

Anestrus in Suckled Beef and Milked Dairy Cattle

Jeffrey S. Stevenson
Dept. of Animal Sciences and Industry
Kansas State University, Manhattan 66506-0201 USA
785-532-1243 (tel)
785-532-7059 (fax)
jstevens@oznet.ksu.edu

Abstract

The factor most limiting early impregnation of suckled cows is the proportion of cows that are not cycling (anestrus) at the beginning of the breeding season. Although prolonged anestrus is not limiting in milked cattle, increased milk production and its subsequent negative energy balances are associated with prolonged periods of anestrus. Continual presence of a suckling calf with their mammary-intact or mastectomized dams delays the reinitiation of estrous cycles. In more than 2,200 beef cows studied, body condition (BC), parity, and days postpartum influenced the proportion of cows cycling (determined by measuring blood progesterone) before the onset of the breeding season. As BC increased from <4 to ≥ 5.5 (1 = thin and 9 = fat), the percentage of cows cycling increased ($P < 0.05$) by $18 \pm 2\%$ for each unit increase in BC. Percentage of cows cycling increased linearly from 25% (<50 days) to a peak after 70 days postpartum (60%). For every 10-day interval from <50 to >80 days, the percentage of cows cycling increased ($P < 0.05$) by $7.5 \pm 0.7\%$. Compared to older cows (53%), fewer ($P < 0.001$) 2-year-old cows with their first calves were cycling (44%), despite calving up to 3 weeks earlier. We have studied dairy cows on three dairy farms in which Holstein cows were milked either 2x or 3x daily. Average milk yields exceeded 10,000 liters. In the first study of 678 cows, the average percentage of cows cycling was 82% by 40 to 68 days in milk. In first lactation, 2-year-old cows, cycling percentages were lower ($P < 0.05$) in one herd (72%) than in the second herd (87%), whereas no difference for older cows (88 vs. 86%) in cycling percentages were detected between herds. Body condition scores (1 = thin and 5 = fat) assessed at time of blood sampling averaged 2.3. Cows in better BC were more likely to be cycling than thinner cows. Cows with more days in milk at the sampling times also were more likely to be cycling. In the second study of 251 cows in one herd, where cows were milked 3x daily, the percentage of cows cycling was only 44% by 47 to 67 days. Again, BC averaged about 2.3. Younger and thinner cows were less likely to be cycling. As BC increased by 0.5 units, the percentage of cows cycling increased ($P < 0.05$) by 24%. Milk yield had no influence on cycling percentages. In the last study of 385 cows in three herds during the summer, the percentage of cows cycling was 84% by 56 to 83 days. In this study, lactation number did not affect cyclicity, but BC (average of 2.4) increased ($P < 0.05$) by 7.2% for every 0.5 unit increase in BC. In both beef and dairy studies, cows not cycling by the beginning of the breeding period conceived at lesser rates and took longer to eventually conceive. Having more cycling cows at the beginning of the breeding season will maximize the proportion of cows that conceive to AI sires. More cows calving early during each successive calving season will enhance AI pregnancy rates, because more cows will be cycling before the breeding period begins.

Introduction

The factor most limiting early impregnation of suckled cows is the proportion of cows that are not cycling (anestrus) at the beginning of the breeding season (Short et

al., 1990). Generally, for milked cattle, the reestablishment of estrous cycles has not been considered to be a problem because most studies indicate that first postpartum ovulations occur by the end of the first month of lactation (Stevenson et al., 1997). The major difference between suckled and milked cattle is the continual presence of a suckling calf that prolongs anestrus and delays the reinitiation of estrous cycles (Williams, 1990). However, calf presence without suckling prolongs interval to first ovulation in intact and mastectomized dams compared to permanent weaning of the calf (Stevenson et al., 1997). Although insufficient energy and protein intake and insufficient body condition at calving are limiting factors, temporary or permanent weaning of the calf from its suckled dam usually initiates estrus within a few days (Williams, 1990). Because calves are removed from milked cattle at birth, calf presence is not a limiting factor. Younger cows nursing calves (Randel, 1990) or milking in their first lactation (Stevenson et al., 1997) generally have more prolonged anestrus because of their additional growth requirement.

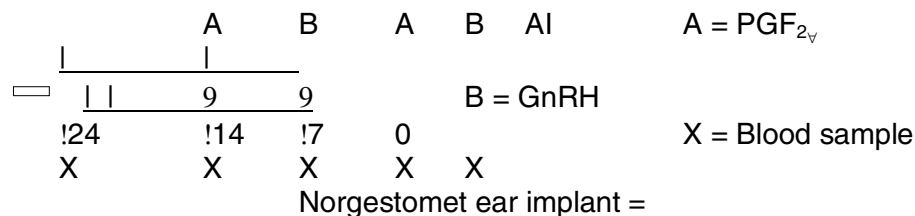
Nutrients are used by cows according to an established priority (Short and Adams, 1989). The first priority is maintenance of essential body functions to preserve life. Once that maintenance requirement is met, remaining nutrients accommodate growth. Finally, lactation and the initiation of estrous cycles are supported. Because older cows have no growth requirement, nutrients are more likely to be available for milk synthesis (first) and estrous cycle initiation (second). Because of this priority system, young, growing cows generally produce less milk and are anestrus longer after calving.

Because suckling occurs rather frequently each day, certainly more often than milking, estrous cycles are delayed longer in suckled than milked cows. Despite that fact, suckled beef cows may have more mothering instinct than the modern dairy cow and will not cycle back as soon even if they are only suckled twice daily by their calf (Stevenson et al., 1997). Dairy cows that are milked four times daily have somewhat longer intervals to first ovulation than cows that are milked twice daily (Stevenson et al., 1997).

Experimental Procedures

Suckled Cattle

Study 1 (1994-1995). Purebred suckled cows (Simmental, Angus, and Hereford; n = 279) at Kansas State University were used. Controls received two injections of PGF_{2 α} (25 mg; Pharmacia & Upjohn, Kalamazoo, MI) on days !14 and 0 and were inseminated at estrus, or in the absence of estrus, at 80 hr after the second PGF_{2 α} . Treated cows received 25 mg of PGF_{2 α} on days !14 and 0 plus 100 μ g of GnRH (Merial Limited, Iselin, NJ) on day !7 and had a norgestomet (NORG) ear implant (Syncro-Mate-B[®]; Merial Limited, Iselin, NJ) in place for 8 days beginning on day !7. Treated cows were inseminated at 72 hr after PGF_{2 α} or 18 hr after a second injection of GnRH given at 54 hr after PGF_{2 α} .



Study 2 (1996). Purebred suckled Angus, Gelbvieh, and Hereford cows and crossbred suckled cows (Simmental, Angus, and Hereford; n = 890) on three private ranches were used. Control cows received two injections of PGF_{2v} on days !14 and 0. A second group of cows received GnRH on day !7 and PGF_{2v} on day 0 (Select Synch). Select Synch + NORG cows also had a norgestomet implant in place for 7 days beginning on day !7. Cows were inseminated after detected estrus. In addition, 164 purebred suckled cows received the Select Synch + NORG treatment at Kansas State University. Cows were either inseminated after detected estrus (one-half) or at 48 hr after PGF_{2v} were given 100 µg of GnRH and then inseminated 16 to 18 hr later.

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Study 3 (1997). Crossbred suckled cows (n = 406; Simmental, Angus, and Hereford crosses) on two private ranches, plus 158 purebred Simmental, Angus, and Hereford suckled cows at Kansas State University were used. Cows were treated with 100 µg of GnRH on day !7 and PGF_{2v} on day 0. They were inseminated after detected estrus, or in the absence of estrus, at 54 hr after PGF_{2v} and given 100 µg of GnRH at the time of AI.

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Study 4 (1998). Purebred Angus, Simmental, and Hereford cows (n = 187) at Kansas State University were used. All cows received 100 µg of GnRH of day !7 and 25 mg of PGF_{2v} on day 0. Half of the cows also received an intravaginal progesterone insert (CIDR-B, InterAg, Hamilton, NZ) on day !7, which was removed on day 0. All were inseminated at 48 hr after PGF_{2v} and given 100 µg of GnRH at the time of AI.

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Study 5 (1999). Purebred Angus, Simmental, and Hereford cows (n = 187) at Kansas State University were used. All cows received 100 µg of GnRH on day !7 and 25 mg of PGF_{2v} on day 0. Half also received an ear implant containing 6 mg of norgestomet on day !7. It was removed on day 0. All cows were inseminated 48 hr after PGF_{2v} and given 100 µg of GnRH at the time of AI.

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Norgestomet implant =

Blood samples were collected prior to hormonal treatments to determine if cows had elevated blood progesterone (functional corpus luteum). At least two blood samples were collected between 7 and 11 days before hormone administration and just before each hormone injection. Progesterone was measured by radioimmunoassay. If either or both samples contained progesterone ≥ 1 ng/ mL, then the cows were assumed to have ovulated and were cycling. If neither sample contained elevated progesterone, then the cow was anestrous. Further blood samples allowed us to determine if hormonal treatments induced ovulation in previously anestrous cows.

Body condition scores (1 = thin, 9 = fat; Whitman, 1975) were assigned to cows on the first day of the breeding season, and days since calving were calculated for each cow. The data were treated by analyses of variance to determine the effects of body condition score, parity, and days postpartum at the beginning of the breeding season on cyclicity. Further, the effects of various hormone treatments on the detection and expression of estrus were determined. In all analyses, herd and year were absorbed into the model.

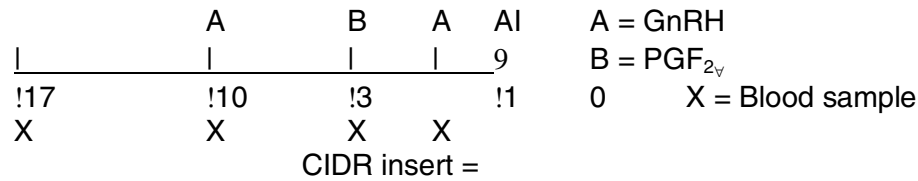
Milked Cattle

During the last 3 years, we have studied more than 1300 dairy cows on three Kansas dairy farms. As part of those studies we have estimated the cycling status of these cows based on blood samples that were collected before synchronization of estrus, ovulation, or both. Upon measuring the hormone progesterone in these samples, we could determine which cows were cycling before the end of the elective waiting period and before first insemination. Two studies were conducted during non-summer months and the third during summer. All herds had annual milk yields exceeding 10,000 liters.

Study 1 (1997-1999). This study was conducted during non-summer months on two dairy farms consisting of 678 Holstein cows milked twice daily. Blood samples were collected before various treatments were used to synchronize ovulation and before timed inseminations were carried out. Treatments were: 1) PGF_{2v} 12 days (day !22) before the Ovsynch protocol (GnRH on days !10 and !1 plus PGF_{2v} on day -3); 2) Ovsynch protocol; and 3) two injections of PGF_{2v} 12 days apart (days !15 and !3) and GnRH on day -1. All cows received one timed insemination on day 0.

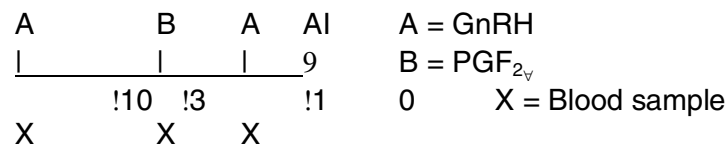
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Study 2 (1999). This study was conducted between January and May on one dairy farm consisting of 251 Holstein cows milked three times daily. Blood samples were collected before various treatments were used to synchronize ovulation and timed inseminations were carried out for all first postpartum services. Treatments were: 1) the Ovsynch protocol (GnRH on days !10 and !1 plus PGF_{2v} on day !3); and 2) Ovsynch protocol + one CIDR insert for 7 days. All cows received one timed insemination on day 0.



A = GnRH
 B = PGF_{2v}
 X = Blood sample

Study 3 (1998-1999). This study was conducted during summer on three dairy farms consisting of 385 Holstein cows milked two or three times daily. Blood samples were collected before various treatments were used to synchronize and timed inseminations were carried out by appointment or after detected estrus. Treatments were: 1) the Ovsynch protocol (GnRH on days !10 and !1 plus PGF_{2v} on day !3) with cows receiving one timed insemination on day 0; and 2) Select Synch protocol (GnRH on days !10 plus PGF_{2v} on day !3) with cows inseminated after detected estrus.



A = GnRH
 B = PGF_{2v}
 X = Blood sample

Blood samples were collected prior to hormonal treatments to determine if cows had elevated blood progesterone (functional corpus luteum). At least two blood samples were collected between 7 and 10 days before hormone administration or just before PGF_{2v}. Again, progesterone was measured by radioimmunoassay and cycling status was determined as described for suckled cattle.

Body condition scores (1 = thin, 5 = fat; Wildman et al., 1982) were assigned to cows before initiating hormonal injections in each study. The data were treated by analyses of variance to determine the effects of body condition score, lactation number, and days in milk on the incidence of cycling status before first insemination.

Results and Discussion

Suckled Cattle

Cycling Status. During the past 6 years, we have treated more than 2,200 beef cows with various hormonal treatments to synchronize estrus, ovulation, or both, in an attempt to achieve conception during the first week of the breeding season and maximize the proportion of cows pregnant to genetically superior AI sires (Stevenson et al., 1997; Thompson et al., 1999; Stevenson et al., 2000). As part of these studies, we measured the incidence of cyclicity at the beginning of the breeding season, both prior to hormonal treatments and in response to these treatments. The risk factors that limit a high rate of cyclicity at the beginning of the breeding season include age of cow, her body condition, and days since calving (Table 1). Despite the fact that 2-year old cows may calve up to 3 weeks before the older cows, their cycling rates were still less than those of older cows (Table 1). Cows with body condition scores less than 5 (1 = thin and 9 = fat) were less likely to be cycling than those at higher body conditions (Table 1). Body condition represents a repeatable visual appraisal of the nutrition program. The literature indicates that suckled cows should calve with a minimum body condition score of 5 (Short et al., 1990) to prevent prolonged postpartum intervals to estrus. Cows that have calved less than 70 days before the beginning of the breeding season also are less likely to be cycling.

In summary, our studies have demonstrated that only 50-60% of suckled beef cows are cycling between 7 and 11 days before the onset of the breeding season. With those

cycling rates, applying an estrus-synchronization system that only controls the onset of estrus in cycling cows, one could only expect 30-40% of the cows to become pregnant if conception rates are 60-70%. In order to maximize pregnancy rates during the first week of the breeding season, one must employ a system that will induce fertile estrus or ovulation in anestrus cows in addition to synchronizing the estrous cycles of cycling cows.

Induction of Ovulation. The scientific literature of the 1970's and 1980's demonstrated that injections of gonadotropin-releasing hormone (GnRH) could induce ovulation of ovarian follicles of dairy (Britt et al., 1974) and suckled beef cows (Schams et al., 1973). Injections of GnRH induced the release of LH (Britt et al., 1974) and FSH (Foster et al., 1980) from the anterior pituitary gland. Resulting elevated blood concentrations of LH and FSH either caused follicular rupture (ovulation), if a follicle(s) was present, or may have induced some new follicular development. In other studies (Entwistle and Oga, 1977; Webb et al., 1977), unless two injections were given 7 to 14 days apart (Webb et al., 1977; Fonseca et al., 1980) and cows were at least 30 days postpartum at the time of treatment, the incidence of ovulation was low. In some cases, cows continued to show regular estrous cycles after induction of ovulation, and in a few cases when the short-lived induced corpus luteum regressed, the cows returned to a state of anestrus.

Based on current knowledge, waves of follicular development occur in suckled cows, beginning as early as 14 days postpartum (Stagg et al., 1995). Because waves of follicles develop and turnover, only to be replaced by a new wave and a new dominant follicle, secretion of FSH is probably not limiting the recurrence of estrous cycles. Reinitiation of sufficient LH pulses to support follicular maturation and ovulation seems to be the most limiting factor to estrus-cycle initiation (Williams, 1990).

In recent studies, injections of GnRH initiated turnover of large follicles or induced the dominant follicle to ovulate when at a proper stage of maturity followed by emergence of a new follicular wave (Twagiramungu et al., 1995; Thompson et al., 1999). Unless a follicle is at least 10 mm in diameter, GnRH-induced LH release will not be effective in stimulating ovulation (Crowe et al., 1993) because once a dominant follicle is selected, it possesses LH receptors and becomes LH-dependent (Lucy et al., 1992). In the absence of progesterone, a dominant follicle will continue to grow until it either matures and ovulates in response to a preovulatory LH surge. In the absence of a corpus luteum and in the presence of an exogenous source of progestin, dominant follicles will continue to grow and "persist" without ovulation until the exogenous source of progestin is removed (Sanchez et al., 1995; Kojima et al., 1995; Smith and Stevenson, 1995). In contrast, in the presence of progesterone produced by the corpus luteum, the dominant follicle will turn over and be replaced by a new dominant follicle (Adams et al., 1992; Smith and Stevenson, 1995).

In our recent studies (Thompson et al., 1999) using transrectal ultrasonography and daily collected blood samples to monitor concentrations of progesterone, we have demonstrated that a single GnRH injection is quite effective in inducing ovulation and formation of a first corpus luteum in late-calving, suckled anestrus cows (34 ± 6 days postpartum). Whether cows were treated with GnRH alone or received GnRH plus a norgestomet implant (Syncro-Mate-B implant), the incidence of induced ovulation was high (>80%).

In further studies, based only on blood samples, the percentage of cows with elevated progesterone (induction of ovulation) 7 days after GnRH. During 1996, the

treatment with GnRH alone increased the rate of induced ovulation. Subtracting the control percentage (that after the second of two injections of PGF_{2v}) of ovulation resulting from spontaneous ovulation (21.3%) from the percentage of cows treated with GnRH (37.8%) showed that 16.5% of the noncycling cows were induced to ovulate. In a second study in 1996, rate of induced ovulation was 28% in anestrous cows treated with GnRH plus a norgestomet implant. From 1997-1999, anywhere from 50 to 67% of the anestrous cows treated with GnRH alone had elevated progesterone 7 days later. In those cows treated with GnRH plus either a norgestomet implant or a CIDR, induction of ovulation was less than that with GnRH alone, based on the incidence of elevated blood progesterone 7 days after GnRH.

Uses of GnRH to Synchronize Estrus. More important than induction of ovulation to the overall pregnancy rates achieved during the first week of the breeding season was the percentage of cows in estrus after PGF_{2v} during the first week of the breeding season in response to these treatments (Table 2). Clearly, GnRH is important to prepare anestrous cows to show estrus after PGF_{2v}, but the ovulation induction ability of GnRH coupled with the addition of a pre-estrus source of progestin, resulted in a greater percentage of cows in estrus during the first week of the breeding season.

In addition, pretreatment of the cows with one norgestomet implant promoted increased size of the dominant follicle in the absence of a CL (Rajamahendran and Taylor, 1991; Smith and Stevenson, 1995) by allowing the LH pulse frequency to increase (Garcia-Winder et al., 1986). Norgestomet treatment before GnRH administration increased the amount of GnRH-induced LH release (Thompson et al., 1999) and the proportion of GnRH-induced ovulations in noncycling, suckled cows (Troxel et al., 1993).

Not only is the source of progestin important for expression of estrus, but it is critical for consistent improvement in pregnancy rates. Table 3 summarizes the detection of estrus for cows and their conception rates based on serum concentrations of progesterone collected 14, 7, and 0 days before PGF_{2v}. Expression of estrus and conception rates were similarly high in all cycling cows of all three treatments when progesterone was elevated at the time of PGF_{2v}. Part of this group included those cows that were anestrous and induced to ovulate (e.g., LLH progesterone profile in Table 3). Among cows that were cycling but had low concentrations of progesterone on day 0, estrus expression was less in controls but conception was greater than that in Select Synch + NORG cows. Among noncycling cows that were not induced to ovulate in response to GnRH on day !7, expression of estrus was greatest if they were pretreated with GnRH and norgestomet compared to controls. Conception rates were greater in both groups that received GnRH than in cows that only received PGF_{2v}.

These results were validated by a subsequent experiment in which cows were treated with GnRH and norgestomet. In this experiment, cows were treated with the Select Synch + NORG protocol and either inseminated after detected estrus or inseminated after detected estrus until 48 hr after PGF_{2v} and then all remaining cows were administered GnRH and inseminated 16 hr later. Pregnancy rates exceeded 50% after both inseminating protocols, but time insemination of noncycling cows produced a lower pregnancy rate.

A further advantage for using a progestin concurrently with GnRH is that most cows (87%) have normal luteal phases after insemination compared to 71% of cows receiving only GnRH or 43% of cows receiving only the norgestomet implant before

PGF_{2v}. Pregnancy rates in the latter three groups were 71, 31, and 15%, respectively (Thompson et al., 1999). These results are consistent with an earlier report in which norgestomet treatment prior to calf weaning reduced the incidence of short estrous cycles (Ramirez-Godinez et al., 1981).

Having more cycling cows at the beginning of the breeding season should maximize the proportion of cows that conceive to AI sires. More cows calving early during each successive calving season will enhance AI-pregnancy rates, because more cows will be cycling before the breeding season begins.

Milked Cattle

In the first study of 678 cows, the average percentage of cows cycling was 82% (Table 4). In the first lactation, 2-year-old cows, cycling percentage was lower in one herd than in the second herd, whereas no difference in cycling percentages were detected between herds for older cows. Body condition scores assessed at time of blood sampling averaged 2.3 ± 0.5 . Cows in better body condition were more likely to be cycling than thinner cows. Cows with more days in milk at the sampling times also were more likely to be cycling.

In the second study of 251 cows in one herd where cows were milked three times daily, the percentage of cows cycling was less at 44% (Table 4). Again, body condition averaged about 2.3 ± 0.5 . Younger and thinner cows were less likely to be cycling. In fact, as body condition increased by 0.5 units, the percentage of cows cycling increased by 24%. Milk yield (150-day energy-corrected milk) had no influence on cycling percentages.

In the last study of 385 cows in three herds during the summer, the percentage of cows cycling was 84% (Table 4). In this study, lactation number did not affect cyclicity, but body condition (average of 2.4 ± 0.5) was very important. For every 0.5 unit increase in body condition for cows in the study, cyclicity increased by 7.2%.

These cycling rates are less than reported for dairy cows in much of the literature (Stevenson et al., 1997). Because most dairy cows ovulate their first or second dominant-wave follicle after calving, average intervals to first ovulation are generally <30 days in most studies. It seems that higher production and increasing frequency of milking from two to three times daily are causing fewer cows to have normal estrous cycles before the end of the elective waiting period. Therefore, a higher percentage of dairy cows are not cycling when programmed breeding systems are initiated to control estrus, ovulation, or both, before inseminations.

An important point learned from these studies: cows that were not cycling by the end of the elective waiting period conceived at lesser rates and took longer to eventually conceive. In each case, body condition (measured sometime between 40 and 83 days) was a very important predictor of when cows began estrous cycles after calving. Body condition scores are undoubtedly a reflection of dry matter intake and efficiency of nutrient utilization in the previous lactation and dry period as well as what is happening in the current lactation. It is no wonder more and more interest and attention is being given to transition cows as they calve and move into lactation. Good health dictates good appetites and greater dry matter intakes by cows, and thus, determines milk production, cyclicity, and reproductive rates.

A study conducted at the University of Florida emphasized the importance of dry matter intake on early cyclicity and milk yield of cows (Staples et al., 1990). Cows with

the greatest dry matter intakes (increase of 24%) produced 17% more milk, cycled back 50% earlier, and lost 50% less body weight. Cows with extra body condition generally lose more weight and consume less feed. Healthy, thinner cows have better appetites and lose less body condition.

Attention to dry matter intakes and body condition is critical. That attention and care begins long before the cow calves, usually during the last 100 days of the previous lactation. At that stage of lactation, there is time to adjust body condition and prepare all cows for their dry period, next lactation, and breeding period. Attention to dry cows and early transition cows can pay big dividends in monitoring subsequent body condition, dry matter intakes, and cyclicity.

In summary, in both beef and dairy studies, cows not cycling by the beginning of the breeding period conceived at lesser rates and took longer to eventually conceive. Having more cycling cows at the beginning of the breeding season will maximize the proportion of cows that conceive to AI sires. More cows calving early during each successive calving season will enhance AI pregnancy rates, because more cows will be cycling before the breeding period begins.

References

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Table 1. Percentage of Suckled Cows Cycling at the Beginning of the Breeding Season Based on Parity, Body Condition Scores, and Days Since Calving

Item	No. of cows	% cycling
Parity ^a		
First	502	44.4
Multiple	1718	54.1
Body condition scores ^b		
#4	453	33.5
4.5	527	44.2
5.0	654	55.4
≥5.5	217	70.0
Days since calving ^c		
#50	201	27.4
51-60	291	39.2
61-70	458	50.4
71-80	641	59.3
>80	629	59.3

^aPercentage of first parity cows cycling was less ($P < 0.05$) than that of older cows.

^bLinear increase ($P < 0.05$) in cycling status with increasing body condition scores.

^cCurvilinear increase ($P < 0.05$) in cycling status with increasing days since calving.

Table 2. Percentage of Suckled Cows Detected in Estrus during the First Week of the Breeding after Various Hormones: Cycling Status before Treatment

Hormonal treatment	No. of cows	% in estrus
GnRH (d !7) + PGF _{2v} (d 0)	454	52.8 ^a
Anestrus	212	34.5
Cycling	242	68.8 ^b
GnRH (d !7) + norgestomet (d !7 to 0) + PGF _{2v} (d 0)	647	67.1
Anestrus	368	60.4
Cycling	279	76.0 ^b
Two injections of PGF _{2v} 14 d apart	366	44.0 ^a
Anestrus	191	27.2
Cycling	175	62.3 ^b

^aDifferent ($P < 0.05$) from anestrus cows within treatment.

^bDifferent ($P < 0.05$) from GnRH + norgestomet + PGF_{2v}.

Table 3. Distribution of cows detected in estrus based on serum concentrations of progesterone on d -14, -7, and 0 before the second (2xPGF_{2v} treatment) or before the only PGF_{2v}

Progesterone profile ^a	Treatment		
	GnRH + PGF _{2v} (Select Synch)	GnRH+ NORG+ PGF _{2v} (Select Synch + NORG)	2xPGF _{2v}
	% in estrus (no.)		
	Cycling + elevated progesterone at PGF _{2v}		
HHH	88.9 (18)	89.5 (19)	88.9 (36)
HLH	86.7 (30)	90.5 (21)	65.2 (23)
LHH	91.2 (36)	83.8 (37)	86.0 (43)
LLH	66.2 (65)	93.6 (31)	83.3 (36)
3 ^b	78.5 ^x (149)	88.8 ^x (108)	83.1 ^x (138)
Conception rate ^c	65.8 ^x	65.3 ^x	65.5 ^x
	Cycling + low progesterone at PGF _{2v}		
HHL	80.0 (25)	100 (26)	20.0 (5)
HLL	75.0 (4)	100 (6)	40.0 (5)
LHL	77.8 (9)	100 (5)	26.7 (15)
3 ^b	78.4 ^x (38)	100 ^x (37)	25.0 ^y (25)
Conception rate ^c	57.1 ^{xy}	41.7 ^x	83.3 ^y
	Noncycling + low progesterone at PGF _{2v}		
LLL ^b	27.9 ^y (111)	51.7 ^x (151)	16.3 ^z (135)
Conception rate ^c	71.4 ^x	59.2 ^x	20.0 ^y

^aBlood serum progesterone was determined on d -14 (first injection of 2xPGF_{2v} treatment; Figure 1), d -7 (injection of GnRH for Select Synch or injection of GnRH + implant of norgestomet for Select Synch + NORG treatment), or d 0 (PGF_{2v} injection for all treatments). Concentrations of progesterone were either low (L; <1 ng/mL) or high (H; ≥1 ng/mL) in each of three serum samples, making up eight permutations (HHH, HHL, HLH, HLL, LHH, LHL, LLH, or LLL).

^bTreatment × progesterone profile interaction (P < 0.001).

^cTreatment × progesterone profile interaction (P < 0.05).

^{xyz}Percentages within a row lacking a common superscript letter differ ($P < 0.05$).

Table 4. Estimates of Cycling Status of Dairy Cows before the Onset of First Insemination Annually Producing >10,000 liters of Milk

Season	Herd	No. of cows	Days in milk	First lactations	Multiple lactations
				----- % cycling -----	
Non summer	1	284	48-68	88	86
	2	394	40-60	72 ^a	87
Summer	1	251	47-67	42	50
	1	66	63-83	96	85
	2	198	57-77	83	87
	3	121	56-76	77	86

^aLess ($P < 0.05$) than first-lactation cows in herd 1.