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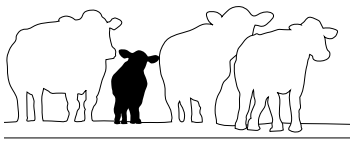

The estrous cycle of heifers and lactating dairy cows: Ovarian and hormonal dynamics and estrous cycle abnormalities

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SUMMARY

Use of ultrasonography and development of sensitive hormonal assays have allowed great progress in understanding the physiology of the estrous cycle in cattle, particularly related to ovarian function. Most of the hormone-based programs for manipulation of the estrous cycle and synchronization of ovulation are based on an understanding of the dynamic processes happening in the ovaries and with the circulating hormones. This chapter reviews these dynamic changes in the ovaries and reproductive hormones to allow practitioners and producers to understand and implement successful reproductive management strategies. First, we examine the estrous cycle from 3 different perspectives: (1) dynamic changes occur in the ovarian structures with growth and selection of a dominant follicle, ovulation of the dominant follicle, and development of the corpus luteum (CL); (2) corresponding changes in circulating reproductive hormones occur, with an increase in estradiol (E2) from the dominant follicle, causing standing heat (estrus), the luteinizing hormone (LH) surge, and eventually ovulation with subsequent increases in progesterone (P4) as the CL develops in preparation for a successful pregnancy; and (3) high milk production causes distinctive alterations in the estrous cycle, circulating hormone concentrations, and in key reproductive traits that need to be managed to produce successful reproductive efficiency on dairy farms. Another key concept related to the estrous cycle is related to cows that do not cycle normally, particularly cows that do not ovulate (termed *anovular cows* in this review). Four perspectives are provided: (1) return to cyclicity after calving, (2) estrous cycle irregularities after first ovulation, (3) classification of anovulation based on physiology of the anovular cow, and (4) treatments for anovular cows.

ESTROUS CYCLE 1: DYNAMIC CHANGES IN OVARIAN STRUCTURES

After puberty, the bovine female enters a period of reproductive cyclicity that continues throughout most of her productive life. These cycles of ovarian activity are called estrous cycles and consist of a series of events beginning at estrus and ending at the subsequent estrus. These cycles are marked by dynamic changes in the structures on the ovary, specifically the growth and regression of follicles and the corpus luteum (**CL**), happening approximately every 3 wk in a nonpregnant cow. Figure 1 shows these ovarian changes for one lactating dairy cow.

The cycle begins with standing heat, termed *estrus*, which is designated as Day 0 of the estrous cycle. As seen in Figure 1, the left ovary has a large preovulatory follicle of 17 mm and the right ovary has a regressed CL. Estradiol (**E2**) concentrations are high (8 pg/mL) due to production by the preovulatory follicle, and progesterone (**P4**) is low. When circulating E2 reaches a sufficient concentration and duration, in the absence of circulating P4, a specific part of the brain—the hypothalamus—will be activated and cause the cow to demonstrate the behaviors typical of estrus, such as standing solidly when mounted, increased physical activity, and more aggressive sexual behavior toward other cows. Close to this time, another area of the hypothalamus is activated by high E2, which elicits a gonadotropin-releasing hormone (**GnRH**) surge that in turn induces a luteinizing hormone (**LH**) surge from the pituitary. The size of the ovulatory follicle varies among cattle, ranging from 13 to 17 mm (diameter) in heifers and from 14 to 20 mm in lactating cows. After the LH surge, circulating E2 concntrations decrease dramatically to basal concentrations during the next 6 to 12 h, which will end behavioral estrus. Ovulation of the follicle happens the next day at 24 to 32 h after the start of estrus. Thus, the estrous cycle begins.

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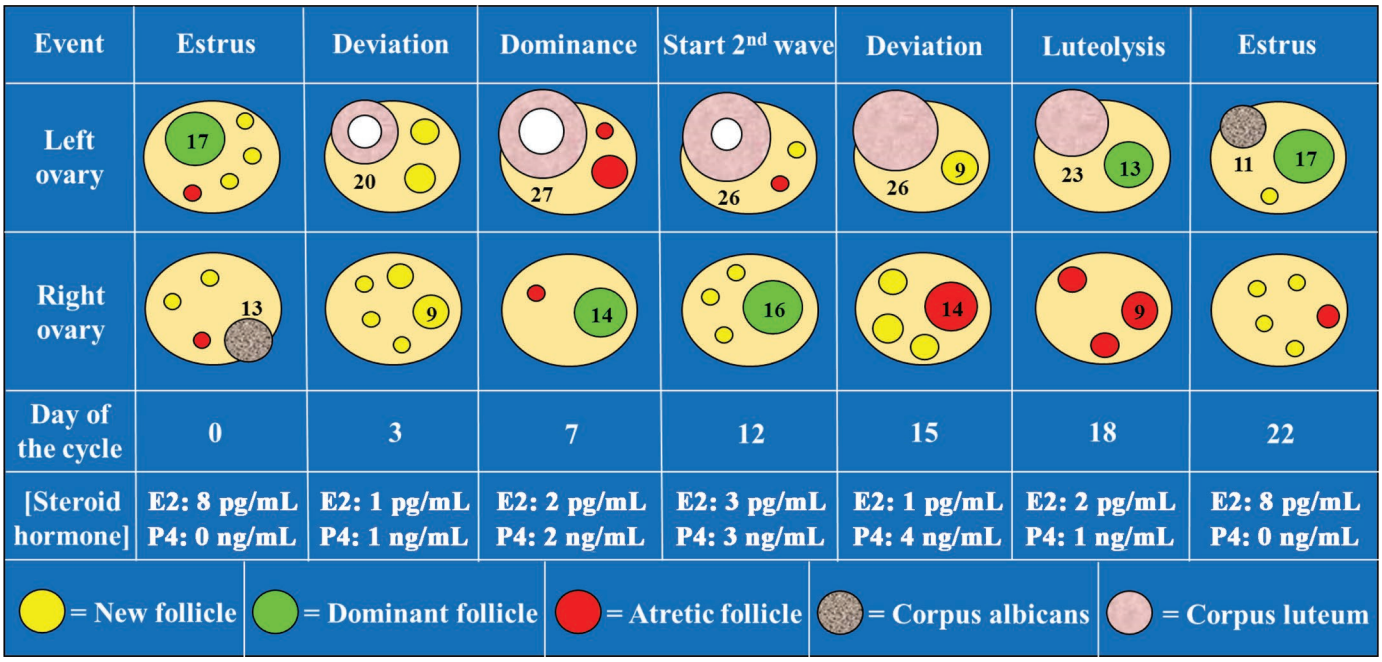

Figure 1. Demonstration of what is observed on the left and right ovaries of a representative lactating dairy cow with 2 follicular waves during an estrous cycle. Estrus occurs on Day 0 and Day 22. The first follicular wave begins near the time of ovulation (Day 1) with the dominant follicle of this wave occurring on the right ovary. The second wave begins on Day 12 with the dominant follicle on the left ovary. The circulating concentrations of estradiol (E2) and progesterone (P4) are also shown for this cow.

After ovulation, the CL forms from the remaining follicular cells. The cow in Figure 1 ovulates the follicle on the left ovary and the CL forms in the same place on the left ovary. Circulating P4 concentrations increase progressively as the CL grows. About 40% of the cows develop a fluid-filled cavity within the CL, which reaches its maximum diameter at Day 7, as shown in Figure 1. Progesterone production by a CL with cavity is similar to that by a CL of the same age without a cavity. Concentrations of P4 remain elevated during the lifespan of the CL, preparing the uterus for an embryo and blocking the action of E2 at the hypothalamus. Thus, circulating P4 is critical for maintaining pregnancy and preventing subsequent spontaneous ovulations. Most of the estrous cycle is under the control of P4, starting after ovulation and formation of the CL and continuing until the time of CL regression in nonpregnant cows. Regression of the CL (luteolysis) during a typical estrous cycle occurs between Days 14 and 19 in heifers and between Days 16 and 24 in lactating cows. In Figure 1, the CL regresses at about Day 18, with CL size and P4 dramatically decreasing. The cow in Figure 1 then shows the next estrus on Day 22, 4 d after the initiation of CL regression. Thus, the timing of CL regression is a key determinant of estrous cycle duration.

To compare estrous cycle length and ovarian function, 7 studies in Holstein heifers (Sirois and Fortune, 1988; Ginther et al., 1989; Knopf et al., 1989; Ko et al., 1991; Lucy et al., 1994; Wilson et al., 1998; Ronchi et al., 2001), and 8 studies in lactating Holstein cows (Savio et al., 1990; Schemm et al., 1990; Taylor and Rajamahendran, 1991; Pursley et al., 1993; Kirby et al., 1997; Trout et al., 1998; Roth et al., 2000; Townson et al., 2002) were selected. By combining the results of all studies, 230 cycles from 224 cows and 104 cycles from 97 heifers were obtained. The average interovulatory interval (or estrous cycle length) was 23.0 d in lactating cows and 20.8 d in heifers. Thus, the average estrous cycle is close to the commonly accepted 21-d interval in heifers but is consistently longer than 21 d in lactating Holstein cows.

Another characteristic feature of the estrous cycle in cattle is the occurrence of wave-like patterns of ovarian follicles. For example, near the time of ovulation in most cows, a new group of small follicles begins synchronous development in the ovaries, starting what is termed the *first follicular wave*. From this group of follicles, a single dominant follicle is selected about 3 to 5 d later and it will continue to grow, whereas other follicles of the first follicular wave will undergo atresia. Because of the presence of a functional CL and high circulating

P4 concentrations, this first dominant follicle does not cause an LH surge or behavioral estrus, and it does not ovulate. The first dominant follicle will become nonfunctional and a second follicular wave begins at about mid cycle. Another dominant follicle is selected from this second follicular wave and, in cows with 2 follicular waves, this follicle will continue to grow until ovulation if it is functional at the time of regression of the CL (Figure 2A). Some cows have 3 waves of follicular growth (Figure 2B), such that the second dominant follicle regresses before endogenous luteolysis, and a third follicular wave is initiated. In this case, the dominant follicle of the third follicular wave is functional at the time of luteolysis and therefore it will be the ovulatory follicle. Cycles with 4 follicular waves have also been reported. Cows or heifers with short cycles will have early CL regression (about Day 7) and the dominant follicle from the first follicular wave will therefore become the ovulatory follicle, producing an estrous cycle with only one follicular wave. In cattle with 2 follicular waves, the time for emergence of the second wave occurs, on average, between d 10 and 12**[AU1: Day 10 and 12, Day 15 and 19, and Day 7 and 10 to be consistent with previous uses (capitalized for specific days of cycle)]** after estrus with consistent timing in most heifers. In cattle with 3 follicular waves, the emergence of the second wave occurs between d 7 and 10 and the emergence of the third wave occurs between d 15 and 19.

A more comprehensive discussion of the estrous cycle in dairy cows is provided at the following link: http://dairycattlereproduction.com/guide/estrous-cycle/.

ESTROUS CYCLE 2: DYNAMIC CHANGES IN CIRCULATING HORMONES

Six key reproductive hormones are discussed in this review (see Table 1). First, circulating P4 comes from the CL and is the prevailing hormone during much of the estrous cycle. The dynamics of circulating P4 are shown for 2 representative lactating cows in Figure 2. As shown, circulating P4 increases as the CL develops and continues to increase until Day 14 to 16, when it reaches a plateau that is maintained until luteolysis occurs. For example, Figure 3 shows an average profile for a cow with 2 follicular waves. The key time when P4 decreases is near the end of the estrous cycle, on Day 18 to 19, when the CL regresses. Progesterone continues to be low for the 1 to 3 d before and during estrus. Circulating P4 concentrations will only increase again after ovulation of the new dominant follicle and as the CL of the next estrous cycle develops. If the cow becomes pregnant, the CL will continue to produce P4 and therefore circulating P4 will remain elevated until about 2 d before calving. Thus, P4 is *progestational* with an essential role in maintaining pregnancy. During the estrous cycle, increased circulating P4 prevents estrus or new ovulations and therefore is the "dominant" hormone of much of the cycle.

The second key hormone of the estrous cycle is E2, which has many effects that are almost opposite those of P4. When P4 decreases, circulating E2 increases as the dominant preovulatory follicle increases in size and produces E2. Thus, during estrus and the period before estrus, termed *proestrus*, E2 is the prevailing hormone. High E2 in the presence of low circulating P4 causes the cow to stand solidly when mounted by a bull or by another cow, a behavior called *estrus*. Estrus is due to an effect of E2 on a part of the brain called the *hypothalamus* that regulates sexual behavior in the cow. Another effect of high E2, in the absence of P4, is to cause secretion of a large amount of GnRH. This effect also is caused by an effect of E2 on a specific part of the hypothalamus. This action of E2 is called the *positive feedback effect* of E2 because high concentrations of E2 lead to a massive secretion of GnRH, termed the *GnRH surge*. The GnRH surge then stimulates cells in the pituitary gland to secrete LH, causing the *LH surge*. The LH surge is the hormonal event that directly causes ovulation of the dominant follicle. Ovulation occurs about 28 h after the peak of the LH surge. Thus, standing estrus and eventual ovulation are both events that occur after the increase in circulating E2 from the preovulatory follicle. Estrus is directly caused by E2 and ovulation is caused by E2 inducing a GnRH surge and then an LH surge.

One question that some producers ask is why does estrus only last 4 to 12 h, whereas ovulation does not happen until about 28 h after the start of estrus? The end of estrus is caused by a decrease in circulating E2 below a threshold concentration. The decrease happens because the follicle stops producing E2, which happens 6 to 9 h after the first standing event of estrus. Thus, cows have low E2 and stop showing standing estrus 12 to 18 h before the follicle ovulates. Artificial insemination (AI) of cows should be performed 4 to 12 h before ovulation so this is generally near the end of estrus or even a few hours after the cow has stopped showing standing heat. However, because estrus duration varies among cows, the time for AI should always be determined in relation to the time of estrus initiation, which coincides with the E2-induced GnRH/LH surge.

Thus, it seems clear that the cow shows estrus due to secretion of E2 from a large dominant follicle. This follicle ovulates, producing a CL that then secretes P4. One other key hormone causes the death or regression of the CL if the cow does not become pregnant. This hormone—prostaglandin $F_{2\alpha}$ (**$PGF_{2\alpha}$**)—comes from

the nonpregnant uterus. The typical pattern of $PGF_{2\alpha}$ secretion from the uterus is pulsatile, with 3 to 5 large pulses of $PGF_{2\alpha}$ occurring during a 36-h period, causing a decrease in circulating P4 (Figure 3). On dairy farms, $PGF_{2\alpha}$ is the most common reproductive hormone used to regulate the reproductive cycle. If a cow is treated with an appropriate dose of $PGF_{2\alpha}$ and has a responsive CL (more than Day 5 after estrus), then the CL will regress and the cow is likely to show estrus and begin a new estrous cycle. Thus, $PGF_{2\alpha}$ treatment mimics what is happening during the normal estrous cycle and causes the cow to potentially have estrus and ovulation at an earlier time and in a more synchronized manner.

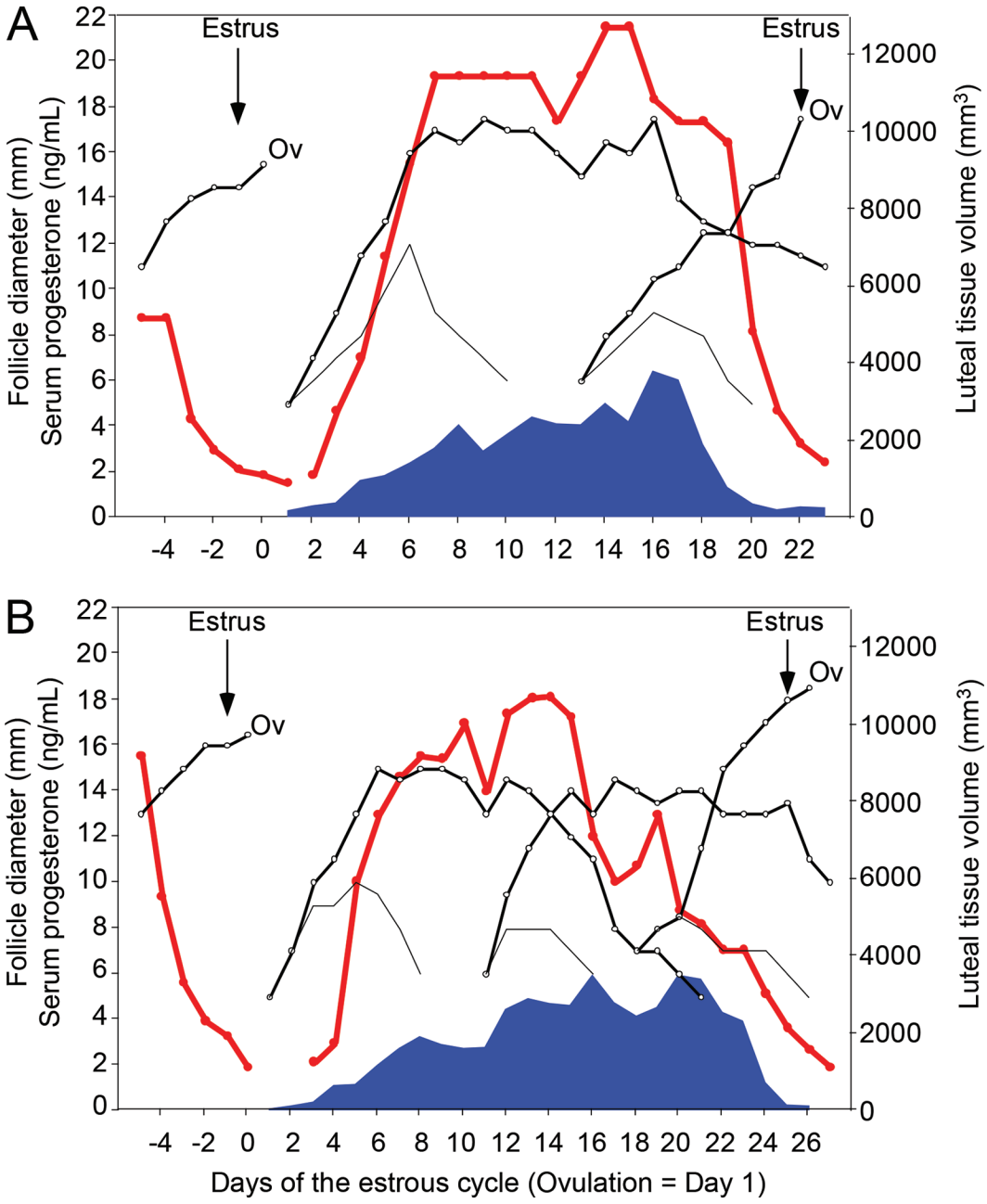

Figure 2. Ovarian dynamics in 2 representative lactating dairy cows with 2 (A), or 3 (B) follicular waves. The dominant follicle of each follicular wave is shown as a line with open circles. The small dark line is the largest subordinate follicle. The large red line with solid circles represents the corpus luteum volume. Circulating serum progesterone (P4) concentrations are shown as the shaded blue area. Times of estrus and ovulation (Ov) are shown. Adapted from Sartori et al. (2004).

Table 1. Summary of 6 reproductive hormones that are essential to the normal estrous cycle

Hormone	Site of production	Action of hormone
Progesterone (P4)	Corpus luteum (CL) on ovary	Maintains uterus compatible with pregnancy Blocks estrus and GnRH surge
Estradiol (E2)	Follicle on ovary	Causes standing estrus Causes GnRH surge Causes uterine tone
Gonadotropin-releasing hormone (GnRH)	Hypothalamus in brain	Causes LH surge
Follicle stimulating hormone (FSH)	Pituitary gland	Causes follicle growth
Luteinizing hormone (LH)	Pituitary gland	LH surge causes ovulation LH pulses cause dominant follicle growth
Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)	Nonpregnant uterus	Regresses CL

The final hormone that is key regulator of follicular waves is circulating follicle-stimulating hormone (**FSH**). Each follicular wave is preceded by an increase in FSH concentrations (Adams et al., 1992). This increase in FSH is essential for initiation of a follicular wave. Time of follicle wave emergence is generally at the peak of the FSH surge. Following emergence, follicles continue to grow and circulating FSH declines to reach a nadir at the time of selection of the dominant follicle. The mechanisms involved in the selection process are complex and associated with acquisition of LH responsiveness (LH receptors) in the granulosa cells of the dominant follicle. Subsequent growth of the dominant follicle, after follicular deviation, appears to be related to small LH pulses from the pituitary gland. These LH pulses occur throughout the estrous cycle, as demonstrated in Figure 3, causing the dominant follicle to grow, and are distinguished from the large LH surge that occurs near the time of estrus that causes the follicle to ovulate. The dominant follicle that is present at the time of CL regression will go on to ovulate. Alternatively, if the CL is functional with high circulating P4 at the time of maximal dominant follicle size, then the dominant follicle undergoes a process termed *follicular atresia* or death of the dominant follicle. Atresia of the dominant follicle allows a new surge in FSH concentrations and initiation of the next follicular wave.

Thus, the estrous cyle is characterized by behavioral changes and specific changes in the follicles and CL on the ovaries. Each of these changes can be explained by changes in specific reproductive hormones in the blood stream. Synchronized or nonsynchronized breeding programs rely on optimizing the circulating patterns of reproductive hormones, ideally producing ovulation of a highly fertile oocyte from an optimally sized follicle. This will lead to development of a functional CL and a circulating P4 pattern that will produce an ideal uterine environment for development of the embryo, maintenance of pregnancy, and eventual delivery of a healthy calf.

ESTROUS CYCLE 3: CHANGES IN DYNAMICS OF THE ESTROUS CYCLE DUE TO HIGH MILK PRODUCTION

When compared with nonlactating heifers, lactating cows have interesting and important changes in reproductive traits, including longer estrous cycles, increased risks of double ovulation and twinning, decreased fertility (pregnancy per AI; **P/AI**), and changes in circulating hormones concentrations and sizes of ovarian structures (Table 2). For example, cows with higher milk production ovulate larger follicles but have lower circulating E2 concentrations (Lopez et al., 2004). In addition, higher-producing dairy cows have a larger volume of luteal tissue but reduced circulating P4 (Lopez et al., 2005). These results are surprising because it would be expected that cows with larger follicles would have greater follicular E2 production, and therefore greater circulating E2. Similarly, cows with a larger luteal tissue volume might be expected to have greater circulating P4. The most likely explanation for this paradox is that lactating dairy cows have increased metabolism of steroid hormones as milk production increases.

The hypothesis that increased liver blood flow (**LBF**), as a result of elevated feed intake, in lactating dairy cows would increase steroid metabolism has been tested (Sangsritavong et al., 2002). The authors found that before feeding, LBF was greater in lactating (1,561 $\pm$ 57 L/h) cows than in nonlactating cows of similar size and age (747 $\pm$ 47 L/h). The LBF and metabolism of P4 and E2 increased immediately after any amount of feed consumption in both lactating and nonlactating cows. The metabolism of E2 and P4 was much greater in lactating than nonlactating cows. There was a strong association ($r^2 = 0.85$) between LBF and metabolism of P4. Nonlactating cows had much lower LBF and much lower metabolism of P4, and cows with greater milk production had greater LBF and P4 metabolism. Thus, the changes in metabolism of E2 and P4 in response

to feeding are immediate and appear to be related to acute changes in LBF. In lactating cows, a continuous high plane of nutrition appears to chronically elevate LBF and metabolism of steroid hormones to almost double the amount observed in nonlactating cows of similar size and age. These results indicate that even with a similar level of hormone production, there would be lower circulating hormone concentrations in lactating dairy cows.

It is clear that low success in detecting estrus is reducing reproductive efficiency on commercial dairy farms. Indeed, Washburn et al. (2002) reported a decrease from 50.9% in 1985 to 41.5% in 1999 for heat detection rates in Holstein dairy herds in the southeastern United States. However, studies have reported both a negative relationship between level of milk production (Harrison et al., 1989) and no relationship (Van Eerdenburg et al., 2002) using visual observation twice a day to mea-

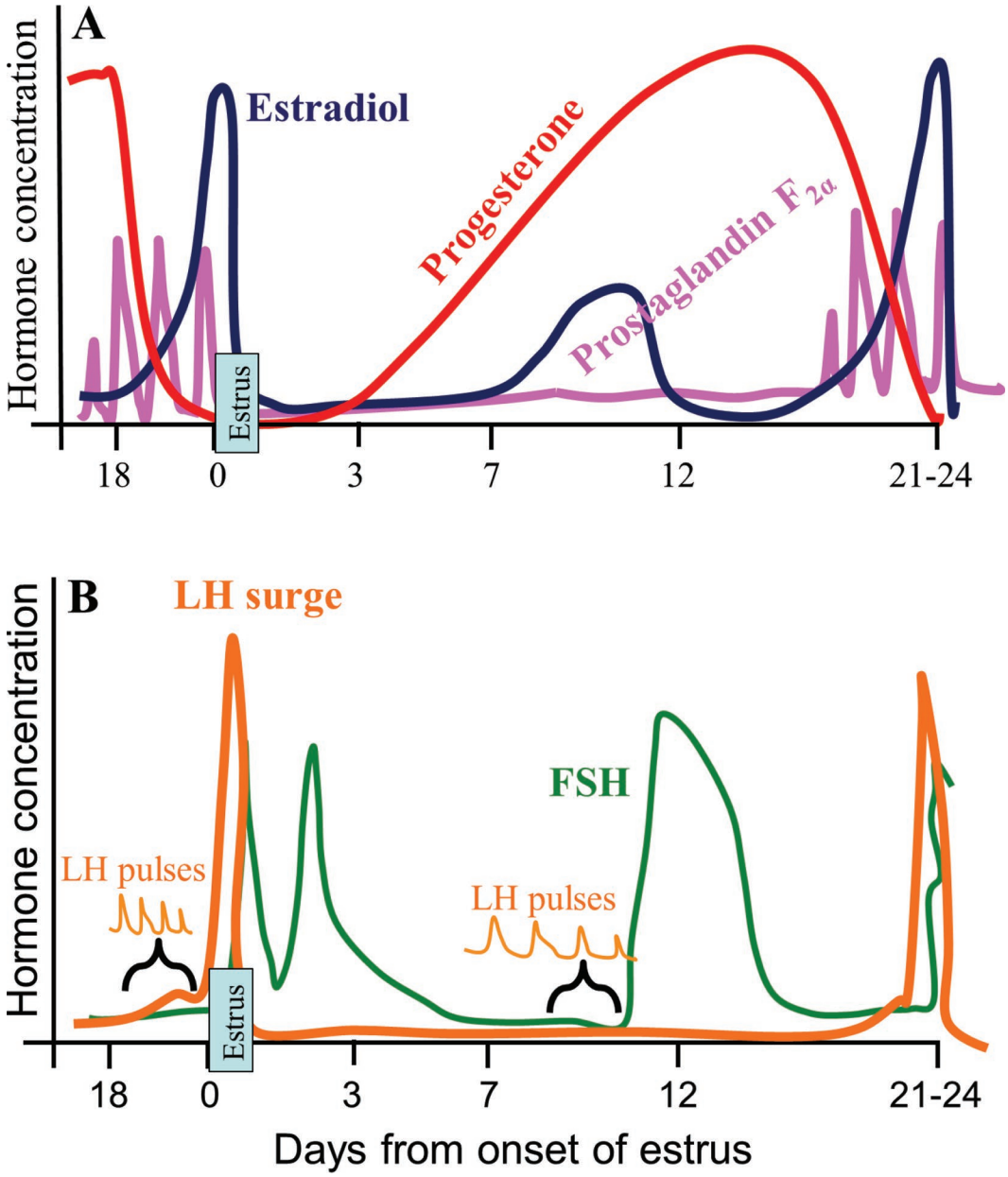

Figure 3. Schematic of the hormonal changes during the estrous cycles of an individual lactating dairy cow with 2 follicular waves. (A) Changes in progesterone, estradiol, and prostaglandin $F_{2\alpha}$; (B) luteinizing hormone (LH) and follicle-stimulating hormone (FSH) surges and a representation of the rapid LH pulses that happen in the proestrous period and slower LH pulses that happen during the mid-luteal phase.

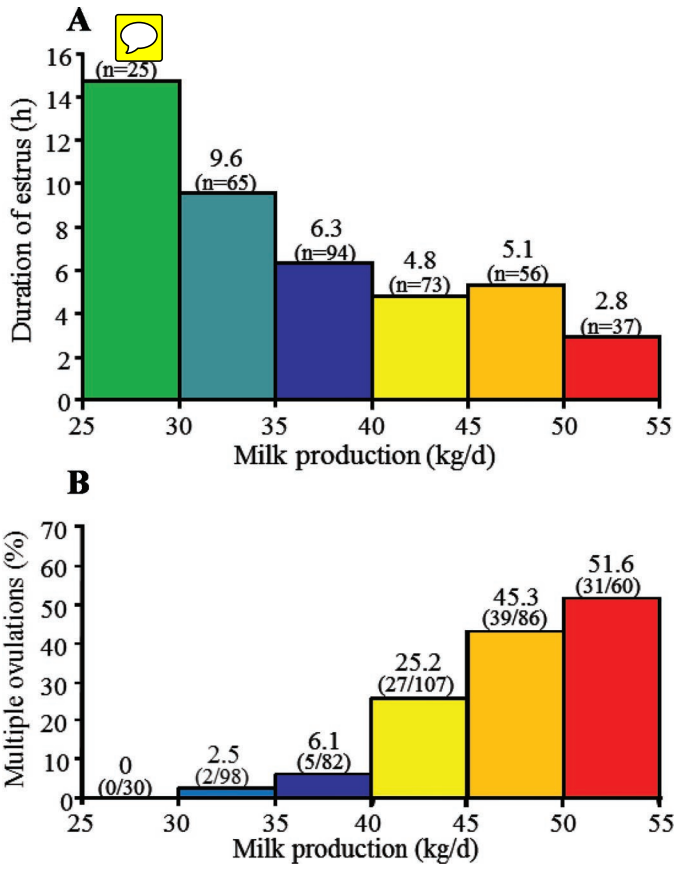

Figure 4. Relationship between level of milk production and duration of estrus (A) or risk of multiple ovulation (%; B). Analysis included all single ovulations (n = 350) except first postpartum ovulations. Average milk production is for the 10 d before estrus. From Lopez et al. (2004, 2005).

sure expression of estrus. Lopez et al. (2004) evaluated the duration of estrus in a group of lactating dairy cows using the HeatWatch system (**[AU2: Add manufacturer name and location (city and country or city and state)]**). This system allows continuous monitoring of mounts for each cow on a 24 h/d basis and can be used to calculate the duration of estrus in individual dairy cows. Cows with milk production above the herd average (~40 kg/d) had shorter duration of estrus (6.2 ± 0.5 h) than cows with lower milk production (10.9 ± 0.7 h). Figure 4A shows the relationship between level of milk production and duration of estrus monitored on a continuous basis with the HeatWatch system.

In a subset of these cows (n = 71), maximal follicular size and circulating E2 concentration on the day of estrus were analyzed. High-producing cows (46.8 kg/d) had larger follicles (18.6 vs. 17.4 mm in diameter) but lower circulating E2 (6.8 vs. 8.6 pg/mL) compared with lower-producing cows (32.3 kg/d). Correlations were evaluated between several different values. As expected, duration of estrus was correlated with peak E2 (r = 0.57) and with milk production (r = −0.51). Level of milk production was also correlated with follicular size (r = −0.45). As discussed above, the theory was that high milk production leads to decreased circulating E2, resulting in a decreased duration of estrus. Decreased E2 could also be the cause of increased follicular size by delaying the time to E2-induction of estrus, GnRH/LH surge, and ovulation in high-producing cows.

The effect of milk production on the risk of double ovulation was also evaluated (Figure 4B). Double ovulation is the primary factor causing the high twinning rate in lactating dairy cows. Cows with lower milk production had a low likelihood of double ovulation (<5%), whereas cows with high milk production had a greatly elevated risk of double ovulation. Cows with the highest milk production had more than a 50% risk of double ovulation in this study. Cows in this study had no hormonal treatments, such as recombinant bovine somatotropin (rbST) or reproductive hormones, during the time of this analysis. Thus, it seems clear that the high risk of twinning in lactating dairy cows, as reported in other studies (Ryan and Boland, 1991), is related to the high double ovulation rate. Recent studies have related the high risk of double ovulation in lactating dairy cows to reduced circulating P4 during

Table 2. Comparison of various reproductive traits of heifers and lactating cows[1]

Trait	Heifers	Lactating cows
Estrous cycle duration, d	21.0	23.0
Day of luteolysis	18.5	18.9
Days from luteolysis to ovulation	4.6	5.2
Maximal size of largest ovulatory follicle, mm	14.9	16.8
Estradiol peak preceding ovulation, pg/mL	11.3	7.9
Duration of estrus, h	13.8	8.7
Maximal luteal tissue volume, cm^3	7.3	11.1
Progesterone peak, ng/mL	7.3	5.6
Double ovulation, %	1.9	17.9
Twinning rate, %	1.0	8.0
Pregnant/AI, %	74.4	39.0

[1]Data from Ryan and Boland (1991), Nebel et al. (1997), Pursley et al. (1997), and Sartori et al. (2004).

the follicular wave that results in the ovulatory follicle (Wiltbank et al., 2014). Thus, changes in economically important reproductive traits due to increased milk production, such as duration of estrus and twinning, seem to be directly related to the changes in circulating hormone concentrations caused by elevated LBF and steroid metabolism in lactating dairy cows. Reproductive management programs on dairy farms with high milk production can be designed to increase insemination rate, maximize fertility, and decrease twinning risk with one important consideration being the optimization of circulating P4 and E2 concentrations.

ANOVULATION 1: RETURN TO CYCLICITY AFTER CALVING

One major factor that can contribute to cows either not receiving AI or having suboptimal P/AI is the prevalence of anovulation in dairy herds. The term *anovulation* is used to identify a situation in which a cow fails to ovulate, usually measured at about 60 to 80 d after calving. Cows that are in the anovulatory condition can be identified by sequential ultrasound exams or measurements of P4. However, in some field situations where the estimation of cyclicity depends on occasional palpation of ovaries per rectum and detection of estrus, cows that are not detected in estrus are sometimes assumed to be anovular. Unfortunately, there are many physiological and environmental reasons why a cow may not show standing estrus even though she has an LH surge and ovulates. The term *anestrus* will not be used in this chapter because it does not clearly define whether cows are not cycling or are simply not displaying estrus.

Time to first postpartum ovulation averaged 33 d in US Holsteins in a compilation of 10 studies reported by Ferguson (1996). Studies at the University of Wisconsin-Madison, with high-producing Holstein cows, observed intervals to first postpartum ovulation of 36 d (n = 13; Haughian et al., 2002) and 51 d (range of 12 to 166 d; n = 266; Lopez et al., unpublished**[AU3: For unpublished data, need names (initials and last names), affiliations, and locations of all contributors. However, if this is likely to be published in the next few months, cite in Refs list as a published paper or book.]**). Similar results have been observed in other countries. For example, a study from Brazil (Meschiatti and Sartori, unpublished**[AU4: See previous query]**) with Holstein (n = 13) or crossbred Holstein × Jersey (n = 38) cows (grazing with supplementation in summer; confined in winter) observed 18% anovular cows at 60 d postpartum, with ovular cows having an average time to first postpartum ovulation of 23 d (range of 9 to 52 d).

The delay in time to first ovulation is surprising because the first follicular wave emerges 4 to 12 d after calving. This first follicular wave after calving is due to a surge in FSH that occurs during the first week, probably due to the dramatic decrease in circulating E2 after loss of the placenta, which produces large amounts of E2 near the end of pregnancy. For example, circulating E2 concentrations are about 500 pg/mL during the last few days before calving, about 50 times the concentration measured near the time of estrus. After calving and loss of the placenta, circulating E2 reaches a nadir in the first few days in milk (DIM) and there is a consistent and large FSH surge during the first week that stimulates emergence of the first follicular wave. This first follicular wave can proceed to ovulation or regress, allowing a new follicular wave, or the dominant follicle may become cystic.For example, pasture-fed dairy cattle had, on average, 4.2 waves of follicle growth before first ovulation, with the maximal size of the largest follicle increasing as first ovulation approached (McDougall et al., 1995). In cows that do not ovulate the follicle of the first wave, the maximal size of the largest follicle generally does not reach ovulatory size. Factors such as negative energy balance, low body condition score (BCS), puerperium disorders, or other diseases can prolong the time to first ovulation.

Another way to think about the anovular problem is to determine the percentage of cows that are anovular near the end of the voluntary waiting period or at about 60 to 70 d after calving. For example, a previous collaboration between several reproductive physiologists and a dairy geneticist, George Shook, evaluated prevalence and heritability of anovulation using data from 13 different studies with a total of 5,818 lactating dairy cows (Bamber at al., 2009). The incidence of anovulation at ~65 d after calving was 23.3% using transrectal ultrasound of ovaries and measurements of circulating P4. This study also found a heritability of 0.171, a high heritability value for a reproductive trait (generally about ~0.05), but much lower than the heritability of milk production and milk component traits. Table 3 summarizes the results from several studies that evaluated anovulation at 60 to 70 DIM in high-producing lactating dairy cows. In studies evaluating over 17,000 lactating dairy cows in US dairy farms, 23.4% of the cows, on average, were anovular at the time breeding programs were initiated. There is substantial variability among farms in percentage of cows that are anovular. Nevertheless, most dairy producers should assume that 20 to 25% of lactating dairy cows are anovular at the time programs for first AI are initiated.

Unlike other reproductive traits, such as duration of estrus and double ovulation risk that are convincingly associated with level of milk production (Figure 4), risk

Table 3. Summary of studies that have evaluated the percentage of cows that are anovular at about 60 to 70 d after calving

Reference	Anovular cows at 60 to 70 d postpartum, % (no./no.)
Wiltbank et al. (2006) (summary of 6 studies)	26.1 (726/2,783)
Stevenson et al. (2008)	25.5 (270/1,060)
Bamber et al. (2009) (summary of 19 studies)	23.3 (1,356/5,818)
Bisinotto et al. (2010) (summary of 8 studies)	22.6 (1,267/5,607)
Herlihy et al. (2012)	24.7 (92/373)
Colazo et al. (2013)	17.6 (107/608)
Vieira-Neto et al. (2014)	22.2 (348/1,569)
Total	23.4 (4,166/17,818)

for anovulation is not strongly associated with level of milk production. Thus, lower-producing cows are generally just as likely to be anovular as high-producing cows. One factor that is related to risk of anovulation is BCS. In general, as BCS decreases, the prevalence of anovular cows increases (Bamber et al., 2009; Santos et al., 2009). In contrast, lactating dairy cows with acceptable BCS at 60 to 70 DIM (2.75 or more) still have a high risk of anovulation, with about 20% being anovular (Bamber et al., 2009). Thus, it is not easy to identify cows that are anovular based on milk production or BCS and therefore careful evaluations of the ovaries are required to identify the anovular cows in a dairy herd.

ANOVULATION 2: OVARIAN DYSFUNCTION AFTER FIRST OVULATION

The incidence of ovarian dysfunction after first postpartum ovulation in dairy cows has been evaluated by several authors, with reports of short estrous cycles (premature CL regression), delayed CL regression (persistent CL), and prolonged interluteal intervals after first ovulation.

The first estrous cycle after calving, regardless of the day after calving when it is initiated, is likely to be an abnormal cycle. Many first ovulations are accompanied by no estrus or a greatly reduced duration of estrus. Many first ovulations are followed by a short estrous cycle of 10 to 11 d. During the postpartum period, the basic mechanisms regulating the maintenance of the CL do not function normally and premature luteolysis can occur. Short estrous cycles are due to premature secretion of $PGF_{2\alpha}$ from the uterus. In cows that have a short estrous cycle, the E2 from the first follicular wave causes the uterus to secrete large amounts of $PGF_{2\alpha}$ and as soon as the developing CL becomes responsive to $PGF_{2\alpha}$ (about Day 7 of the cycle), the CL will regress. The cow will then come into standing estrus 3 to 4 d after CL regression or about Day 10 to 11 of the first cycle. Studies reported incidences of 11 to 54% for short cycles in the postpartum dairy cow. For example, Royal et al. (2000) observed 27.3% (176 of 645 cycles) and 9.2% (45 of 490 cycles) incidences of short cycles after the first and second ovulations postpartum, respectively. Obviously, breeding cows that have a short cycle will not result in pregnancy.

Delayed luteolysis or persistent CL is another reproductive dysfunction often observed in the postpartum cow and it is one of the causes of prolonged interovulatory intervals. Persistent CL has been defined in a variety of ways, including milk P4 >3 ng/mL for ≥19 d or for >30 d with variability in reported incidence. In general, persistent CL is not very common (reported incidence ranges from 1.5 to 7.3%). Exceptionally, Ball and McEwan (1998) reported a greater incidence of prolonged luteal function, especially in cows that began ovulations less than 25 d after parturition (18 of 73 cows = 25%) compared with cows that first ovulated between 25 and 45 d after parturition (0 of 34 cows). This result is consistent with the association between a persistent CL and uterine abnormalities or uterine infection. It seems likely that lack of uterine involution, uterine infection, or other uterine abnormalities may reduce $PGF_{2\alpha}$ secretion or $PGF_{2\alpha}$ transport to the ovary, resulting in a prolonged CL lifespan.

The interval between the demise of the CL (milk P4 <3 ng/mL) of the previous cycle and the beginning of the next luteal phase (milk P4 >3 ng/mL) is called the *interluteal interval*; it lasted 7.8 d when data from 5,204 interluteal intervals were evaluated (Darwash and Lamming, 1995). An interluteal interval of >12 d was classified as a prolonged interluteal interval and has been described in several studies. Authors**[AU5: Darwash and Lamming?]** reported prolonged interluteal intervals in about 10% of dairy cows after a previous estrous cycle. It seems likely that ovulation failure of the dominant follicle that is present at the time of luteolysis is the major cause for prolonged interluteal interval, probably due to lack of an adequate LH surge or inadequate LH responsiveness in the follicle. Two examples of this type of anovulation are shown in Fig-

ure 5. Following an apparently normal estrous cycle, CL regression is followed by a prolonged period with lack of ovulation, in spite of 1 or 2 periods of standing estrus. Follicles grow to ovulatory size but do not undergo ovulation. In this study[AU6: Darwash and Lamming?], a high proportion (12/26) of cows did not ovulate the dominant follicle that was present at the time of CL regression.

ANOVULATION 3: CLASSIFYING ANOVULATION BASED ON PHYSIOLOGY

We have suggested that anovulation be classified into physiological categories based on maximal size of the largest growing follicle and circulating E2 (Wiltbank et al., 2002). In 2 studies, weekly ultrasound exams were combined with P4 concentrations to evaluate cyclicity. Incidences of anovulation of 20.2% (64 of 316 cows;

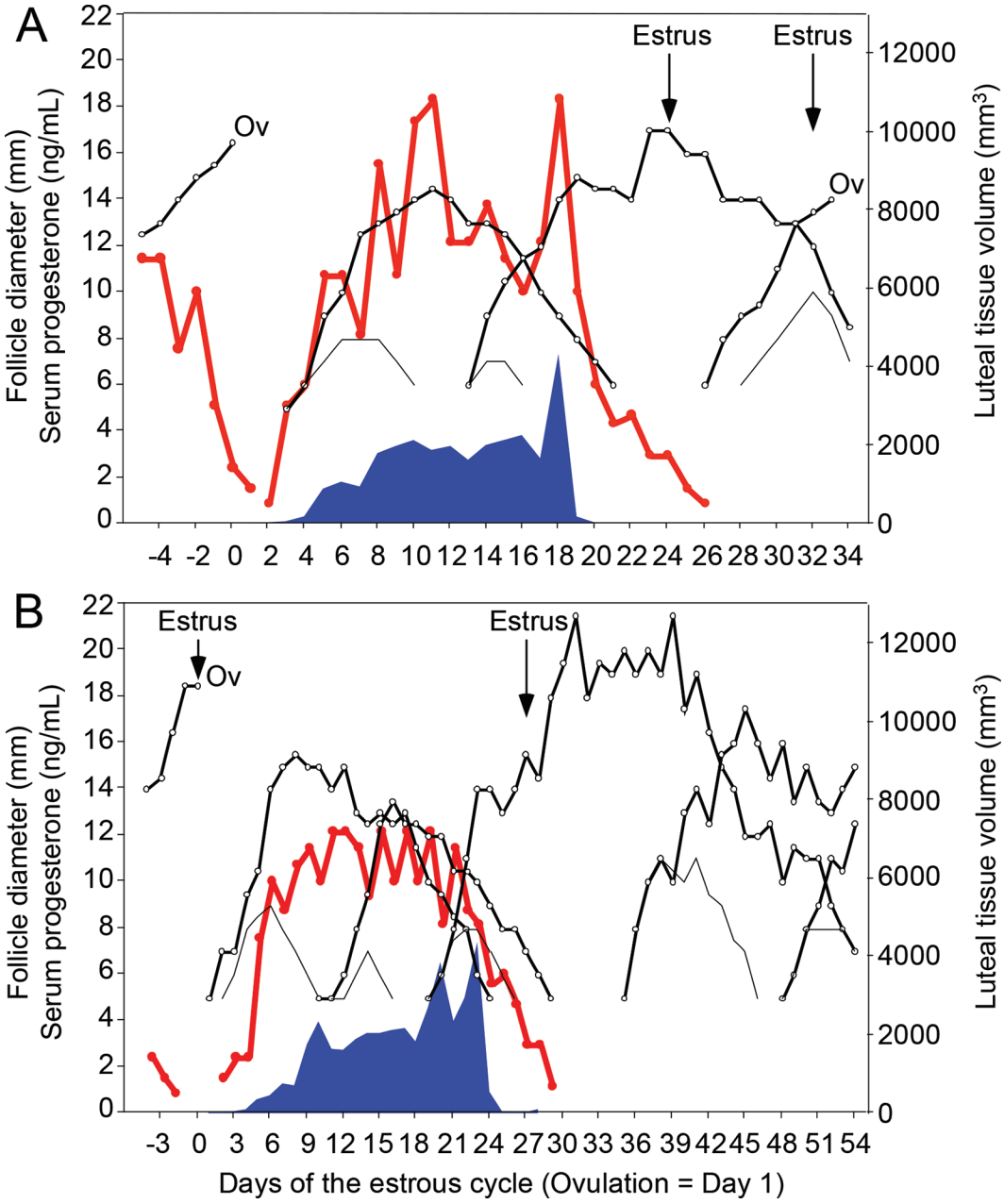

Figure 5. Patterns of follicular (black lines: dominant follicles = thick lines with open circles; subordinate follicles = thin lines) and luteal development (red lines with solid circles), and serum progesterone concentration (shaded blue area) of 2 lactating cows that developed anovular follicles after regression of the corpus luteum. Ov = ovulation. Adapted from Sartori et al. (2004).

Gümen et al., 2003) and 28.5% (76 of 267 cows) were observed by 60 and 71 DIM, respectively. Table 4 shows the maximal size of anovular follicles from these 2 studies of lactating dairy cows. Only one cow did not grow follicles to at least 9 mm; that is, past the point of follicular deviation. Thus, "inactive" ovaries are a rare occurrence because, in most anovular cows, follicular waves occur with growing follicles >9 mm in diameter. About 20% of cows did not grow a follicle to ovulatory follicle size (<15 mm) and these cows had, on average, lower BCS than the cows that had larger anovular follicles. About 20% of cows grew "cystic" follicles (>25 mm). Surprisingly, most anovular cows (~60%) grew follicles to about ovulatory size or larger (15 to 25 mm) but were not large enough to be considered a "cystic" follicle. Anovular cows with good BCS (2.75 or more) had, on average, larger anovular follicles. These results demonstrate how cows that remain anovular could easily be mistaken for cows that might be cycling because anovular follicles are large but not cystic.

Figure 6 illustrates the physiology that underlies the 2 major types of anovulation in lactating dairy cows. Figure 6B illustrates follicle growth and hormonal patterns in a cow undergoing normal estrous cycles. Figure 6A illustrates the physiology of anovular cows with the "small follicle anovular phenotype." These cows are characterized by growth of dominant follicles (>8 mm) but not to ovular size. As illustrated in Figure 6A, follicle growth is limited because of inadequate numbers of LH pulses to grow the follicle to ovulatory size. A small amount of E2 from the dominant follicle is very inhibitory to the GnRH pulse center in the hypothalamus and GnRH pulses are inhibited, leading to inadequate numbers of LH pulses, which limits follicle growth. Alternatively, cows with the "large follicle anovular phenotype" (Figure 6C) grow follicles larger than ovulatory size and produce elevated amounts of E2 but high circulating E2 does not induce a GnRH surge from the GnRH surge center of the hypothalamus. These cows may or may not show signs of estrus. However, due to lack of a GnRH surge, there is no LH surge, and no ovulation in these cows. Thus, 2 main types of anovulation occur in lactating dairy cows. Some cows grow follicles but not to ovulatory size, probably due to negative energy balance and inadequate numbers of LH pulses. Other anovular cows grow large follicles, sometimes to "cystic" size, but these cows do not ovulate due to lack of a GnRH/LH surge.

Table 4. Incidence of types of anovulation in 583 lactating dairy cows (n = 140 anovular cows; 24%)

	Anovular cows	
Maximal diameter of follicle[1]	No.	%
4 to 8 mm	1	0.7
9 to 14 mm	27	19.3
15 to 25 mm	85	60.7
>25 mm	27	19.3
Total	140	100

[1]Follicle diameter was evaluated 3 to 6 times (55 to 95 d postpartum) using weekly transrectal ultrasound of the ovaries.

ANOVULATION 4: TREATMENTS FOR ANOVULAR COWS

Subsequent chapters will deal with the breeding management programs that have been developed and validated for treatment of anovular and cycling cows. These programs are based on the normal physiology of the estrous cycle, as discussed above, and on research that has evaluated treatment of anovular cows. In general, the main treatment for anovular cows is to increase circulating P4 concentrations. Increasing P4 is effective in treating anovular cows with either the small or large anovular follicle phenotypes. In cows with small follicle anovular phenotype, removal of P4 will stimulate LH pulses that generally cause follicle growth to ovulatory size, increased E2, and induction of the GnRH/LH surge that will cause ovulation. In cows with the large follicle anovular phenotype, the presence of high circulating P4 will cause the GnRH surge center to become responsive to E2 so that increased circulating E2 during growth of the next ovulatory-sized follicle will induce a GnRH/LH surge with subsequent ovulation. Cows need to have elevated P4 for at least 3 d to be induced back to cyclicity. Two practical methods are used to increase circulating P4 in anovular cows.

One way to increase P4 in anovular cows is to ovulate a follicle. In one study, most anovular cows ovulated in response to treatment with GnRH (88%; 29/33). Thus, programs that use GnRH during the protocol, such as Ovsynch, can induce anovular cows to begin cyclicity. However, fertility is generally lower in anovular cows that are treated with Ovsynch compared with cycling cows treated with Ovsynch. In addition, pregnancy loss can be greater in anovular than in cyclic cows that become pregnant to Ovsynch. Nevertheless, programs that use GnRH can be effective in stimulating cyclicity in anovular cows. Programs that use only $PGF_{2\alpha}$ are not effective in inducing cyclicity because there is no CL that can be regressed in anovular cows.

Another method to increase circulating P4 is to treat cows with an intravaginal P4 insert such as a controlled internal drug-releasing insert (**CIDR**). The P4 insert can be used independently or in conjunction with Ovsynch programs to treat anovular cows. Several studies has demonstrated improvements in P/AI in anovular cows that are treated with a P4 insert. One issue

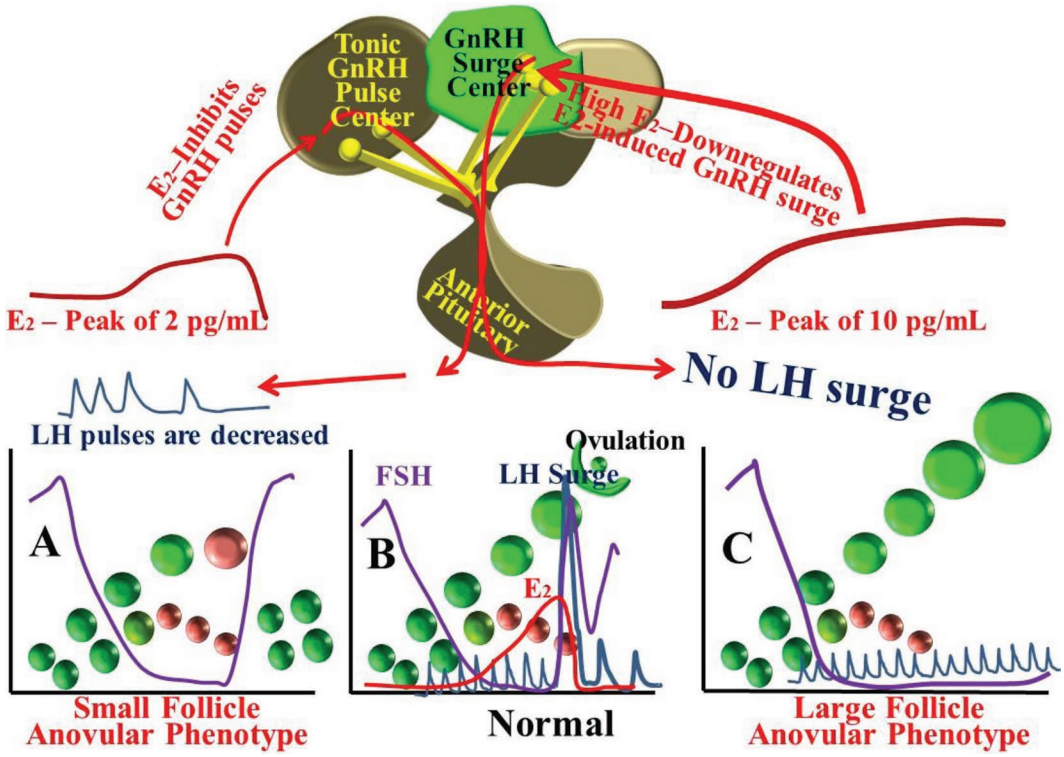

Figure 6. The middle panel (B) illustrates the hormonal changes during the normal preovulatory period. Circulating follicle-stimulating hormone (FSH) decreases with growth of the follicular wave, FSH reaches a nadir at the time a dominant follicle is selected, luteinizing hormone (LH) pulses due to gonadotropin-releasing hormone (GnRH) pulses from the tonic GnRH pulse center of the hypothalamus cause the follicle to continue growing and producing high estradiol (E2). High circulating E2 eventually leads to estrus, a GnRH surge from the GnRH surge center of the hypothalamus, producing an LH surge and ovulation. The left panel (A) shows follicle growth and circulating hormones in an anovular cow that only grows smaller follicles. Although FSH is normal, as E2 increases after selection of the dominant follicle, the GnRH pulses from the tonic GnRH pulse center are suppressed, LH pulses are reduced, and the dominant follicle stops growing and producing E2. In contrast, in a cow with large follicle anovular phenotype (C; right panel), growth of a dominant follicle and high E2 production do not induce a GnRH surge due to some type of block of E2 positive feedback at the GnRH surge center.

with use of a single CIDR in high-producing lactating Holstein cows is the marginal increase in concentrations of P4, only 0.8 to 1.1 ng/mL (Cerri et al., 2009). When 2 CIDR inserts were used during the timed-AI protocol in cows without CL, P4 concentrations increased by 2.5 ng/mL and P/AI became similar to that of cows with CL, and both treatments had P/AI that were 17 percentage units greater than those of untreated cows that did not have CL (Bisinotto et al., 2013). Thus, programs to treat anovular cows should use methods to either induce a new CL (GnRH) or deliver P4 from an exogenous source such as an intravaginal P4 insert. Subsequent chapters will provide detailed information on reproductive management programs that can be used to both synchronize cycling cows and treat anovular cows.

REFERENCES

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

Ball, P. J. B., and E. E. A. McEwan. 1998. The incidence of prolonged luteal function following early resumption of ovarian activity in post partum dairy cows. Page 187 in Br. Soc. Anim. Sci.**[AU7: Is this a meeting proceedings? If so, need location of meeting and name and location of proceedings publisher. If it's a book, need title of book and name and location of publisher.]**

Bamber, R. L., G. E. Shook, M. C. Wiltbank, J. E. P. Santos, and P. M. Fricke. 2009. Genetic parameters for anovulation and pregnancy loss in dairy cattle. J. Dairy Sci. 92:5739–5753.

Bisinotto, R. S., R. C. Chebel, and J. E. Santos. 2010. Follicular wave of the ovulatory follicle and not cyclic status influences fertility of dairy cows. J. Dairy Sci. 93:3578–3587.

Bisinotto, R. S., E. S. Ribeiro, F. S. Lima, N. Martinez, L. F. Greco, L. Barbosa, P. P. Bueno, L. F. S. Scagion, W. W. Thatcher, and J. E. P. Santos. 2013. Targeted progesterone supplementation improves fertility in lactating dairy cows without a corpus luteum at the initiation of the timed artificial insemination protocol. J. Dairy Sci. 96:2214–2225. http://dx.doi.org/10.3168/jds.2012-6038.

Cerri, R. L. A., H. M. Rutigliano, R. G. S. Bruno, and J. E. P. Santos. 2009. Progesterone concentration, follicular development and induction of cyclicity in dairy cows receiving intravaginal progesterone inserts. Anim. Reprod. Sci. 110:56–70. http://dx.doi.org/10.1016/j.anireprosci.2007.12.005.

Colazo, M. G., A. Dourey, R. Rajamahendran, and D. J. Ambrose. 2013. Progesterone supplementation before timed AI increased

ovulation synchrony and pregnancy per AI, and supplementation after timed AI reduced pregnancy losses in lactating dairy cows. Theriogenology 79:833–841.

Darwash, A. O., and G. E. Lamming. 1995. To define and quantify atypical ovarian-function in untreated postpartum cows. Biol. Reprod. 52:72. (Abstr.)

Endo, N., K. Nagai, T. Tanaka, and H. Kamomae. 2012. Comparison between lactating and non-lactating dairy cows on follicular growth and corpus luteum development, and endocrine patterns of ovarian steroids and luteinizing hormone in the estrous cycles. Anim. Reprod. Sci. 134:112–118.**[AU8: Reference is not cited in the text. Please add an in-text citation or delete the reference.]**

Ferguson, J. D. 1996. Diet, production and reproduction in dairy cows. Anim. Feed Sci. Technol. 59:173–184.

Ginther, O. J., L. Knopf, and J. P. Kastelic. 1989. Temporal associations among ovarian events in cattle during oestrous cycles with two and three follicular waves. J. Reprod. Fertil. 87:223–230.

Gümen, A., J. N. Guenther, and M. C. Wiltbank. 2003. Follicular size and response to Ovsynch versus detection of estrus in anovular and ovular lactating dairy cows. J. Dairy Sci. 86:3184–3194.

Harrison, R. O., J. W. Young, A. E. Freeman, and S. P. Ford. 1989. Effects of lactational level on reactivation of ovarian function, and interval from parturition to first visual oestrus and conception in high-producing Holstein cows. Anim. Prod. 49:23–28.

Haughian, J. M., R. Sartori, J. N. Guenther, A. Gumen, and M. C. Wiltbank. 2002. Extending the postpartum anovulatory period in dairy cattle with estradiol cypionate. J. Dairy Sci. 85:3238–3249.

Herlihy, M. M., J. O. Giordano, A. H. Souza, H. Ayres, R. M. Ferreira, A. Keskin, A. B. Nascimento, J. N. Guenther, J. M. Gaska, S. J. Kacuba, M. A. Crowe, S. T. Butler, and M. C. Wiltbank. 2012. Presynchronization with Double-Ovsynch improves fertility at first postpartum artificial insemination in lactating dairy cows. J. Dairy Sci. 95:7003–7014. http://dx.doi.org/10.3168/jds.2011-5260.

Kirby, C. J., M. F. Smith, D. H. Keisler, and M. C. Lucy. 1997. Follicular function in lactating dairy cows treated with sustained-release bovine somatotropin. J. Dairy Sci. 80:273–285.

Knopf, L., J. P. Kastelic, E. Schallenberger, and O. J. Ginther. 1989. Ovarian follicular dynamics in heifers: test of two-wave hypothesis by ultrasonically monitoring individual follicles. Domest. Anim. Endocrinol. 6:111–119.

Ko, J. C. H., J. P. Kastelic, M. R. Del Campo, and O. J. Ginther. 1991. Effects of a dominant follicle on ovarian follicular dynamics during the oestrous cycle in heifers. J. Reprod. Fertil. 91:511–519.

Lopez, H., D. Z. Caraviello, L. D. Satter, P. M. Fricke, and M. C. Wiltbank. 2005. Relationship between level of milk production and multiple ovulations in lactating dairy cows. J. Dairy Sci. 88:2783–2793.

Lopez, H., L. D. Satter, and M. C. Wiltbank. 2004. Relationship between level of milk production and estrous behavior of lactating dairy cows. Anim. Reprod. Sci. 81:209–223.

Lucy, M. C., J. C. Byatt, T. L. Curran, D. F. Curran, and R. J. Collier. 1994. Placental lactogen and somatotropin: Hormone binding to the corpus luteum and effects on the growth and functions of the ovary in heifers. Biol. Reprod. 50:1136–1144.

McDougall, S., C. R. Burke, K. L. MacMillan, and N. B. Williamson. 1995. Patterns of follicular development during periods of anovulation in pasture-fed dairy cows after calving. Res. Vet. Sci. 58:212–216.

Nebel, R. L., S. M. Jobst, M. B. G. Dransfield, S. M. Pandolfi, and T. L. Bailey. 1997. Use of a radiofrequency data communication system, Heat Watch, to describe behavioral estrus in dairy cattle. J. Dairy Sci. 80:151. (Abstr.)

Pursley, J. R., J. S. Stevenson, and J. E. Minton. 1993. Ovarian follicular waves in dairy cows after administration of gonadotropin-releasing hormone at estrus. J. Dairy Sci. 76:2548–2560.

Pursley, J. R., M. C. Wiltbank, J. S. Stevenson, J. S. Ottobre, H. A. Garverick, and L. L. Anderson. 1997. Pregnancy rates per artificial insemination for cows and heifers inseminated at a synchronized ovulation or synchronized estrus. J. Dairy Sci. 80:295–300.

Ronchi, B., G. Stradaioli, A. Verini Supplizi, U. Bernabucci, N. Lacetera, P. A. Accorsi, A. Nardone, and E. Seren. 2001. Influence of heat stress or feed restriction on plasma progesterone, oestradiol-17β, LH, FSH, prolactin and cortisol in Holstein heifers. Livest. Prod. Sci. 68:231–241.

Roth, Z., R. Meidan, R. Braw-Tal, and D. Wolfenson. 2000. Immediate and delayed effects of heat stress on follicular development and its association with plasma FSH and inhibin concentration in cows. J. Reprod. Fertil. 120:83–90.

Royal, M. D., A. O. Darwash, A. P. F. Flint, R. Webb, J. A. Woolliams, and G. E. Lamming. 2000. Declining fertility in dairy cattle: Changes in traditional and endocrine parameters of fertility. Anim. Sci. 70:487–501.

Ryan, D. P., and M. P. Boland. 1991. Frequency of twin births among Holstein-Friesian cows in a warm dry climate. Theriogenology 36:1–10.

Sangsritavong, S., D. K. Combs, R. Sartori, and M. C. Wiltbank. 2002. High feed intake increases blood flow and metabolism of progesterone and estradiol-17β in dairy cattle. J. Dairy Sci. 85:2831–2842.

Santos, J. E. P., H. M. Rutigliano, and M. F. Sa Filho. 2009. Risk factors for resumption of postpartum estrous cycles and embryonic survival in lactating dairy cows. Anim. Reprod. Sci. 110:207–221.

Sartori, R., J. M. Haughian, R. D. Shaver, G. J. Rosa, and M. C. Wiltbank. 2004. Comparison of ovarian function and circulating steroids in estrous cycles of Holstein heifers and lactating cows. J. Dairy Sci. 87:905–920.

Savio, J. D., M. P. Boland, N. Hynes, and J. F. Roche. 1990. Resumption of follicular activity in the early post-partum period of dairy cows. J. Reprod. Fertil. 88:569–579.

Schemm, S. R., D. R. Deaver, L. C. Griel Jr., and L. D. Muller. 1990. Effects of recombinant bovine somatotropin on luteinizing hormone and ovarian function in lactating dairy cows. Biol. Reprod. 42:815–821.

Sirois, J., and J. E. Fortune. 1988. Ovarian follicular dynamics during the estrous cycle in heifers monitored by real-time ultrasonography. Biol. Reprod. 39:308–317.

Stevenson, J. S., D. E. Tenhouse, R. L. Krisher, G. C. Lamb, J. E. Larson, C. R. Dahlen, J. R. Pursley, N. M. Bello, P. M. Fricke, M. C. Wiltbank, D. J. Brusveen, M. Burkhart, R. S. Youngquist, and H. A. Garverick. 2008. Detection of anovulation by heat-mount detectors and transrectal ultrasonography before treatment with progesterone in a timed insemination protocol. J. Dairy Sci. 91:2901–2915. http://dx.doi.org/10.3168/jds.2007-0856.

Taylor, C., and R. Rajamahendran. 1991. Follicular dynamics, corpus luteum growth and regression in lactating dairy cattle. Can. J. Anim. Sci. 71:61–68.

Townson, D. H., P. C. W. Tsang, W. R. Butler, M. Frajblat, L. C. Griel Jr., C. J. Johnson, R. A. Milvae, G. M. Niksic, and J. L. Pate. 2002. Relationship of fertility to ovarian follicular waves before breeding in dairy cows. J. Anim. Sci. 80:1053–1058.

Trout, J. P., L. R. McDowell, and P. J. Hansen. 1998. Characteristics of the estrous cycle and antioxidant status of lactating Holstein cows exposed to heat stress. J. Dairy Sci. 81:1244–1250.

Van Eerdenburg, F. J. C. M., D. Karthaus, M. A. M. Taverne, I. Merics, and O. Szenci. 2002. The relationship between estrous behavioral score and time of ovulation in dairy cattle. J. Dairy Sci. 85:1150–1156.

Vasconcelos, J. L. M., S. Sangsritavong, S. J. Tsai, and M. C. Wiltbank. 2003. Acute reduction in serum progesterone concentrations after feed intake in dairy cows. Theriogenology 60:795–807.**[AU9: Reference is not cited in the text. Please add an in-text citation or delete the reference.]**

Vieira-Neto, A., R. O. Gilbert, W. R. Butler, J. E. Santos, E. S. Ribeiro, M. M. Vercouteren, R. G. Bruno, J. H. Bittar, and K. N. Galvão. 2014. Individual and combined effects of anovulation and cytological endometritis on the reproductive performance of dairy cows. J. Dairy Sci. 97:5415–5425. http://dx.doi.org/10.3168/jds.2013-7725.

Washburn, S. P., W. J. Silvia, C. H. Brown, B. T. McDaniel, and A. J. McAllister. 2002. Trends in reproductive performance in southeastern Holstein and Jersey DHI herds. J. Dairy Sci. 85:244–251.

Wilson, S. J., C. J. Kirby, A. T. Koenigsfeld, D. H. Keisler, and M. C. Lucy. 1998. Effects of controlled heat stress on ovarian function of dairy cattle. 2. Heifers. J. Dairy Sci. 81:2132–2138.

Wiltbank, M., H. Lopez, R. Sartori, S. Sangsritavong, and A. Gumen. 2006. Changes in reproductive physiology of lactating dairy cows due to elevated steroid metabolism. Theriogenology 65:17–29.

Wiltbank, M. C., A. Gumen, H. Lopez, and R. Sartori. 2008. Management and treatment of dairy cows that are not cycling or have follicular cysts. Cattle Pract. 16:14–19.**[AU10: Reference is not cited in the text. Please add an in-text citation or delete the reference.]**

Wiltbank, M. C., A. Gümen, and R. Sartori. 2002. Physiological classification of anovulatory conditions in cattle. Theriogenology 57:21–52.

Wiltbank, M. C., A. H. Souza, P. D. Carvalho, A. P. Cunha, J. O. Giordano, P. M. Fricke, G. M. Baez, and M. G. Diskin. 2014. Physiological and practical effects of progesterone on reproduction in dairy cattle. Animal 8(Suppl. 1):70–81.