Physiology of the Estrous Cycle: The Why's and How's of Controlling Estrus and Ovulation

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Introduction

During the last 20 years, our understanding of reproductive processes in the cows has increased tremendously. With the advent of laboratory techniques such as the radioimmunoassay, detection of minute quantities of reproductive hormones and their receptors in blood serum, plasma, tissue, and milk has become possible. Precise and specific assays of hormones allow us to describe patterns of hormonal secretion and changes in specific hormone receptors during the estrous cycle. Since the discovery and synthesis of hypothalamic peptides, including gonadotropin-releasing hormone (GnRH) that induces the secretion of the pituitary gonadotropins, luteinizing hormone (LH), and follicle-stimulating hormone (FSH); and the elaboration of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$ from the uterus as the putative luteolysin in cattle), reproductive physiology emerged as a major discipline in Animal and Veterinary Sciences. Only recently have we been able to understand the interaction of FSH, ovarian follicular dynamics, and the turnover of dominant follicles during the estrous cycle via use of transrectal ultrasonography as it relates to timing of estrus after $PGF_{2\alpha}$ treatments. We have at our disposal several of the necessary tools to understand more fully the physiologic controls of reproduction in the bovine.

Mini-Review of the Estrous Cycle[1]

The cow is a polyestrous mammal undergoing estrous cycles of approximately 21 to 22 days. Once puberty occurs, estrous cycles continue unless interrupted by pregnancy (greatest cause of anestrus in the bovine), in which case the cycles resume at variable intervals after parturition depending on management of the cow (suckled versus milked), body condition, nutritional status and energy balance, parity or age and, to a lesser degree, season of the year (Short et al., 1990; Stevenson et al., 2003).

The period of estrus or sexual receptivity may last from 2 to 50 hours, but more typically averages 12 to 18 hours in duration. The day of estrus usually is referred to as day 0, with ovulation occurring 24 to 30 hours after the onset of estrus or on day 1 of the cycle.

The cyclic nature of the estrous cycle occurs as a result of repeated changes in follicular development in the ovaries, ovulation and formation of a corpus luteum, a temporary structure that is maintained only if a pregnancy is established. All is intricately controlled by a complex of mechanisms involving the interaction of hormones secreted by the hypothalamus, pituitary, ovary, and uterus.

Waves of follicular growth occur throughout the bovine estrous cycle. Either two, three, or four waves of follicular growth occur (Ginther et al., 1989; Pursley et al., 1993; Fortune, 1994). An example of one cow with two follicular waves (most common) is illustrated in Figure 1. Each

[1] The reader is referred to an excellent earlier review by Smith et al. (2010) covering physiologic principles underlying synchronization of estrus in beef cattle.

wave consists of a group (cohort) of follicles that begin to increase in diameter from the 1- to 2-mm size, one of which becomes dominant or largest follicle, by continuing its growth trajectory while the others degenerate and undergo death or atresia. The dominant follicle undergoes three phases of development: growth (increasing diameter); static (little change in diameter); and regression (decreasing diameter [atresia]). The first wave consistently begins around day 1 of the cycle. The second, third, or fourth wave begins at more variable times, with more waves occurring in cows with longer estrous cycles. Any dominant follicle can mature and ovulate if the corpus luteum is regressed at the appropriate time. In other words, if $PGF_{2\alpha}$ were given during the growing phase of the dominant follicle, the dominant follicle could proceed to ovulation. The ovulatory follicle increases in diameter, and matures during estrus, reaching a peak diameter of 12 to 16 mm. The follicle destined to ovulate cannot be identified until a few days before estrus. Ovulation generally occurs about 24 to 30 hours after the onset of estrus or about 6 to 18 hours after the end of estrus.

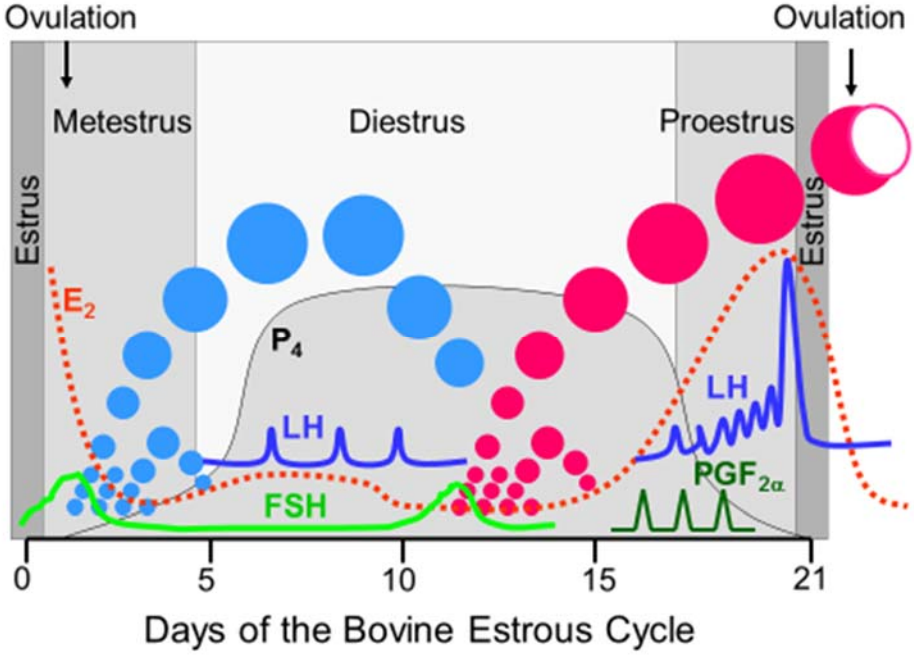

Figure 1. Hormonal characteristics during four stages of the estrous cycle: estrus, metestrus (time of ovulation), diestrus, and proestrus. Blue- and pink-colored circles represent the first and second dominant follicles that develop during the first and second wave of follicle growth, respectively. Increases in follicle-stimulating hormone (FSH) cause waves of follicles to grow resulting in one dominant follicle per wave. Final follicular maturation occurs in the presence of increased pulse secretion of luteinizing hormone (LH). The mature follicle secretes estradiol (E2), which peaks at the onset of estrus, initiates the large increase in LH (preovulatory LH surge) that causes the follicle to ovulate 24 to 30 hours later. After ovulation, the follicle differentiates into a corpus luteum (CL) that secretes progesterone (P4; gray shaded area). Pulses of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) from the uterus initiates death of the corpus luteum in the absence of a viable embryo.

The cavity of the ruptured follicle following ovulation is invaded by proliferating cells of the granulosa and theca layers. Capillaries and the basement membrane are disturbed at ovulation, accounting for some hemorrhage, and thus allowing both capillaries and theca cells to pervade the ruptured follicle. The theca and granulosa cells differentiate (luteinize) into luteal cells that form the corpus luteum (CL; Fitz et al., 1982; Farin et al., 1988). Theca cells seem to develop into smaller-diameter cells, known as small luteal cells. The granulosa cells become larger luteal cells or so-called blossom cells.

Both luteal cell types secrete progesterone, but the small cells seem to have nearly all of the LH receptors and are six times more responsive to LH in vitro than the large luteal cells in terms of progesterone secretion. The small luteal cells contribute about 15% of the progesterone secreted by the corpus luteum, whereas the remainder is derived from the large luteal cells. The large cells, however, have nearly all of the receptors for $PGF_{2\alpha}$ (Braden et al. 1988). Concentrations and affinity of highly specific $PGF_{2\alpha}$ receptors in bovine CL are similar on days 2, 4, 6, and 10 of the estrous cycle (Wiltbank et al., 1995), failing to explain the lack of luteolytic response of the corpus luteum to $PGF_{2\alpha}$ before day 5 or 6 of the cycle.

Various steroid hormones are secreted by the ovary, of which estrogen and progesterone are most important. The principal estrogen is estradiol-17ß, although estrone and estriol also are secreted in lesser concentrations. Plasma concentrations of estradiol are generally low but fluctuate in parallel with the diameter and steroidogenic capabilities of the dominant follicles of the cycle (Figure 1). Concentrations of estradiol begin to rise about 4 days before estrus, reach a peak at the onset of standing estrus. The rise in estradiol is correlated with the increasing diameter of the dominant follicle, probably the only source of the estradiol in the bovine.

Progesterone, the other principal ovarian steroid, is secreted by the CL. Concentrations of progesterone begin to rise on days 3 or 4, reach a peak between days 8 and 16, and then decrease to basal concentrations before the next estrus in response to uterine secretion of $PGF_{2\alpha}$ and in the absence of a viable embryo in the uterus (Figure 2). Progesterone is the dominant steroid secreted from about day 4 until day 17, constituting the diestrous stage of the estrous cycle (Figure 1). Both progesterone and estradiol concentrations can be detected in milk as well as in blood plasma or serum. Concentrations in milk generally parallel those in plasma or serum and can be used to monitor luteal and ovarian function during the cycle. One drawback in monitoring steroids in milk samples, however, is their concentrations reflect cumulative filling of the mammary gland and are static reflections of a dynamic pool of steroids subject to metabolic changes in the peripheral circulation.

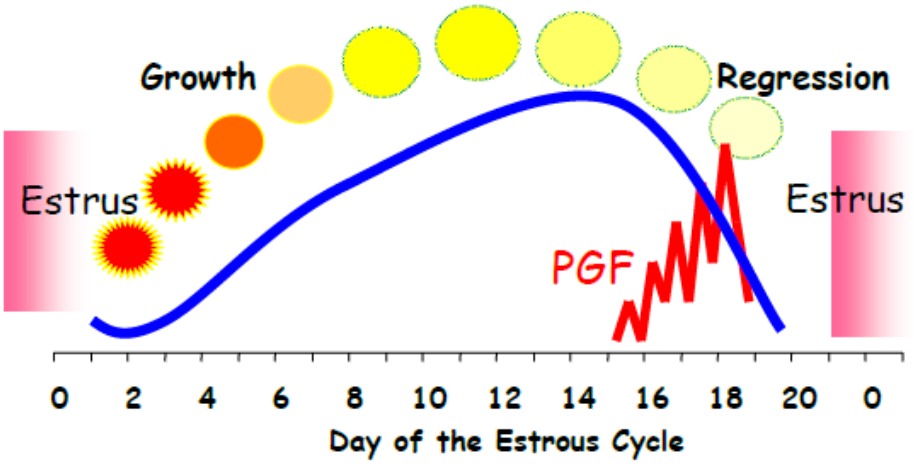

Figure 2. Change in corpus luteum (CL) growth (circular structures), blood concentrations of progesterone and prostaglandin $F_{2\alpha}$ (PGF) during the estrous cycle. Progesterone secretion by the CL inhibits expression of estrus and ovulation. In the absence of a viable embryo, $PGF_{2\alpha}$ is secreted as pulses to cause regression of the CL and rapid decrease in progesterone (Smith et al., 2010).

Role of GnRH in Ovulation

Injection of GnRH will induce a surge of LH secretion from the pituitary (prolonged high concentrations) in the blood stream. The consequences of the GnRH-induced LH release on follicles depends whether a receptive dominant follicle is present (follicle ≥ 10 mm in diameter) and the amount of LH released (Figure 3).

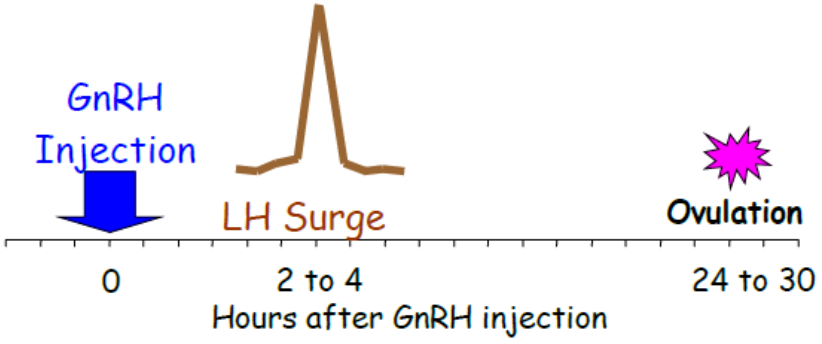

Figure 3. Injection of GnRH (e.g., Cystorelin, Factrel, Fertagyl, OvaCyst, or GONABreed) induces a surge of LH during 2 to 4 hours and ovulation of a viable follicle (≥ 10 mm) between 24 and 30 hours later (Smith et al., 2010).

Generally, a dominant follicle will ovulate in response to GnRH on days 4 through 10 and days 14 through 20 of the cycle (Figure 4), resulting in ovulation. Let's use the example of a standard CO-Synch + CIDR insert program (Figure 5) for further explanation. Generally, three conditions are prominent in suckled beef cows that are exposed to a CO-Synch + CIDR insert program, which is initiated with a GnRH treatment (GnRH-1) and progesterone exposure (intravaginal CIDR insertion).

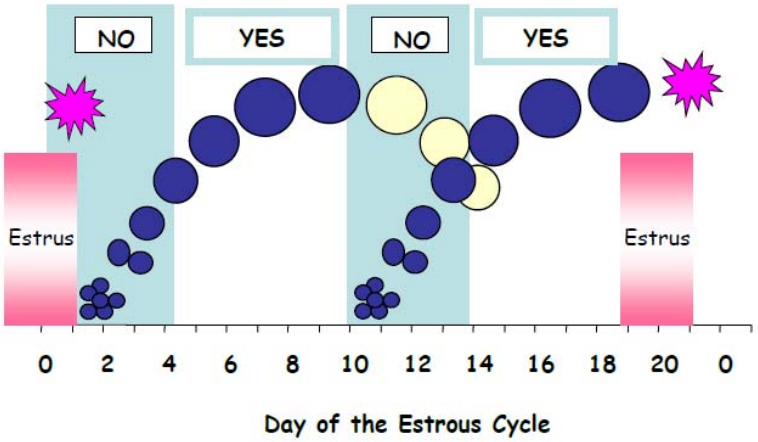

Figure 4. Treatment with GnRH induces ovulation of a dominant follicle (≥ 10 mm) during days 4 through 10 (yes) and days 14 through 20 of the cycle (yes); otherwise, ovulation is unlikely (no) in response to GnRH. Dark-colored blue circles represent growing viable follicles and light-colored yellow circles represent dying (atretic) follicles (Smith et al., 2010).

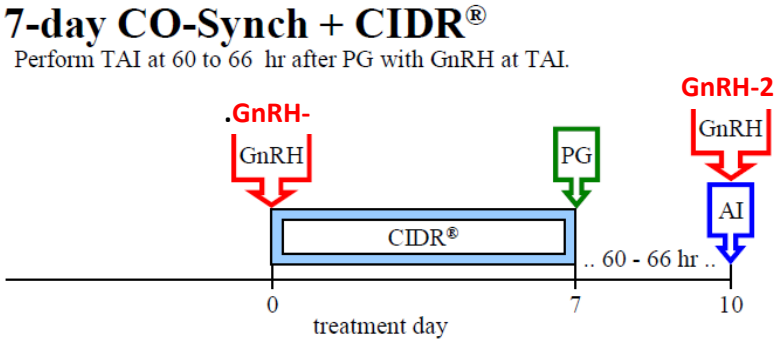

Figure 5. The basic "workhorse" program for synchronizing estrus (no GnRH-2 after PG, AI after heat detection) or synchronizing ovulation (GnRH-2 at 60 to 66 hours after PG concurrent with timed AI) in suckled beef cows. Abbreviations: GnRH = gonadotropin-releasing hormone; CIDR = intravaginal progeserone insert (control internal drug release); PG = prostaglandin $F_{2\alpha}$ (e.g., estroPLAN, Estrumate, In-Synch, Lutalyse, Lutalyse HighCon, ProstaMate, SYNCHSURE). Source: ARSBC website (2017).

The ovulation response to GnRH-1 varies with the luteal status of the cow: (1) cycling cows with a CL (these cows may ovulate form a second new CL); (2) cycling cows with no CL (these cows may ovulate to form a new CL); and (3) noncycling (anestrous) cows that have not begun their estrous cycles since calving (these cows may ovulate to form their first post-calving CL), thus initiating their first postpartum estrous cycle.
Ovulation response to GnRH-1 also depends on two factors: (1) presence of a dominant follicle capable of ovulation; and (2) concentrations of progesterone and estradiol that modulate the amount of LH that is secreted in response to GnRH (Stevenson, 2016), which varies according to the stage of the estrous cycle or condition of anestrus at the time of treatment. Cows with a CL and larger concentrations of progesterone (progesterone inhibits the amount of ovulation hormone (LH) that is released in response to GnRH) are less likely to ovulate than cows with a

CL, but with lesser concentrations of progesterone. Therefore, three outcomes are possible 7 days after GnRH-1 when cows are treated with $PGF_{2\alpha}$. Cows may have: (1) a new CL (often the first CL after calving); (2) an original CL that was present before GnRH-1 (original CL); or (3) an original CL that was present before GnRH-1 plus a new CL (original + new CL). When ovulation to GnRH-1 occurs, growth of a new wave of follicles is initiated approximately 36 to 48 hours after GnRH-1 so at the time of $PGF_{2\alpha}$, a new dominant follicle will exist that is approximately 5 days old. When ovulation after GnRH-1 does not occur, the dominant follicle is less uniform in size and function (less synchronous) with the forthcoming $PGF_{2\alpha}$ injection and subsequent luteal regression (death of the CL).

Role of Progestins

Two progestin products commercially available for synchronization of estrus include the orally active melengestrol acetate (MGA) and the CIDR (control internal drug release) or progesterone insert. In cycling cows and heifers, administration of MGA or a CIDR does not affect the time of CL regression. The CL will regress according to its programmed time, and in the presence of progestin administration such as MGA or a CIDR, estrus and ovulation generally are prevented (Figure 6). Consequently, progestin administration in cows in which the CL has regressed will delay the expression of estrus and ovulation until after progestin treatment withdrawal and blood concentrations of progestin have returned to basal concentrations. The effect of MGA treatment (14 days) on heifers in different stages of the estrous cycle is illustrated in Figure 6.

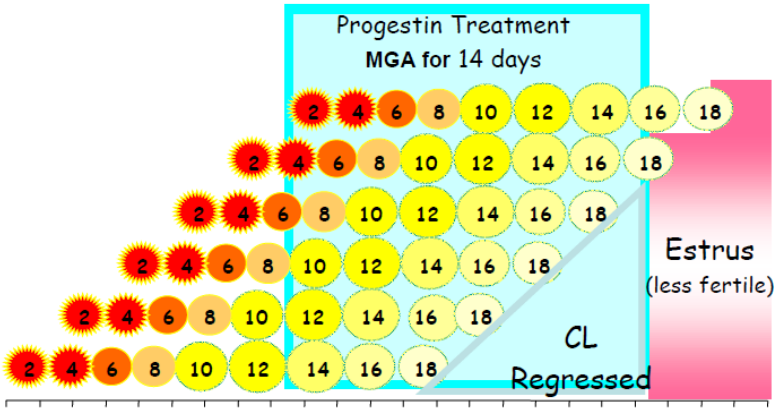

Figure 6. Effect of 14 days of melengestrol acetate (MGA) feeding on synchronization of estrus for heifers in different stages of the estrous cycle at the onset of treatment feeding. Each row of circles represents growth and regression of corpora lutea (CL) for an individual heifer. Numbers inside each circle represent days of the cycle. In this diagram, spontaneous luteal regression occurs around day 17 to 18 of the cycle. Note that at the end of a 14-day progestin treatment all CL have regressed or are in the process of regressing (Smith et al., 2010).

At the start of a breeding season, most herds consist of a mixture of cycling and anestrous cows or heifers. An effective program to control the estrous cycle must be capable of inducing a fertile estrus or ovulation in both anestrous and cycling heifers and cows. A short diestrous period usually occurs in prepuberal heifers and postpartum beef cows (Perry et al., 1991) after their first ovulation. This short exposure to progesterone is believed to be necessary for reprogramming the reproductive axis to resume normal expression of heat and regular estrous cycles. Therefore, in herds that have a large proportion of prepuberal heifers or anestrous cows, progestin pretreatment before induction of ovulation may initiate estrous cycles and reduce the occurrence of short estrous cycles that follow first insemination.

Administering small doses of a progestin (i.e., daily dose of 0.5 mg MGA) in the absence of a CL can result in the formation of a persistent follicle. Persistent follicles are characterized by an extended dominant follicle life span and increased estradiol production. Administering small doses of progestin does not induce persistent follicle formation in early anestrous postpartum beef cows (Perry et al., 2002). In contrast, treatment of cycling heifers or cows with small doses of a progestin during and after CL death (luteolysis) results in the formation of persistent follicles. Formation of persistent follicles is associated with increased LH pulse frequency, and infusion of exogenous LH induced persistent follicle formation, and results in ovulation of an egg that is capable of fertilization but the resulting embryo does not survive to reach the uterus (Inskeep, 2004).

Limitations to Successful Synchronization

Corpus Luteum Regression

We expect pregnancy outcomes to be less when: (1) luteal regression is incomplete or does not occur; and (or) (2) the dominant follicle is not responsive to GnRH-2 treatment as evidenced by timely ovulation and shedding of the egg into the oviduct. As a result of an ovulation after GnRH-1, the age of the CL can be quite variable in age and alter responsiveness to $PGF_{2\alpha}$.

In general, the CL is responsive to $PGF_{2\alpha}$ and regresses beginning at 5 days of age, but its maximal response to $PGF_{2\alpha}$ does not occur until approximately day 12. Note in Table 1 (data from lactating dairy cows) that regression of the original CL exceeds 95%, but the new CL regression is much less successful at 64%. In contrast, when a new CL and the original CL co-exist, regression success approaches that of the original CL. When a new CL co-exists with the original CL as a result of ovulation after GnRH-1, CL regression is much more successful, thus explaining, in part, why fertility is always improved in cows that ovulate in response to GnRH-1. The greatest limitation to successful CL regression is when a new CL exists by itself as the only CL.

Response of the CL to an exogenous luteolytic dose of $PGF_{2\alpha}$ is age-dependent (Figure 7A), and interval to estrus after $PGF_{2\alpha}$ is dominant-follicle dependent. In general, once the CL begins to regress at the end of the cycle, heat will occur in approximately 3 days (Figure 7B). Examples of varying intervals to heat are illustrated in Figure 7C and 7D depending on dominant follicle function.

Table 1. Age and number of corpus luteum (CL) at the $PGF_{2\alpha}$ injection (7 days after GnRH-1) and outcome of CL regression (Source: Stevenson, 2016)

Age and number of CL at $PGF_{2\alpha}$ treatment	Proportion of cows with CL regression (%)
New CL	64
Original CL	97
Original CL + new CL	92

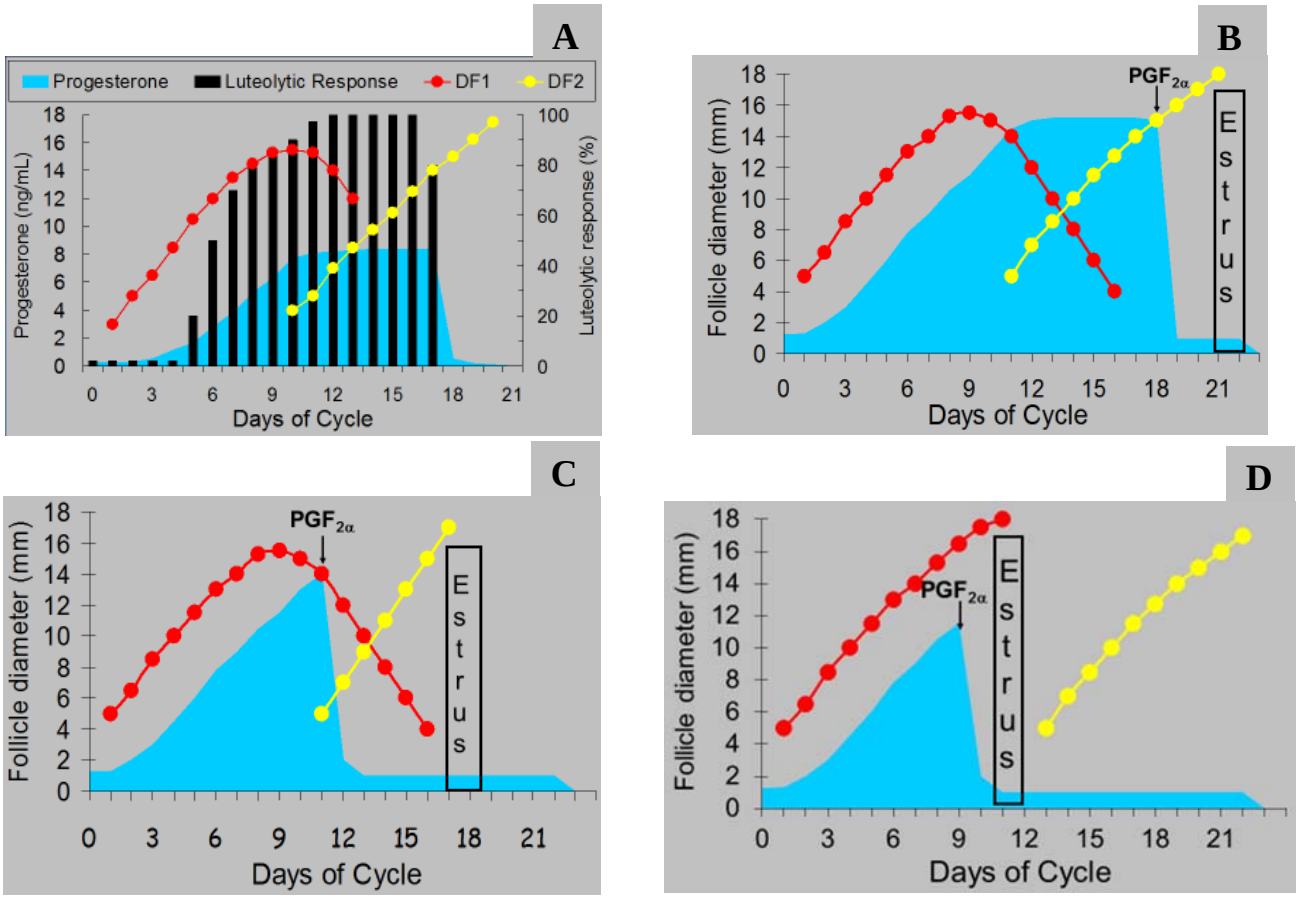

Figure 7. Blood progesterone concentrations (blue-shaded area), first- (red circles) and second- (yellow circles) wave dominant follicles, luteolytic response to $PGF_{2\alpha}$ (black vertical bars), and onset of estrus after $PGF_{2\alpha}$. **A.** Successful complete regression of the CL (luteolysis) increases gradually from day 5 and reaches a threshold on day 12. **B.** During a normal estrous cycle, $PGF_{2\alpha}$ causes CL regression at approximately day 18 (day 0 = heat), and in the absence of a viable embryo, heat occurs in approximately 3 days. **C.** If $PGF_{2\alpha}$ is administered on a day when the first dominant follicle is no longer growing and healthy (e.g., days 11 – 13), but before the second dominant follicle is sufficiently mature, the onset of estrus is much longer than 3 days. **D.** If $PGF_{2\alpha}$ is administered when the first dominant follicle is still functional (e.g., day 9), then estrus occurs in less than 3 days.

Ovulation

Ovulation response to GnRH-2 will occur nearly 100% of the time if luteal regression occurs successfully or if progesterone concentrations are very low at the time of AI (Stevenson, 2016). Timing of the GnRH-2 injection is a critical component to maximizing pregnancy outcomes in dairy cows, but little information is available testing that concept in suckled beef cows. Only one study (Geary et al., 2001) in suckled beef cows has compared the timing of GnRH-2 (48 [Ovsynch; n = 237] vs. 72 [CO-Synch; n = 236] hours) with timed AI at 72 hours after $PGF_{2\alpha}$, which also included 48-hour calf removal starting at the time of $PGF_{2\alpha}$. Timing of GnRH had no effect on pregnancy outcomes and calf removal for 48 h improved conception regardless of the timing of GnRH-2 treatment. To reduce cow-calf handling, use of CO-Synch for ovulation synchronization of suckle beef cows has proved to be practical for most applications.

Why might it be better to administer GnRH-2 approximately 16 hours before insemination? That sequence best mimics what occurs at spontaneous estrus. When a cow starts into heat and stands for the first time in response to a mounting herd mate, two significant hormonal events occur. Heat is caused by estradiol (estrogen) secretion from a mature, preovulatory follicle. Blood concentrations of estradiol peak at the onset of standing heat. The peak of estradiol is the trigger to initiate the surge release of GnRH from the hypothalamus in the brain that results in the release (surge) of the LH from the pituitary gland. Concentrations of estradiol and LH peak at or near the onset of standing heat. We expect ovulation of the preovulatory follicle to occur in 24 to 30 hours after the onset of heat, peak in estradiol, and the LH surge. Ideally, according to the a.m.-p.m. rule, one inseminates cows approximately 12 hours after the onset of heat. In practice, for beef cattle, the CO-Synch + CIDR insert program has proven to be most practical.

In timed AI programs, the trigger to initiate ovulation is GnRH-2, if estrus had not occurred before GnRH-2. Surge release of LH peaks approximately 1 hour after GnRH-2 and we expect ovulation to occur 24 to 30 hours later. So injecting GnRH-2 before insemination mimics the natural events associated with a spontaneous estrus and shortens the time to ovulation relative to semen placement. Sperm transport requires up to 10 hours from the uterine body or horns to the utero-tubal junction where sperm concentrate and await ovulation. By administering GnRH-2 approximately 16 hours before AI allows for ovulation to occur approximately 8 to 14 after sperm have formed a reservoir at the utero-tubal junction. This timing matches nicely what occurs naturally when cows come into heat spontaneously and ovulate in response to their own estradiol-GnRH-LH signals.

Further work is warranted to test the timing of GnRH-2 relative to time of AI in suckled beef cows because in dairy cows, administering GnRH-2 at approximately 56 hours after $PGF_{2\alpha}$ and inseminating approximately 16 hours later (72 hours after $PGF_{2\alpha}$) produced greater pregnancy outcomes than administering GnRH-2 at the time of AI. Any potential improvement in pregnancy outcome that might occur must outweigh additional time and handling cost.

Conclusions

Significant benefits to genetic improvement and reproductive management can be achieved from implementing programs to control estrus and ovulation before AI of heifers and postpartum beef cows. Artificial insemination in beef cattle is much more practical because of advancements in timed AI programs. Identifying sires with highly accurate EPDs and a market structure that can identify and reward producers for the quality of their cattle are beneficial consequences of employing AI. Understanding the limitations of how to control the estrous cycle can led to more uniform and consistent pregnancy outcomes.

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