

Regulation of the Bovine Estrous Cycle

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General Introduction

Figure 1 summarizes some key aspects of the bovine estrous cycle. Changes in the 2 key structures on the ovary, the preovulatory follicle and the corpus luteum, are emphasized in this figure. The function of 4 important hormones is also summarized in this figure. Estradiol-17 β from the preovulatory follicle causes the cow to manifest estrus behaviour and have an LH surge. The LH surge causes ovulation of the preovulatory follicle about 28 h later. The cells that remain from the preovulatory follicle develop into the corpus luteum. The corpus luteum grows in size during the first part of the estrus cycle and then reaches a plateau phase in which it maintains a large size (20-25 mm diameter). The major hormone coming from the corpus luteum is progesterone and the increase in size of the corpus luteum is reflected in increased concentrations of progesterone in the blood. If the cow becomes pregnant the corpus luteum maintains a large size and progesterone concentrations remain elevated. These high progesterone concentrations prevent the cow from coming into estrus or having a subsequent ovulation. If the cow does not become pregnant then the corpus luteum will decrease at about day 17-20 of the estrous cycle (day of estrus = 0). The reason the corpus luteum regresses is because of secretion of prostaglandin F_{2 α} (PGF_{2 α}) from the non-pregnant uterus. After exposure to PGF_{2 α} there is a decrease in circulating progesterone concentrations as well as a subsequent decrease in size of the corpus luteum. Thus, a major feature of the estrous cycle is the development and regression of the corpus luteum.

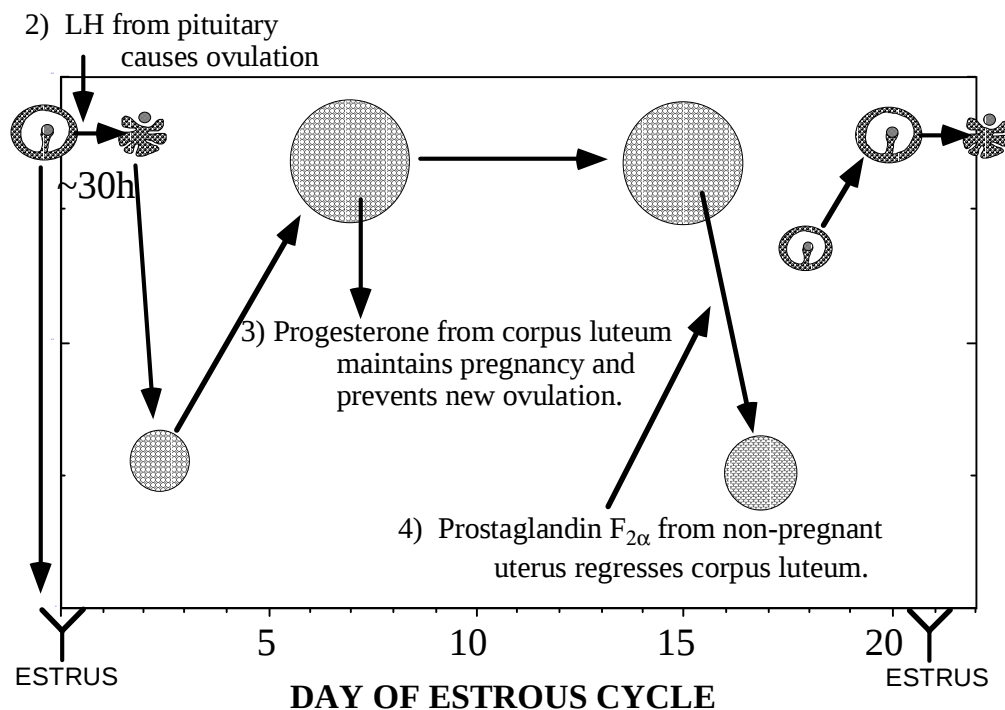


Figure 1. General features of the bovine estrous cycle emphasizing 2 key ovarian structures (preovulatory follicle and corpus luteum) and 4 key hormones.

The information in Figure 1 has been well known for some time. However in the last few years some important data has become available regarding growth of follicles and the relationship to Follicle-Stimulating Hormone (FSH) concentrations. An understanding of this new information is important for understanding recent synchronization protocols and therefore a brief summary of this information is provided below.

A number of recent studies have used transrectal ultrasound to analyze the final stages of follicular growth in cattle. Near the time of ovulation a group of small follicles begins to grow on the ovaries and this growth has been termed a follicular wave. From this group of follicles a single dominant follicle is selected to continue growth; whereas, other follicles of the follicular wave undergo regression. Due to the presence of a functional corpus luteum and high progesterone concentrations, this first dominant follicle does not cause estrus behaviour and does not continue to ovulation. The first dominant follicle will become non-functional and a second follicular wave begins at about mid-cycle. Again a dominant follicle is selected from this second follicular wave and this follicle continues to ovulation because its growth corresponds to the time of regression of the corpus luteum. Some cows also show 3 waves of follicular growth such that the second dominant follicle regresses, a third follicular wave is initiated, and the third dominant follicle becomes the ovulatory follicle. Both the first and second follicular waves are preceded by an increase in FSH concentrations (Adams et al., 1992). These increases in FSH are essential for the initiation of a follicular wave. The subsequent decrease in FSH is essential for selection a single dominant follicle. There is also a FSH surge in association with the LH surge that causes ovulation. This FSH surge occurs near the onset of estrus and is shorter of duration than the other FSH surge. The physiological function of this LH surge-associated FSH surge is not defined at this time.

This manuscript will be divided into 3 sections. The first will discuss the hormonal changes near the time of ovulation. The second section will deal with some functional aspects of the corpus luteum and factors that determine the circulating progesterone concentration. The third section will focus on follicular waves and mechanisms for selection of a single dominant follicle.

I. Key events around estrus:

Artificial insemination programs are mainly built around detection of estrus and breeding the cow in relation to the time of that detected estrus. The pregnancy that results from this breeding is obviously not due to the estrous behavior of the cow but is due to ovulation of an oocyte near the time of this estrous behavior. The timing of the hormonal and ovarian events around the time of estrus have been very well characterized using hormonal assays and observation of the ovaries with transrectal ultrasound. Figure 2 shows a simplification of key events around estrus: the increase in estradiol, standing estrus, the luteinizing hormone (LH) surge, and ovulation. All of the events are initiated by high circulating estradiol concentrations. The elevated estradiol is due to growth of a large preovulatory follicle on the ovary. After regression of the corpus luteum the dominant follicle grows and produces increasing amounts of estradiol. The cow becomes sexually active prior to the onset of standing estrus due to the increasing amounts of estradiol in the absence of circulating progesterone. Progesterone is low due to regression of the corpus luteum. If the corpus luteum does not regress and progesterone remains elevated some or all of the subsequent events (LH surge, estrus, ovulation) do not occur even when estradiol is elevated.

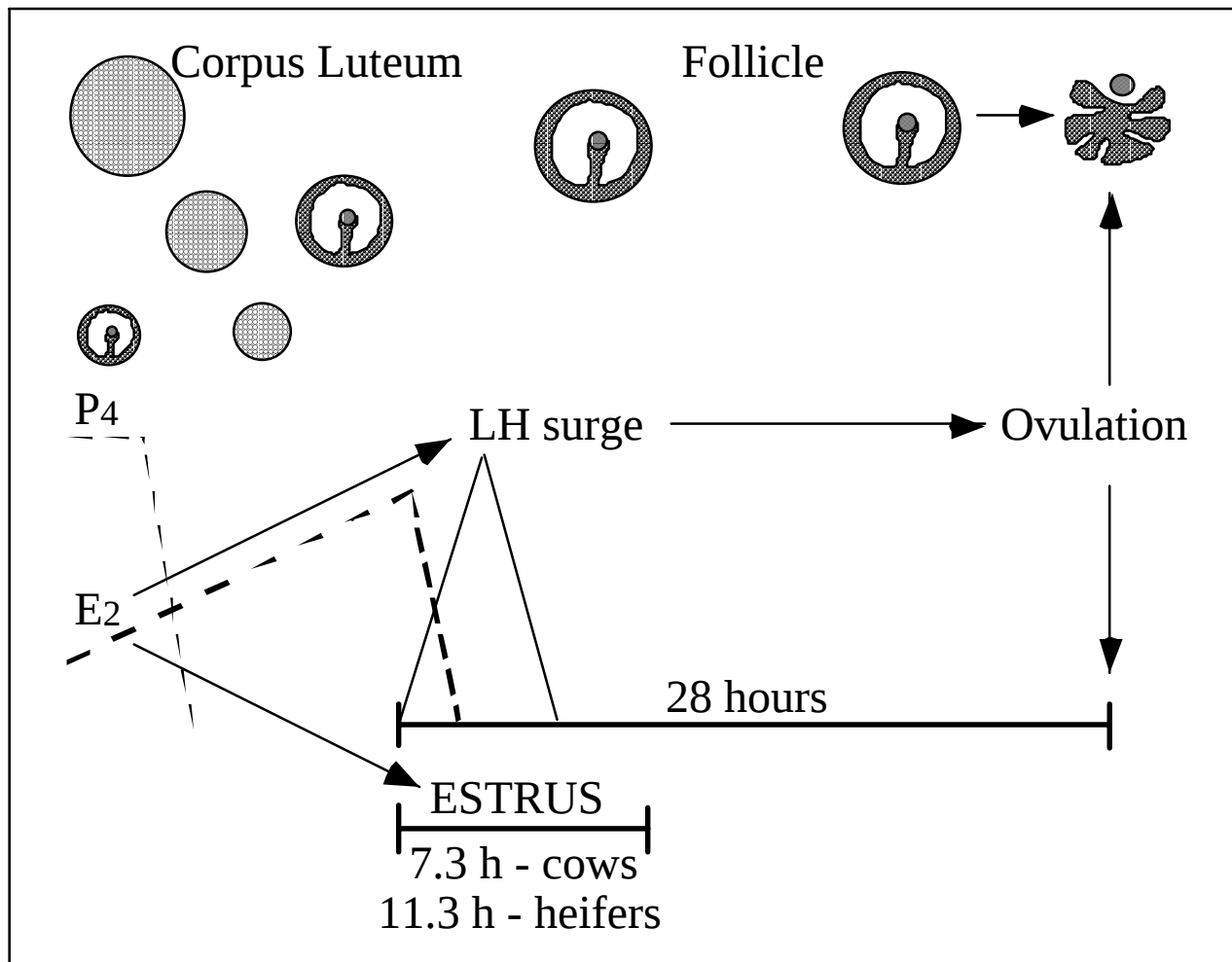


Figure 2. Diagram of the key events near estrus. High circulating estradiol causes the LH surge and standing estrous behavior. Estrus lasts longer, on average, in heifers than in lactating cows. Cows and heifers ovulate about 28 h from the onset of estrus and the LH surge.

After estradiol concentrations have reached sufficient concentrations for a certain time period there is a change in the brain that causes the cow to begin to stand solidly during mounting (onset of estrus). There is also secretion of the hormone gonadotropin releasing hormone (GnRH) in large amounts from a part of the brain called the hypothalamus. The secretion of GnRH is what causes the LH surge. These 2 events (estrus and GnRH/LH surge) happen very close to the same time but are actually due to 2 distinct events in the brain that are generally synchronized. It is possible to have estrus occur in some cows without a corresponding LH surge. For example, some cows that are cystic will show standing estrous behavior without any LH surge or ovulation. Using daily ultrasound on lactating cows ($n = 175$) we have observed estrus without an ovulation in about 11% of lactating cows (Sartori and Wiltbank, unpublished results). In general, these cows did not have follicles that were sufficiently large to qualify as cystic cows. The lack of ovulation is probably due to lack of an estradiol-induced LH surge. Alternatively, there are also times in lactating cows that no standing estrous behavior is demonstrated but the cows have ovulation due to an LH surge (~9%). Thus, although estrus and the LH surge normally happen almost simultaneously, there are instances when either of these 2 events can occur independently.

The onset of estrus is due to the high circulating estradiol concentrations. Estrous behavior ends prior to ovulation in cattle. The end of estrus may be due to decreased circulating estradiol because estradiol production in the follicle is dramatically reduced following the LH surge. As shown in Figure 1 the duration of estrus is greater in heifers than in lactating cows (Nebel et al., 1997). We have also found that circulating peak estradiol concentrations near the time of estrus is lower in lactating cows than heifers (Sartori et al., 2000). Thus, the reduced intensity and duration of estrus in lactating cows may be due to lower circulating estradiol concentrations.

The time from the onset of estrus until ovulation is between 25 and 34 h (Walker et al., 1996). The time of the LH surge is the event that sets the time of ovulation. Induction of an LH surge and ovulation with a GnRH injection prior to the normal time of ovulation (Pursley et al., 1995) will also cause an ovulation in about 28 h after the GnRH treatment (range of 24-32 h).

It is critical to note that AI to estrous behavior is based on the idea that estrus is a good sign of the time of ovulation. If there is no ovulation, there will be no fertilization or pregnancy even if the cow showed very clear signs of estrus. A commonly used procedure in commercial dairy operation, the Ovsynch protocol, was developed to allow breeding of cows to a synchronized ovulation without the need for detection of estrus (Pursley et al., 1995; Pursley et al., 1997). This protocol relies on an injection with GnRH to induce an LH surge and subsequent ovulation. This protocol has also allowed extensive studies on the optimal time of AI in relation to an induced ovulation. It is possible to compare the optimal time of AI between studies using estrus and studies using GnRH because the onset of estrus should correspond to the time of the GnRH/LH surge. Thus, the time of injection of GnRH will approximate the time of onset of estrus and both will be about 28 h from the time of ovulation.

II. The corpus luteum and circulating progesterone.

A. What determines size of the corpus luteum

It seems obvious that the size of the ovulatory follicle will have an effect on size of the corpus luteum. This relationship was clearly demonstrated in a study that we recently published (Vasconcelos et al., 2001). We used the Ovsynch protocol (GnRH-7 days- PGF2a-2 days-GnRH) to synchronize an ovulation but we reduced the size of the ovulatory follicle in some cows by aspirating the dominant follicle at day 3 or 4 after the first GnRH. At the time of the second GnRH injection the follicle size was much less in the aspirated group (11.5 ± 0.2 mm) than the non-aspirated group (14.5 ± 0.4 mm). The volume of the CL was dramatically reduced by ovulation of the smaller follicle when measured either at day 7 ($2,862 \pm 228$ vs. $5,363 \pm 342$ mm³) or at day 14 (4652 ± 283 vs. 6526 ± 373 mm³) after the second GnRH injection. Thus, size of the ovulatory follicle can dramatically alter size of the corpus luteum, probably due to a greater number of granulosa and/or thecal cells contributing to the corpus luteum.

The amount of LH support can also regulate the growth of the corpus luteum. In Figure 3 is shown data from Peters et al., 1994. These investigators treated heifers with a GnRH antagonist on different days of the estrous cycle to evaluate the effect on subsequent luteal function and serum progesterone concentrations. Treatment with the GnRH antagonist eliminated LH pulses but it did not cause regression of the corpus luteum. It appears that the corpus luteum did not grow as well when the cows were treated with GnRH antagonist during the early luteal phase. This is reflected in the serum progesterone concentrations shown in Figure 3. Thus, in addition to an effect of size of the ovulatory follicle, there also is a stimulatory effect of

LH on growth of the corpus luteum and these 2 factors appear to be important in determination of size of the corpus luteum.

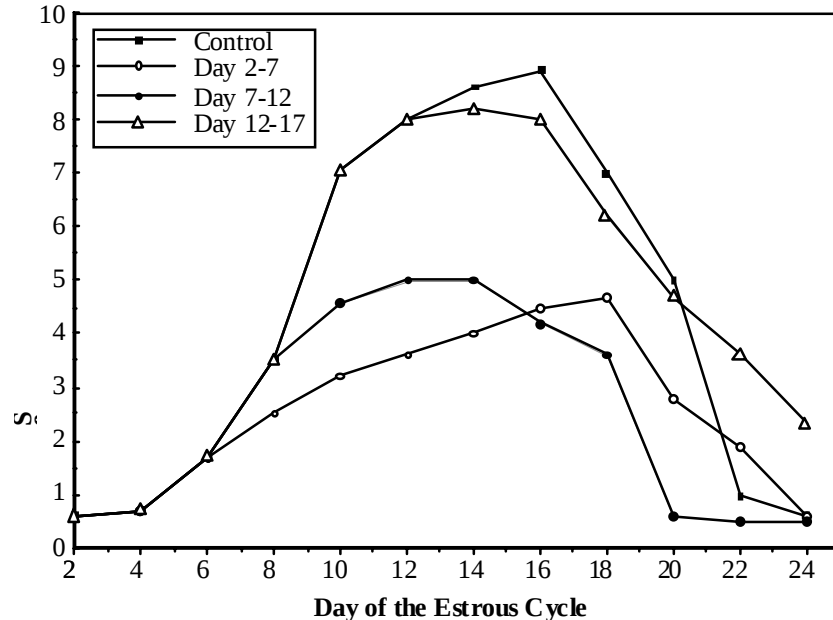


Figure 3. Graph of serum progesterone concentrations in heifers treated with GnRH antagonist from days 2-7, 7-12, or 12-17 of the estrous cycle or left untreated (Control). The serum progesterone concentrations were reduced in heifers treated on days 2-7 or days 7-12 compared to the other 2 groups (data from Peters et al., 1994).

B. What determines circulating progesterone concentrations?

The amount of luteal tissue volume is probably a key determinant of progesterone production from the corpus luteum. In the experiment discussed above (Vasconcelos et al., 2001) in which aspiration was used to reduce the size of the ovulatory follicle, a reduction in size of the corpus luteum also was reflected in reduced circulating progesterone concentrations. However, in most data sets with cows that naturally ovulated there appears to be weak or no relationship between size of the corpus luteum and circulating progesterone concentrations. Although, at first glance, it seems likely that these 2 parameters should be closely correlated, it must be remembered that the circulating concentration of a hormone is determined not only by the rates of production but also by the rates of degradation of the hormone.

Our recent studies have shown that lactating cows have much greater rates of progesterone metabolism than heifers or non-lactating cows. This appears to be due to the very high rates of liver blood flow that are present in lactating dairy cows. The physiological scenario appears to be that high milk production requires very high levels of feed intake. This high level of feed intake increases blood flow to the gut and subsequently increases blood flow to the liver. Steroids, such as progesterone and estradiol are primarily metabolized in the liver and therefore elevated liver blood flow leads to increased rates of steroid metabolism. In recent studies we have found that lactating dairy cows have much larger corpora lutea but lower circulating progesterone concentrations than found in heifers. This is probably due to the high rates of progesterone metabolism in lactating dairy cows. The reason for the larger corpora lutea appears to be due to ovulation of larger follicles in lactating dairy cows. Ovulation of larger follicles may be due to the high estradiol metabolism in lactating dairy cows not allowing circulating estradiol

to reach critical levels that will induce an LH surge until follicular estradiol production is greatly elevated. Thus, lactating dairy cows may grow larger follicles but circulating estradiol may not be correspondingly increased due to high estradiol metabolism. Similarly, larger corpora lutea formed due to ovulation of these larger follicles may not produce elevated circulating progesterone concentrations because progesterone is metabolized at a greater elevated rate in lactating dairy cows.

C. Why does the corpus luteum regress too early or too late?

Regression of the corpus luteum is due to a physiological sequence of events that involves progesterone, estradiol, oxytocin, and prostaglandin (PG) $F_{2\alpha}$ (Silvia et al., 1991; Salfen et al., 1999). It has been known for many years that regression of the corpus luteum is primarily due to uterine secretion of $PGF_{2\alpha}$. Short estrous cycles appear to be primarily due to premature secretion of $PGF_{2\alpha}$ from the uterus. Figure 4 shows a summary of data from various studies on short estrous cycles. In the absence of prior progestin treatment, beef cows will have a short luteal phase following first ovulation. In contrast, prior treatment with progestin results in normal serum progesterone concentrations following first ovulation. The reason for the short luteal phase is premature secretion of $PGF_{2\alpha}$ (Hunter, 1991). Probably most instances of either early or late regression of the corpus luteum ultimately relate to an alteration in uterine $PGF_{2\alpha}$ secretion.

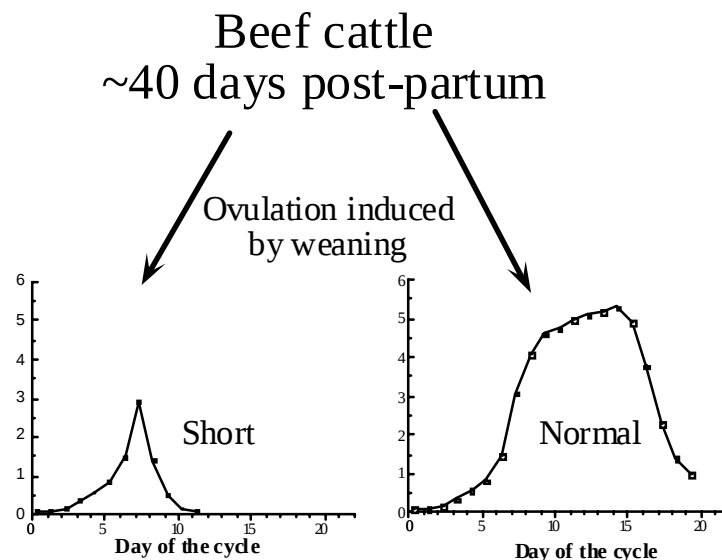


Figure 4. Summary of serum progesterone concentrations observed following the first ovulation of beef cattle that was preceded or not preceded by progestin treatment.

III. Follicular Waves and Selection of a Single Dominant Follicle

A. General Aspects of Follicular Waves

A number of recent studies have used transrectal ultrasound to analyze the final stages of follicular growth in cattle. In Figure 5 is shown a schematic of follicle growth and FSH for a cow that has 2 follicular waves during a 21 d estrous cycle. Near the time of ovulation a group of small follicles begins to grow on the ovaries and this growth has been termed a follicular wave. From this group of follicles a single dominant follicle is selected to continue growth; whereas,

other follicles of the follicular wave undergo regression. Due to the presence of a functional corpus luteum and high progesterone concentrations, this first dominant follicle does not cause an LH surge, behavioral estrus, and does not continue to ovulation. The first dominant follicle will become non-functional and a second follicular wave begins at about mid-cycle. Again a dominant follicle is selected from this second follicular wave and this follicle continues to ovulation because this dominant follicle is functional at the time of regression of the corpus luteum. Some cows also have 3 waves of follicular growth such that the second dominant follicle regresses, a third follicular wave is initiated, and the third dominant follicle is functional at the time of luteolysis and therefore is the ovulatory follicle. The pattern of circulating FSH concentrations has an important functional relationship to the pattern of follicular growth. Both the first and second follicular waves are preceded by an increase in FSH concentrations (Adams et al., 1983). Near the time of estrus there are 2 surges in FSH that are difficult to discriminate because they are temporally adjacent. The first surge corresponds to the GnRH/LH surge that induces ovulation and a second occurs near the time of ovulation and is associated with emergence of the first follicular wave. This increase in FSH is essential for initiation of a follicular wave. Emergence of the follicular wave has generally been retrospectively determined as the time when the first follicles of the follicular wave reached ≥ 4 mm. The time of emergence is generally at the peak of the FSH surge. Following emergence, follicles continue growth and circulating FSH begins to decline up until the time of follicular deviation.

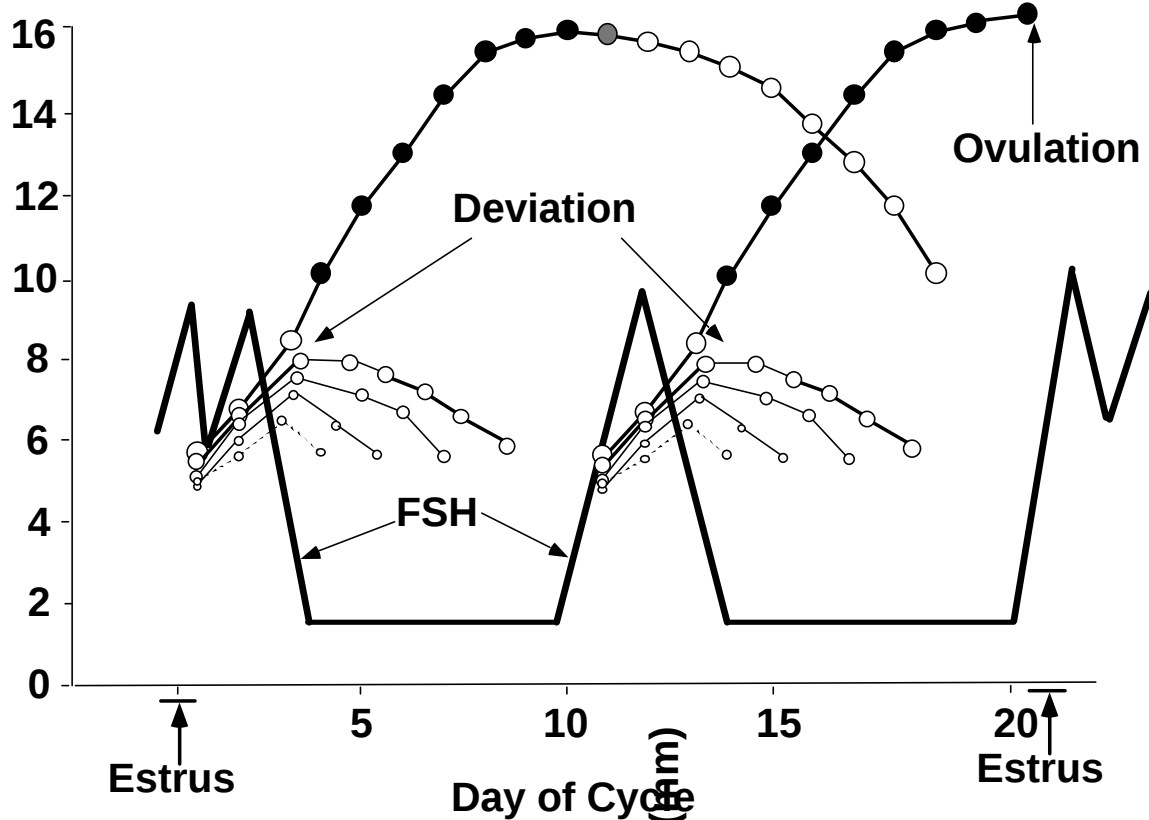


Figure 5. Schematic of follicle growth and FSH for a cow that has 2 follicular waves during a 21 d estrous cycle.

Follicular deviation has been identified in many studies in which follicular growth was followed on a regular basis using transrectal ultrasound. Follicular deviation has been defined as

the beginning of the greatest difference in growth rates (diameter changes between successive ultrasound examinations) between the largest follicle (i.e., dominant follicle) and the second largest follicle (i.e., largest subordinate follicle) at or before the examination when the second largest follicle reached its maximum diameter (Ginther et al., 1996). A representation of the time of follicular deviation is shown in Figure 5. At the time of follicular deviation the diameter of the future dominant follicle averages 8.5 mm and the future largest subordinate follicle averages 7.2 mm (Ginther et al., 1996). The circulating FSH concentrations reach a nadir near the time of follicular deviation and this decrease is probably essential for selection of a single dominant follicle. Selection of the dominant follicle either occurs at the time of follicular diameter deviation or is closely associated with this process. Later sections will discuss the potential mechanisms involved in follicular selection and growth of the follicle after follicular selection.

Follicular waves do not occur only in cycling cattle (Table 1). Follicular waves are present in prepubertal calves by 2 months of age (Evans et al., 1994) and are also present in dairy or beef cattle prior to onset of cyclicity. Follicular waves also occur during most of pregnancy (Ginther et al., 1996a). The maximal diameter of the largest follicle decreases from 15.7 mm in wave 1 to 13.1 mm in wave 2 of pregnancy (Ginther et al., 1994). There is a subsequent decrease in the maximal size of the largest follicle from 12.7 mm at Day 90 of pregnancy to 10.3 mm at the third wave after day 90 and further decreases down to a maximal size of 8.5 mm by the ninth month (Ginther et al., 1996a). As in other physiological states there is an FSH surge that, on average, corresponds to emergence of each follicular wave during pregnancy. The time of follicular selection appears to be similar in most of these states; although, selection may occur at slightly smaller follicles during the first 4 weeks after birth (Evans et al., 1994) and in the last months of pregnancy (Ginther et al., 1996a). The regulation of follicular waves in some of these anovulatory states will be discussed in the next section of this manuscript.

Table 1. Physiologic states in which follicular waves have been found.

Physiologic state	Follicular waves	Length of follicular wave	FSH surge before wave
Estrous Cycle	Yes	9-14 days	Yes
Postpartum Anestrus	Yes	7-12 days	Not evaluated
Prepubertal	Yes	7 days	Yes
Pregnancy	Yes	6-12 days (vary by stage)	Yes
Follicular Cysts	Yes	7-25 days	Yes

B. Follicle Growth from Emergence to Selection

One critical interaction occurring during almost all phases of reproduction in cattle is an interaction between secretion of pituitary FSH and secretion of ovarian inhibitors of FSH. This has been termed two-way functional coupling between FSH and follicles (Ginther et al., 2000). In the absence of any follicles following ovariectomy of cattle there is a rapid increase in circulating FSH that reaches maximum by 24 h with a subsequent continuous maximal secretion (Gibbons et al., 1997). Aspiration of all follicles greater than 3 mm results in a similar elevation in FSH indicating that the follicular FSH inhibiting activity is coming from follicles greater than 3 mm in diameter (Gibbons et al., 1997). The 2 primary inhibitors of FSH that are secreted by the follicle are inhibin and estradiol. Inhibin appears to be secreted by follicles of all sizes; however, circulating estradiol only appears to increase after a dominant follicle has been selected following deviation. Maximal FSH concentrations are observed at the time of emergence of a new follicular wave probably due to low circulating concentrations of inhibin and estradiol. As the follicular wave progresses, larger follicles appear to produce greater FSH inhibitory activity,

probably inhibin, reducing circulating FSH. On average, the nadir in circulating FSH is reached at the time of follicular selection with the dominant follicle of approximately 8.5 mm in diameter. Diminished FSH continues until a few days before emergence of a subsequent follicular wave. Circulating estradiol increases from ~0.2 to ~1 pg/ml near the time of follicle selection (Kulick et al., 1999) and this is probably responsible for the final depression in circulating FSH at this time. Estradiol alone is a very weak inhibitor of FSH secretion but synergizes with inhibin to strongly inhibit FSH secretion.

Figure 1 shows a simplified diagram of the interaction that exists between FSH and the follicle. Circulating FSH is elevated at the time of follicular emergence due to lack of the circulating inhibitors of FSH, inhibin and estradiol. As the follicles grow, circulating FSH declines due to increasing circulating inhibin. Some smaller follicles do not continue growth in the lower FSH environment (Ginther et al., 1996). Near the time of follicular selection there is a further depression in FSH probably due to the combined actions of circulating inhibin and low amounts of estradiol. One critical aspect of this postulated model is that follicle growth up to the time of deviation only involves an interaction between the pituitary and follicle with no involvement of the hypothalamus. Thus, this pituitary-follicle interaction can continue to occur in the presence of widely divergent ovulatory or anovulatory physiological states. Obviously, specific events can set the timing of follicle wave emergence such as removal of dominant follicle inhibitory activities at the time of ovulation or inhibition of post-deviation follicular growth in prepubertal calves or early postpartum cows. The critical concept is that an underlying physiological motif that is present during almost all physiological states is a dynamic interaction between pituitary FSH and follicular growth from emergence to follicular selection.

C. Changes in LH Receptor during Selection

There are probably multiple cellular mechanisms involved in the deviation process including increased free IGF-1, decreased IGF binding proteins, and increased follicular estradiol production. This section will focus on the data related to increased LH receptor near the time of diameter deviation because of the wealth of information on this subject and because of the well documented importance of LH action in follicular growth after deviation (see section E). Beg et al., 2001 compared the diameter of the largest and second largest follicles from the first follicular wave using slaughterhouse ovaries to estimate diameter deviation and determined granulosa cell LH receptor mRNA by quantitative RT-PCR. The increased difference in LH receptor mRNA expression between the 2 largest follicles occurred, on average, an equivalent of 8 h before any increased difference in either follicle diameter (follicular selection) or follicular-fluid estradiol concentration (Beg et al., 2001). In other studies follicular diameter deviation in individual cows was not determined but similar changes in LH receptor mRNA or binding have been noted near the time of follicular selection. For example, Table 2 shows the results of Xu et al., 1995. There was an increase in LH receptor mRNA on granulosa cells from non-detectable on Day 2 (average of 6.7 mm) to highly expressed levels on Day 4 (average of 10.8 mm; Day 0 = day of wave emergence). Near the expected time of follicular selection, there was also a 4-fold increase in LH receptor mRNA in theca cells.

Table 2. Expression of mRNAs for LH receptor (LHr) and FSH receptor (FSHr) in theca and granulosa cells of bovine follicles collected on different days of the first follicular wave. No FSHr was detected in theca cells (data not shown) (data from Xu et al., 1995).

Day of wave	No. heifers (No. of follicles)	Size of follicle	Theca LHr	Granulosa FSHr	Granulosa LHr
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0	3 (8)	5.3 ± 0.4	15 ± 7 ^a	15 ± 5	0 ^a
2	4 (12)	6.7 ± 0.5	13 ± 6 ^a	25 ± 4	0 ^a
4	4 (4)	10.8 ± 0.8	59 ± 10 ^b	31 ± 11	26 ± 10 ^b
6	5 (5)	12.9 ± 0.6	21 ± 10 ^a	14 ± 4	24 ± 8 ^b
8	4 (4)	11.1 ± 1.0	28 ± 10 ^a	13 ± 6	9 ± 7 ^a
10	3 (3)	11.7 ± 0.3	10 ± 7 ^a	6 ± 3	0 ^a

^{a,b}Values with different superscripts differ within columns (P<0.05)

Another study (Bao et al., 1997) also found an increase in detectable (by in situ hybridization) LH receptor mRNA in granulosa cells at 10.8 mm, and LH receptor mRNA increased nearly 2-fold as the follicle grew to 13.2 mm and another 2-fold as the follicle grew to 15.0 mm. Although not statistically significant, Bodensteiner et al., 1996 reported approximately 3-fold greater granulosa cell LH receptor numbers (measured by [¹²⁵I]-hCG binding) from the largest follicle on Day 2 after ovulation (average of 8.5 mm) compared to the largest follicle on Day 4 after ovulation (average of 13.0 mm). In contrast, Evans and Fortune, 1997 reported no increase in detectable LH receptor mRNA (by in situ hybridization) in granulosa cells from follicles on Day 2 (~9 mm) compared to Day 3 (~11 mm; Day 0 = day of wave emergence); but reported that differences in estradiol concentrations between the dominant and largest subordinate follicle were already detectable on both Day 2 and Day 3. However, Jolly et al., 1994 measured the in vitro cAMP response to LH in granulosa cells from follicles of different sizes and found a clear increase in LH responsiveness as follicles grew to greater than 9-10 mm. We have recently completed a study in which we evaluated when the follicle acquired the ability to ovulate after an injection of LH. We found that follicles just after deviation (10 mm) had the ability to ovulate to a large dose of LH (40 mg). Follicles before deviation (7.0 or 8.5 mm) could not ovulate to this high dose of LH. Thus, at the time of follicular selection (~8.5 mm) there are a variety of measures that indicate that after follicular selection there is acquisition of LH responsiveness in the follicle (LH-induced ovulation) and LH receptors in the granulosa cells (cAMP production, LH binding, LH receptor mRNA).

D. Follicle Growth from Selection to Ovulatory Size

Follicle growth past the time of deviation as well as post-deviation follicular estradiol production appear to be regulated by LH pulses. Gong et al., 1995 found that follicles failed to grow beyond ~9 mm in diameter (size of largest follicle at deviation) in cows in which LH pulses had been suppressed by chronic treatment with a GnRH agonist. Fike et al., 1997 used a more straightforward animal model by treating cows with a GnRH antagonist to reduce LH pulses. Cows treated on days 2-7 after estrus, i.e. during the first follicular wave, had the largest follicle grow to an average diameter of only 8.0 mm and persisted for only 6.3 d as compared to maximal diameter of 11.8 mm and persistence of 10.4 d in control heifers. There was also a reduction in circulating estradiol following inhibition of LH pulses (Fike et al., 1997). In a different study, inhibition of LH concentrations by treatment with progesterone did not alter the time or diameter characteristics at the time of follicular selection (Ginther et al., 2001). However, the growth rate of the developing dominant follicle was reduced when the follicle reached ~10 mm in diameter. Thus, follicle growth past deviation appears to require LH pulses and maximal size of the follicle may be decreased by reducing LH pulses.

There is additional evidence for the role of LH pulses in post-selection growth from examination of results from studies that increased LH pulses. A reduction in circulating progesterone concentrations was found to increase numbers of LH pulses and to prolong growth and increase maximal diameter of the dominant follicle. This persistent dominant follicle can also be induced by treatment with small exogenous LH pulses (Taft et al., 1996). Of possible physiological importance is the finding that a transient increase in mean circulating LH concentrations encompasses the expected time of follicular deviation, although this transient increase apparently is not required for diameter deviation (Ginther et al., 2001). Thus, maximal growth of the dominant follicle may be regulated by numbers of LH pulses.

E. Model of Follicular Growth

We propose a follicular growth model based on a plethora of data that shows the importance of FSH and LH in follicular growth. A particularly interesting recent study (Crowe et al., 2001) used heifers that were immunized against GnRH and given either FSH (single bolus of 1.5 mg pFSH), LH pulses (150 µg pLH i.v. every 4 h), or both FSH + LH treatment. In heifers treated with LH alone, there was no growth of follicles to a diameter greater than 5 mm. Treatment with FSH alone caused follicle growth from 5 mm to 9.5 mm. Follicular growth past the expected time of follicular selection (to 10 mm or greater) occurred only in heifers treated with both FSH and LH. The small (< 5mm) and medium (5-9.5 mm) were not estrogen active (only 3 of 131 follicles with Estradiol:Progesterone > 1.0); whereas, almost all larger follicles (> 10 mm) were estrogen active (18 of 21 follicles all in FSH + LH group). Thus, follicle growth before follicular selection requires FSH, but after follicular selection, LH pulses are required. Figure 2 shows a simplified model for the growth of follicles from emergence until ovulation. The emergence of the follicular wave and growth until the time of follicular selection is primarily regulated by circulating FSH. The FSH concentrations are progressively inhibited until they reach a nadir at follicular selection. At this time, continued growth of the follicle and follicular estradiol production requires LH pulses. The dominant follicle continues growth until sufficient circulating estradiol is achieved to induce an LH surge and ovulation of the dominant follicle.

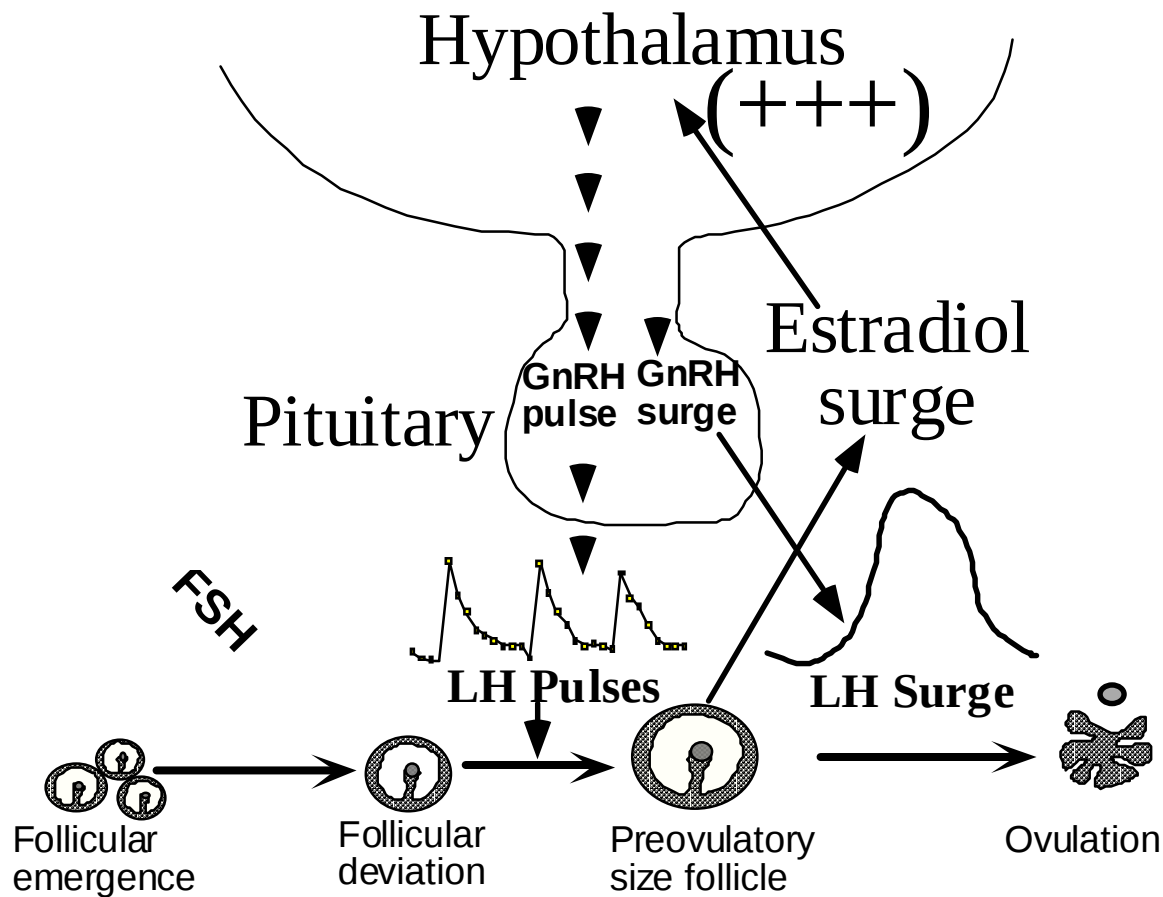


Figure 6. Simplified model for the relationship between the action of hormones and the growth of follicles from emergence until ovulation.

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