the hypothalamus (Schally et al., 1971). Pulses of LH occur in synchrony with pulses of GnRH from the neurons of the hypothalamus of sheep (Clarke and Cummins, 1982) and cattle (Rodriguez and Wise, 1989). Primary factors modulating changes in the pulsatile secretion of LH are the steroid hormones from the ovary that function at the hypothalamic-pituitary axis during the estrous cycle of cattle (Rahe et al., 1980; Ireland and Roche, 1982a). The extensive feedback mechanisms by ovarian steroids are, therefore, associated with the neuroendocrine function of the hypothalamus and endocrine function of the pituitary to regulate ovarian functions during the estrous cycle.

Concentrations of Gonadotropins in Circulation: Luteinizing Hormone (LH). Rahe et al. (1980) characterized the changes in the pattern of LH in circulation during the estrous cycle of cattle. Pulses of LH were less frequent and of greater amplitude when progesterone was elevated during the mid- to late-luteal phases of the estrous cycle, whereas pulses were of greater frequency and less amplitude during the early luteal and follicular phases of the estrous cycle when greater concentrations of 17 β -estradiol were secreted from the ovarian follicle. The relationship between pulsatile patterns of LH and the typical profile of concentrations of progesterone during the estrous cycle of cattle is presented in Figure 1 (Kojima and Patterson, 2002).

During the mid-luteal phase of the estrous cycle, pulses of progesterone are more frequent than pulses of LH (Hixon et al., 1983). During this period, pulses of LH are released concomitantly with pulses of FSH, and all LH pulses and FSH pulses are secreted either concomitantly with or followed by a pulse of progesterone (Walters et al., 1984). Other reports suggest, however, that there is no apparent relationship between concentrations of progesterone and pulsatile release of LH in cows (Rhodes et al., 1995), since pulses of LH and secretion of progesterone are not associated during the early luteal phase of the estrous cycle. Peters et al. (1994) reported that stimulation by LH pulses is required for the normal progesterone production by corpora lutea from d 2 to 12, but not from d 12 to 17 of the estrous cycle of cattle. Furthermore, Quintal-Franco et al. (1999) reported that LH pulses are necessary for both structural development (corpora lutea with typical diameter) and normal function (production of progesterone) of corpora lutea 48 h before the time of preovulatory surge of LH (d 0) to d 7 of the estrous cycle in cattle.

In addition to changes in the frequency of LH pulses, mean concentrations and pulse amplitude of LH also change during the luteal phase of the estrous cycle (Schams et al., 1977; Cupp et al., 1995b). Mean concentrations of LH are fairly constant during the early- to mid-luteal phase of the estrous cycle (d 0 to 10) after ovulation (Cupp et al., 1995b). Increases in amplitude of LH pulses are observed between d 8 and 11 of the estrous cycle (Cupp et al., 1995b). Subsequently, mean concentrations and pulse amplitude of LH decline until the time of luteolysis around d 18 to 19 (Schams et al., 1977; Cupp et al., 1995b).

During the follicular phase of the estrous cycle, which ranges 3 to 5 d in length, mean concentrations of LH increase linearly (Chenault et al., 1975; Imakawa et al., 1986) with an increase in pulse frequency (Imakawa et al., 1986). Increases in LH pulse frequency stimulate follicular maturation, causing the ovarian follicle to secrete pulses of 17 β -estradiol with greater amplitude which results in an increase in circulating concentrations of 17 β -estradiol that increase simultaneously with the decline in concentrations of progesterone (Schallenberger, 1984; Walters et al., 1984). Greater concentrations of 17 β -estradiol, in turn, enhance the amplitude of LH pulses (Stumpf et al., 1989) and is clearly the stimulus for the surge release of LH (Walters et al., 1984; Stumpf, et al., 1991). The preovulatory surge of LH occurs at or near the onset of estrus in the cow (Swanson and Hafs, 1971; Chenault et al., 1975; Schams et al., 1977) with a duration of 7 to 10 h (Chenault et al., 1975; Britt et al., 1981; Kojima, 1991). Ovulation occurs 25 to 35 h following the onset of the preovulatory surge of LH (Chenault et al., 1975; Christenson et al., 1975; Bernard et al., 1983). The preovulatory surge of gonadotropins is necessary for completion of the first meiotic division of the oocyte, final follicular maturation, and subsequent ovulation.

Regulation of Luteinizing Hormone (LH) Secretion. An intricate relationship exists between circulating concentrations of progesterone, frequency of LH pulses, and secretion of 17 β -estradiol from ovarian follicles in cattle. Ovariectomy of cows results in greater concentrations of LH in circulation and an enhanced frequency of LH pulses (Hobson and Hansel, 1972; Short et al., 1973;

Beck et al., 1976; Schallenberger and Peterson, 1982). During the mid- to late luteal phases of the estrous cycle, when greater amounts of progesterone are secreted from the corpus luteum (Wettemann et al., 1972; Echternkamp and Hansel, 1973; Walters et al., 1984), LH pulse frequency is relatively low compared to the early luteal phase (Rahe et al., 1980), and relatively low concentrations of 17 β -estradiol are secreted from the ovary compared to other phases of the estrous cycle (Echternkamp and Hansel, 1973; Walters et al., 1984; Cupp et al., 1995b). Administration of progesterone to ovariectomized cows suppresses pulsatile secretion of LH (Beck et al., 1976; Stumpf et al., 1993). Varying concentrations of progesterone during the luteal phase of the estrous cycle result in alterations in the LH pulse frequency (Ireland and Roche, 1982a). As a consequence of the inhibitory effect of progesterone on pulsatile secretion of LH, the final stages of maturation of an ovulatory follicle are suppressed during the luteal phase of the estrous cycle. Administration of physiological concentrations of 17 β -estradiol in the absence of progesterone increases mean concentrations (Cupp et al., 1995a) and amplitude of LH pulses (Kinder et al., 1991). Whereas bolus administration of 17 β -estradiol (supraphysiological concentration) has a biphasic effect on LH secretion, pulsatile secretion of LH is initially inhibited for several hours followed by a preovulatory-like LH surge in cattle (Kesner et al., 1981). A combination of progesterone and 17 β -estradiol is more effective in inhibiting pulsatile secretion of LH than administration of either alone (Beck et al., 1976; Stumpf et al., 1993). The combined effects of gonadal steroids and the presence of the corpus luteum, therefore, appear to be responsible for the decreased frequency of LH pulses observed during the luteal phase of the bovine estrous cycle (Roberson et al., 1989; Kojima et al., 1992; Stumpf et al., 1993). This indicates a role for 17 β -estradiol in modulating LH secretion during the luteal phase of the estrous cycle. The ovarian steroids, progesterone and 17 β -estradiol regulate LH secretion by working independently or in concert during the estrous cycle of cattle (Beck et al., 1976; Kinder et al., 1991; Stumpf et al., 1993).

Concentrations of Gonadotropins in Circulation: Follicle-Stimulating Hormone (FSH).

Follicle-stimulating hormone (FSH) has been implicated to have an important role in the control of ovarian follicular recruitment and development in cattle (reviewed by Ireland, 1987), although the temporal relationship between circulating concentrations of FSH and recruitment and development of ovarian follicles is not completely understood.

Circulating concentrations of LH and FSH are not coupled during the follicular phase to the early luteal phase of the bovine estrous cycle, suggesting that these times may be critical for the proper stimulation of follicular development by appropriate concentrations of each gonadotropin. Following luteolysis, increasing mean concentrations and pulse frequency of LH (Rahe et al., 1980) are accompanied by decreased concentrations of FSH (Butler et al., 1983; Schallenberger et al., 1984). Concentrations of FSH in circulation during the follicular phase of the estrous cycle are less than during the luteal phase until the time of the preovulatory surge of FSH (Goodman et al., 1981). Before and during the preovulatory surge of gonadotropin, pulses of LH are almost always secreted concomitantly with pulses of FSH (Akbar et al., 1974; Rahe et al., 1980) and the surges of LH and FSH are apparently composed of very frequent pulses of higher amplitude (Rahe et al., 1980). Basal concentrations and pulse amplitudes of FSH increase 4 to 12 h after the preovulatory surge, resulting in a distinct secondary surge of FSH (Dobson, 1978; Peters et al., 1981; Ireland and Roche, 1982b), whereas pulses of LH are not detected for 4 to 12 h after the preovulatory surge (Walters and Schallenberger, 1984). A relationship between the secretory pattern of FSH and ovarian follicular development during the estrous cycle is discussed in the later section.

Regulation of Follicle-Stimulating Hormone (FSH) Secretion. The most potent inhibitor of FSH secretion in cattle is thought to be 17 β -estradiol. Removal of gonads causes a well-described increase in circulating concentrations of FSH in all mammalian species (Wettemann et al., 1972; Butler et al., 1983; Pierson and Ginther, 1986; NAHMS, 1998). Administration of 17 β -estradiol at a supraphysiological concentration resulted in decreased FSH secretion in ovariectomized heifers (Butler et al., 1983; Kesner and Convey, 1982). A combined administration of luteal phase levels of 17 β -estradiol and progesterone to ovariectomized heifers resulted in concentrations of FSH similar to those detected during the luteal phase of the estrous cycle (Price and Webb, 1988). A negative correlation between concentrations of estradiol and FSH was also observed during the follicular phase of the estrous cycle in cattle (Butler et al., 1983; Quirk and Fortune, 1986; Kaneko et al., 1991; Sutherland et al., 1994).

Administration of charcoal-stripped bovine follicular fluid results in suppressed concentrations of FSH in ovariectomized or intact heifers (Ireland et al., 1983; Quirk and Fortune, 1986; Beard et al., 1989; Beard et al., 1990). These effects are most likely attributable to a gonadal glycoprotein hormone, inhibin, which is secreted from granulosa cells of the follicle and selectively inhibits synthesis and release of FSH by the anterior pituitary (de Jong and Sharpe, 1976; Henderson and Franchimont, 1981; Ying, 1988; Beard et al., 1990). Beard et al. (1990) demonstrated that highly purified bovine inhibin has the ability to suppress FSH secretion in ovariectomized heifers. Active immunization of heifers against synthetic peptides of the bovine inhibin α subunit (Glencross et al., 1992; Morris et al., 1993; Scanlon et al., 1993; Wood et al., 1993; Glencross et al., 1994) and against ovine recombinant inhibin α subunit (O'Shea et al., 1994) has been found to increase the number of ovulations. These observations suggest that inhibin is involved in the regulation of FSH secretion in intact cattle.

While inhibin and 17 β -estradiol are probably involved in the control of FSH release, their relative importance was not clear until recent studies utilized passive immunoneutralization of inhibin to evaluate the short-term effect of inhibin on FSH secretion. Passive immunoneutralization of inhibin during the mid-luteal phase of the estrous cycle in cows produced a marked increase in concentrations of FSH, indicating that inhibin has an important role in the regulation of FSH secretion when 17 β -estradiol secretion is low (Kaneko et al., 1993). The same procedure during the early luteal phase of the estrous cycle in cows increased FSH secretion and stimulated follicular growth and 17 β -estradiol secretion (Kaneko et al., 1997). Passive immunoneutralization of inhibin during the follicular phase of the estrous cycle in cows resulted in a drastic increase in concentrations of FSH with a coincident increase in the number of antral follicles, providing clear evidence that inhibin is an important regulator for FSH secretion during this period (Kaneko et al., 1995). These observations suggest that inhibin is a potent regulator of FSH secretion even in the presence of high concentrations of 17 β -estradiol and progesterone. Collectively, inhibin is of physiological significance in the regulation of FSH secretion at any stage of the estrous cycle in cattle (Kaneko et al., 1993; Kaneko et al., 1997).

Recently, several other factors have been implicated as selective regulators of FSH secretion. Activin, a functional opposite of inhibin, enhances FSH secretion (Ying, 1988). Follistatin, a binding protein of activin and inhibin, neutralizes activin but not inhibin action (Robertson, 1992). Studies indicated that activins, inhibins, and follistatins are produced not only at the gonad but also at the pituitary and, therefore, have the potential to exert local modulatory effects on FSH secretion (reviewed by Knight and Glister, 2001; Padmanabhan and McNeilly, 2001). The negative feedback mechanisms regulating FSH secretion in domestic animals involve very complex and delicate mechanisms and are not completely understood.

Concentrations of Progesterone in Circulation. Concentrations of progesterone in circulation are low immediately after ovulation through the early luteal phase (d 2 to 3; d 0 = estrus), then begin to increase by d 4 of the estrous cycle coincident with an increase in the corpus luteum weight. Luteal concentrations of progesterone are maximal by d 7 to 12 of the estrous cycle (Donaldson and Hansel, 1965). Concentrations of progesterone are maintained at relatively greater levels throughout the remainder of the luteal phase of the estrous cycle until luteolysis (d 18 to 19 or 2 to 3 d before estrus). With the regression of the corpus luteum (luteolysis), concentrations of progesterone in circulation decline rapidly and are low throughout the follicular phase as compared to the luteal phase of the estrous cycle (Wetteman et al., 1972; Echterkamp and Hansel, 1973). The typical profile of concentrations of progesterone in blood during the estrous cycle of cattle is depicted in Figure 2 (Kojima and Patterson, 2002).

Secretion of progesterone from the corpus luteum appears to occur in a pulsatile fashion during the early to mid-luteal phases of the estrous cycle of cattle (Schallenberger et al., 1984; Walters and Schallenberger, 1984). During the early to mid-luteal phase of the estrous cycle, pulse amplitude and basal concentrations of progesterone are at their greatest. Frequency of progesterone pulses is greater during the early luteal phase and declines during the mid-luteal phase of the estrous cycle.

Concentrations of 17 β -Estradiol in Circulation. Increasing serum concentrations of 17 β -estradiol by an increase in both frequency and amplitude of 17 β -estradiol pulses from the ovary were observed during the early luteal phase of the estrous cycle, corresponding to the greater frequency of LH pulses (discussed in the next section) observed during this same period (Walters et al., 1984). Generally, greater secretion of 17 β -estradiol is associated with a greater frequency of LH pulses (McNeilly et al., 1982; Walters et al., 1984; Kojima et al., 1992; Kojima et al., 1995; Rhodes et al., 1995), supporting the conclusion of Walters et al. (1984) that 17 β -estradiol production is regulated by the frequency of LH pulses. The increasing concentrations of circulating 17 β -estradiol in cows during the early luteal phase of the estrous cycle are accompanied by the concurrent appearance of the large estrogen-active follicle in the ovary (Holst et al., 1972; Glencross et al., 1973; Ireland and Roche, 1982b). Concentrations of 17 β -estradiol during the early luteal phase of the estrous cycle are often observed to be comparable to concentrations observed just before the preovulatory surge of LH (Glencross et al., 1973; Walters and Schallenberger, 1984). The preovulatory surge of LH and subsequent ovulation, however, are blocked by the presence of smaller amounts of progesterone in circulation during the early luteal phase (Short et al., 1979).

During the early- and mid-luteal phases of the estrous cycle, all LH/FSH pulses are followed by distinct pulses of 17 β -estradiol (Walters et al., 1984), but pulses of FSH independent of LH pulses are not associated with pulsatile secretion of 17 β -estradiol (Walters et al., 1984). The amplitude of 17 β -estradiol pulses decreases from the early to mid-luteal phase (Walters et al., 1984). Similarly, Rhodes et al. (1995) reported that there is a significant increase in concentrations of 17 β -estradiol following each LH pulse with concentrations being maximal between 15 and 30 min after the peak of LH pulses. The temporal relationship between the release of LH and 17 β -estradiol, therefore, remains unchanged as time progresses from the early to the mid-luteal phases of the estrous cycle, and the amount of 17 β -estradiol secretion is determined by the frequency of the LH pulses in cattle.

During the mid- to late luteal phases of the estrous cycle, concentrations of 17 β -estradiol remain relatively low, but fluctuate throughout the luteal phase (Wetteman et al., 1972; Hansel et al., 1973; Chenault et al., 1975). These fluctuations in circulating concentrations of 17 β -estradiol observed in earlier studies were probably corresponding to the growth of dominant follicles during a wave-like pattern of ovarian follicular development (discussed in the later section) as reported in recent years

(Kaneko et al., 1991; Rhodes et al., 1995). The typical profile of concentrations of 17β -estradiol in blood during the estrous cycle of cattle having three waves of ovarian follicular development is illustrated in Figure 3 (Kojima and Patterson, 2002).

Maximum concentrations of 17β -estradiol are observed following luteolysis, and before and during the preovulatory surge of gonadotropins (Walters and Schallenberger, 1984), where it is closely associated with the onset of behavioral estrus (Chenault et al., 1975; Schams et al., 1977; Swanson and Hafs, 1971). The surge release of gonadotropins is a result of 17β -estradiol being secreted from the preovulatory ovarian follicle in very frequent (≈ 1 pulse/h) pulses of relatively higher amplitude (Walters and Schallenberger, 1984). After the preovulatory surge of gonadotropins, 17β -estradiol declines rapidly (Chenault et al., 1975; Kojima, 1991), and no pulses have been observed in cattle (Walters and Schallenberger, 1984).

The precipitous decline in 17β -estradiol may be important for proper transport of the zygote in the reproductive tract. Greenwald (1967) reported that administration of physiological doses of estrogens accelerate egg transport through the oviduct during the first few days after ovulation in the hamster, rabbit, guinea pig, and rat. Hawk and Bolt (1974) found that in the ewe, the majority of uterine contractions in early estrus travel toward the oviduct, whereas at 48 h post estrus the majority of uterine contractions are directed toward the cervix. Hawk (1974) has also shown that the injection of small amounts of 17β -estradiol after the onset of estrus prevents the change in direction of contractions that normally occurs 36 to 48 h after onset of estrus. Therefore, the rapid decrease in 17β -estradiol after the surge of LH may be necessary for proper synchrony of gamete transport pre- and postovulation. The rapid drop in plasma 17β -estradiol following the preovulatory surge of LH may also be related to other physiological changes in the uterus or vagina such as blood flow (Greiss and Anderson, 1970) and/or thermal changes (Abrams et al., 1971; Abrams et al., 1972; Abrams et al., 1973; Gwazdauskas et al., 1974). The association between uterine temperature and fertility at the time of insemination may be related to changes in endogenous secretion of 17β -estradiol on the day of estrus (Gwazdauskas et al., 1973).

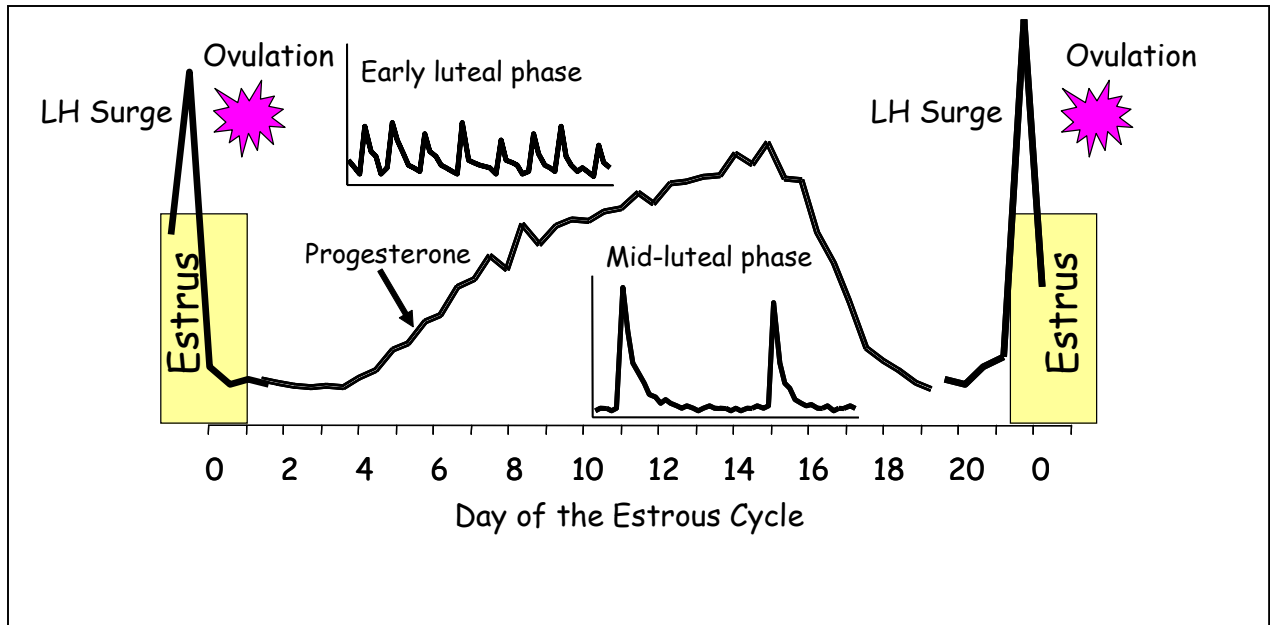


Figure 1. A relationship between pulsatile patterns of LH (solid line) and the typical profile of progesterone (double line) concentrations during the estrous cycle of cattle (modified from Kojima and Patterson, 2002).

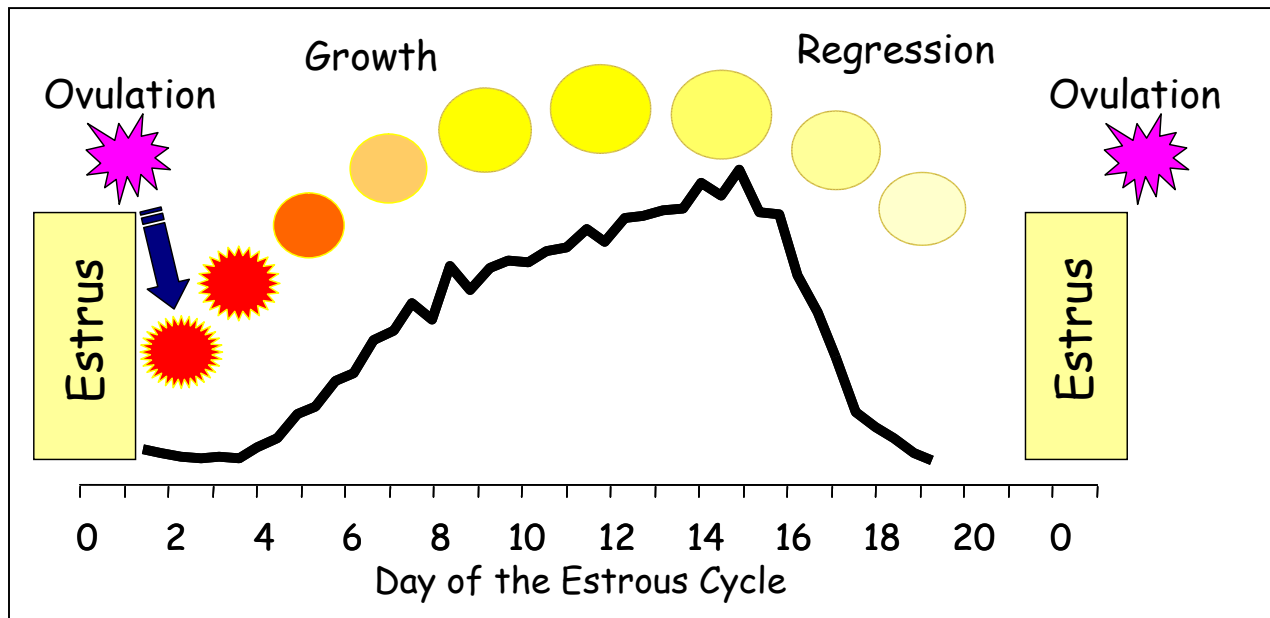


Figure 2. The typical profile of progesterone concentrations and schematic of corpus luteum growth and regression during the estrous cycle of cattle (modified from Kojima and Patterson, 2002).

Ovarian Follicular Development

Folliculogenesis. Folliculogenesis is defined as the formation of Graafian (mature or preovulatory) follicles from a pool of primordial follicles. The primordial follicles are established in

the ovary during embryonic development. During the lifetime of the female, primordial follicles enter a pool of growing follicles in response to a stimulus that remains undefined. Early follicular development up to a certain stage (antral formation in rodents and after antral formation in humans and ruminants) occurs continuously under basal hormonal conditions and this stage of development is probably independent of gonadotropin support (Fortune, 1994; Greenwald and Roy, 1994). Further follicular growth requires specific gonadotropin support. Two terms, recruitment and selection, have been used to describe the late stages of follicular development. Recruitment is the process by which a cohort of follicles is stimulated to grow beyond the early stage. From among this cohort, one (in monotocous species) or more (in polytocous species) follicles are selected for continued growth and ovulation, whereas the remaining follicles in the cohort regress (Greenwald and Roy, 1994). The development of large follicles has been found to be a dynamic process, in which large follicles appear on the surface of the ovary, then regress and are replaced by other large follicles. In some species (rodents, primates, and pigs), recruitment and selection occur only during the follicular phase, whereas in other species (cattle and sheep), ovulatory-size follicles develop throughout the estrous cycle (Fortune, 1994).

Dynamics of Follicular Development in Cattle: Follicular waves. Normal ovarian function depends on the continual process of growth and development of follicles to ensure the presence of a follicle capable of ovulation during each estrous cycle. From the classic histological work, Rajakoski (1960) was the first to suggest that ovarian follicular growth during the estrous cycle of the cow occurred as at least two waves of development. Matton et al. (1981) later supported this hypothesis by direct follicle-marking studies. A follicle starts to grow a few days after estrus (d 0 = estrus), reaches an ovulatory size of more than 10 mm, and subsequently undergoes atresia between d 6 to 12 of the estrous cycle. The second wave of follicular development begins around d 12 to 14 of the estrous cycle; the dominant follicle grows to ovulatory size during the follicular phase of the estrous cycle following luteolysis (around d 18 to estrus), and ovulates on d 1 of the following estrous cycle (Rajakoski, 1960; Matton et al. 1981). This hypothesis was subsequently supported by the observations of Swanson et al. (1972) and Ireland et al. (1979).

The use of real-time ultrasonography has unequivocally demonstrated that development of an ovulatory or dominant follicle from the small antral stage during the bovine estrous cycle is characterized by a wave-like pattern of follicular development, referred to as "follicular waves" (Pierson and Ginther, 1984; Pierson and Ginther, 1986; Quirk et al., 1986; Pierson and Ginther, 1987a; Pierson and Ginther, 1987b; Fortune et al., 1988; Sirois and Fortune, 1988; Ginther et al., 1989a; Ginther et al., 1989b; Ginther et al., 1989c; Knopf et al., 1989; Sirois and Fortune, 1990). Each wave occurs in several distinct phases: "Recruitment", the entrance of a cohort of small antral follicles into the acutely gonadotropin dependent stage; "Selection", a reduction of the number of recruited excess follicles to one follicle (i.e. the number that would normally ovulate); and "Dominance", the inhibition of additional follicular recruitment and development by the selected follicle, which escapes initial atresia and continues to grow until ovulation or atresia (Goodman and Hodgen, 1983; Ireland and Roche, 1987; Lucy et al., 1992; Fortune, 1994).

Turzillo and Fortune (1990) reported that treatment with charcoal-extracted bovine follicular fluid from 12 to 60 h after the onset of behavioral estrus abolishes the latter part of the secondary surge of FSH, resulting in a delay in the emergence of the first wave of ovarian follicular development in the estrous cycle of cattle. From these results, Turzillo and Fortune (1990) suggested that the secondary surge of FSH may have an important role in regulating the pattern of ovarian follicular development in cattle. Adams et al. (1992) suggested that there is a temporal

relationship between surges of FSH and the emergence of follicular waves; a surge of FSH was detected 1 to 2 d before the emergence of a new wave of follicles and may be necessary for the emergence of a wave of ovarian follicular development. Similarly, Rhodes et al. (1995) reported that concentrations of FSH during the plateau phase of the first wave of ovarian follicular growth of the estrous cycle of cattle are greater than during the regression phase. Sunderland et al. (1994) also reported that the selection periods of follicular growth, during d 1-3 (first wave) and d 10-12 (second wave) of the estrous cycle, are preceded by a transient rise in circulating concentrations of FSH.

Cupp et al. (1995b) have performed an intensive evaluation of changes in concentrations of FSH during the luteal phase of the bovine estrous cycle and also detected subtle fluctuations in concentrations of FSH. Concentrations of FSH change during the luteal phase of the bovine estrous cycle with increases occurring around the time of the emergence of new waves of ovarian follicular development (Adams et al., 1992; Rhodes et al., 1995) or selection periods for developing follicles (Sunderland et al., 1994). These results suggest that increases in circulating concentrations of FSH initiate the development of follicular growth in cattle, but further investigation is required to elucidate the exact function of FSH secretion during the emergence or selection of ovarian follicles. The relationship between secretion profile of FSH and ovarian follicular development during the estrous cycle of cattle having three follicular waves is depicted in Figure 4 (Kojima and Patterson, 2002).

The fate of a dominant follicle is dependent upon whether it is exposed to endogenous or exogenous concentrations of luteinizing hormone (LH) sufficient to induce ovulation. The absence of a preovulatory surge of LH results in atresia of the dominant follicle and reinitiation of follicular recruitment. Studies utilizing ultrasonography to monitor follicular dynamics in cattle indicate that 2 to 4 follicular waves occur during each estrous cycle, with animals predominantly having 2 or 3 waves (Savio et al., 1988; Sirois and Fortune, 1988; Ginther et al., 1989a; Ginther et al., 1989b). In cattle having two waves of ovarian follicular development during the estrous cycle, the first wave begins between d 3 and 4, and the second wave (from which develops the ovulatory follicle) starts between d 12 and 14 (Pierson and Ginther, 1984; Pierson and Ginther, 1986; Pierson and Ginther, 1987a; Pierson and Ginther, 1987b). For cattle with three waves of follicular development, waves of follicles occur at an average interval of 7 d: the first, second, and third waves starting, on the average, on d 2, 9, and 16 of the estrous cycle, respectively. The preovulatory follicle is not the largest follicle until the day of estrus (Quirk et al., 1986; Sirois and Fortune, 1988). Cows exhibiting three waves of follicular development have an estrous cycle of approximately 20 to 23 d, while cows having two waves of follicular development have a slightly shorter estrous cycle of approximately 18 to 20 d in both dairy (Ginther et al., 1989c; Taylor and Rajamahendran, 1991) and beef cattle (Kojima et al., unpublished observations: University of Missouri, 1998).

The growth rate of the dominant ovulatory follicle of the third wave is greater than (Kojima et al., 1994) or not different (Sirois and Fortune, 1988) from that of the nonovulatory follicle of the first wave. In contrast, the dominant nonovulatory follicle of the second wave may have a slower growth rate and smaller maximum size compared with follicles of other waves (Fortune et al., 1988; Sirois and Fortune, 1988). Differences observed in the second dominant follicles may be in response to changing secretory pattern of LH pulses caused by higher concentrations of progesterone during the mid-luteal phase of the estrous cycle (Figure 1). Sirois and Fortune (1988) have also suggested that the length of the follicular phase of the estrous cycle is influenced by the size of the preovulatory follicle at the beginning of this period. Heifers with a small preovulatory follicle at the beginning of the follicular phase had a longer interval from the time progesterone reached less than

1 ng/ml to the preovulatory surge of LH than heifers with a larger preovulatory follicle. This observation supports the previous findings of Scaramuzzi et al. (1980) and Macmillan and Henderson (1984) who reported that the size, and presumably the degree of maturity, of the largest follicle present at the time of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)-induced luteolysis influenced the interval from $PGF_{2\alpha}$ injection to estrus. The length of the follicular phase may be associated with differences observed in the length of the estrous cycle between cattle having either two or three waves of follicular development.

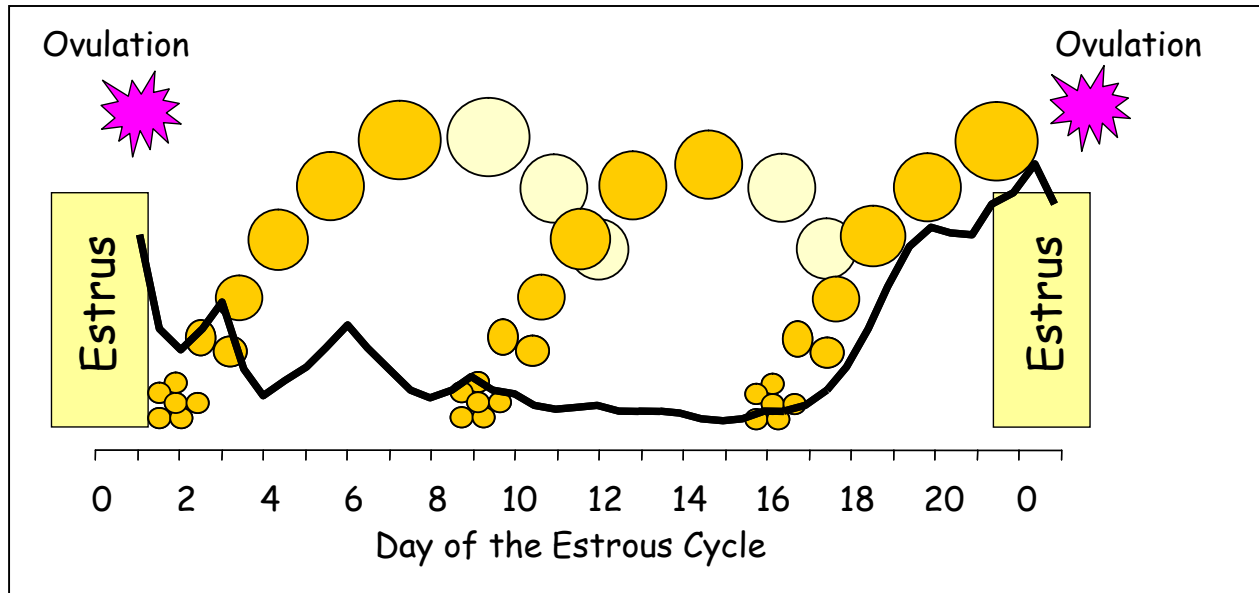


Figure 3. The typical profile of 17β -estradiol concentrations and schematic of ovarian follicular growth and regression during the estrous cycle of cattle having three waves of follicular development (modified from Kojima and Patterson, 2002).

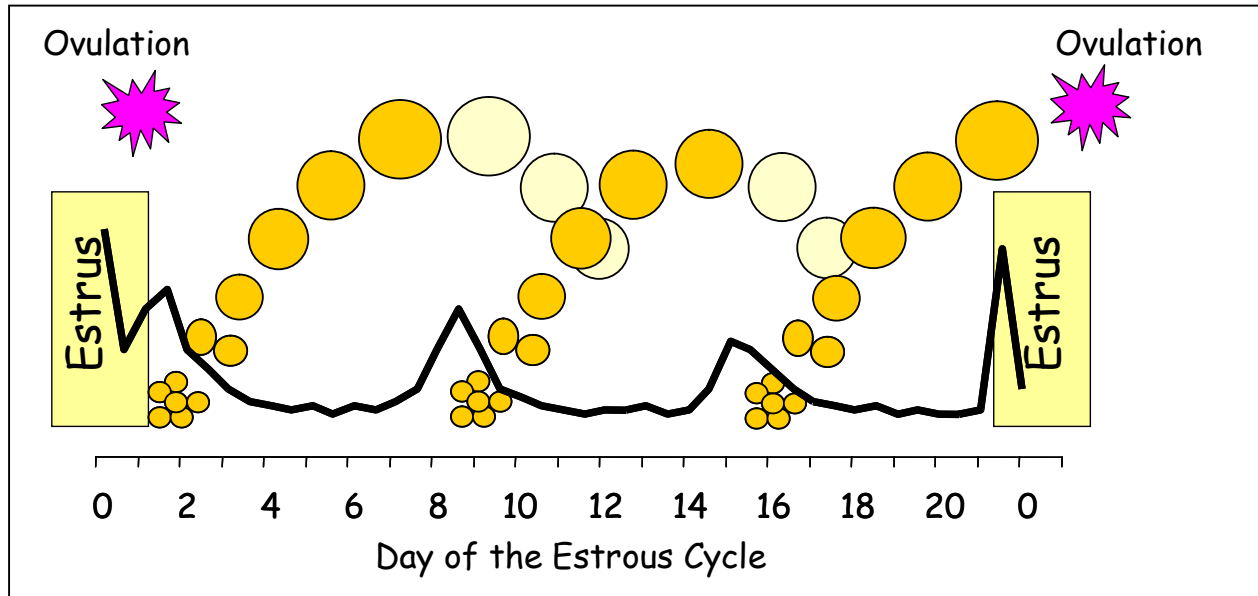


Figure 4. A relationship between secretion profile of FSH and schematic of ovarian follicular growth and regression during the estrous cycle of cattle having three waves of follicular development (modified from Kojima and Patterson, 2002).

The Corpus Luteum

Morphology. During the luteal phase of the estrous cycle, progesterone is the predominant ovarian steroid secreted from the corpus luteum. The corpus luteum of sheep and cows is composed primarily of luteinized granulosa and thecal cells (Rothchild, 1981; Fritz and Speroff, 1982). The primary function of the corpus luteum is the production of progesterone which prepares the uterine endometrium for implantation and maintains early pregnancy. If fertilization does not occur, ovulatory cycles resume only after luteal function ceases. Another important role of the corpus luteum is the blocking of ovulation.

The classic study by Hammond in 1927 described distinct changes in morphology of the corpus luteum during the estrous cycle of the cow. During the early luteal phase (d 1 to 4 of the estrous cycle [d 0 = estrus]), the point of follicular rupture remains uncovered by epithelium and the corpus luteum appears red, occasionally filled with blood. Internally, cells are loosely organized (referred to as *Corpus Hemorrhagicum*). After the point of rupture has closed, the apex of the corpus luteum remains red or brown and remainder of corpus luteum is orange during the early to mid-luteal phases (d 5 to 10 of the estrous cycle). The corpus luteum rapidly increases weight and progesterone content during this period. During the mid-luteal phase (d 11 to 17 of the estrous cycle), a vascular network appears on the surface of corpus luteum, and the bisected corpus luteum appears bright orange or yellow (referred to as *Corpus Luteum*). During the late luteal phase (d 18 to 20 of the estrous cycle), the vascular network on the surface of the corpus luteum disappears and the corpus luteum appears fading yellow to white (referred to as *Corpus Albicans*). At this point, bisection of the corpus luteum results in no bleeding, whereas bisection at all other phases results in bleeding (Ireland et al., 1980; McNutt, 1924).

The corpus luteum of many species, including the sheep (Warbritton, 1934; Fitz et al., 1982; Rogers and O'Shea, 1982) and cow (Foley and Greenstein, 1958; Donaldson and Hansel, 1965; Koos and Hansel, 1981), is formed by two morphologically and biochemically distinct

steroidogenic cell types, termed large and small luteal cells. These two types of cells appear to have a different origin. Large luteal cells are derived from granulosa cells of the preovulatory follicle, whereas small luteal cells develop from the theca internal cells and then differentiate into large luteal cells as the corpus luteum progresses through its life cycle (Donaldson and Hansel, 1965; McClellan et al., 1975; O'Shea et al., 1980; Alila and Hansel, 1984). The differentiation of small luteal cells into large luteal cells is induced by LH (Farin et al., 1988; Farin et al., 1990). The injection of LH into ewes during the mid-luteal phase of the estrous cycle increased the number of large luteal cells and concomitantly reduced the number of small luteal cells in the ovine corpus luteum. Large luteal cells are almost nonexistent in corpora lutea early in the ovine estrous cycle (d 4), but as the estrous cycle progresses (d 8 and 12), the number of large luteal cells increases markedly. Small luteal cells also increase in number as the corpus luteum matures, but not to the same degree as large luteal cells (Farin et al., 1986; Farin et al., 1989). The large:small cell ratio changes constantly from early (1:3.7), mid (1:3.0), and late (1:2.0) luteal phase of the ovine estrous cycle (Schwall et al., 1986; Harrison et al., 1987).

Prostaglandin F_{2α} and Luteolysis. Prostaglandin F_{2α} (PGF_{2α}) is responsible for luteolysis in domestic animals. In sheep (McCracken et al., 1972; McCracken et al., 1981) and cattle (Hixon and Hansel 1974), PGF_{2α} is synthesized in the uterus and secreted into the circulation, where via local counter current exchange it is transported to the ovary. Uterine PGF_{2α} can also be transferred from uterine lymphatic vessels to the adjacent ovary and ovarian vein in sheep (Heap et al., 1985).

Administration of PGF_{2α} by various routes (intrauterine, intra-muscular, or subcutaneous) can induce functional and morphological regression of the corpus luteum in domestic animals. In cattle, this induction can occur between d 5 and 18 of the estrous cycle. Active immunization against PGF_{2α} results in cessation of the normal luteolytic mechanism in cyclic ewes (Scaramuzzi et al., 1973) and prevents premature regression of the first postpartum corpus luteum in cows (Copelin et al., 1989). These observations strongly suggest that PGF_{2α} of uterine origin has a definite physiological role in regulating luteal function in ruminants.

Measurement of PGF_{2α} or its major circulating metabolite, 13,14-dihydro-15-keto-PGF_{2α} (PGFM), in peripheral or utero-ovarian venous blood in ewes (Thorburn et al., 1973; Baird et al., 1976; Peterson et al., 1976) and cows (Fairclough and Payne, 1975; Kindahl et al., 1976; Guilbault et al., 1984; Cooper et al., 1991) has revealed pulses of secretion during luteolysis with a concomitant decrease in circulating progesterone. Cooper et al. (1991) suggested that the measurement of PGFM may not be adequate to detect all subtle changes in PGF_{2α} in certain physiological states in cows. Only 31% of [³H]PGF_{2α} is present as PGFM after one pulmonary passage in the cow (Davis et al., 1985) and after three circulations, 16% of the injected PGF_{2α} remains unmetabolized. Concentrations of PGFM have been utilized as an indicator of uterine release of PGF_{2α} in the cow by several investigators (Kindahl et al., 1976; Guilbault et al., 1984). The rate of pulmonary metabolism of PGF_{2α} to PGFM is lower in cows than in ewes (Davis et al., 1979; Milvae and Hansel, 1980; Davis et al., 1985) and the half-life of PGFM (Kindahl et al., 1976; Meyer et al., 1989) is longer than that of PGF_{2α} (Ferreira and Vane, 1967).

Conclusions

The percentage of beef cattle inseminated artificially is predicted to substantially increase with the advent of sexed semen (Seidel, 1998). However, surveys in 1994 indicate that fewer

than 5 % of the beef heifers and cows in the U.S. are bred by AI and only half of the cattlemen that practice AI use any form of estrous synchronization to facilitate their AI programs (NAHMS, 1994). The inability to predict time of estrus for individual females in a group often makes it impractical to use AI because of the labor required for estrus detection (NAHMS, 1998). Expanded use of AI and/or adoption of emerging reproductive technologies for beef heifers and cows require precise methods of estrous cycle control. Effective control of the estrous cycle requires the synchronization of both luteal and follicular functions. A better understanding of the physiology and endocrinology of the estrous cycle, therefore, will improve reproductive management of beef cattle and facilitate the successful application of estrous synchronization protocols. The development of an economical method of artificially inseminating beef heifers and cows at a fixed time with high fertility, eliminating the need to detect estrus, would result in a dramatic increase in the adoption of AI in the U.S. beef herds.

Implications

A better understanding of physiology and endocrinology of the estrous cycle will improve reproductive management of beef cattle and facilitate the successful application of estrous synchronization protocols. This, in turn, will increase use of AI and hasten genetic improvement in beef cattle production systems in the United States.

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