Changes in Cisternal Compartment Based on Stage of Lactation and Time Since Milk Ejection in the Udder of Dairy Cows

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ABSTRACT

Two experiments were conducted to study changes induced by stage of lactation and milk ejection in the cisternal compartment of the udder in dairy cows. In experiment 1, 18 cows grouped according to stage of lactation were used 12 h after milking for measuring alveolar and cisternal milk volumes (by cannula) and cisternal area (by ultrasonography) in the front quarters. Cisternal milk and cisternal area were correlated ($r = 0.74$ to 0.82) for all stages of lactation. As lactation advanced, volumes of alveolar and cisternal milk and cisternal area decreased. Proportion of cisternal milk varied between stages (early, 33.2%; mid, 23.1%; and late, 42.6%). In experiment 2, 7 cows were used to show return of milk from cisternal to alveolar compartments when milk ejection was induced without milking. Cisternal area was measured before (0 min) and after (3, 15, 30, and 60 min) an i.v. oxytocin (OT) injection administered immediately before normal a.m. and p.m. milking times. Cisternal area increased dramatically from 0 to 3 min (98%) and decreased slowly thereafter. The 0- and 3-min data provide clear evidence of milk ejection, and their difference indicated cistern elasticity. Maximum cisternal area in each cow was similar for the 8- and 16-h milking intervals, indicating that in both cases the cistern was completely full of milk. Decrease in cisternal area after 3 min was significant at 15, 30, and 60 min. Decreased cisternal area was interpreted as the reflux of cisternal milk to the alveolar compartment. We termed this 'cisternal recoil.' In conclusion, ultrasonography was a useful method to evaluate dynamic changes in cisternal milk throughout lactation and after udder stimulation in dairy cows. Evidence exists that udder cisterns decrease when lactation advances and milk returns to the alveolar compartment when cows remain unmilked after milk ejection.

(**Key words:** milk back-flush, cisternal milk, ultrasonography, dairy cow)

Abbreviation key: OT = oxytocin.

INTRODUCTION

Milk storage in the udder is explained by using a simplistic model of 2 compartments (Wilde et al., 1996; Stelwagen, 2001) corresponding to cisternal (milk stored within the large ducts and the gland and teat cisterns) and alveolar (secreted milk stored within the small ducts and alveoli) milk fractions. Several reports demonstrated differences in distribution and accumulation of cisternal and alveolar milk in dairy cows (Knight et al., 1994; Stelwagen et al., 1996; Davis et al., 1998). A new approach for studying cisternal milk in a non-invasive way was reported (Ayadi et al., 2003a) in dairy cows using direct cisternal scanning by ultrasonography.

Volume of cisternal and alveolar milk decreases throughout lactation in dairy cows (Dewhurst and Knight, 1993; Pfeilsticker et al., 1996; Bruckmaier and Blum, 1998). Rovai et al. (2002) also reported that cisternal milk volume and cisternal area measured by ultrasonography decreased in dairy ewes as lactation advanced. Ultrasonography has not been used before to evaluate cisternal changes during lactation in dairy cows.

Importance of milk ejection for effective machine milking was demonstrated in dairy cows (Schams et al., 1984; Mayer et al., 1991) in which cisternal milk and total milk yield were greater with previous stimulation (Pfeilsticker et al., 1996). Oxytocin (OT) is mainly responsible for alveolar to cisternal milk transfer during milk ejection by contracting the myoepithelial cells surrounding the alveoli (Lefcourt and Akers, 1983; Blum et al., 1989; Bruckmaier, 2001). Milk ejection causes a sudden pressure increase within the teat cistern (Mayer et al., 1991; Bruckmaier et al., 1994b) and an enlargement of the cisterns measured by ultrasonography

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(Bruckmaier and Blum, 1992; Ayadi et al., 2003a). If milk is not withdrawn after stimulation, intramammary pressure remains stable for at least 10 min before decreasing slowly thereafter (Bruckmaier et al., 1991).

Linzell (1955) was first to demonstrate the reflux of milk from ducts into alveoli of mice after milk ejection, but he found no evidence for an equivalent phenomenon in dairy cows. Reflux of milk to the ductal and alveolar compartments of the udder following completion of milk ejection was implied in the mathematical model of milk distribution presented by Knight et al. (1994). It was not confirmed, however, at different times after stimulation in dairy cows (Pfeilsticker et al., 1996) or in dairy goats (Salama et al., 2004). A delay between milk ejection and milk evacuation may negatively affect milk yield. Losses in milk yield ranging from 5 to 30% are reported in dairy cows when milking was delayed after teat stimulation (Murray and Lightbody, 1962; Labussière, 1981; Mayer et al., 1984), but not by others (Phillips, 1984; Pfeilsticker et al., 1996). Lack of milk removal after milk ejection is currently of more interest because of the occasional failure of prompt attachment of teat cups and the possible rejection of milk-ejected cows subject to robotic milking. Failed or interrupted milkings can increase residual milk in the udder and impair milk yield and milking efficiency of the milking equipment, especially for robotic milking.

Knowing more about changes in cisternal compartmental volume during lactation and reduction in milk yield when time between milk ejection and milk evacuation are prolonged may be useful for optimizing milking routines and the study of milk synthesis. The aims of this work were 1) to evaluate changes of udder cisterns according to stage of lactation and 2) to study the reflux of milk from the cisternal to the alveolar compartments when milk is not removed after milk ejection in dairy cows.

MATERIALS AND METHODS

Experiment 1. Cistern Differences According to Stage of Lactation

Animals, feeding, and milking routine. Eighteen British Friesian cows (4 primiparous and 14 multiparous) from the Hannah Research Institute (Ayr, UK) were used. Cows were grouped according to DIM into 3 stages of lactation: early ($n = 6$; 56 ± 13) mid ($n = 5$; 151 ± 18), and late ($n = 7$; 311 ± 21). Milk yield varied according to stage (early, 27.1 ± 1.5 ; mid, 22.0 ± 1.8 ; and late, 9.8 ± 2.0 kg/d). Cows grazed natural pasture for 8 h/d, were housed in free stalls by night, and were fed ad libitum a TMR containing 4.5 MJ NE_L and 15% CP (DM basis). Milking occurred twice daily at an 8- and 16-h milking interval (0700 and 1500 h) in a her-

ringbone milking parlor (Gascoigne Melotte, Ayr, UK) equipped to collect milk separately from half udders (i.e., diagonally opposed glands). Milk yield was recorded at each milking to a precision of 100 g.

Experimental procedures. Milk partitioning in the udder and cisternal areas was evaluated for each cow on 3 consecutive d. Randomly chosen cows were separated from the herd when returning from grazing to apply the experimental procedure at a 12-h milking interval. Cows were moved to a tie stall barn to prevent spontaneous milk ejection during udder manipulation by placing the cows in unhabitual surroundings (Bruckmaier et al., 1993). Cisternal area was measured by ultrasonography according to Ayadi et al. (2003a). Udder scans for the left and the right front quarters were performed in duplicate 12 h after the a.m. milking (1900 h) by using a real time B-mode ultrasonograph (Ultra Scan 900; Ami Medical Alliance Inc., Montreal, Canada) equipped with a 5-MHz sectoral probe (2 dB power; 80° scanning angle, 0.5-mm axial and 1.5-mm lateral resolution). Images were transmitted to a portable computer and processed in triplicate using image treatment software (MIP4 Advanced System; Microm España, Barcelona, Spain). Cisternal area was measured for each triplicate and converted to cm² (1 cm² = 1024 pixels). Individual milk yield was recorded at each milking during the experimental period to a precision of 100 g. After the scans, cisternal milk was drained from each quarter using a teat cannula (100-mm length, 2.77-mm o.d., and, 1.88-mm i.d.; Portex; Hythe, Kent, UK), and weights were recorded. Finally, alveolar milk was machine milked after injecting 40 IU i.m. of OT (Intervet, Cambridge, UK).

Experiment 2. Cisternal Recoil

Animals, feeding, and milking routine. A total of 7 multiparous Friesian dairy cows varying in stage of lactation (215 ± 49 DIM) and milk yield (23.0 ± 3.2 L/d) were used. Three cows were British Friesian from the Hannah Research Institute (Ayr, UK) and were housed, managed, and milked as described in Experiment 1. The remaining 4 cows were Holstein Friesian from the S1GCE (Servei de Granges i Camps Experimentals) of the Universitat Autònoma de Barcelona (Bellaterra, Barcelona, Spain) and were housed in tie stalls. Cows spent 6 h/d in an exercise paddock and were fed ad libitum a TMR (5.02 MJ NE_L and 16.1% CP, DM basis). Water and TMR also were available to cows while in the paddock. The S1GCE cows were milked in their tie stalls at the a.m. (0800 h) and p.m. (1900 h) milking intervals using a high pipeline milking system (Westfalia Surge Ibérica, Granollers, Barcelona, Spain). Milk yield was recorded at each milking to a

precision of 200 g. Routine milking included teat cleaning, machine stripping, and teat dipping (P3-cide plus; Henkel Hygiene S.A., Madrid, Spain).

Experimental procedures. Cows were submitted randomly to an OT challenge, and cisternal area was measured by using a real time B-mode ultrasonograph with a 5-MHz sectoral probe as described in experiment 1. Duplicated scans of the left and right front quarters were made before (0 min) and after (3, 15, 30, and 60 min) an i.v. injection of OT (5 IU per cow; Intervet, Cambridge, UK and Veterin Lobulor, Laboratorio Andreu, Barcelona, Spain) in the jugular vein. Cows in the Hannah Research Institute were moved to unhabitual surroundings to prevent spontaneous milk ejection during udder manipulation as previously described. Cows at the Universitat Autònoma de Barcelona facility were maintained in their stalls and injected in the epigastric vein with an OT-receptor blocking agent (10 µg/kg BW; Atosiban, Ferring Laboratory, Malmö, Sweden) before scanning to prevent spontaneous milk ejection (Knight et al., 1994; Bruckmaier et al., 1997; Wellnitz et al., 1999). Cisternal measurements were repeated randomly in each cow for the long (16 h) and short (8 h) milking interval. Scanning images of the udder cisterns were transmitted to a portable computer and processed (MIP4 Advanced System) in triplicate as indicated (Ayadi et al., 2003a).

Statistical Analyses

Data from experiment 1 were analyzed by using ANOVA (GLM procedure; SAS Inst., Inc., Cary, NC; version 8.1). The model included the fixed effect of stage of lactation (early, mid, and late) and the random effect of animal (cows 1 to 18), udder side (left and right), the respective interactions, and the residual error. When the probability of the interaction term was nonsignificant ($P > 0.20$), it was deleted from the model. Differences among least square means were separated using the Newman-Keules test.

Repeated measurements of experiment 2 were analyzed by ANOVA (mixed model procedure for repeated measurements in SAS). The model included fixed effects of location (Hannah Research Institute and Universitat Autònoma de Barcelona), treatment time (0, 3, 15, 30, and 60 min), and milking interval (8 and 16 h) and random effects of animal (cow 1 to 7), front quarters (left and right), respective interactions, and the residual error. Differences among least square means were separated using the PDIF test in SAS. When the probability of the interaction term was nonsignificant ($P > 0.20$), it was deleted from the model. Pearson's correlation coefficients between cisternal milk and cisternal area also were calculated.

Table 1. Milk partitioning in the udder of dairy cows according to stage of lactation in experiment 1.¹

Item	Stage of lactation		
	Early	Mid	Late
Cows, no	6	5	7
DIM	56 ± 13	151 ± 18	311 ± 21
Milk yield, L/d	27.10 ^a ± 1.51	22.04 ^b ± 1.49	9.80 ^c ± 1.42
Milk partitioning ²			
Alveolar, L	2.45 ^a ± 0.18	2.06 ^a ± 0.15	0.66 ^b ± 0.11
Cisternal, L	1.22 ^a ± 0.16	0.62 ^b ± 0.09	0.49 ^b ± 0.06
Total, L	3.67 ^a ± 0.24	2.68 ^b ± 0.20	1.15 ^c ± 0.12
Cisternal area, ³ cm ²	12.10 ^a ± 1.30	8.00 ^b ± 1.70	4.20 ^c ± 0.52
Cisternal:total, %	33.2 ^b ± 6.6	23.1 ^c ± 4.5	42.6 ^a ± 5.0

^{a,b,c}Means with different superscript letters within a row differ ($P < 0.05$).

¹Values are means ± SEM.

²Measured in front quarters 12 h after milking.

³Average of front quarters measured at 12 h after milking by ultrasonography according to Ayadi et al. (2003a).

RESULTS AND DISCUSSION

Experiment 1. Cistern Differences According to Stage of Lactation

Changes in milk yield and milk partitioning in the udder at various stages of lactation are summarized in Table 1. No differences were observed between research stations or between left and right front udder quarters; therefore, their values were averaged and discussed jointly. Milk yield decreased ($P < 0.01$) as lactation advanced. Cisternal area and volumes of cisternal and alveolar milk per quarter for individual cows ranged from 1.98 to 16.9 cm², 0.19 to 2.09 L, and 0.23 to 3.20 L, respectively. On average, volume of cisternal milk in front quarters represented 33% of the milk stored in the udder at 12-h milking intervals, in agreement with previous observations in dairy cows (Ayadi et al., 2003a). No differences were observed between left and right front quarters in cisternal area ($P = 0.51$), cisternal milk volume ($P = 0.60$), and alveolar milk volume ($P = 0.53$).

The correlation between cisternal area and volume of cisternal milk was significant ($P < 0.01$) and positive at early ($r = 0.82$), mid ($r = 0.74$), and late lactation ($r = 0.80$), as well as when pooled data were analyzed ($r = 0.80$). The high correlation between cisternal milk and cisternal area at different stages of lactation in our study confirms that the 12-h milking interval used was adequate for obtaining representative images of the udder cistern in dairy cows as indicated by Ayadi et al. (2003a). Moreover, the positive correlation between the scanning area and the volume of cisternal milk agrees with those previously reported (Bruckmaier et al., 1994a; Ayadi et al., 2003a) in dairy cows. Linear regression analysis indicated a significant relationship be-

tween daily milk yield and cisternal area; the regression equation was

$$\text{Milk yield (L/d)} = 6.83 + 1.33 \times \text{cisternal area (cm}^2\text{)};$$

$$r = 0.70 \text{ (} P < 0.01; \text{SD} = 1.66\text{)}$$

The positive correlation between cisternal area measured by ultrasonography and daily milk yield observed in our study was similar to the correlation between udder volume and milk yield reported by Knight and Dewhurst (1994) in dairy cows. Moreover, Davis et al. (1998) indicated that cisternal compartment is responsible for approximately one-half of the functional ability of the udder to accumulate milk. Ayadi et al. (2003b) observed a strong negative correlation between the reduction in milk yield attributable to omitting one milking weekly and cisternal area measured by ultrasonography. These results indicate that animals with large cisterns should be more tolerant to extended milking intervals.

As lactation advanced, alveolar and cisternal milk volumes and cisternal area measured by ultrasonography decreased ($P < 0.01$; Table 1). Moreover, alveolar milk volume did not change between early and mid lactation, but decreased ($P < 0.01$) by 68% between mid and late lactation. Both cisternal milk and cisternal area decreased when lactation advanced, but the decline in the cisternal area was more evident (Table 1). Thus, although cisternal milk volume decreased ($P < 0.01$) by 49% between early and mid lactation and remained unchanged thereafter, cisternal area only decreased ($P < 0.01$) by 34% between early and mid lactation and by 48% ($P < 0.01$) between mid and late lactation. These decreases confirm previous reports (Dewhurst and Knight, 1993; Pfeilsticker et al., 1996; Bruckmaier and Blum, 1998) in which it was observed that cisternal milk decreased during lactation in dairy cows. Moreover, Rovai et al. (2002) reported that cisternal milk volume and area of cisterns in dairy ewes (measured by ultrasonography using 5-MHz sectoral probe at 8-h milking intervals) decreased throughout lactation. This decrease in cisternal milk stored in the udder can be explained by the decrease in milk yield as a consequence of the decline in secretory tissue during lactation by apoptosis (Dewhurst and Knight, 1993; Wilde et al., 1997).

Percentages of cisternal milk were high and represented 33% of total milk stored in the udder during early lactation, whereas it decreased during mid lactation (23%) and increased during late lactation (43%; Table 1). These values were similar to those previously reported for a 12-h milking interval (20 to 35%) in dairy cows using different methodologies to prevent spontaneous milk letdown (Bruckmaier et al., 1994a; Knight et al., 1994; Pfeilsticker et al., 1996). Nevertheless, percentages of cisternal milk were greater than measured

by Ayadi et al. (2003a) at the same 12-h milking interval (17%) using an oxytocin-blocking agent. Differences between reports may be a consequence of milk ejection during teat manipulation for the drainage of cisternal milk. We suspect that cisternal milk was overestimated in the previously cited references as well as in our case because milk ejection was incompletely blocked by using catecholamine or by placing them in unhabitual surroundings. Despite the high percentages of cisternal milk obtained during late lactation (43%), no adaptation to the experimental conditions was possible because different cows were studied during each of the 3 stages of lactation.

Dewhurst and Knight (1993) and Wilde et al. (1996) observed an incremental increase in the proportion of cisternal milk as lactation advanced in dairy cows as a consequence of the great reduction of alveolar milk. This effect was more marked between mid and late lactation in our results (Table 1).

Experiment 2. Cisternal Recoil

During ultrasonography, the udder cistern filled with milk was clearly evident as a dark area (anechogenic) and the glandular parenchyma as a gray-white area (echogenic), in agreement with other reports (Cartee et al., 1986; Bruckmaier and Blum, 1992; Ruberte et al., 1994). Ultrasonographic images obtained in our research (Figure 1) were similar to those previously reported in cows (Bruckmaier and Blum, 1992; Bruckmaier et al., 1994a; Ayadi et al., 2003a).

Cisternal area before OT injection ranged from 2.8 to 27.7 cm² and differed ($P < 0.001$) among cows and between milking intervals (8 h, 7.6 ± 1.5 cm²; 16 h, 11.1 ± 2.4 cm²; $P < 0.01$). No effect ($P = 0.33$) in cisternal area change was detected between locations despite differences in methodology used to prevent involuntary stimulation of the udder and milk ejection during the scanning in the British cows (Hannah Research University, by unfamiliar surroundings) vs. Holstein cows (Universitat Autònoma de Barcelona, by Atosiban).

Mean cisternal area increased dramatically (98%) and reached a maximum 3 min after OT injection (Figure 2). These results agree with others (Bruckmaier and Blum, 1992; Ayadi and Caja, 2000) in which cisternal area increased by 41 and 120%, respectively, after OT injection. Variation in the increase of cisternal area between experiments may be due to differences in methodology (i.e., use of linear vs. sectoral probe) or differences in udder morphology (i.e., cisternal area). The increase in cisternal area after OT injection was a consequence of milk transfer from alveoli to cistern when myoepithelial cells contracted.

Figure 1. Ultrasonography of the left front quarter cistern of a dairy cow at 8-h milking interval according to minutes since a 5 IU oxytocin i.v. injection (A, 0 min; B, 3 min; C, 15 min; D, 30 min; and E, 60 min). Udder cisterns filled with milk appear as dark areas, and the glandular parenchyma appear as a gray-white areas.

Figure 2. Changes in cisternal area measured by ultrasonography in the front quarters of dairy cows in experiment 2 at 8-h (—▲—) and 16-h (---□---) milking intervals based on time after oxytocin injection. Values are means $\pm$ SE for 7 cows.

At 3 min after OT injection, average cisternal area increased ($P < 0.001$) dramatically (0 min, 8.7 cm² and 3 min, 16.8 cm²) and decreased slowly thereafter (14.6, 13.5, and 12.8 cm² at 5, 30, and 60 min, respectively). Change from 0 to 3 min was greater ($P < 0.05$) for 8-h (136%) than for 16-h (60%) milking intervals, but their maximum values did not differ (Figure 2). These results show that maximum udder distension was observed at 3 min after OT injection, regardless of the quantity of milk stored in the udder. As a consequence, maximum elasticity of the cistern can be calculated by difference between the areas before and after the OT injection at a fixed milking interval.

After 3 min, cisternal area decreased ($P < 0.01$) for both milking intervals: 8 h, 13.5, 22.5, and 28.6% and 16 h, 7.9, 13.2 and 25.7%, respectively, at 15, 30, and 60 min. This variation in the decrease of cisternal area between the 8- and 16-h milking intervals could be due to the fact that volume of cisternal milk after 16 h was greater than that after 8 h. At the 8-h milking interval, cisternal milk only represented between 10% (Bruck-

maier et al., 1994a) and 14% (Ayadi et al., 2003a) of total milk yield, and alveoli were not totally full of milk. On the other hand, cisternal milk as a percentage of total milk was 20% for 10 to 14 h after milking (Bruckmaier et al., 1994a; Knight et al., 1994) and 32% for 16 h (Ayadi et al., 2003a). Ducts contained more milk; therefore a lower percentage of alveolar milk moved to the cisterns when OT was injected at 16-h milking intervals, according to the model proposed by Stelwagen (2001).

Decreased cisternal area was only significant ($P < 0.05$) after 15 min, consistent with time necessary for relaxation. Reduction in cisternal area, as a consequence of the decrease of cisternal milk volume in our results, did not agree with the results of Pfeilsticker et al. (1996), who reported nonsignificant differences in the amount of cisternal milk measured at 10-h milking intervals when teats were stimulated 15, 60, or 120 min before milking. Nevertheless, because they did not use an OT blocking agent, their results could have been influenced by milk ejection resulting from renewed OT secretion during the drainage of cisternal milk. Moreover, the contraction strength produced by the myoepithelial cells under the effect of a supraphysiological OT dose, as was used in our experiment, might have been greater in comparison with that produced by spontaneous milk ejection in their study (Pfeilsticker et al., 1996). The stronger and longer alveolar contraction expected under our experimental conditions enabled the visualization of differences in the cisternal area when the myoepithelial cells relaxed. Salama et al. (2004) did not observe changes in cisternal area at any point after maximum distention produced by an OT challenge as a consequence of the high cisternal: alveolar milk ratio.

Decreased cisternal area reported herein was interpreted to be a consequence of the reflux of milk to the ductal and alveolar compartments of the udder following the completion of milk ejection. We call this 'cisternal recoil.' This cisternal recoil effect may be explained by the suction of cisternal milk when myoepithelial cells that surround the alveoli and the smooth muscular fibers of ducts are relaxed. Our results confirm the cisternal recoil effect in dairy cows previously implied in the mathematical model proposed by Knight et al. (1994).

Decreased cisternal area after OT injection at the 16-h milking interval also may be related to increased permeability of tight junctions between mammary cells observed at 16 to 17 h (Stelwagen et al., 1997). Transfer of lactose from milk to blood plasma may reduce osmotic pressure, which partly induces return of milk richer in lactose from cisternal to alveolar compartments to maintain a constant osmotic pressure within the alveoli.

A delay between the activation of the milk ejection reflex and milk evacuation from the udder can negatively affect milk yield. Labussière (1981) reported that milk yield decreased by 5, 16, and 30% when milking was delayed by 2, 6, and 13 min, respectively. Several reports in dairy cows confirm this phenomenon when milking was delayed after milk ejection had occurred (Murray and Lightbody, 1962; Mayer et al., 1984). Nevertheless, Phillips (1984) did not observe milk losses when teat cup attachment was delayed by 3 or 12 min compared with immediate attachment after udder stimulation. Pfeilsticker et al. (1996) did not observe losses in milk when teats were stimulated 15, 60, or 120 min before milking.

In practice, to profit from the short and beneficial effects of endogenous OT (3 to 4 min) on milk ejection, it is important to reduce the time between initial preparation of the udder and evacuation of milk by machine milking (Labussière, 1993; Bruckmaier and Blum, 1998; Bruckmaier, 2001). Milk returned to the alveoli would become residual milk when no further milk ejection is renewed by OT injection. Conditioned reflexes (entrance in the holding pen and milking parlor, presence of sounds and smells associated with the milking process, etc.), udder preparation (teat washing and drying, extraction of first milk squirts), and teat cup attachment stimulate the sympathetic nervous system, and OT release produces milk ejection. As a consequence, milk could return to alveoli if time between milk ejection and milk evacuation is prolonged (i.e., delayed teat cup attachment or premature teat cup removal). Recently, introduction of the automatic milking systems in some intensive farms may reduce time between milk ejection and milking or, on the contrary, may dramatically increase time after stimulation when cluster attachment has failed or the cow is rejected from being milked until the next opportunity (Lind et al., 2000).

CONCLUSIONS

Ultrasonography was a useful method for evaluating changes in cisternal milk throughout lactation in dairy cows and served to prove that cisternal area decreases as lactation advances. Clear evidence exists that milk returns to the ductal and alveolar compartments when cows are not milked promptly after milk ejection. We termed this effect 'cisternal recoil.' Moreover, maximum distension of the cistern was observed at the same time (3 min after oxytocin injection), regardless of the quantity of milk stored in the udder. This technique makes it possible to evaluate the maximum cisternal size of dairy cows at any milking interval to choose the optimal milking frequency in practice.

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