Introduction

The dry period length is very important because it is directly related to subsequent milk production and income. During the dry period mammary glands undergo a number of changes that are necessary to stimulate maximal milk production following parturition. The time period needed for involution is the major factor determining the optimum length of the dry period. This is a very important topic. Yet, few studies have been completed and published to evaluate the problem. Although nonexperimental and experimental data suggested that 7 to 10 wk of dry period was necessary to maintain maximal production, recent experiments specially designed to compare 30 and 60 d of dry periods indicate that shorter dry periods are possible without a decrease in milk production. Thus, it is important to understand the changes that occur during the involution process, which leads to the necessity for a dry period, and compare nonexperimental studies with recent experimental studies to draw meaningful conclusions about shorter dry periods.

Involution

After frequent periodic milk removal from the mammary gland is discontinued, the individual mammary glands undergo involution. Three types of involution have been described (1). Gradual involution occurs during the declining phase of lactation after the peak MY had been reached. Senile involution occurs at the end of the reproductive life of the animal. Finally, initiated involution is described by the regression of the lactation function.

Abstract: The most critical problem for the evaluation of different dry period lengths is the relationship between days dry and subsequent milk production. It is assumed that when a cow is dried off, the loss in the current lactation will be compensated for by greater milk production during the following lactation. However, the process of parturition and initiation of lactation are extremely important events and they are associated with many problems that may result in removal of the individual cow from the herd or in greatly reduced milk production, especially early in the lactation. Therefore, identification of the shortest possible dry period that would allow maximum milk production after parturition is crucial. Indeed there are only a limited number of studies, experimental and observational, that have been conducted to establish the association between minimum dry period and maximum milk yield (MY) in the lactation that follows the dry period. This review will try to address both observational and experimental data to give an understanding about the topic.

Key Words: Dry period length, milk yield, dairy cow, lactation
with sudden cessation of milk removal, either natural or enforced by the dairy producer.

Initiated involution of mammary glands occurs after cessation of milking. Regression of mammary secretory tissue accompanies dramatic changes in secretion composition during the transition from lactation to a non-functional gland. It has been shown that dairy cows require a nonlactating period prior to the next lactation to achieve maximal milk production during that lactation (2). Adequate proliferation and differentiation of mammary secretory epithelium during the nonlactating period are essential for optimal secretory function during the subsequent lactation and duration of the nonlactating interval is related significantly to milk production (3).

Smith and Todhunter (4) suggested that there are three important stages during a typical dry period. The first stage is the active involution, which begins with cessation of milking and is completed within 21 to 28 d. This stage is characterized by engorgement of cisternal spaces, ducts and alveoli with milk constituents, gradual changes in mammary secretion composition, and regression of mammary tissue. The second stage is steady state involution representing fully involuted mammary gland. The final stage represents colostrum formation and initiation of lactation, which begins about 14 d before parturition. Near parturition, mammary glands undergo significant changes characterized by intense growth, rapid differentiation of secretory epithelial cells, and synthesis and secretion of proteins, fat and carbohydrates leading to accumulation of colostrum. Thus, according to this view, a 45- to 60-d dry period would represent an active period of involution until the involution phase was completed, followed by redevelopment of mammary gland beginning 21 to 28 d prior to parturition (5,6).

Much of the information on involution has been based on research conducted using laboratory animals. In rodents, continuous milk production during lactation is dependent upon a complex interplay of lactogenic hormones and the suckling stimulus exerted by the young. Involution can be initiated in the mouse mammary gland at any stage of lactation by removing the pups (7). Cessation of milking causes accumulation of milk in alveoli and ducts, and this increases intramammary pressure, which causes degeneration of secretory cells and subsequent disruption of alveolar and lobular structures. In rats, involution is associated with a massive engorgement of the gland with milk followed by apoptosis of secretory epithelial cells and destruction of the gland. Involution remains reversible for about 30 to 36 h after it has been initiated (7).

After weaning, the decline in lactogenic hormones and milk stasis lead to involution, a process that is mainly characterized by three events: (i) downregulation of milk protein gene expression, (ii) loss of epithelial cells by apoptosis, and (iii) tissue remodeling and preparation of the gland for a new lactation. Each of these processes is likely to depend upon the activity of specific sets of transcription factors in the mammary epithelium and stroma that ensure the timely and spatially coordinated expression of critical gene products (8).

In the mammary gland, secretory epithelial cells are removed by apoptosis during involution. A number of transcription factors were found to control apoptosis. These include c-Fos, c-Jun, p53, E2F, Myc/Max and STAT3 (9). STAT3 was reported to be activated during pregnancy and lactation at the start of involution. This activation was characterized by removal of epithelial cells by apoptosis (9,10). In addition to STAT3, STAT1 also is activated during latter stages of involution when remodeling of the mammary gland occurs (9). The timely breakdown of extra-cellular matrix is essential for remodeling (11). The cell loss coincides with matrix metalloproteinase (MMP) activation and basement membrane degradation in rats.

Using in vitro culture, it was demonstrated that first passage epithelial cells isolated from pregnant mouse mammary gland die by apoptosis (12). On the other hand, cell death was suppressed by basement membrane suggesting the requirement of basement membrane for cell survival (12). In the same experiment, blockage of integrin with anti-beta 1 integrin antibody doubled the rate of apoptosis, whereas expression of Bcl-2 did not correlate with cell survival. However, increased levels of Bax were associated with apoptosis. Thus, basement membrane provides a survival stimulus for epithelial cells in vivo, and loss of interaction between cells and this type of matrix may act as a control point for cell deletion during mammary gland involution.

In the rat, mouse and rabbit, autophagocytic and heterophagocytic mechanisms play a very important role in tissue degeneration during mammary involution (13). Formation of lysosomes and structures containing cytoplasmic organelles (cytosegrosomes) has been
interpreted as evidence of autophagocytosis of alveolar cells. This often was accompanied by dissociation of epithelial cells from the basement membrane (7), whereas infiltration of mononuclear leukocytes into involuting tissue has been associated with heterophagocytosis of degenerating cells and cellular debris (7). Thus, involution in the rodent mammary gland is distinguished by the separation of epithelial cells from the basement membrane and extension of myoepithelial cells to fill the gaps left by the discarded epithelial cells (13).

Ruminant mammary epithelial cells, on the other hand, apparently do not regress to the same extent as occurs in rodent mammary glands. They appear to maintain some synthetic and secretory activity throughout the nonlactating period. This conclusion is based upon conditions of mammary involution in dairy cows that generally are considerably different from those in laboratory species (13,14). Dairy cows are still producing large quantities of milk and most often they are pregnant at the time of milk cessation by initiated involution or stoppage of milk removal (15). Even in the absence of pregnancy, mammary involution in dairy animals occurs at a slower rate than in rodents (16). Alveolar structure is maintained for several weeks and lactation can be reinitiated after 4 wk or more of involution. Although apoptosis in the mammary tissue of ewes appears to be initiated within a similar time frame to that in rodents, beginning about 2 d after cessation of milking or postweaning with a peak at about 4 d, the maximum proportion of apoptotic epithelial cells appears to be less than in rodents, and apoptosis may be accompanied by an initial increase in cell proliferation (17). Alveolar structure of cows is largely maintained and little or no loss of cells occurs with cessation of milking. On the other hand, there is increased apoptosis and cell proliferation in freshly dried off mammary glands, relative to those of lactating glands during the same stage of gestation. Thus, it appears that a nonlactating period serves to promote cell turnover prior to the next lactation (17). In contrast to the view that suggests an active involution process during a dry period requiring 45 to 60 d in dairy cows (4), more recent studies (16-18) imply that the dry period is important for replacement of damaged cells before the next lactation starts but not for extensive degeneration of mammary gland structure and apoptosis. This suggests that 45 to 60 d of dry period may not be the optimal time interval for maximum production of dairy cows and that the length of the dry period may be shortened without a negative effect on the cow’s subsequent lactation performance.

Sordillo and Nickerson (14) examined the morphologic changes in the mammary glands of 5 cows during mammary involution. Mammary tissue samples were obtained weekly beginning the day milking was discontinued through next parturition. As involution progressed, a gradual reduction in synthetic and secretory activity of alveolar epithelium was noted under light and electron microscopic evaluation. During the first 2 wk of involution they observed increased stroma and nonactive secretory epithelium with concomitant decreases in epithelium, lumen, and fully active secretory epithelium. There was a decrease in the number of organelles associated with milk synthesis and secretion at the alveolar epithelium level. These changes were gradually reversed beginning 2 wk before parturition and by the time of calving occurred cell structure was typical of the lactating mammary gland.

Free fatty acid level in milk increased more than 10-fold in cows during mammary involution (19). Their appearance did not immediately follow the cessation of milking but followed the increase in permeability of the mammary epithelium, which is parallel with the changes in the electrolyte content of the milk. However, the concentration of free fatty acids did not remain high throughout the dry period but declined to low levels before the change in permeability was reversed at the next parturition. It has been concluded that the high level of free fatty acids in milk during mammary involution most likely arises from the breakdown of triglycerides remaining in the gland and this may be accelerated in some manner by the increase in permeability of the mammary epithelium (19).

Lactating gland studied morphologically by Li et al. (16) in goat showed tightly packed secretory alveoli with columnar shaped alveolar cells that retained a large apical secretory vesicle. Secretory alveoli were separated by small amounts of interstitial connective tissue. After drying off, the unmilked gland showed a lactating morphology for 3 d with reduced number of alveolar cells and more intralobular stromal tissue around the alveolus. However, less than 1% of alveolar epithelial cells underwent apoptosis as determined by both presence of TUNEL-positive cells and DNA fragmentation in tissue extracts. The lumen of unmilked alveoli contained residual
secretion and polymorphic neutrophils that infiltrated into the parenchyma. Morphology of the unmilked gland changed 7 d after milk removal ceased. Alveolar cells lost their columnar shape and the cytoplasm contained a large apical vesicle with a compressed ill-defined nucleus. Apoptic bodies were observed next to alveolar cells (2-3% of alveolar cells) within some alveoli. During the second week, the myoepithelial cells were pronounced and formed a band around each alveolus. Body defense cells were present among the structurally deficient alveolar cells, which had indistinct cell membranes with an intensely stained pyknotic nucleus. Apoptic bodies were observed next to alveolar cells (2-3% of alveolar cells) within some alveoli. During the second week, the myoepithelial cells were pronounced and formed a band around each alveolus. Body defense cells were present among the structurally deficient alveolar cells, which had indistinct cell membranes with an intensely stained pyknotic nucleus.

Apoptic cell rate was estimated to be 5%. Three weeks after the cessation of milking, fibrocollagenous tissue separated the ducts and ductules. Apoptotic bodies were present in most ductal structures in and around the mammary parenchyma with masses of body defense cells. In this study (16), apoptosis was not limited to the dry glands. Infrequent TUNEL-positive cells and low level of DNA fragmentation were also observed in milked glands, suggesting apoptic cells could have accounted for the net decrease in mammary cell numbers of the goat during declining lactation.

Capuco et al. (18) concluded that, in contrast to rodents, net loss of mammary cells did not occur during the dry period in dairy cows. In their experiment, dry and lactating cows were sacrificed on days corresponding to different number of days of the dry period and mammary tissues were sampled for total DNA and RNA and morphological analyses. Neither parenchymal weight nor DNA content differed in glands of lactating or dry cows. On the 7th day of the dry period, DNA content was identical in glands of dry and lactating cows and subsequently increased more rapidly in dry glands than in lactating glands. More epithelial cells (96%) were labeled with [3H]Tdr in dry glands than in lactating glands (86%). Based upon morphology, 62% of epithelial cells in lactating glands contained secretory vesicles and lipid droplets and these were not affected by day of gestation. However, if cows had been dried for 7 d, 25% of epithelial cells appeared to be secretory. At 35 d prepartum (cows dry for 25 d), none of the epithelial cells were secretory. Cells showing secretory activity increased to 78% and 98% after d -35 and d -7, respectively. They concluded that processes of proliferation and cell turnover seemingly increased the percentage of epithelial cells in dry mammary glands prior to parturition (18).

Earlier studies suggested that along with the changes in composition of mammary secretions, mammary gland was fully involuted during the steady state involution phase in cows (4). During this process, it was believed that total destruction of the gland is completed. However, as a result of more recent studies, it has been suggested that mammary involution is an inappropriate term to describe the changes that occur in the bovine mammary gland during the dry period. Current results do not support a net loss of mammary cells during the characteristic 60-d dry period in dairy cows (18). Apparently, the bovine mammary gland does not degenerate to the virgin state as it does in rodents during the dry period. The retention of alveolar structures still can be seen 30 d following milk stasis (13,18). However, it has been suggested that the dry period is necessary to allow replacement of damaged or senescent epithelial cells prior to the succeeding lactation (18). Because mammary gland completed involution by 25th d of dry period (18), it may not be necessary to have a 60-d dry period in dairy cows. Thus, it is possible that the dry period could be shortened to 30 to 35 d without an effect on subsequent milk production. However, this conclusion implies that they have regained adequate body condition score (BCS) and replenishment of body tissue reserves that will be needed during the subsequent lactation.

Plasmin and Involution

Throughout lactation, milk contains a number of proteases such as leukocyte derived proteases (20). Plasmin and its inactive zymogen, plasminogen, are two of the several significant proteases in bovine milk and the plasmin system is believed to have a role in the involution of mammary gland. Most proteolytic activity found in milk is stimulated by plasmin (21). Plasmin is an extracellular serine protease and it exists mainly in its inactive form in bovine milk. Plasmin is formed by cleavage of a peptide bond in the single polypeptide chain of the inactive proenzyme plasminogen (22). Plasmin generation in the extracellular area is initiated by cellular release of the single-chain forms of the plasminogen activators (PA) and their subsequent activation is responsible for degradation of fibrin (23). Components of the plasmin system are often associated with casein micelles but also are present in the serum and cream phases of milk (24). Plasmin appears to be responsible for fibrinolysis and thrombolysis, as well as for biological...
processes involving breakdown of cellular matrix and basement membranes such as cell migration, invasion, organ involution, tissue remodeling and destruction (25).

Increased plasmin and PA in milk are correlated during the declining phase of lactation. Stage of lactation affects plasmin with late lactation associated with higher concentrations of plasmin (21). Potential mechanisms responsible for increased milk plasmin include an inflow of plasminogen from blood (26). In the rodent mammary gland, PA converts plasminogen to plasmin during late lactation and this is associated with the onset of involution (27). Activation of plasminogen in bovine milk increases as lactation progresses and plasminogen activity increases further by d 3 after drying off (28). Elevated plasmin activity during involution of the bovine mammary gland is responsible for increased hydrolysis of casein, and increased activities of lactoferrin and protease other than those of plasmin do not seem to play a major role in protein hydrolysis during involution (20). It has been suggested that the increased plasmin activity seen during late lactation may be involved in subsequent mammary gland involution (28) and estrogen accelerated plasmin activity in the mammary gland (29).

**Dry Period**

The lactation cycle begins with a period of mammary gland development followed by lactogenesis. Milk synthesis and secretion occurs after parturition. After the peak in milk production, a declining phase follows until milk removal is stopped either at weaning or due to the management practices enforced by the dairy producer. This lactation cycle of the female continues several times during her reproductive life. The period between successive lactations after periodic milk removal is stopped is called the dry period. This dry period allows remodeling of mammary tissue and the reinitiation of lactation at a maximal level after next calving (30).

Many factors must be considered to determine the appropriate length of the dry period for individual cows. In a genetic and environmental study, Schaeffer and Henderson (31) examined the lactation records of Holstein cows. They concluded that age and month of calving significantly affected the length of the dry period. O'Connor and Oltenacu (32) included parity, month of calving and time of conception as factors that affect optimum time of drying off. Dias and Allaire (33), after analyzing data from 8981 Holstein cows, reported that the time required for dry period decreased as the lactation number increased from first through fourth lactations. They stated that cows with calving intervals greater than 365 d required fewer days dry than cows with shorter calving intervals except those in first lactation. Effect of dry period length on MY was greater for younger cows and the optimal number of days dry declined from 65 to 23 d as age at calving increased from 24 to 83 mo in the same study. They also stated that calving interval and daily MY at 100 d before calving were significant factors affecting total days needed for the dry period.

**Dry Period Length**

Establishing optimum length of the dry period is critical to achieve maximum milk production during the next lactation. Since 1936 many observational and experimental data have been generated to establish an optimal drying off time for cows. Fifty-five to 60 d dry period length has been recommended based on the fact that this would maximize production in the following lactation (2,31,33,34). Arnold and Becker (35) evaluated Jersey cows with dry periods of 30 d or less (10 cows), 31 to 60 d (54 cows), 61 to 90 d (45 cows) and 91 d or more (56 cows) preceding the lactation. In their study a dry period of 31 to 60 d allowed the maximum MY in the subsequent lactation.

Earlier recommendations were that dry periods should not be less than 50 d. Klein and Woodward (34) utilized 1139 lactation records to study dry period length. They found that the optimum dry period was 55 d for cows producing ~5000 kg of 4% fat corrected milk (FCM) with 12-mo calving interval. They made this recommendation even though average milk production for 40 to 49 and 60 to 69 d of dry periods did not differ significantly from the 55-d dry period. Schaeffer and Henderson (31) concluded that cows with dry periods of 50-59 d had the highest milk production during the subsequent lactation. Moreover, Funk et al. (36) reported that cows dry for 60 to 69 d produced significantly more milk (~459 kg) in the subsequent lactation than cows dry for 40 d or less. Sorensen and Enevoldsen (37) evaluated the effect of different dry periods on subsequent MY. Cows were dried off at 4, 7 or 10 wk before expected calving. They found a 2.8 kg decrease in yield of 4%
FCM/d when dry period length was decreased from 7 to 4 wk, whereas there was a 0.4 kg/d increase in milk production when dry period was increased from 7 to 10 wk. Effects of days dry on MYs of first (n = 11,583), second (n = 7143) and third (n = 6102) lactation of Holstein cows from Zimbabwe and North Carolina were evaluated by Makuza and McDaniel (38). Milk yields of cows dry for 30-39, 40-49 and 50-59 d were 610, 633 and 202 kg less than those of dry for 60 d in both locations, and little advantage was observed for dry periods longer than 60 d.

Observational data will be affected by many factors, in addition to dry period length, that are highly related to subsequent milk production. For example, data from existing records often will not include the reason why a specific cow was dried off earlier than other cows or why cows were dried off late (<60 d). Some cows cease lactation spontaneously or the dairy producer will dry off cows early because of insufficient milk production. Thus, the reason why cows had shorter dry periods most often cannot be learned from the MY records. Cows with short dry periods also may include those cows that calved early due to physiological problems, sickness or exposure to heat stress, among others. This would bias the estimated effect of days dry on MY in the subsequent lactation because of potential or actual problems during early lactation associated with early calving; this would affect the lactational performance. As a result, flaws in record analysis may produce a bias in the milk production records and this may result in insufficient information to estimate the true effects of dry period length adequately (39).

Studies using a designed experimental protocol and utilizing larger numbers of cows have also been conducted. For example, Coppock et al. (2) carried out a 42-mo field trial to evaluate the effects of dry period length on later milk production. Cows were assigned to treatments of 20, 30, 40, 50 or 60 d of dry periods. Although the cow numbers were high (n = 1019), only 305 cows (~30 %) completed the 42-mo study. At the end of 42 mo they concluded that cows averaging less than a 40-d dry period produced 450 to 680 kg less milk in the subsequent lactation compared to cows having dry periods of 40 d or longer. However, dry period lengths were allowed to have ±10 d range in each group. Therefore, a cow in 30-d dry group could have had a dry period ranging from 20 to 40 d. Importantly, the average length of days dry between cows assigned to 20 and 50 d dry could have been only 10 d. This could have seriously biased the estimated effect of the dry period length on subsequent milk production because the actual days dry varied greatly within the individual groups (2).

In another experimental trial, Swanson (40) used five pairs of identical twin dairy cows. One of each pair of identical twins was given at least an 8 wk of dry period, whereas other pairmates were milked continuously for two consecutive lactations. Average MY of the continuously milked twins in the second and third lactations was 75% and 62% of the control twins that had ~60 d dry period. In another study, two quarters of each mammary gland of 2 cows were milked continuously while the other two quarters within the same cow were dried off for ~60 d before expected parturition. The quarters dried for 8 to 9 wk produced 40% more milk in the subsequent lactation (41). However, a small number of cows were utilized in both trials and these only showed the need for a dry period not the dry period length. The only conclusion from many of these studies would be that mammary gland benefits from a dry period. Gulay et al. (42) also used half-udder model. Two quarters allowed a 70-d dry period produced more milk (38.3 kg/d) than the other two quarters allowed a 30-d dry period (32.2 kg/d). Their results suggested that impaired production by quarters given a 30-d dry period in the reason of the difference in milk production. They concluded that half-udder model had intricate flaws that invalidate its use to compare efficiently the efficacy of different lengths of dry periods in dairy cattle.

As described previously, there is a little doubt that cows need a dry period to reach maximum possible milk production. The exact length of time needed for the dry period has not been established definitively and dry period length is likely influenced by the time needed for mammary involution. The time course and degree of mammary involution that occurs in cows differs noticeably from that seen in rodents, which makes it difficult to model and evaluate the correct dry period length for cows by using a rapidly reproducing short lactation species. Involution of the mammary gland occurs at a slower rate and alveolar structure is maintained for a greater portion of the period of involution in dairy cows than in rodents (17). Moreover, it has been proposed that the process of mammary involution is completed by day 25 of the dry period in dairy cows. A nonsecretory state
was achieved at 35 d prepartum (no epithelial cells containing secretory vesicles or fat droplets and mammary luminal area decreased to its minimum)(18). This finding differs greatly from previously held view on speed and extent of involution in dairy cows but supports the results obtained from the most recent studies based upon lactation performance.

In one of the most recent studies, 84 Holstein cows were utilized to evaluate the effects of dry period (30 and 60 d) with or without Estradiol Cypionate (ECP) to accelerate mammary gland involution (39). Cows with 30-d dry period (5950 ± 117 kg; n = 28) and ECP injected 30-d dry period (5857 ± 150 kg; n = 29) produced as much milk as cows with 60-d dry period (5835 ± 144 kg; n = 27) at 150 days in milk (DIM). Although estradiol 17β injections have been suggested as a way to increase rate of mammary involution in cows (29), no benefits of ECP injection were seen when 30-d dry period was coupled with ECP injections. Shorter dry periods did not negatively affect subsequent lactational performance compared to cows provided a 60-d dry period. Thus, they did not detect a benefit in setting the dry period at 60 d. Moreover, 30-d dry groups produced an additional ~510 kg milk during the extended 30-d lactation period before they were dried off (39). These results agreed with those reported previously (43,44). In their studies cows (n = 15; 34 d dry) produced as much milk as their herdmates that had 57-d dry period (n = 19). The overall MY for both short and long dry periods were about 9125 and 8986 kg at 305 DIM (43). Moreover, 10 cows produced about 11,194 kg of milk following a 32-d dry period, whereas 9 cows with 61-d dry period from the same herd produced 10,551 kg (44). These authors indicated that shorter dry periods can be a profitable practice for dairy farmers. On the other hand, follow-up research should be done to study the effects of short dry period on long term health and longevity of these cows.

Summary and Conclusions

One of the most important objectives of the dairy producer is to keep cows producing milk as much as possible throughout the year. During the dry period, cows do not produce milk and to achieve maximal milk production during the next lactation with the least number of dry days becomes important. Establishing optimum length of the dry period is critical to achieve maximum milk production during the next lactation. If one can verify that cows with ~30-d dry period will produce just as much milk as those with 60-70-d dry periods during the next lactation, with no other negative effects on the cow as a consequence of reducing dry period length, then there is an opportunity of extra milk income being generated for each cow during a lactation. Certainly, favorable results of recent studies strongly support the need for further research efforts in this area to evaluate potential health effects, any changes in calving intervals, and cow turnover (culling rates) that may occur. Of course, in order to perform this short dry period practice, cows should produce adequate amounts of milk at -60 d prepartum when they traditionally dried off. They also should be provided the required nutrients via an excellent feeding program to support their needs. On the other hand, there is an extra feed cost to be accounted for if cows are to have shorter dry periods and it is very unlikely that all cows would qualify for a shorter dry period. Lactation diets are relatively more expensive than normal cost of diets for dry cows. Thus, income coming from the increased MY and the cost of additional feed need to be determined carefully to achieve economically sound management.

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References


