Reproductive Endocrinology of the Cow: Part 1 Endocrinology of the Estrous Cycle

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Introduction

Veterinarians active in herd reproductive health programs will invariably become responsible for assessment of ovarian status, for therapy to alter ovarian activity, and for programs designed to manipulate the estrus cycle of groups of cattle. Effectively completing these procedures requires an increasingly detailed understanding of normal reproductive events and hormonal changes. This is a two part series aimed at a description of basic bovine reproductive endocrinology. Part 1 details the endocrinological changes that occur in the normal bovine estrus cycle. The effects and limitations of prostaglandin administration are described. In Part 2, the methods used to effect a superovulatory response and the hormonal events of superovulation in the bovine are presented.

Components of the Bovine Estrous Cycle

The duration of the estrous cycle is variable but it is generally accepted that an average cycle length is 20 days in heifers and 21 days in cows (1). The estrous cycle of the cow is commonly divided into a luteal phase, which occupies approximately 17 days, and a follicular phase of 3-4 days (2). The duration of estrus is approximately 18h (3,4) and ovulation occurs 10-12h after the end of estrus (5) or 25-30h after the preovulatory surge of luteinizing hormone (LH) (6).

For the following description of the endocrine changes which regulate the sequence of events outlined above, it is convenient to divide the estrous cycle into a number of components:

I. **Follicular phase**
   (a) Period from luteal regression to the preovulatory surge of LH (pre-surge)
   (b) Period between the preovulatory surge of LH and ovulation (post-surge)
   (c) Follicular rupture and release of oocyte
II. *Luteal phase*—the period over which the corpus luteum produces progesterone; this includes growth, maintenance and regression of the corpus luteum.

I. Follicular Phase

(a)Pre-surge period.

Various reports (7,8,9) have demonstrated that the hypothalamic, pituitary and ovarian hormones are largely secreted in a pulsatile manner. In cows, following natural or prostaglandin $PGF_{2}\alpha$-induced luteolysis the concentrations of LH increased peripheral serum (9,10). The decrease in progesterone concentration following luteolysis allows a 2-3 fold increase in both the frequency and amplitude of LH pulses and a 2-3 fold increase in basal concentration. This increase in LH release probably results from the relaxation of the negative feedback effect of progesterone as the corpus luteum undergoes functional regression (11). Progesterone is a potent inhibitor of LH secretion (12).

In sheep, it has been shown that, at this time, each pulse of LH is followed by an increase in estradiol concentrations in ovarian venous blood (13). Similarly in cows, the frequency of estradiol pulses increased in parallel with those of LH, and resulted in higher mean estradiol concentrations, without significantly increased pulse amplitude (14). During 10h immediately before the onset of LH surge, the amplitude of estradiol pulses increased steadily and the concentration of this hormone reached a maximum (14).

Follicle-stimulating hormone (FSH) is normally required for the induction of LH receptors in the granulosa cells of ovarian follicles (15). During the follicular phase in the cow, however, no significant changes in circulating FSH occur until the time of the preovulatory surge of FSH (9). Presumably, acquisition of a greater number of LH receptors in the granulosa cells of preovulatory follicles is responsible for the increased amplitude of estradiol pulses (16). While this may be an FSH-coordinated event, substantial evidence for this in the cow is not available.

The increasing estradiol concentrations in the circulation result in an increased frequency of hypothalamic gonadotrophin-releasing hormone (GnRH) release and an

increased ability of the anterior pituitary gland to release LH and FSH in response to GnRH. An increase in both GnRH secretion and pituitary responsiveness to GnRH are necessary to elicit a full preovulatory surge of gonadotrophins (17).

It has been demonstrated that the pituitary gland acquires maximum responsiveness to GnRH 10-16h prior to the onset of the LH surge (17). During this period of maximum responsiveness, high concentrations of estradiol suppress the amplitude of LH and FSH pulses below those found during the luteal phase (18). Since there is a strong correlation between the amplitude of endogenous GnRH pulses and the amplitude of corresponding LH pulses (19), it appears that elevated estradiol concentrations acting on the hypothalamus reduce the pulse amplitude of LH and FSH (14). This is thought to occur from a decrease in the amplitude of endogenous GnRH pulses as demonstrated in rats (20).

Since the exogenous administration of estradiol induced a preovulatory-like LH surge in cows and because inhibition of estradiol during the pre-surge period inhibited the preovulatory surges of FSH and LH (21), it has been traditionally considered that the increase in estradiol concentration during the late follicular phase triggered the LH surge (11). Recently, however, researchers have demonstrated that there is a decrease in estradiol concentrations approximately one hour before the surge of LH (14). This could trigger the preovulatory gonadotrophin surge by reducing the inhibitory effect of estradiol on the hypothalamus. These authors hypothesized that falling estradiol concentration prior to LH surge would allow the amplitude of the GnRH pulses to increase which would result in a higher amplitude of LH pulses and the cascade of GnRH and gonadotrophin release to become established. After this release, a preovulatory rise in estradiol is responsible for the hormonally-driven components of estrous behaviour (11).

The pulsatile pattern of LH release is maintained throughout the surge. Pulse amplitude is higher during the ascending phase than during the descending phase when the frequency of release resembles that found during the early luteal phase (7). Pulses of LH and FSH are concomitant throughout. Presumably GnRH alone controls their release during this period (9). Termination of the LH and FSH surge occurs because the pituitary gland becomes refractory to GnRH, and not because of exhaustion of the store of gonadotrophins within the gland (22).

It has been demonstrated that in preovulatory follicles, binding of LH to the granulosa and theca cells increases during the LH surge (12). A maximum number of granulosa cells and maximum concentrations of androstenedione were also recorded in follicular fluid. It appears, therefore, that at the onset of the preovulatory LH surge, large estrogen-active follicles are equipped with optimal availability of substrate, a maximum number of granulosa cells and aromatase activity to convert androstenedione into estradiol.

(b) *Post Surge period.*

Immediately after the preovulatory gonadotrophin surge, basal concentrations of LH fall below those observed during the pre-surge period. Concentrations of FSH, however, increased 4-12h after the LH surge (9). This secondary increase in FSH concentration was once thought to be the result of a decrease in the negative feedback effect of estradiol (18), but failure of estradiol administration to abolish this has led to the idea that a decrease in inhibin concentrations may allow FSH to rise (9,23). A marked reduction in plasma concentrations of estradiol also occurs after the preovulatory LH surge in cows (24) and LH concentrations are unaffected if ovariectomy is performed during this time (25).

Theca cells produce androgens which are aromatised to estrogens by the granulosa cells (26). Since LH binding to theca and granulosa cells decreases after the preovulatory LH surge (27), it appears that estradiol biosynthesis in the preovulatory follicle is terminated by the inhibitory action of the preovulatory LH peak on androgen production and a slow decrease in aromatase activity (28,29).

(c) *Follicular rupture and release of oocyte.*

The characteristic preovulatory change in the follicular wall has been described as edema formation (30). This edema occurs due to increased membrane permeability of the peri-follilcular blood vessels (31). This change in the vascular bed of the follicle is closely coincident with the capillary invasion of the granulosa layer after breakdown of the basement membrane (32). It appears, therefore, that increased permeability of the perifollicular vasculature may play an important role in ovulation (31). Fluid accumulates and causes edema of the ovary and its preovulatory follicle (32). A short time before ovulation a clear, avascular spot develops on the surface of the Graafian follicle which is known as the "stigma" and is the site of the impending rupture (33,34). It has been demonstrated that in the cow, formation of the stigma occurs only an hour before ovulation (35).

The hydrostatic pressure in the antrum which remains constant during ovulation seems to play an important role in the ovulation process (36). Weakening of follicle wall at the stigma occurs until the pressure is sufficient to cause rupture (33). It has been suggested that the lysosomal enzymes and other unknown components of the follicle cells released near the time of ovulation cause weakening and actual breakdown of the follicle wall (37). However, other reports, based on the fact that rabbit follicle wall contains dense connective tissue with abundant collagen,

have indicated that the enzymatic dissociation of collagenous tissue results in follicular rupture (38,39).

At the endocrine level, increased binding of LH to theca and granulosa cells of the preovulatory follicle occurs at the time of the LH surge (27). The preovulatory gonadotrophin surge also promotes a gradual change in the preovulatory follicle from predominantly estrogen secretion to progesterone secretion (40). This increase in progesterone concentration in the follicular fluid may promote breakdown of the basement membrane and modulate final enzymatic changes in the follicle wall (32).

A number of reports had strongly indicated that prostaglandins play a critical role in the process of ovulation. The concentrations of prostaglandins increase in follicular fluid prior to ovulation (41) and treatment with inhibitors of prostaglandins, such as indomethacin, in late pro-estrus blocks ovulation in rats (42). It was suggested that prostaglandins facilitate the ovulation process by changing the chemical pathways or contractile activity of ovarian tissue (32). A recent report, however, has demonstrated that smooth muscle contractions may not be essential for the process of follicular rupture but simply accompany the ovulatory process (31). In this report, it was demonstrated that the degree of maturity of ovulated ova is dependent upon the presence of LH or hCG in the perfusate and also on the duration of exposure of the oocyte to hCG. In ovaries not exposed to hCG, the ovulated ova were immature. Oocyte maturation appears to be gonadotrophin dependent.

Finally, with or without prior formation of the stigma, nonviscous fluid is released rapidly followed by an oozing of the viscous portion of follicular fluid together with the cumulus mass enclosing the egg (32). In rats and humans, after initial rupture the follicular wall collapses slowly (33,43). In heifers, however, collapse of the follicle wall has always been found to occurr rapidly (35).

II. Luteal Phase

After ovulation, the remnants of the newly vascularised follicle hypertrophies and proliferates rapidly to form the structure commonly known as the corpus luteum (2). During first week post-estrus, the weight of the corpus luteum increases and it attains mature size by day 7 of the estrous cycle (44).

A mature corpus luteum of the cow contains small and large luteal cells (45,46). Several studies (44,45) have demonstrated that after ovulation, both the theca and granulosa cells contribute to corpus luteum formation. Small luteal cells originate from the theca cells and large luteal cells are derived from the granulosa cells. Since granulosa-derived large luteal cells have limited lifespan, their number decreases as the age of the corpus luteum increases. These cells disappear almost completely after 150 days of gestation (45).

Although both types of luteal cell (small and large) are capable of producing progesterone, the small luteal cells produced 6 times more progesterone than the large luteal cells when LH was added to the dissociated cell preparations (46). Exogenous administration of LH during mid-luteal phase lengthens the estrous cycle because functional lifespan of the corpus luteum is prolonged (47). The corpus luteum regresses and LH-stimulated progesterone synthesis by luteal cells is inhibited if endogenous production of LH is neutralized with LH antiserum (11,48,49). Based on these observations, it is believed that the LH is the major luteotrophic hormone in the cow (11).

Two to three days after estrus, the concentrations of progesterone begin to increase steadily and reach a maximum concentration on, approximately, day 10 (11). During the early luteal phase when progesterone concentrations are low, episodic LH release has a lower frequency than during the mid-luteal phase. At this time, increases in LH pulse amplitude were also associated with an increase in the amplitude of estradiol pulses (16). The presence of a large estrogen-active follicle in the ovaries of the cows at this time is the most likely source of this estrogen (27). It was suggested that this increase in estrogen pulse amplitude may be due to an increased responsiveness of the estrogen-active follicle to LH (16). These workers also demonstrated that during the mid-luteal phase, although pulse frequency of LH was reduced, amplitude and basal concentrations of LH did not differ from those observed during the early luteal phase. A corpus luteum during the mid-luteal phase contains maximum number of the small luteal cells (45). It appears, therefore, that either the large number or the increased responsiveness of these cells to LH maintains the higher concentrations of progesterone during the mid-luteal phase when LH release is less frequent.

Regression of the corpus luteum:

The degeneration of the corpus luteum, which occurs around day 18, is associated with a decline in circulating concentrations of progesterone. The lifespan and function of the corpus luteum are prolonged when an animal becomes pregnant. Prolonged function of the corpus luteum in the form of continuous supply of progesterone is essential for successful implantation and embryonic development. High concentrations of progesterone also ensure that the cyclic changes which occur during the estrous cycle do not persist during the period of gestation (3). In cows, it has been demonstrated that the administration of oxytocin during the early luteal phase (day 2 to 6) of the estrous cycle inhibits full development of the corpus luteum and induces premature estrus and ovulation (50). This effect was not observed in hysterectomized animals or in animals in which the uterine horn ipsilateral to the corpus luteum

was removed (51). Premature estrus occurred in cows if uterine irritants were placed into the horn of the uterus ipsilateral to the corpus luteum (52). Based on these observations, it was proposed that, as in sheep, a substance of uterine origin acts locally to induce regression of the corpus luteum (3).

In sheep, prostaglandin-$F_{2\alpha}$ ($PGF_{2\alpha}$) of uterine origin reaches the corpus luteum as a result of counter current exchange between the utuerine vein and the ovarian artery and luteolysis is induced (53,54,55). In cows, however, conclusive proof for this veno-arterial transfer mechanism has not been found (11).

In contrast, a number of reports indicate that the concentrations of $PGF_{2\alpha}$ in the endometrium and in uterine vein increased between day 15 and 21 of the estrous cycle (56). A concomitant increase was not, however, detected in the ovarian arterial blood. In addition, there has been no evidence of a counter-current exchange of $PGF_{2\alpha}$ (57). These reports indicate that local transfer may not be essential. A recent report suggests that the corpus luteum regression is not entirely dependent on $PGF_{2\alpha}$ of uterine orign (58). Bovine corpora lutea can produce both $PGF_{2\alpha}$ and prostacyclin (PGI_2). In vivo and in vitro studies indicated that administration of PGI_2 stimulates progesterone secretion by the corpus luteum (57). They proposed that PGI_2 may exert a luteotropic effect in the normal cycling cow. Since the ratio of PGI_2 to $PGF_{2\alpha}$ steadily decreases as the age of the corpus luteum progresses, the events such as development, maintenance and regression of corpus luteum appear to be regulated by changing a balance between the amounts of luteolytic and luteotrophic prostaglandin (58).

Oxytocin, like other hypothalamic hormones, is secreted in a pulsatile manner in the cycling cow (16). It is present in high concentrations in bovine corpora lutea and is contained in the electron-dense granules commonly found in all large luteal cells (59). The peripheral concentrations of this hormone become undetectable either when the CL is not present on the ovary (follicular phase) or when ovaries are removed from the cow (9).

Both active and passive immunization against oxytocin results in prolonged estrous cycles in sheep (60). It is also known that the corpus luteum of the cow releases large amounts of oxytocin in response to $PGF_{2\alpha}$ injection (9) and that treatment of the cows with ocytocin results in increased secretion of $PGF_{2\alpha}$ from the uterus (57). These facts have led to the belief that a positive feedback system may exist between the ovary and the uterus which controls luteolysis in cattle (9).

Roberts and his co-workers (61) demonstrated that, in sheep, endometrial and myometrial tissues vary in their capacity to synthesize $PGF_{2\alpha}$ according to the stage of the estrous cycle. In their experiment, endometrial tissue isolated on the day of estrus, produced significantly less $PGF_{2\alpha}$ than that isolated on day 13 or 15 of the estrous cycle. They further demonstrated that although both endometrial and myometrial tissues are capable of producing $PGF_{2\alpha}$, in vitro, the presence of oxytocin in culture medium enhanced the release of $PGF_{2\alpha}$ from the endometrium only. Numerous studies have indicated that estradiol is a potent luteolytic agent in the cow (3,62). Since destruction of follicles at day 10 post-estrus in the cow results in extended luteal function, the follicles present during mid to late diestrus determine the duration of diestrus (63). The luteolytic action of growing follicles appears to be exerted after day 15 of the estrous cycle when estradiol produced by growing follicles initiates luteal regression (64). The luteolytic effects of estradiol and $PGF_{2\alpha}$ may be additive (11) but the ultimate effector component appears to be $PGF_{2\alpha}$ secreted by the uterus (64).

It has been suggested that the ovarian steriods at high concentrations (estradiol in the follicular phase and progesterone during the luteal phase) stimulate the proliferation of oxytocin receptors in the uterus. These receptors, upon stimulation by oxytocin, promote the release of increased amounts of $PGF_{2\alpha}$ from the uterus. From this information, steroid (estradiol and progesterone) dependent stimulation of oxytocin receptors in the uterus appear to be the initial component in the events of luteal regression. If the release of luteal oxytocin and uterine $PGF_{2\alpha}$ are controlled by a positive feedback mechanism as proposed by Schallenberger (9), then following stimulation of uterine oxytocin receptors, greater secretion of both $PGF_{2\alpha}$ and oxytocin would be expected. It would also be expected that either refractoriness of oxytocin receptors in the uterus or of $PGF_{2\alpha}$ receptors in the corpus luteum may limit the total release of either hormone.

In summary, it is clear that the regression of the corpus luteum in the cow is induced by a local action of uterine $PGF_{2\alpha}$ on the ovary; it is not clear, however, if there are additional mechanisms acting in association with these local regulators.

Synchronization of estrus using Prostaglandin $F_{2\alpha}$

Exogenous administration of $PGF_{2\alpha}$ (PG) or its analogues induces luteolysis in most farm mammals (11). Since PG and its analogues are readily available at reasonable cost and since normal fertility is observed at the induced estrus, their use has become common in controlling the estrous cycles of cattle (65). The higher potency and negligible post-treatment effects of PG analogues offers some advantage over the use of naturally occurring PG (66).

In cows, the administration of PG during the mid-luteal phase of the estrous cycle depresses plasma concentrations of progesterone below 1 ng/ml within 24h (67) and estrus is normally observed after an interval of 48 to 96h (68,69).

Since the administration of PG is ineffective in inducing luteolysis before day 5 (70,71) or after day 18 of the estrous cycle, approximately 40% of cycling cows may fail to exhibit estrus within 5 days of a single injection of PG (66). To over-come this problem, two injections of PG 10-12 days apart would ensure that all the cows possess a corpus luteum which could be regressed with the second PG injection (72,73).

Early studies indicated that the rate of plasma progesterone decline, the interval to the onset of estrus and the LH peak, the time of ovulation and the subsequent cycle length were not different when cows were treated with PG on either day 7, 11 or 15 of the estrous cycle (71,74). More recent studies employing single or double injections (11 days apart) of PG, have demonstrated that estrus occurred earlier and was more synchronized after the second injection of PG than in cows treated with a single injection on day 8 of their cycle (75). Similarly, an influence of the stage of the cycle on the incidence of the preovulatory surge of gonadotrophin has been demonstrated (76). They observed that the interval from PG injection to the peak of LH concentrations (73h) was significantly longer in a group of heifers injected on day 12-14 than in heifers injected on day 7 or 8 of their cycles (59h). Concentrations of LH at the peak did not differ among the various groups (d 7-8, d 12-14, and d 15-16). In an even more recent study, the onset of estrus ranged from 24-72h ($\bar{x}$=48h) for heifers treated on day 7, compared with 32h-104h ($\bar{x}$=72h) in heifers treated on either day 11 or day 15 (77). Similar findings have been reported by researchers in Minnesota (90).

The large variation associated with the timing of the onset of estrus and the LH surge following PG injection on different days of the estrous cycle probably reflects the waves of follicular development in the bovine ovary. The time taken by the follicle present at PG injection to develop and secrete enough estradiol will primarily determine the time to the onset of the LH surge and estrus (9). At least two periods of follicular growth and atresia (day 3-7 and 9-13 of the cycle) have been demonstrated during the luteal phase (78). Thus, in order to obtain the most precise synchronization it is necessary to treat cows with PG either on day 7 or day 15-16 of the estrous cycle (65).

The duration of estrus is approximately 18h (3,4); although it may last up to 26h (79). Estrus varies in intensity from pronounced standing estrus to silent estrus (80,81). Most studies indicate that ovulation without signs of estrus occurs infrequently (82), except in the early post-partum period (81). Factors which may affect intensity or expression of estrus include group size, housing facilities, number of females in estrus together, proper observation of estrus, frequency of observation and proper use of records (81,83,89). Major reasons associated with failure of cows to express estrous signs in reponse to PG were that either the animal was not cycling or was injected too early in the cycle (84).

Time of ovulation and artificial insemination after PG:

In normally cycling cows, ovulation occurs 10-12h after the end of estrus (5). Several workers have reported that overt signs of estrus may occur before the onset of the LH surge (10,85) or after the onset of the LH surge (86, 87). For this reason the onset of estrus would not precisely predict the time of ovulation (35). On the other hand, ovulations have been shown to occur at a fixed interval after the LH surge (35). Plasma concentrations of LH were measured using a rapid radiommunoassay system and ovulation was observed through a laproscope. Ovulations occurred at 27.3 $\pm$ 1.6h and 17.0 $\pm$ 1.5h after the onset and the end of LH surge, respectively (35). Therefore, the preovulatory surge of LH could be used to predict the time of ovulation and, of greater importance, the optimal time for insemination.

A system called the "double-double system" is often used for estrous synchronization and artificial insemination (66). In this system, cows are synchronized with two injections of PG given 10-12 days apart, followed by inseminations at 72h and 96h after the administration of PG. A recent report indicates that synchronization of cows using two injections of PG (10 days apart) and inseminations at 72h and 96h after second PG injection resulted in the highest conception rates (50%) (88). The results of a single insemination performed at observed estrus or a double insemination at 72h and 96h after synchronization of cows with a single PG injection were inferior (25% and 33% respectively). Thus, double insemination at fixed times after double PG treatment may be recommended as it eliminates the problems of estrus detection by farmers and ensures effective coordination with the inseminator. Factors such as method of observation, cost of semen, housing and other environmental factors must be considered in the design of an estrus synchronization program.

Conclusions

The intricate hormonal changes and relationships which make up the estrus cycle of the cow have been described. A knowledge of these normal events is a basic requirement for understanding the pathophysiology of abnormal ovarian activity such as ovarian cystic degeneration. This knowledge is also crucial to effective control of bovine reproductive programs and to efficient use of techniques designed to manipulate the cycle. Part 2 will describe the methods used to effect a super-ovulatory response in the cow and the endocrinological changes which occur during superovulation.

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