Effects of Dry Period Length on Milk Yield and Mammary Epithelial Cells*

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ABSTRACT

A dry period, typically 40 to 60 d, between lactations is believed to be required to maximize milk yield in the subsequent lactation. Several hypotheses have been proposed to explain the requirement for the dry period, including (1) replenishment of body reserves, (2) regeneration of mammary tissue, and (3) optimization of benefits from endocrine events near the time of parturition. Continuously milked cows or glands have depressed milk yields but no differences in mammary DNA content or cell number. Nutritional status and endocrine hormones are not factors in reduced milk yield in continuously milked glands. Data from continuous lactation studies suggest that depressed milk yields are due to reduced functionality of mammary parenchyma. There is a need to reevaluate effects of continuous lactation on milk yield in today's high-producing dairy cow because studies on this topic were done using cows achieving peak milk production of 18 to 30 kg/d compared with 45 to 50 kg/d in today's dairy cows. Another factor that has not been considered in conjunction with current milk production levels is the use of bovine somatotropin (bST). Supplementation with bST increases milk yield, improves lactation persistency, and may improve milk yield in continuously milked cows. Future research goals are to examine the effects of continuous lactation in high-producing cows and to determine the effects of bST on milk yield and mammary functionality in continuously milked cows.

(Key words: dry period length, management, mammary development, bovine somatotropin)

Abbreviation key: CM = continuous milking; MEC = mammary epithelial cells, TGF- β 1 = transforming growth factor β 1.

INTRODUCTION

The optimal dry period length was discussed as early as 1805, when some English farmers believed that a dry period of 2 mo was optimal, whereas others believed in dry periods as short as 10 d (Dix Arnold and Becker, 1936). The literature during the mid- to late-1800s favored a dry period of 6 to 8 wk. A review of annual dairy records in the early 1900s (Carrol, 1913; Hammond and Sanders, 1923; ref. by Dix Arnold and Becker, 1936) suggested that cows need a dry period greater than 35 to 40 d, with a 2-mo dry period being optimal. In 1926, Woodward and Dawson (ref. by Dix Arnold and Becker, 1936) suggested that dry period length should be determined based on productivity and body condition. Dickerson and Chapman (1939) also believed in a nutritional influence on dry period length after determining that depressed milk yields following a shortened dry period were more pronounced in undernourished herds. World War II resulted in the adoption of the 305-d lactation and 60-d dry period in the United Kingdom to provide maximum production and accelerate genetic progress during a time of food shortage (Knight, 1998). The 60-d dry period was also adopted in the United States. Currently, average days dry in the United States is 60.6 and more than 74% of US dairies are using a dry period of 60 d or greater (USDA, 2002). Since adoption of the 60-d dry period, the US dairy industry has improved milk production by 5000 kg per lactation per cow, placed a larger emphasis on profit, accelerated genetic progress by AI and embryo transfer, adopted the TMR, increased milking frequency, altered photoperiod, and adopted bST. These changes have led to investigations regarding effects of lengthening lactation and delaying breeding, but reevaluation of the optimal dry period length has received little attention.

Few controlled studies have been conducted to directly evaluate the effect of dry period length. Results from these studies demonstrated that production losses of 18 to 29% occurred in the lactation following an omitted dry period (Rémond et al., 1997). Other studies demonstrate that a dry period less than 40 d reduces milk yield in the subsequent lactation, and an 8-wk dry

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Figure 1. Illustration of the effects of genetic gain and management practices on peak milk yield and lactation persistency (Adapted from Knight, 1998).

period is optimal (Klein and Woodward, 1943; Wilton et al., 1967; Schaeffer and Henderson, 1972; Coppock et al., 1974; Funk et al., 1987; Sørensen and Enevoldsen, 1991). The majority of these conclusions are derived from surveys of dairy records (from herds managing for a 60-d dry period) rather than controlled studies. Thus, there may be bias caused by mismanagement, health status, aborted pregnancies, twinning, or inaccurate breeding dates in cows with shortened dry periods. Others have shown no production losses following a 30-d dry period (Rémond et al., 1997; Bachman, 2002; Gulay et al., 2003).

Objectives of this paper are to review the literature regarding physiological and production responses to a shortened or omitted dry period, evaluate changes in the lactation curve since adoption of a 60-d dry period, and discuss prospective roles of management technologies introduced since World War II that improve milk yield, some of which may address the issue of reduced milk yield following a shortened or omitted dry period.

SHORTENED OR OMITTED DRY PERIOD

Hypotheses Regarding Dry Period Requirements

Discussion of milk yields following a shortened or omitted dry period will be limited primarily to controlled studies. For reviews of retrospective data collected from dairy records, see Klein and Woodward (1943), Wilton et al. (1967), Schaeffer and Henderson (1972), and Funk et al. (1987).

Prior to 1960, the dry period was thought to be necessary for replenishment of body reserves, regeneration of mammary tissue, and for maximal benefits from lactogenic endocrine events near the time of parturition (Smith et al., 1967). The nutritional or replenishment

of body reserves hypothesis began with observational data from Woodward and Dawson (1926) (ref. by Dix Arnold and Becker, 1936). They suggested that body condition should be a factor in determining dry period length. Dickerson and Chapman (1939) indicated greater milk yield reductions in undernourished herds that used a shortened dry period. This hypothesis was first tested by Swanson (1965) with a between animal comparison model using 5 pairs of identical twins. Twins were assigned to either control or continuous milking (CM; no dry period) at the end of their first lactation. The study followed the twins through 3 consecutive lactations after assignment to treatment. The first lactation data were used to demonstrate similar milk yields in the twins before treatment application. The control (60-d dry) twins were fed only a roughage diet during the dry period, whereas the CM twins received both roughage and concentrates in their diet. Body weight was recorded weekly as a measure of nutritional status. In the second and third lactations, the CM twins only produced 75 and 62% as much milk as the control twins, respectively (Table 1). Body weights were heavier in the CM twins throughout the second and third lactations. If BW is an indicator of improved nutritional status, the nutritional theory would have predicted that CM cows should have enhanced milk yield. Prior to the fourth lactation, all animals were given a 60-d dry period, and production was slightly better in the CM twins than controls. Others have also demonstrated that returning CM cows to a 60-d dry period results in normal or nonsignificant increases in milk yield (Coppock et al., 1974; Rémond et al., 1997). Swanson (1965) concluded that reduced milk yield in CM cows in the subsequent lactations was more likely due to an inhibitory effect on the mammary gland or the factors regulating the mammary gland and milk synthesis. To put these results in perspective compared with today's herds, at peak milk production, the controls were only producing 18 to 20 kg/d (Table 1).

Lotan and Alder (1976) addressed the nutritional effects of a shortened dry period by comparing milk yield, BW, and circulating concentrations of glucose and NEFA in cows given a 30-d dry period compared with cows given a 60-d dry period (controls). Results demonstrated that cows given a 30-d dry period had 305-d FCM yield equal to the controls (Table 1). However, the 30-d dry group produced less milk during the first 2 mo of lactation and lost less BW than 60-d dry cows during the first 3 mo of lactation. In addition to greater weight loss, 60-d dry cows had reduced blood glucose concentrations and slightly greater circulating NEFA after calving. These data suggested that cows with a 60-d dry period were in a greater negative energy balance in the early postpartum period, most likely due to higher milk

Table 1. Summary of results from controlled experiments evaluating the effects of continuous milking (CM) or shortened dry periods on milk production.

Reference	Model	Dry period length	Production effects	Milk yield in controls
Swanson, 1965	Identical twins	CM	<ul style="list-style-type: none"> • 25% reduction in milk yield in the second lactation • 38% reduction in the third lactation • Improved BW throughout the study 	Peak milk yield of 18–20 kg/d
Smith et al., 1967	Half-udder (contralateral quarters)	CM	23% reduction in milk yield in CM glands	Peak milk yield of 20–25 kg/d
Coppock et al., 1974	Between animal	20, 30, 40, and 50d	<ul style="list-style-type: none"> • 20-, 30- and 40-d dry periods reduced milk yield by 10, 7, and 1% • Dry periods greater than 40 d did not alter subsequent milk production 	6726 kg of 305-d mature equivalent milk yield
Lotan and Alder, 1976	Between animal	30 d	Reduced milk yield for 2 mo post partum, but no difference in 305-dFCM yield	Peak milk yield less than 34 kg/d
Sørensen and Enevoldsen, 1991	Between animal	4 wk	4-wk dry period reduced subsequent milk yield by 10%	Average milk yield/d for 84 DIM 25 kg/d
Rémond et al., 1992	Between animal	CM	Reduced milk yield by 4 kg/d (17% of average daily milk)	Peak milk yield less than 30 kg/d
Bachman, 2002	Between animal	30 d	No difference in 305-d milk yield	Peak milk yield of 46 kg/d
Gulay et al., 2003	Between animal	30 d	No difference in daily milk yield or 3.5% FCM yield	Peak milk yield of 44 kg/d

yields. By the end of the lactation, the 30-d dry group had the same production as the controls. The 30-d dry cows may have retained more body reserves during early lactation, which would have contributed to the improved milk production later in lactation. Another possible explanation for these results is delayed mammary growth in the 30-d dry cows resulting in lower milk yields in early lactation, but equivalent milk production by the end of lactation. The authors concluded that the prepartum conditions of a shortened dry period and reduced time on an all-roughage, dry-cow diet programmed postpartum energy partitioning. These data add further support to the concept that reduced milk yield following a shortened or omitted dry period is not caused by nutritional limitations.

Rémond et al. (1992) also studied the effects of nutrition on milk yield in the subsequent lactation with 15 primiparous CM cows and 11 primiparous control cows. The CM cows had milk yields that were 4 kg/d less than controls in the subsequent lactation (Table 1). However, increased BW in late gestation, higher postpartum milk-protein content, and equal plasma glucose and NEFA concentrations in CM cows compared with controls, suggest improved energy balance in CM cows. These results further disprove the hypothesis that nutritional status is the limiting factor in reduced postpartum milk yield of CM cows.

Another proposed hypothesis was that CM altered endocrine events around calving and initiation of lactation causing reduced milk yields in the next lactation. Smith et al. (1967) used the within-animal (half udder)

model to test this hypothesis. The study utilized 5 cows and measured milk production from the CM and control udder halves for 14 wk to demonstrate similar production in the udder halves, then dried contralateral quarters 10 wk before expected calving dates. This experimental model has been criticized because milk stasis in one gland or inhibition of secretion in a gland by colchicine treatment has been shown to cause compensatory mammary growth and milk production in the quarters that remain lactating (Capuco and Akers, 1999). Further, involution of the control quarters may be delayed due to the influence of galactopoietic hormones and stimuli from continued milking in the other glands. The advantages of this model are (1) udder half rather than cow is the experimental unit, reducing between-animal variation and animal numbers required to achieve adequate statistical power, and (2) each udder half is exposed to the same nutrients, circulating growth factors, and other endocrine hormones. In this study, Smith et al. (1967) demonstrated that milk production in CM quarters during the last 10 wk of gestation declined throughout the remainder of gestation without evidence of compensatory milk production or mammary growth. Further, the authors noted that control quarters dried and regressed in size in a progression typical of dried udders. Milk yield in the subsequent lactation was reduced dramatically in the CM quarters by 33 to 44% in 3 of the 5 cows. However, 2 of the cows produced 97 and 99% of the production in control quarters. In the absence of information on parity, age, and DIM at the start of the study, reasons for

Table 2. Summary of changes in mammary epithelial cells (MEC) from control and continuously milked (CM) cows (Capuco et al., 1997).

	53 d prepartum (7-d dry)	35 d prepartum (25-d dry)	7 d prepartum (53-d dry)
Control	MEC in a nonsecretory state	MEC proliferation increasing Luminal area at a minimum and stromal elements at a maximum	98% of MEC in a secretory state MEC account for 83% of total mammary cells
CM	50% of MEC in a secretory state	Luminal area and stromal elements did not vary with stage of gestation in CM glands	75% of MEC in a secretory state MEC account for 74% of total mammary cells

the inconsistent results are not known. The average loss in milk yield in CM halves for all cows was 23% (Table 1). These data confirm that the effect of CM is not due to nutritional factors or endocrine hormones, as they were common to all quarters. It is possible that CM may affect hormonal regulation of lactation, involution, and proliferation in an autocrine or paracrine manner. Smith et al. (1967) hypothesized that CM maintains activity of the secretory tissue and may inhibit renewal of mammary epithelial cells (MEC) that occurs during lactogenesis just prior to parturition.

Effects of CM on mammary-cell numbers and functionality were first studied by Swanson et al. (1967) using a within-animal model in which lateral halves were either dried 60 d prior to expected calving date or CM. The CM halves were dried 1 wk prior to slaughtering the animals. The 6 wk difference in dry period length did not result in differences in dry fat-free tissue weight, DNA concentration, total DNA content, or number of alveoli. Results suggested that reduced milk yields following CM may be due to secretory activity per unit of mammary tissue and the physiological factors that affect the cells during lactogenesis, but not due to MEC numbers. These data also suggested little or no alveolar involution occurs during the normal 60-d dry period.

Thirty years later, the effects of CM on MEC were evaluated by Capuco et al. (1997) using 26 multiparous cows; 13 were dried 60 d prior to their expected calving date, and 13 were milked continuously. A subset from each group was slaughtered at 53, 35, 20, and 7 d prepartum, and mammary tissue was harvested for analysis of DNA content, parenchyma content, and proliferation in MEC. Total mammary DNA content increased 2-fold from 53 to 7 d prepartum but was not affected by lactation status. Using [³H]-thymidine incorporation to evaluate mammary cell proliferation, they demonstrated 80% greater incorporation in mammary tissue from control (60-d dry) cows compared with CM cows. Cells from CM glands were proliferating but at a reduced rate. Thus, proliferation was decreased but not entirely inhibited. Others have reported a greater mitotic frequency in nonlactating tissue than in lactating tissue (Altman, 1945; Knight and Peaker, 1984). By the

last week of gestation, increased MEC proliferation and turnover resulted in an enhanced number of epithelial cells in control glands (Table 2). These results suggested that control glands had greater replacement of senescent cells, but mammary growth or cell replacement was not prevented in CM glands.

Data from Capuco et al. (1997) also add merit to the conclusions of Swanson et al. (1967) that widespread mammary involution does not occur in dairy cows because DNA content was not affected by lactation status during the last 60 d of gestation. This conclusion is also supported by histological data demonstrating intact alveoli throughout the dry period (Holst et al., 1987). Mammary DNA content of nonlactating glands was at a nadir after 25-d dry, and MEC were in a nonsecretory state by 7-d dry, suggesting that mammary involution was complete before 25 d after milk stasis (Capuco et al., 1997). In addition, increased MEC proliferation began by 25 d of the dry period, suggesting regression of the gland was complete and mammary growth was progressing. The time during which secretory activity has ceased and luminal volume is at a minimum may be a critical point for mammary development during the dry period (Table 2) (Capuco et al., 1997). Also at this pivotal time, there were increases in incorporation of [³H]-uridine and hydroxyproline content in dried glands, suggesting an increase in RNA synthesis and remodeling of the extracellular matrix. These changes may be the foundation for MEC turnover and proliferation later in the dry period. The authors hypothesized that the benefit of the dry period may be replacement of senescent MEC during late gestation and improved cell turnover (Capuco et al., 1997).

Bachman (2002) addressed the decline in replacement of senescent MEC and reduced milk yield in the subsequent lactation by studying a shortened dry period (30 d) and attempting to hasten involution by treatment with estrogen. The 305-d ME did not differ between 30-d dry and 60-d dry groups (Table 1). There was also no effect of estrogen treatment on 305-d ME regardless of dry period length. Based on the finding that mammary involution is complete by 25 d of the dry period (Capuco et al., 1997), a 60-d dry period may have been adequate for involution to occur without es-

trogen treatment and also alleviate reduced MEC proliferation compared with CM glands. More recently, Gulay et al. (2003) examined the effects of a 30-d dry period, low-dose (143 mg/14 d compared with the FDA-approved 500 mg/14 d dose) administration of POSILAC (bST) during the last 28 d of gestation and first 60 d postpartum, and estrogen on postpartum milk production and DMI. Shortening the dry period and estrogen treatment at the time of dry-off did not alter milk yield during the 21-wk study (Table 1). Bovine somatotropin administration improved milk yield by 8%, but there was no interaction between dry period length and bST supplementation. The DMI tended ($P < 0.08$) to be greater during the early postpartum period for cows with a 30-d dry period, regardless of bST treatment. Control cows experienced a larger decrease in BCS after calving that persisted throughout the study, and BCS was not affected by bST treatment. The animals in these studies (Bachman, 2002; Gulay et al., 2003) had milk yields (Table 1) representative of today's dairy cow and further supports the need for research on the optimal dry period length with current production levels and management strategies in the dairy industry.

The effect of days dry on subsequent milk yield has also been examined in field trials. Coppock et al. (1974) conducted a field trial to evaluate dry periods of 20, 30, 40, 50, and 60 d. They concluded that a dry period greater than 40 d resulted in production equal to cows dry 50 d or longer, but a dry period less than 40 d resulted in significant production losses (Table 1). In the lactation following shortened dry periods, a traditional dry period was utilized, and milk production was similar to cows with 50- to 60-d dry periods throughout the study. Others have also demonstrated the absence of a carryover effect from shortened or omitted dry periods (Swanson, 1965; Rémond et al., 1997). Another field trial comparing 4-, 7-, and 10-wk dry periods determined that a 4-wk dry period decreased milk yield by 2.7 kg/d during the first 168 d of the subsequent lactation (Table 1) (Sørensen and Enevoldsen, 1991). Cows with a 10-wk dry period had similar production compared with cows given a 7-wk dry period.

Continuous lactation also has been studied in goats, rats, and humans. Omitting the dry period in goats did not result in reduced milk yield in the subsequent lactation (Fowler et al., 1991). Fowler et al. (1991) used the within-animal model and found that CM glands were visibly smaller at the time of parturition compared with control glands, but this difference did not continue past parturition. At 18-wk postpartum, mammary parenchyma weight and secretory cell number were not different between CM and control glands. In rats, omission of the dry period resulted in a 28% decrease in litter weight gain, compared with rats given a 4-d dry

period (Paape and Tucker, 1969). The CM rats had greater total DNA than 4-d dry rats on the first day of lactation; however, the inverse was true on d 8 and 16 of lactation. Further, collagen content was reduced in CM rats on d 1 of lactation. If collagen is required for future mammary development, its reduction at the start of lactation may contribute to reduced cell numbers later in lactation. As mentioned previously, the importance of the extracellular matrix on mammary development also has been suggested in cattle (Capuco et al., 1997). Pitkow et al. (1972) also demonstrated reduced milk yield in CM rats, but provided evidence that omitting the dry period resulted in an increase in mammary cell carryover from one lactation to the next. In humans, women who nursed through the last trimester of pregnancy had infants with a 15% reduction in weight gain during the first month postpartum (Marquis et al., 2002). Further, the infants from CM women breastfed longer per feeding and longer in a 24-h time period than infants from women that had not breastfed during pregnancy. Despite the longer feeding times, infants from CM women had a tendency for lower intakes (Marquis et al., 2002). Results suggest a reduced milk yield in CM women, possibly due to reduced MEC function or number.

The difference in a dry-period requirement in cattle, rats, and humans compared with goats may be related to the fact that, prior to peak milk yield, cell activity plays a major role in determining milk yield in goats, and post peak milk yield is more dependent on cell number (Knight and Wilde, 1987). Goats that are continuously milked do not demonstrate reduced production in subsequent lactation. Conversely, in rodents (and possibly in cattle and humans), cell number is more important in early lactation (Knight and Wilde, 1987) and omission of the dry period decreases milk yield.

In summary, the studies reviewed have used identical twins (between animal comparisons), half-udder models (within-animal comparisons), and across-animal comparisons to demonstrate CM animals, or specific glands have depressed milk yield but no differences in mammary DNA content, cell numbers, or BW when compared to animals or specific glands given a 60-d dry period. Data from these continuous lactation studies suggest that depressed milk yields are due to reduced functionality of mammary parenchyma rather than nutritional status, endocrine regulation, or cell number. Collectively, our interpretation of the literature is that reduction of subsequent milk yield in CM animals is caused by reduced MEC turnover and larger carryover of "old" MEC to the next lactation, resulting in reduced secretory capacity of mammary tissue and perhaps more resting MEC in CM glands. Continuous milking

may alter cell turnover through reduced MEC proliferation (reduces new MEC) and apoptosis (increases old MEC), thus maintaining DNA content equal to glands with a dry period but with different populations of old and new cells. Data on the effects of a 30-d dry period on subsequent milk yield and mammary functionality are equivocal. Shortened dry periods have recently been extensively reviewed by Bachman and Schairer (2003). The majority of research on this topic was done with low-producing cows (Table 1) and needs to be reexamined using today's genetically superior cows in conjunction with modern management and feeding practices.

Proposed Effects of CM on MEC Turnover

For CM to alter MEC turnover, the process of apoptosis and proliferation must be altered compared to events occurring during a normal 60-d dry period. Effects of milk removal and milk stasis are better understood for apoptotic stimuli than for mitogenic events. There are both systemic and local effects on MEC apoptosis during lactation. However, the effects of CM are most likely at a local level. Systemic effects are thought to be governed by the galactopoietic hormones, prolactin, somatotropin, and insulin-like growth factors (IGF), based on data collected in rodents (Wilde et al., 1999). Prolactin is believed to inhibit mammary apoptosis by repression of insulin-like growth-factor binding protein 5 (IGFBP5), which antagonizes the effects of IGF-I on the survival of MEC (Wilde et al., 1999). According to this scenario, the role of ST is believed to be through elevation of IGF-I.

Local control of apoptosis can overcome the antiapoptotic effects of galactopoietic hormones, as demonstrated by apoptosis of MEC during the decline phase of lactation, and by involution following milk stasis in individual glands (unilateral milk stasis in goats, teat sealing in rats), while lactation is maintained in other glands (Wilde et al., 1999). The within-animal model studies previously discussed disprove systemic endocrine theories, but do not address autocrine or paracrine control of involution. Involution under local control of the mammary gland is believed to be the result of an accumulation of apoptotic factors in stored milk and/or MEC and due to physical distention on the mammary epithelium during milk stasis (Wilde et al., 1999).

The apoptotic factors known to increase in milk after cessation of milk removal are IGFBP5 and transforming growth factor- β 1 (TGF- β 1) (Wilde et al., 1999). Inhibition of IGF-I by IGFBP5 was discussed previously. The role of TGF- β 1 is not well known, but it is upregulated in involuting tissues and during *in vitro* conditions that stimulate apoptosis (Wilde et al., 1999). Others also have demonstrated that forced involution

following weaning or cessation of milking reduces MEC responsiveness to IGF-I activation of the PI3-kinase and Akt survival pathways, regardless of overexpression of IGF-I or adequate IGF-I receptor numbers (Hadsell et al., 2001). Physical distention may induce apoptosis by disrupting cell shape, changing intramembrane composition, altering intracellular free-calcium content, and changing gene expression, which have implications on the function of the mammary epithelium (Wilde et al., 1999). Progressive induction of mammary distention without milk stasis has been shown to inhibit milk secretion, indicating that both physical and chemical feedback contribute to mammary involution and tissue remodeling (Wilde et al., 1999).

The physical changes in MEC during involution in dairy cattle have been described (Holst et al., 1987). Within 24 h of milk stasis, there is a reduction in secretory vesicles fused to the apical membrane, and thus an accumulation of secretory vesicles and fat droplets occurs within MEC (Holst et al., 1987). These vesicles appear to fuse, forming large stasis vacuoles within 48 to 72 h. The accumulation of vacuoles and secretory products results in engorged MEC and inhibition of milk secretion by end-product inhibition of milk synthesis (Holst et al., 1987; Capuco and Akers, 1999). Engorgement of MEC changes cell shape and may alter intracellular calcium homeostasis and membrane composition to favor apoptotic signals. Continuous milking may reduce apoptosis through local effects of milk removal, minimizing the buildup of apoptotic factors and/or physical distention in the mammary gland.

The other aspect of MEC turnover, proliferation, has not been well studied in late-gestation cattle. A normal dry period between lactations increases cell renewal in cattle (Capuco et al., 1997) and improves cell turnover in rats (Paape and Tucker, 1969) when compared with lactating tissue from CM animals. Nonlactating tissue also has a greater mitotic index than lactating tissue (Knight and Peaker, 1984). The mechanism by which CM alters MEC proliferation is unknown. Interestingly, IGF-I, which is elevated in plasma during lactation, stimulates MEC proliferation *in vitro* (McGrath et al., 1991) and *in vivo* (Collier et al., 1993). The IGFBP (BP1-5) that control availability of IGF-I to target tissues are secreted locally by MEC. Perhaps continuous lactation alters the profile of IGFBP in late gestation to decrease the rate of MEC proliferation. Together, the hypothesis that a dry period is required for complete replacement of senescent or old cells with new cells is supported by observations on the reduction in apoptosis and proliferation associated with continuous lactation. This hypothesis assumes that old cells have less secretory ability than new cells and have less proliferative capability than new cells, causing a premature decline of MEC

during lactation (Capuco and Akers, 1999). These assumptions are supported by the findings of Paape and Tucker (1969) that demonstrate reduced numbers of secretory cells in midlactation in CM rats as well as by findings of decreased cell number and activity during the decline phase of lactation on conventionally managed goats and cattle (Knight and Peaker, 1984; Wilde and Knight, 1989; Capuco and Akers, 1999).

Effects of Dry Period Length on Milk Composition

Because many milk pricing plans and quota systems pay the dairy producer not only for the quantity of milk produced, but also for the composition of that milk, the effects of CM on milk composition are important. Further, changing milk composition could have implications at the consumer level and poses issues regarding salable milk. Changes in colostrum quality following omission of the dry period are also important due to the importance of good quality colostrum on calf morbidity and mortality.

Milk fat content in CM cows increases over the last 2 mo of gestation (Wheelock et al., 1965; Rémond et al., 1992; Rémond et al., 1997). Wheelock et al. (1965) demonstrated that this gradual increase occurs until 7 to 10 d before calving and then remains elevated through parturition. Postpartum milk-fat percentage is similar in CM and control cows or glands (Wheelock et al., 1965; Rémond et al., 1992, 1997). Rémond et al. (1997) reported a decrease in long-chain fatty acids in milk from CM cows. The reduced milk yield in CM cows most likely improved energy balance and decreased mobilization of adipose tissue. This effect may have reduced LCFA incorporation into milk fat and increased *de novo* synthesis of short- and medium-chain fatty acids.

Similar to fat, milk protein increased gradually throughout the last 2 mo of gestation and remained elevated through parturition (Wheelock et al., 1965; Rémond et al., 1992, 1997). As milk yields decreased to very low levels (<6 kg/d), the ratio of casein to total protein decreased (Rémond et al., 1992, 1997). The change in the casein to total protein ratio was affected by a greater increase in soluble (whey) proteins compared with casein. Postpartum milk protein percent was greater (Rémond et al., 1992, 1997) or unchanged (Smith et al., 1967) in CM cows when compared with controls. An increase in milk protein percent may be the result of reduced milk yield, improving energy balance and thereby sparing amino acids and energy for protein synthesis (Rémond et al., 1997). Casein content of milk protein production in the subsequent lactation was not different between CM and control glands (Rémond et al., 1997).

The lactose content of milk has been shown to increase during the last 2 mo of gestation in a similar, but inverse, temporal pattern as observed with fat and protein (Rémond et al., 1997). Postpartum lactose content in milk from CM glands or cows decreased in some experiments (Rémond et al., 1992, 1997) and was unaffected by length of dry period in others (Wheelock et al., 1965; Smith et al., 1967). Lactose is the major osmoregulator of milk, and a decrease in lactose production from CM cows or glands would explain reduced milk yield. However, not all experiments with decreased milk yield following an omitted dry period demonstrate reductions in lactose content. Reduced milk yield without reduced lactose synthesis would suggest fewer cells in the secretory state in CM glands.

During CM and late gestation, Wheelock et al. (1965) observed a decrease in the potassium (K) to lactose ratio, which was accompanied by an increase in sodium (Na) and chloride (Cl) concentrations. It was suggested that these changes were caused by the very low milk yields in late gestation and a shift from a primary secretion of milk to transudate of plasma. Further, these changes suggest a decrease in metabolic activity of secretory cells because maintenance of the normal K gradient between intracellular fluid and the alveolar lumen is an energy-dependent process (Wheelock et al., 1965). Postpartum levels of K, Na, and Cl in milk were not different in CM glands compared with controls (Wheelock et al., 1965; Smith et al., 1967).

A review of the literature suggests that colostral Ig and protein content are reduced in CM cows (Rémond et al., 1997). Enhanced protein and Ig concentrations in colostrum are believed to be the result of an accumulation of secretion in the udder prior to calving (Wheelock et al., 1967; Rémond et al., 1997). Continuously milked cows may have reduced colostrum quality due to the lack of a secretion-accumulation period. Interestingly, cows that were given a dry period of 1 to 10 d produced colostrum with Ig concentrations that are 60 to 70% of colostrum from cows given a 60-d dry period (Rémond et al., 1997). If colostral Ig concentrations in CM cows are reduced, the use of colostrum supplements to achieve desired Ig concentrations would be necessary.

The typical pattern of SCC during a traditional lactation cycle is characterized by high concentrations at freshening, a nadir at peak milk and midlactation, and concentrations gradually rising in late lactation (Peters, 2002). Rémond et al. (1997) discussed the impact of omitting the dry period on SCC using data from several studies (controlled and commercial field trials) conducted by their laboratory. During the last 60 d of gestation, there was a gradual increase in SCC until 4 wk prepartum and a more substantial increase during

the last 4 wk of gestation. These trends are similar to SCC pattern observed in traditional lactation cycles (Peters, 2002). In subsequent lactations, Rémond et al. (1997) observed a tendency for elevated SCC in milk from cows given a shortened or omitted dry period. This increase in SCC was not accompanied by an increase in clinical mastitis cases. Further, the 60-d dry cows were all given intramammary antibiotic treatment at the time of milk stasis. No preventive therapy was used in CM cows and in many of the shortened dry-period animals (Rémond et al., 1997). One could speculate that continuous milking of cows could reduce clinical mammary infections by eliminating new infections associated with cessation of milking and the dry period.

NEW OPPORTUNITIES FOR CONTINUOUS LACTATION

Dramatic production increases and improved persistency of lactation in today's dairy cow provide new opportunities for reinvestigating omission of the dry period (Figure 1). The first opportunity is improved milk yield at the time of milk stasis. Many cows are producing more than 30 kg/d at milk stasis and have the potential to milk through the last 60 d of gestation at a profitable production level. Additional days of lactation maximize income generated per cow per lactation and decrease the number of replacement animals needed to keep a dairy at desired cow number capacity. Further, such high yields at milk stasis result in extreme changes in metabolic and physiological state complicated with dramatic diet changes to add essentially a second transition period to the lactation cycle. Another opportunity lies at the other end of the lactation curve in the "traditional" transition period (3 wk prepartum to 3 wk postpartum) when parturition stress, initiation of lactation, and dramatic diet changes occur. These 2 transition periods within 60 d of each other offer the cow a relatively short time period to adapt to many extreme changes. One could speculate that CM would keep the animal adapted to lactation and associated diets and may further reduce the risk of metabolic disorders. A third and, perhaps, most important opportunity arises from new management technologies that were introduced since World War II that improve milk yield (Figure 1). Some of these management technologies, including increased milking frequency, bST, and photoperiod regulation are known to increase MEC functionality and, therefore, may address the issue of reduced functionality in MEC without a rest period between lactations.

Bovine somatotropin is of particular interest and will be the focus of this discussion. Exogenous bST increases milk yield by 10 to 15% and improves lactation persis-

tency (Bauman et al., 1999). Most importantly, bST has been shown to impact MEC by all or a combination of the following mechanisms: (1) improved synthetic activity on a per cell basis (increased RNA content), (2) increased number of cells in a secretory state and reduced number of cells in a resting state (increased parenchyma volume without increased DNA synthesis), and (3) reduced cell loss (low levels of plasmin during bST treatment) (Bauman and Vernon, 1993).

The effects of bST treatment on milk yield are believed to be indirect (McDowell et al., 1987; Davis et al., 1989; Prosser et al., 1990; Bauman and Vernon, 1993). Production of IGF-I occurs in mammary stromal cells, and IGFBP may localize IGF-I to the MEC (Cohick, 1998). During bST treatment, IGFBP3 is elevated and IGFBP2 is decreased. Such alterations in the IGFBP profiles could regulate the galactopoietic effects of bST. In addition to these galactopoietic effects, IGF-I has been shown to be an important regulator of cell survival (Hadsell et al., 2001) and may contribute further to reduced cell loss in bST-supplemented animals. However, a reduction in apoptotic indices has not been observed in bST-treated animals (Capuco et al., 2001; Baldi et al., 2002). Perhaps small numbers of apoptotic cells make it difficult to detect a difference due to bST treatment. Reduced apoptosis in MEC has been demonstrated in transgenic mice over-expressing IGF-I or IGFBP3 (Hadsell et al., 1996; Neuenschwander et al., 1996). Hadsell et al. (1996) found similar results in transgenic mice over-expressing truncated IGF-I (des-IGF-I). As previously discussed, the anti-apoptotic properties of IGF-I are diminished during forced involution due to local factors within the mammary gland (Cohick, 1998; Hadsell et al., 2001). This ability of local signals to mediate MEC apoptosis may be responsible for different responses to bST, compared with IGF-I on cell survival during lactation.

In addition to the possible antiapoptotic effects of elevated IGF-I, bST supplementation may reduce MEC loss by reducing plasmin in the mammary gland. Plasmin is a serine protease that is elevated in milk during the declining phase of lactation (gradual involution) (Politis et al., 1990). Bovine ST treatment prevented the increase in milk plasmin, suggesting a role in decreasing the conversion of inactive plasminogen to plasmin. This decrease in plasmin may correlate with reduced mammary involution in bST-treated cows (Politis et al., 1990). These effects of bST on plasmin activity in milk have also been shown in ewes (Chiofalo et al., 1999). Others have not been able to demonstrate an effect of bST on plasmin concentrations in milk (Stelwagen et al., 1994; Baldi et al., 2002).

Proliferative effects of bST in nonlactating tissue from prepubertal heifers have been exhibited both in

vivo (Sejrsen et al., 1986; Berry et al., 2003) and in vitro (Weber et al., 2000). In vitro studies have demonstrated proliferative properties of IGF-I in MEC (Shamay et al., 1988; Baumrucker and Stemberger, 1989; Winder et al., 1989; McGrath et al., 1991; Peri et al., 1992; Collier et al., 1993; Purup et al., 1995). Concentrations of IGF-I are elevated in milk, and the numbers of high-affinity IGF-I receptors are increased in mammary tissue during pregnancy, further demonstrating a potential role of IGF-I in mammary proliferation (Collier et al., 1989, 1993).

Bovine ST supplementation in nonlactating, pregnant cows had no effect on postpartum milk yield (Bachman et al., 1992). Intramammary infusion of bST in late-pregnant heifers was mammatogenic but did not increase postpartum milk yield (Collier et al., 2002). The mammatogenic effect observed in this study may not have been sustained through calving. Intramammary infusion of IGF-I in late-pregnant heifers was also mammatogenic but resulted in decreased postpartum milk yield (Collier et al., 2002). In ewes, bST treatment during the dry period increased MEC number and improved postpartum milk yield (Stelwagen et al., 1993). Recently, a mammatogenic response to bST has been demonstrated in lactating cows (Capuco et al., 2001). However, the increase in MEC proliferation was not accompanied by a change in apoptotic frequency, resulting in a reduced rate of mammary regression rather than a net increase in mammary parenchyma (Capuco et al., 2001). Treatment of late-gestation (last 60 d of gestation) lactating cows with bST has not been evaluated.

The effects of bST supplementation and the resulting indirect effects mediated by the IGF system have not been investigated in CM cows. The fact that MEC proliferation is reduced in CM glands without a reduction of total DNA content compared with control glands suggests that CM alters MEC turnover to favor an increase in the number of "old" MEC in the subsequent lactation (Capuco et al., 1997). Also, it is important to recognize that MEC turnover exists throughout lactation (Knight and Peaker, 1984; Capuco et al., 2001); thus, "new" cells do not only originate during or following a dry period. These suggested changes in cell turnover have been implicated in reduced milk yields in CM glands. Bovine ST may have a role in alleviating reduced milk yields in CM cows through the previously mentioned effects on MEC. It is possible that bST treatment would enhance secretory capacity and survival of "old" MEC to establish milk yields equivalent to those derived from glands with a larger population of "new" MEC. Bovine ST could also improve milk yield in CM cows with more old MEC by decreasing the number of cells in a resting state. Further, mitogenic properties of bST/IGF-I may reverse the reduction in proliferative rates in MEC of CM

glands and improve mammary cell turnover during late gestation.

We conducted a commercial trial examining the effects of CM in combination with bST supplementation on milk yield and composition. Treatments included: (1) 60-d dry period, label bST (control; label = bST started at 57 to 70 DIM to end of lactation), (2) 30-d dry period, label bST, (3) CM, label bST, and (4) CM with continuous bST. Average milk yield was reduced in primiparous cows during the first 17 wk postpartum for treatments 2, 3, and 4, compared with controls (38.1, 35.1, 37.5 vs. 44.1 kg/d; $P < 0.01$, $n = 56$) (Annen et al., 2003). Milk production in multiparous cows was not affected by treatment (46.6, 43.4, 46.5, 47.7 kg/d; $P > 0.5$, $n = 39$). These data suggest that a shortened dry period and CM have minimal effects in multiparous cows ($\geq$ second lactation during previous lactation) but result in reduced production in the subsequent lactation in primiparous cows. We hypothesize that a shortened or omitted dry period impedes mammary growth in primiparous animals and may have minimal production effects in multiparous cows treated with bST.

Rémond et al. (1997) reviewed a large commercial trial (Brittany Survey) that included data from 252 lactations after the dry period was omitted and 139 lactations following a shortened dry period. In this study, primiparous cows milked continuously had larger production losses in the next lactation (1525 kg) than CM multiparous cows (1342 kg). They concluded that continued body and mammary growth in primiparous cows resulted in larger production losses after a shortened or omitted dry period. Further, several studies analyzing dairy records also detected a greater sensitivity of a reduced or omitted dry period between the first and second lactations than in older cows (Wilton et al., 1967; Dias and Allaire, 1982). Dias and Allaire (1982) determined that as age at calving in the lactation before a shortened dry period increased from 24 to 83 mo, the optimal number of dry days decreased from 65 to 23 d. As mentioned previously, data from such observational studies may have biased interpretations because animals were not actually being managed for shortened or omitted dry periods. Sørensen and Enevoldsen (1991) were not able to demonstrate a parity effect in their evaluation of a shortened (4 wk) dry period.

Our evaluation of dry period length with concomitant use of bST, demonstrated improved profitability in multiparous cows through increased net milk income generated by omitting the dry period (Annen et al., 2003). For example, CM multiparous cows and continuous bST resulted in a cumulative gain of \$50/cow over control (60-d dry) cows during the first 17 wk of the subsequent lactation. The cows in this study were representative of today's high-producing cow. Control multiparous cows

reached peak milk yields of 52 kg/d and control primiparous cows reached peak milk yields of 47 kg/d in the subsequent lactation.

CONCLUSION

Continuous milking has been shown to reduce milk yield in subsequent lactations. A shortened dry period has been shown to cause both reductions and no change in subsequent milk yields. Much of these data were collected from cows with dramatically lower milk yields than today's genetically superior cow and without the use of many of the management tools used in the dairy industry today, such as bST. It is hypothesized that reduced milk yield after continuous lactation is caused by reduced MEC proliferation and turnover rather than reduced MEC numbers. New research is required to examine the effects of CM in high-producing cows in combination with bST supplementation.

REFERENCES

- Altman, A. D. 1945. Udder changes in lactating cows. *Dairy Sci. Abstr.* 9:287.
- Annen, E. L., M. A. McGuire, J. L. Vicini, and R. J. Collier. 2003. Effect of POSILAC (bST) and dry period management strategy on milk yield. *J. Dairy Sci.* 86(Suppl. 1):154. (Abstr)
- Bachman, K. C. 2002. Milk production of dairy cows treated with estrogen at the onset of a short dry period. *J. Dairy Sci.* 85:797–803.
- Bachman, K. C., and M. L. Schairer. 2003. Invited review: Bovine studies on optimal lengths of dry periods. *J. Dairy Sci.* 86:3027–3037.
- Bachman, K. C., D. H. Wilfond, H. H. Head, C. J. Wilcox, and M. Singh. 1992. Milk yields and hormone concentrations of Holstein cows in response to Somatitrope (Somatotropin) treatment during the dry period. *J. Dairy Sci.* 75:1883–1890.
- Baldi, A., S. Modina, F. Cheli, F. Gandolfi, L. Pinotti, L. B. Scesi, F. Fantuz, and V. Dell'Orto. 2002. Bovine somatotropin administration to dairy goats in late lactation: Effects on mammary gland function, composition, and morphology. *J. Dairy Sci.* 85:1093–1102.
- Bauman, D. E., R. W. Everett, W. H. Weiland, and R. J. Collier. 1999. Production responses to bovine somatotropin in northeast dairy herds. *J. Dairy Sci.* 82:2564–2573.
- Bauman, D. E., and R. G. Vernon. 1993. Effects of exogenous bovine somatotropin on lactation. *Annu. Rev. Nutr.* 13:437–461.
- Baumrucker, C. R., and B. H. Stemberger. 1989. Insulin and insulin-like growth factor-I stimulate DNA synthesis in bovine mammary tissue in vitro. *J. Anim. Sci.* 67:3503–3514.
- Berry, S. D. K., P. M. Jobst, S. E. Ellis, R. D. Howard, A. V. Capuco, and R. M. Akers. 2003. Mammary epithelial proliferation and estrogen receptor expression in prepubertal heifers: Effects of ovariectomy and growth hormone. *J. Dairy Sci.* 86:2098–2105.
- Capuco, A. V., and R. M. Akers. 1999. Mammary involution in dairy animals. *J. Mammary Gland Biol. Neoplasia* 4:137–144.
- Capuco, A. V., D. L. Wood, R. Baldwin, K. McLeod, and M. J. Paape. 2001. Mammary cell number, proliferation, and apoptosis during a bovine lactation: Relation to milk production and effect of bST. *J. Dairy Sci.* 84:2177–2187.
- Capuco, A. V., R. M. Akers, and J. J. Smith. 1997. Mammary growth in Holstein cows during the dry period: Quantification of nucleic acids and histology. *J. Dairy Sci.* 80:477–487.
- Chiofalo, V., A. Baldi, G. Savoini, F. Polidori, V. Dell'Orto, and I. Politis. 1999. Response of dairy ewes in late lactation to recombinant bovine somatotropin. *Small Ruminant Res.* 34:119–125.
- Cohick, W. S. 1998. Role of the insulin-like growth factors and their binding proteins in lactation. *J. Dairy Sci.* 81:1769–1777.
- Collier, R. J., J. C. Byatt, M. F. McGrath, J. L. Vicini, P. J. Eppard, and C. Steining. 2002. Effect of growth factors and hormones on mammosgenesis and lactogenesis. *J. Dairy Sci.* 85(Suppl. 1):53. (Abstr)
- Collier, R. J., M. F. McGrath, J. C. Byatt, and L. L. Zurfluh. 1993. Regulation of bovine mammary growth by peptide hormones: Involvement of receptors, growth factors and binding proteins. *Livest. Prod. Sci.* 35:21–33.
- Collier, R. J., S. Ganguli, P. T. Menke, F. C. Buonomo, M. F. McGrath, C. E. Knotts, and G. G. Krivi. 1989. Changes in insulin and somatomedin receptors and the uptake of insulin, IGF-I and IGF-II during mammary growth, lactogenesis, and lactation. Pages 153–163 in *Biotechnology in Growth Regulation*. R. B. Heap, C. G. Prosser, and G. E. Lamming, eds. Butterworths, London, UK.
- Coppock, C. E., R. W. Everett, R. P. Natzke, and H. R. Ainslie. 1974. Effect of dry period length on Holstein milk production and selected disorders at parturition. *J. Dairy Sci.* 57:712–717.
- Davis, S. R., P. D. Gluckman, S. C. Hodgkinson, V. C. Farr, B. H. Breier, and B. D. Burleigh. 1989. Comparison of the effects of administration of recombinant bovine growth hormone or n-met insulin-like growth factor-I to lactating goats. *J. Endocrinol.* 123:33–39.
- Dias, F. M., and F. R. Allaire. 1982. Dry period to maximize milk production over two consecutive lactations. *J. Dairy Sci.* 65:136–145.
- Dickerson, G. E., and Chapman, A. B. 1939. The effect of age and dry period on production at different levels of producing ability. *Am. Soc. Anim. Prod.* 1939:73–76.
- Dix Arnold, P. T., and R. B. Becker. 1936. Influence of preceding dry period and of mineral supplementation on lactation. *J. Dairy Sci.* 19:257–266.
- Fowler, P. A., C. H. Knight, and M. A. Foster. 1991. Omitting the dry period between lactations does not reduce subsequent milk production in goats. *J. Dairy Res.* 58:13–19.
- Funk, D. A., A. E. Freeman, and P. J. Berger. 1987. Effects of previous days open, previous days dry, and present days open on lactation yield. *J. Dairy Sci.* 70:2366–2373.
- Gulay, M. S., M. J. Hayen, K. C. Bachman, T. Belloso, M. Liboni, and H. H. Head. 2003. Milk production and feed intake of Holstein cows given short (30-d) or normal (60-d) dry periods. *J. Dairy Sci.* 86:2030–2038.
- Hadsell, D. L., N. M. Greenberg, J. M. Fligger, C. R. Baumrucker, and J. M. Rosen. 1996. Targeted expression of des(1–3) human insulin-like growth factor I in transgenic mice influences mammary gland development and IGF-binding protein expression. *Endocrinology* 137:321–330.
- Hadsell, D. L., T. Alexeenko, Y. Klemintidis, D. Torres, and A. V. Lee. 2001. Inability of overexpressed des(1–3) human insulin-like growth factor I (IGF-I) to inhibit forced mammary gland involution is associated with decreased expression of IGF signaling molecules. *Endocrinology* 142:1479–1488.
- Holst, B. D., W. L. Hurley, and D. R. Nelson. 1987. Involution of the bovine mammary gland: Histological and ultrastructural changes. *J. Dairy Sci.* 70:935–944.
- Klein, J. W., and T. E. Woodward. 1943. Influence of length of dry period upon the quantity of milk produced in the subsequent lactation. *J. Dairy Sci.* 26:705–713.
- Knight, C. H. 1998. Extended lactation. Pages 30–39 in *Hannah Research Institute Yearbook 1998, Hannah Interactions*, Glasgow, UK.
- Knight, C. H., and C. J. Wilde. 1987. Mammary growth during lactation: Implications for increasing milk yield. *J. Dairy Sci.* 70:1991–2000.
- Knight, C. H., and M. Peaker. 1984. Mammary development and regression during lactation in goats in relation to milk secretion. *J. Exp. Physiol.* 69:331–338.
- Lotan, E., and J. H. Alder. 1976. Observations on the effect of shortening the dry period on milk yield, body weight, and circulating glucose and FFA levels in dairy cows. *Tijdschr. Diergeneesk.* 101:77–82.

- Marquis, G. S., M. E. Penny, J. M. Diaz, and R. M. Marin. 2002. Postpartum consequences of an overlap of breastfeeding and pregnancy: Reduced breast milk intake and growth during early infancy. *Pediatrics* 109:e56–e63.
- McDowell, G. H., I. C. Hart, and A. C. Kirby. 1987. Local intra-arterial infusion of growth hormone into the mammary glands of sheep and goats: Effects on milk yield and composition, plasma hormones and metabolites. *Aust. J. Biol. Sci.* 40:181–189.
- McGrath, M. F., R. J. Collier, D. R. Clemmons, W. H. Busby, C. A. Sweeny, and G. G. Krivi. 1991. The direct *in vitro* effect of insulin-like growth factors (IGFs) on normal bovine mammary cell proliferation and production of IGF binding proteins. *Endocrinology* 129:671–678.
- Neuenschwander, S., A. Schwartz, T. L. Wood, C. T. Roberts, Jr., L. Henninghausen, and D. LeRoith. 1996. Involution of the lactating mammary gland is inhibited by the IGF system in a transgenic mouse model. *J. Clin. Invest.* 97:2225–2232.
- Paape, M. J., and H. A. Tucker. 1969. Influence of length of dry period on subsequent lactation in the rat. *J. Dairy Sci.* 52:518–522.
- Peri, I., A. Shamay, M. F. McGrath, R. J. Collier, and A. Gertler. 1992. Comparative mitogenic and galactopoietic effects of IGF-I, IGF-II, and des-3-IGF-I in bovine mammary gland *in vitro*. *Cell Biol. Int. Rep.* 16:359–368.
- Peters, R. R. 2002. Evaluating herd milk quality using DHI somatic cell counts. Pages 57–73 in *Proceedings: Arizona Dairy Production Conference*, Tucson.
- Pitkow, H. S., R. P. Reece, and G. L. Waszilycsak. 1972. The integrity of mammary alveolar cells in two consecutive lactations. *Proc. Soc. Exp. Biol. Med.* 139:845–850.
- Politis, I., E. Block, and J. D. Turner. 1990. Effect of somatotropin on the plasminogen and plasmin system in the mammary gland: Proposed mechanism of action for somatotropin on the mammary gland. *J. Dairy Sci.* 73:1494–1499.
- Prosser, C. G., I. R. Fleet, A. N. Corps, E. R. Froesch, and R. B. Heap. 1990. Increase in milk secretion and mammary blood flow by intra-arterial infusion of insulin-like growth factor-I into the mammary gland of the goat. *J. Endocrinol.* 126:437–443.
- Purup, S., K. Sejrsen, and R. M. Akers. 1995. Effect of bovine GH and ovariectomy on mammary tissue sensitivity to IGF-I in prepubertal heifers. *J. Endocrinol.* 144:153–158.
- Rémond, B., J. Kérouanton, and V. Brocard. 1997. Effets de la réduction de la durée de la période sèche ou de son omission sur les performances des vaches laitières. *INRA Prod. Anim.* 10:301–333.
- Rémond, B., A. Ollier, and G. Miranda. 1992. Milking cows in late pregnancy: Milk production during this period and during the succeeding lactation. *J. Dairy Res.* 59:233–241.
- Schaeffer, L. R., and C. R. Henderson. 1972. Effects of days dry and days open on Holstein milk production. *J. Dairy Sci.* 55:107–112.
- Sejrsen, K., J. Foldager, M. T. Sorensen, R. M. Akers, and D. E. Bauman. 1986. Effect of exogenous bovine somatotropin on pubertal mammary development in heifers. *J. Dairy Sci.* 69:528–535.
- Shamay, A., N. Cohen, M. Niwa, and A. Gertler. 1988. Effect of insulin-like growth factor I on deoxyribonucleic acid synthesis and galactopoiesis in bovine undifferentiated and lactation mammary tissue *in vitro*. *Endocrinology* 123:804–809.
- Smith, A., J. V. Wheelock, and F. H. Dodd. 1967. Effect of milking throughout pregnancy on milk secretion in the succeeding lactation. *J. Dairy Res.* 34:145–150.
- Sørensen, J. T., and C. Enevoldsen. 1991. Effect of dry period length on milk production in subsequent lactation. *J. Dairy Sci.* 74:1277–1283.
- Stelwagen, K., D. G. Grieve, J. S. Walton, J. L. Ball, and B. W. McBride. 1993. Effect of prepartum bovine somatotropin in primigravid ewes on mammary development, milk production, and hormone concentrations. *J. Dairy Sci.* 76:992–1001.
- Stelwagen, K., I. Politis, J. H. White, B. Zavizion, C. G. Prosser, S. R. Davis, and V. C. Farr. 1994. Effect of milking frequency and somatotropin on the activity of plasminogen activator, plasminogen, and plasmin in bovine milk. *J. Dairy Sci.* 77:3577–3583.
- Swanson, E. W. 1965. Comparing continuous milking with sixty-day dry periods in successive lactations. *J. Dairy Sci.* 48:1205–1209.
- Swanson, E. W., F. E. Pardue, and D. B. Longmire. 1967. Effect of gestation and dry period on deoxyribonucleic acid and alveolar characteristics of bovine mammary glands. *J. Dairy Sci.* 50:1288–1292.
- United States Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services. 2002. Dairy 2002. Part 1: Reference of Dairy Health and Management in the United States, 2002. USDA, Anim. Plant Health Inspection Serv., Vet. Serv., Ctr. Epidemiol. Anim. Health, Natl. Anim. Health Monitoring Syst., Ft. Collins, CO. Info. Sheet No. N377.1202.
- Weber, M. S., S. Purup, M. Vestergaard, R. M. Akers, and K. Sejrsen. 2000. Regulation of local synthesis of insulin-like growth factor-I and binding proteins in mammary tissue. *J. Dairy Sci.* 83:30–37.
- Wheelock, J. V., J. A. F. Rook, and F. H. Dodd. 1965. The effect of milking throughout the whole pregnancy on the composition of cow's milk. *J. Dairy Res.* 32:249–254.
- Wilde, C. J., and C. H. Knight. 1989. Metabolic adaptations in the mammary gland during the declining phase of lactation. *J. Dairy Sci.* 72:1679–1692.
- Wilde, C. J., C. H. Knight, and D. J. Flint. 1999. Control of milk secretion and apoptosis during mammary involution. *J. Mammary Gland. Biol. Neoplasia* 4:129–136.
- Wilton, J. W., E. B. Burnside, and J. C. Rennie. 1967. The effects of days dry and days open on the milk and butterfat production of Holstein Friesian cattle. *Can. J. Anim. Sci.* 47:85–90.
- Winder, S. J., A. Turvey, and I. A. Forsyth. 1989. Stimulation of DNA synthesis in cultures of ovine mammary epithelial cells by insulin and insulin-like growth factors. *J. Endocrinol.* 123:319–326.