

Mammary Response to Exogenous Prolactin or Frequent Milking During Early Lactation in Dairy Cows

E. H. Wall,* H. M. Crawford,† S. E. Ellis,‡ G. E. Dahl,† and T. B. McFadden*¹

*Lactation and Mammary Gland Biology Group, Department of Animal Science, University of Vermont, Burlington 05405

†Department of Animal Science, University of Illinois, Urbana 61801

‡Animal and Veterinary Science Department, Clemson University, Clemson, SC 29634

ABSTRACT

Frequent milking of dairy cows during early lactation results in a persistent increase in milk yield; however, the mechanism underlying this effect is unknown. We hypothesized that increased exposure of the mammary gland to prolactin (PRL) mediates the milk yield response. Fifteen multiparous Holstein cows were assigned to 3 treatments for the first 3 wk of lactation: twice daily milking with (2× + PRL) or without (2×) supplemental exogenous PRL, or 4 times daily milking (4×). Mammary biopsies were obtained at 7 DIM, and rates of [³H]thymidine incorporation into DNA in vitro were determined. Mammary expression of suppressors of cytokine signaling (SOCS)-1, -2, and -3; the long form of PRL-receptor; and α -lactalbumin mRNA was measured by real-time reverse-transcription PCR. Incorporation of [³H]thymidine into DNA was not affected by frequent milking or PRL treatment; however, analysis of autoradiograms revealed that stromal cell proliferation was greater in 4× cows. Mammary expression of SOCS-1 was not affected by milking frequency or PRL treatment. Expression of SOCS-2 mRNA was increased with frequent milking or PRL treatment, whereas expression of SOCS-3 mRNA was reduced by frequent milking or exogenous PRL. Abundance of PRL-receptor mRNA was reduced, whereas α -lactalbumin mRNA was increased with PRL treatment. These results demonstrate that the bovine mammary gland is responsive to exogenous PRL during early lactation. In addition, differences in the response to frequent milking or exogenous PRL during early lactation indicate distinct effects of PRL and milk removal on the mammary function of dairy cows.

Key words: frequent milking, mammary gland, prolactin, suppressor of cytokine signaling

INTRODUCTION

Regular removal of milk from the mammary gland is required for the maintenance of established lactation.

Indeed, frequency of milk removal is positively correlated with milk yield (Ludwin, 1942; Erdman and Varner, 1995). Cows responded to an increase in milking frequency by producing more milk within 1 d of treatment (Morag, 1973; Svennersten et al., 1990). During middle to late lactation, resuming a reduced milking frequency resulted in an immediate decrease in milk yield (Morag, 1973; Svennersten et al., 1990). In contrast, increasing milking frequency from 2 to 4 times or from 3 to 6 times daily during early lactation elicited an increase in milk yield that persisted even after lower milking frequency was resumed (Bar-Peled et al., 1995; Hale et al., 2003; Dahl et al., 2004). The mechanism by which frequent milking during early lactation elicits a persistent effect on milk yield is unknown; however, Hale et al. (2003) suggested that it may be because of greater mammary cell proliferation in frequently milked cows.

Concentrations of prolactin (PRL) in circulation increase transiently by approximately 3-fold in response to milking (Koprowski and Tucker, 1973). As lactation progresses, milking-induced PRL release declines, coinciding with a decrease in milk yield (Koprowski and Tucker, 1973). In target tissues, PRL binds to its receptor, activates several pathways including the Janus kinase/signal transducers and activators of transcription pathway, and thereby regulates transcription of PRL-responsive genes (Hennighausen et al., 1997). In the mammary gland, these genes are associated with proliferation, differentiation, and lactogenesis. Cellular response to PRL is regulated by the inverse relationship between PRL concentrations and prolactin receptor expression (Hennighausen et al., 1997), as well as the recently discovered suppressors of cytokine signaling (SOCS; Lindeman et al., 2001).

The SOCS are induced in response to PRL, growth hormone, and leptin, as well as cytokines including IL and IFN- γ (Aman and Leonard, 1997; Larsen and Ropke, 2002). They act through negative feedback to modulate the signaling of cytokines that use the Janus kinase/signal transducers and activators of transcription pathway (Aman and Leonard, 1997; Larsen and Ropke, 2002).

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¹Corresponding author: thomas.mcfadden@uvm.edu

The SOCS function in the mammary gland has only recently been investigated and is still not fully understood. Expression of SOCS-3 mRNA in the mammary gland of suckled rats is limited, but was shown to increase within 16 h of pup removal (Tam et al., 2001). Response of SOCS-3 to milk stasis is thought to be a function of mammary gland fill with milk and may suppress PRL signaling during milk stasis (Tam et al., 2001). In addition, we previously reported that SOCS-1, -2, and -3 and cytokine-inducible SH2-containing protein are expressed in the bovine mammary gland and may play a role in mammary development and function during the transition period of dairy cows (Wall et al., 2005a).

We hypothesized that PRL release associated with frequent milking would increase mammary cell growth, secretory activity, or both. Our objectives were to determine effects of frequent milking or exogenous PRL during early lactation on mammary cell proliferation, apoptosis, and expression of PRL-regulated genes in the mammary gland, as well as to differentiate between the effects of PRL exposure and milk removal.

MATERIALS AND METHODS

Animals and Treatments

Cows used in this study were a subset ($n = 5$ per treatment) of cows from a larger study (Crawford et al., 2004). Multiparous Holstein cows housed at the University of Illinois were assigned randomly to 3 treatments during the first 3 wk of lactation: 2× milking (2×), 4× milking (4×), or 2× milking plus intravenous injection of bovine PRL (**bPRL**) (2× + PRL; 1 $\mu\text{g}/\text{kg}$ of BW; provided by John Byatt, Monsanto Co., St. Louis, MO). All cows were milked at 0600 and 1700 h daily, and 4× cows were milked again at 1000 and 2100 h. For the 2× + PRL group, bPRL injections were administered at 1000 and 2100 h, coincident with the 2 extra milkings of the 4× cows. Cows calved between September and November were housed in a ventilated free-stall barn under ambient lighting and received water, hay, and TMR ad libitum. The University of Vermont and University of Illinois Institutional Animal Care and Use Committees approved all animal use.

Prolactin Assay

Blood (10 mL) from the jugular vein of cows was collected on d 4, 7, 14, and 21 of lactation between 0900 and 1000 h into sterile Vacutainer tubes containing sodium heparin (Becton Dickinson and Co., Franklin Lakes, NJ). Plasma was harvested from whole blood after centrifugation ($1,850 \times g$, 20 min, 4°C) and stored at -20°C until assayed. Plasma PRL concentrations

were determined in a single assay by RIA as described by Miller et al. (1999). The intraassay coefficient of variation was <10%.

Mammary Biopsies

Mammary biopsies were obtained at 7 ± 2 DIM between 0800 and 0930 h from the right rear quarter of the mammary gland as described previously (Wall et al., 2005b). Biopsy samples (approximately 500 mg; about 70×40 mm) were trimmed of extraparenchymal tissue and a portion (about 200 mg) was immediately frozen in liquid nitrogen for subsequent isolation of RNA. Remaining parenchyma was diced into explants that were used to measure incorporation of [^3H]thymidine into DNA (about 100 mg), or were fixed in 10% buffered formalin for subsequent histological analysis (about 5 mg).

Mammary Proliferation Assay

Mammary parenchyma was diced into explants and approximately 100 mg was incubated in a shaking water bath for 1 h at 37°C in 3 mL of medium 199 (Sigma, St. Louis, MO) supplemented with 1 $\mu\text{Ci}/\text{mL}$ of [^3H]thymidine (33 Ci/mmol; ICN, Irvine, CA) to determine incorporation of [^3H]thymidine into DNA. After incubation, explants were rinsed in PBS (Sigma) and 5 to 6 explants were fixed in 10% buffered formalin overnight for histology. Remaining explants were blotted, weighed, and frozen in liquid nitrogen. Incorporation of [^3H]thymidine into DNA was determined as described by Wall et al. (2005b).

DNA Assay

Total DNA in tissue homogenates was measured as described by Labarca and Paigen (1980), but was modified for assay in a 96-well plate as described previously (Wall et al., 2005b). Briefly, duplicate 2- μL aliquots of homogenate were pipetted into wells. Then, 98 μL of DABS-E (0.5 M dibasic NaPO_4 , 0.5 M monobasic NaPO_4 , 2 M NaCl, and 0.1 M EDTA) buffer and 100 μL of Hoechst 33528 dye (2 $\mu\text{g}/\text{mL}$; Sigma) were added to each well. Fluorescence was determined using a fluorimeter spectrophotometer (KC4; BioTek Instruments, Winooski, VT). The DNA concentration of homogenates was determined by comparison with a standard curve from serial dilutions of calf thymus DNA (Sigma) and was used to calculate the total amount of DNA in the original homogenate.

Autoradiography

Autoradiograms were prepared as described (Wall et al., 2005b). Briefly, explants were embedded in Poly-

sciences Immunobed (Polysciences Inc., Warrington, PA), and sections were cut at approximately 1.5- μ m thickness. Slides were dipped in NTB-2 track nuclear emulsion (Kodak, Rochester, NY) and exposed for 5 d at 4°C, then developed in Dektol (Kodak) and fixed (Kodak Fixer; Kodak). After preparation of the autoradiograms, slides were stained with 0.5% azure II and 0.025% basic fuchsin in a 0.5% Na-borate solution, and cover slips were mounted with Cytoseal.

Terminal Deoxynucleotidyl Transferase dUTP Nick-End Labeling

Detection of apoptotic cells in situ was performed using terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL), as described by Wall et al. (2005b). Briefly, explants were embedded in paraffin, sectioned at approximately 4- μ m thickness, and mounted onto silanized slides. A commercial TUNEL kit (ApopTag Plus Peroxidase; Chemicon International, Temecula, CA) was used in accordance with the manufacturer's protocol. After the labeling assay, cover slips were mounted with Cytoseal.

Quantification of Autoradiograms and TUNEL Slides

Tissue sections were viewed by light microscopy with an Olympus BX41 light microscope (Olympus America Inc., Melville, NY) to quantify labeled cells. Details are described in Wall et al. (2005b). For both autoradiograms and TUNEL preparations, cells were classified as epithelial, stromal, labeled epithelial, or labeled stromal cells. For autoradiograms, all cells in 2 separate fields were counted (at least 4,000 cells) and were classified as labeled if they were overlaid with more than 10 silver grains per nucleus. For TUNEL, all cells in one field were counted (at least 2,000 cells), and labeled brown nuclei were readily visible. A cell was classified as labeled when the nuclear staining was at least twice as intense as the background. Slides were coded for counting to prevent observer bias.

RNA Isolation and Reverse Transcription

Total RNA was isolated from each biopsy sample (approximately 200 mg of tissue) using Trizol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Additional details on RNA processing can be found in Wall et al. (2005a).

Total RNA (5 μ g) was primed with 1 μ L of oligo dT primer (0.5 μ g/ μ L; Invitrogen) and cDNA was synthesized using the SuperScript II reverse transcription kit (Invitrogen) according to the manufacturer's protocol.

Real-Time Quantitative PCR

Relative mRNA expression profiles were determined by real-time quantitative PCR using a PE 7700 thermal cycler (Applied Biosystems, Foster City, CA). Details on PCR can be found in Wall et al. (2005a,b). Primers (Table 1) were designed for bovine SOCS-2, the long form of PRL-receptor (**PRL-R1**) and α -LA using Primer 3 software (http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi). Primers and probes for SOCS-1 and -3 were designed using Primer Express (version 1.5; Applied Biosystems). After primer design, the predicted product was BLAST searched against the bovine database to ensure specificity of the primers. All gene expression values were normalized to that of β -actin in the same sample.

Statistical Analyses

Statistical analyses were performed using the GLM procedure (version 8.2; SAS Inst. Inc., Cary, NC). The model included treatment as a fixed effect and cow as a random effect. Fisher's protected LSD test was used to detect effects of treatment.

RESULTS AND DISCUSSION

Crawford et al. (2004) reported milk yield, milk composition, and DMI of all cows in the study. Milk yield for the first 6 wk of lactation was greater for 2 \times + PRL (46.9 kg/d; $P < 0.10$ compared with 2 \times) and 4 \times (45.7 kg/d; $P < 0.05$ compared with 2 \times) cows than for 2 \times cows (40.5 kg/d). Therefore, treatment with exogenous PRL during early lactation elicited an increase in milk yield similar to that observed with increased milking frequency.

Blood samples were collected immediately before milking or administration of exogenous PRL to confirm that PRL concentrations did not differ at 12 h after treatment. Concentrations of PRL in the plasma of biopsied cows during the first 21 DIM were not affected by time, so values were averaged for each treatment and are presented in Figure 1. Concentrations of PRL in circulation at the time of biopsy were not affected by treatment (Figure 1).

Mammary cell proliferation, determined by incorporation of [³H]thymidine into DNA in vitro, was not affected by frequent milking or exogenous PRL (Figure 2), but was numerically greater in 4 \times cows than 2 \times cows (Figure 2). Although not significant, this response is similar to that reported by Hale et al. (2003), who concluded that frequent milking was associated with a 2-fold increase in [³H]thymidine incorporation into DNA on d 7 of lactation. In the current study, we observed slightly greater cow variation than did Hale et al.

Table 1. Primer sequences for bovine suppressors of cytokine signaling (SOCS)-1, -2, and -3; prolactin receptor (PRL-R1, long form); α -LA; and β -actin¹

Target	GenBank no.	Primer	Sequence 5' to 3'	Slope	R
SOCS-1	CB460055	Forward	CACAGCAGAAAAATAAAGCCAGAGA	-3.27	0.99
		Reverse	CTCGTACCTCCTACCTCTTCATGTT		
		Probe	TCCCCAACCCCTGGTTTGTGCAA		
SOCS-2	AY183452	Forward	GGGACTGCCTTTACCAACAA	-3.20	0.97
		Reverse	GTGCTGGGACCTTTTCACCTA		
SOCS-3	AY026859	Forward	GGCCACTCTCCAACATCTCTGT	-3.39	0.99
		Reverse	TCCAGGAACTCCCGAATGG		
		Probe	CGTCAACGGCCACCTGGACTCCTA		
PRL-R1	NM_174155	Forward	GATGACTGTGAGGACCAGCA	-3.50	0.99
α -LA	NM_174378	Forward	AAGGCCATGTGGAAGATTG	-3.30	0.99
		Reverse	GAGTGCTTTATGGCCAACC		
β -Actin	AY141970	Forward	GCTCTCTTCCAGCCTTCCTT	-3.38	0.98
		Reverse	GGACTCATCGTACTCCTGCTT		

¹Gene-specific primers were designed using Primer 3 (http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi) or Primer Express software based on the available sequences in GenBank (accession numbers indicated). Amplicons were purified and sequenced, and product sequences were then BLAST searched against the National Center for Biotechnology Information database to confirm identity of the product.

(2003), and this may explain why we did not observe a significant increase in proliferation. Prolactin is thought to enhance mammary growth in rodents, in part by stimulating transcription of IGF-II, a mammary mitogen and survival factor (Hovey et al., 2003). We previously reported that elevated PRL was associated with increased mammary expression of IGF-II mRNA in nonlactating dairy cows (Wall et al., 2005b). In the current study, exogenous PRL had no effect on mammary tissue proliferation (Figure 2). However, because PRL was injected twice daily to mimic episodic milking-induced PRL release, its concentration or duration of

treatment may not have been sufficient to elicit a proliferative response. Diurnal changes in mammary cell proliferation of rodents have been reported (Borst and Mahoney, 1980). If this also occurs in dairy cows, it is possible that the timing of biopsy in the current study influenced our results.

To localize proliferating cells, autoradiograms were prepared. As is typical of early lactation (Capuco et al., 2001; Wall et al., 2005b), the percentage of proliferating cells in mammary epithelium and stroma was very small (Figure 3A and 3B). In agreement with the results

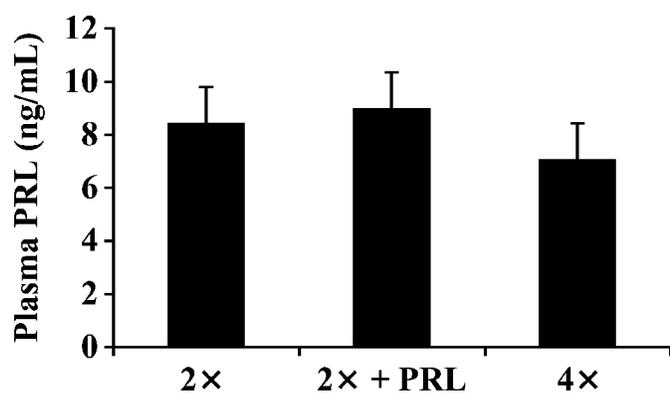


Figure 1. Effects of frequent milking or exogenous prolactin (PRL) on concentrations of PRL in the plasma of cows. Concentrations of PRL in the plasma of cows milked twice daily with (2x + PRL) or without (2x) supplemental bovine PRL or milked 4 times daily (4x) during the first 3 wk of lactation. Blood samples were obtained 12 h after PRL injection. Each bar represents the least squares mean \pm pooled SE concentration of plasma PRL (ng/mL; n = 5 cows/treatment) averaged over the first 21 DIM. Concentrations of PRL in plasma were not affected by time or treatment.

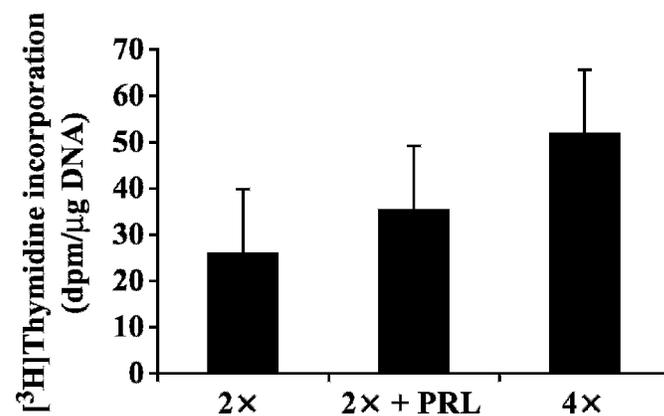


Figure 2. Effects of frequent milking or exogenous prolactin (PRL) on incorporation of [3H]thymidine into DNA in vitro by the mammary tissue of lactating cows. Incorporation of [3H]thymidine by the mammary tissue of cows milked 2 times daily with (2x + PRL) or without (2x) supplemental bovine PRL or 4 times daily (4x) for the first 3 wk of lactation. Mammary biopsies were taken at 7 \pm 2 DIM (n = 5 cows/treatment). Each bar represents the least squares mean \pm pooled SE incorporation (dpm/ μ g of DNA). Incorporation of [3H]thymidine into DNA was not affected by treatment.

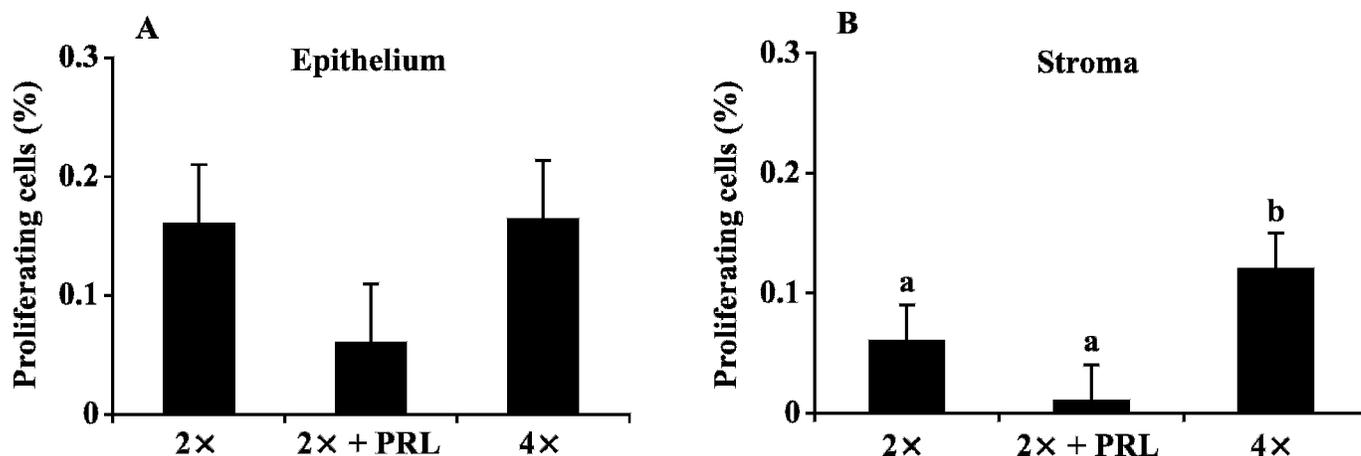


Figure 3. Effects of frequent milking or exogenous prolactin (PRL) on proliferation of epithelial (Figure 3A) or stromal (Figure 3B) cells in the lactating bovine mammary gland. Proliferation of mammary cells of cows milked 2 times daily with (2x + PRL) or without (2x) supplemental bovine PRL or 4 times daily (4x) for the first 3 wk of lactation. Mammary biopsies were taken at 7 ± 2 DIM ($n = 5$ cows/treatment). Each bar represents the least squares mean \pm pooled SE percentage of proliferating cells. Epithelial cell proliferation was not affected by treatment, but stromal cell proliferation was greater ($P < 0.02$) in 4x cows than in 2x + PRL cows and tended ($P < 0.10$) to be greater in 4x than in 2x cows. ^{a,b} $P \leq 0.10$.

of the thymidine assay, the labeling index for epithelial cells was not affected by frequent milking or exogenous PRL (Figure 3A). The pattern of stromal cell proliferation was generally similar to that of the epithelium, but the effects of frequent 4x milking tended to differ ($P < 0.10$), compared with 2x, or differed ($P < 0.02$), compared with 2x + PRL (Figure 3b), only in stroma. It is possible that the stromal response may have induced subsequent proliferation in the epithelium as shown to occur in mice (Shyamala and Ferenczy, 1984). Stromal cell proliferation was increased in 4x cows, but not in 2x + PRL cows relative to 2x cows, indicating a direct effect of milk removal, distinct from effects of exogenous PRL.

Mammary epithelial (Figure 4A) and stromal (Figure 4B) cell apoptosis was not affected by frequent milking or exogenous PRL, although it was numerically reduced in the 4x and 2x + PRL cows compared with 2x cows. Prolactin has been associated with enhanced mammary cell survival in dairy cows (Accorsi et al., 2002) and in rodents (Tonner et al., 1997). It is possible that the dosage of exogenous PRL or duration of treatment in the current study may not have been sufficient to inhibit cellular apoptosis. In addition, the timing of biopsy relative to treatment may have affected our observations.

Mammary expression of SOCS-1 mRNA was not affected by frequent milking or exogenous PRL (Figure 5A), whereas expression of SOCS-2 mRNA tended ($P < 0.10$) to increase with frequent milking or was increased ($P < 0.03$) by exogenous PRL (Figure 5B). Expression of SOCS-3 mRNA was decreased ($P < 0.05$) by frequent milking, and tended ($P < 0.10$) to be decreased by exoge-

nous PRL (Figure 5C). Decreased expression of SOCS-3 mRNA after treatment with exogenous PRL was unexpected, considering our previous observation that elevated concentrations of PRL in blood were positively associated with expression of SOCS-3 mRNA in the mammary gland of nonlactating dairy cows (Wall et al., 2005a). However, cows in that study were exposed to different photoperiods, which induced altered concentrations of PRL in blood for the entire 60-d dry period (Wall et al., 2005a). Cows in the current study were injected with PRL twice daily; thus, they were exposed to episodic pulses of PRL rather than a chronic increase in PRL concentrations. Our data also contrast with findings in rodents, in which expression of SOCS-1, -3, or both was increased by treatment with exogenous PRL (Tam et al., 2001; Le Provost et al., 2005). In the present study, blood samples and mammary biopsies were obtained nearly 12 h after PRL injection; thus, it is possible that any transient increase in SOCS-1 or -3 mRNA expression after treatment with exogenous PRL was missed by our tissue sampling regimen. Indeed, Le Provost et al. (2005) showed that PRL stimulation of SOCS-3 expression in the mammary tissue of mice returned to basal concentrations between 4 and 24 h postinjection.

Our results on the effect of frequent milking on SOCS-1 and -3 mRNA expression in the mammary gland of dairy cows are consistent with the observation by Tam et al. (2001) that milk removal in rats had no effect on expression of SOCS-1, but was associated with decreased expression of SOCS-3 in the mammary gland. Changes in SOCS-2 and -3 mRNA expression (Figure

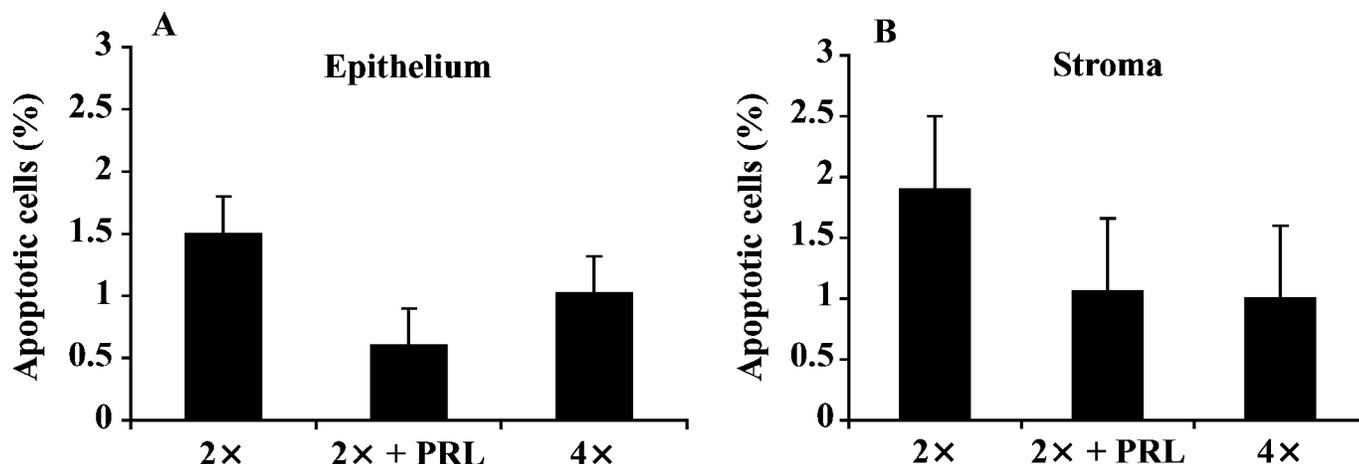


Figure 4. Effects of frequent milking or exogenous prolactin (PRL) on apoptosis of epithelial (Figure 4A) or stromal (Figure 4B) cells in the lactating bovine mammary gland. Apoptotic cells in the mammary tissue of cows milked 2 times daily with (2x + PRL) or without (2x) supplemental bovine PRL or 4 times daily (4x) for the first 3 wk of lactation. Mammary biopsies were taken at 7 ± 2 DIM ($n = 5$ cows/treatment). Each bar represents the least squares mean \pm pooled SE percentage of apoptotic cells. Epithelial or stromal cell apoptosis was not affected by treatment.

5B and 5C) in response to frequent milking or exogenous PRL indicate that expression of these genes is regulated both locally by milk accumulation and systemically by PRL. The SOCS-3 may be involved in the inhibition of milk secretion between milkings in the bovine mammary gland (Tam et al., 2001), and this may have contributed to the enhanced milk yield reported by Crawford et al. (2004) in the 2x + PRL and 4x cows, compared with 2x cows.

Mammary expression of PRL-R1 mRNA was greater ($P < 0.04$) in 2x cows than in 2x + PRL cows (Figure 6A). An increase in the systemic concentration of PRL has been associated with a decrease in PRL-R1 expression in the lymphocytes, liver, and mammary gland of calves (Auchtung et al., 2003) as well as in the lymphocytes and mammary gland of cows (Auchtung et al., 2005). Although concentrations of PRL in circulation were not different across treatments at the time of biopsy, it is apparent that negative feedback of exogenous PRL on PRL-R1 mRNA expression was still present. The relative abundance of PRL-R1 mRNA was not affected by milking frequency (Figure 6A). Dahl et al. (2002) reported that frequent milking during early lactation was associated with an increase in the expression of PRL-R1 mRNA in lymphocytes of dairy cows. In that study, blood samples were obtained immediately after milking so that acute responses to milking could be detected. Kim et al. (1997) reported that suckling induced an acute increase in PRL-R1 mRNA and protein expression in the mammary glands of mice. Our sampling scheme would not have detected such acute effects of milking on the expression of PRL-R1 mRNA.

The relative abundance of α -LA mRNA was measured as an indicator of milk protein gene expression and secretory cell activity. Mammary expression of α -LA mRNA tended ($P < 0.10$) to be twice as great in 4x cows as in 2x cows (Figure 6B), and was greater ($P < 0.04$) in 2x + PRL cows than in 2x cows. Our observations are consistent with those of Plaut et al. (1987), who reported that administration of exogenous PRL to lactating cows resulted in an increase in α -LA concentrations in milk.

Plaut et al. (1987) suggested that the lactating bovine mammary gland is unresponsive to exogenous PRL, perhaps because PRL receptors are saturated. However, cows in their study did not receive exogenous PRL until they had reached 21 DIM (Plaut et al., 1987). In the current study, cows received exogenous PRL during the first 21 DIM. Our gene expression data clearly demonstrate a response to exogenous PRL by the bovine mammary gland during early lactation.

One of our primary objectives in conducting this experiment was to differentiate the response of the mammary gland to exogenous PRL from the response to frequent milking in dairy cows. Another aim was to further elucidate the regulation of SOCS genes in the mammary gland with respect to exogenous PRL and milk removal. Our results indicate that mammary stromal cell proliferation is regulated by milk removal (Figure 3B), whereas expression of PRL-R1 mRNA is regulated by both exogenous PRL and milk removal (Figure 6A). Expression of SOCS-2, SOCS-3, and α -LA mRNA seems to be principally regulated by exogenous PRL (Figures 5B, 5C, and 6B). Although regulation of SOCS

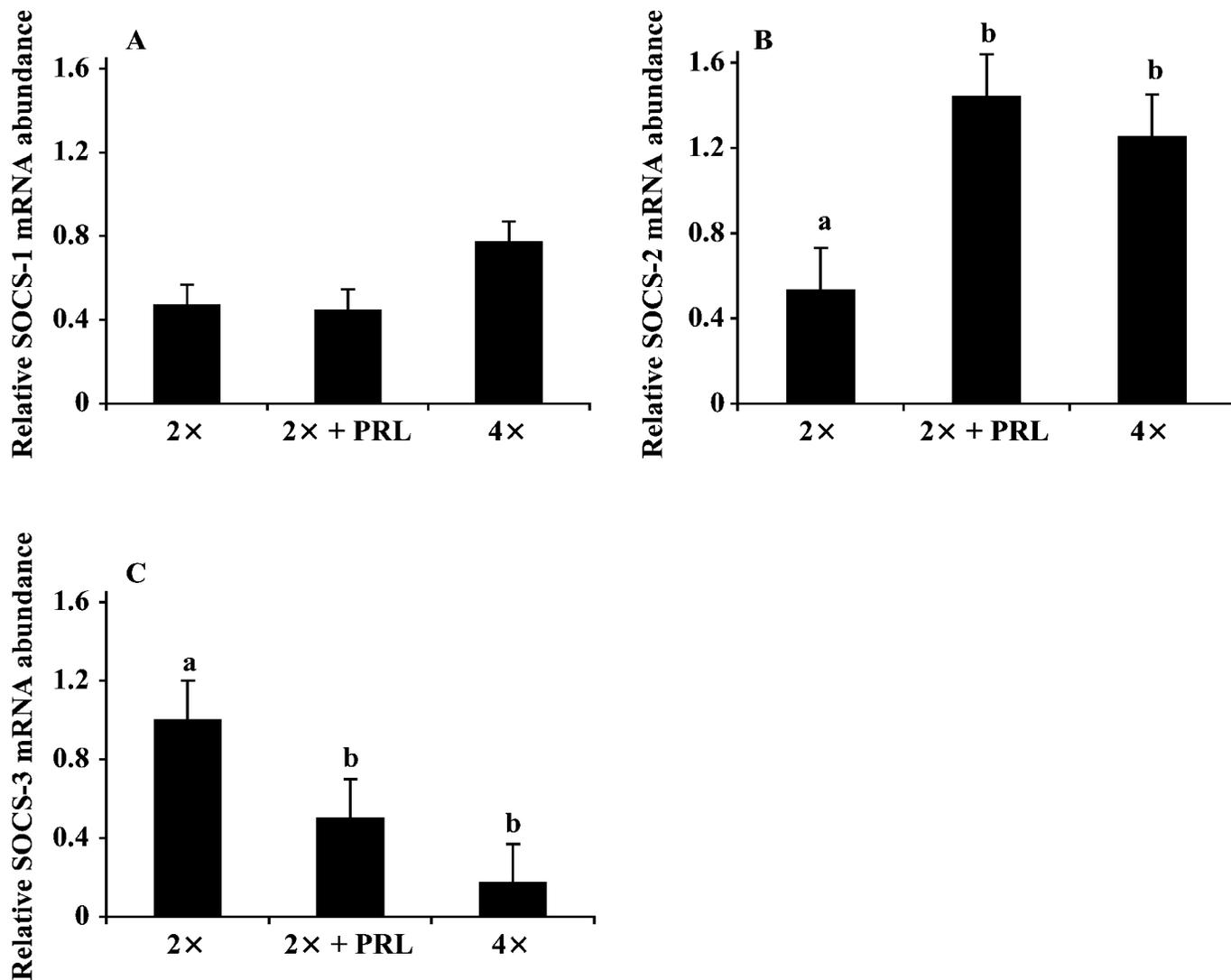


Figure 5. Effects of frequent milking or exogenous prolactin (PRL) on the expression of suppressors of cytokine signaling (SOCS)-1 (Figure 5A), -2 (Figure 5B), and -3 (Figure 5C) mRNA in the lactating bovine mammary gland. Expression of SOCS mRNA in the mammary tissue of cows milked 2 times daily with (2x + PRL) or without (2x) supplemental bovine PRL or 4 times daily (4x) for the first 3 wk of lactation. Mammary biopsies were taken at 7 ± 2 DIM ($n = 5$ cows/treatment). Each bar represents the least squares mean \pm pooled SE relative expression normalized to β -actin. Expression of SOCS-1 was not affected by treatment. Expression of SOCS-2 was greater ($P < 0.03$) in 2x + PRL cows and tended ($P < 0.10$) to be greater in 4x than in 2x cows. Expression of SOCS-3 tended ($P < 0.10$) to be greater in 2x cows than in 2x + PRL cows and was greater ($P < 0.05$) in 2x than in 4x cows. ^{a,b} $P \leq 0.10$.

mRNA expression in the bovine mammary gland is apparent from our results, it is not consistent across different SOCS (Figure 5). Previous research has indicated that SOCS-1 and -3 share common functions and regulation, whereas SOCS-2 and cytokine-inducible SH2-containing protein share common functions and regulation (Larsen and Ropke, 2002). In the current study, differences in the response of SOCS to exogenous PRL or milk removal indicate clear distinctions in SOCS function and regulation in the bovine mammary gland. Further mechanistic studies with more frequent sam-

pling will be necessary to fully understand the regulation and function of SOCS in the mammary gland and how this relates to mammary function and milk yield in dairy cows.

CONCLUSIONS

We conclude that the bovine mammary gland is responsive to exogenous PRL or frequent milking during early lactation. Observed responses to exogenous PRL or frequent milking indicate some similar and some

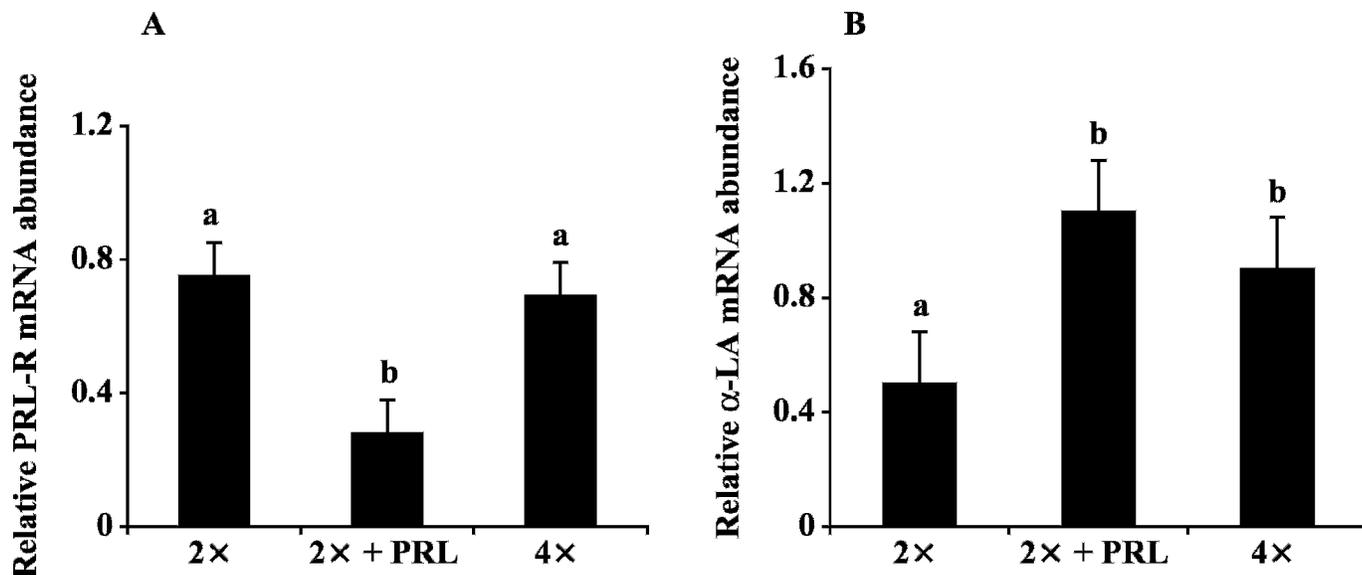


Figure 6. Effects of frequent milking or exogenous prolactin (PRL) on the expression of PRL-receptor (PRL-R1 long form; Figure 6A) and α -LA (Figure 6B) mRNA in the lactating bovine mammary gland. Expression of PRL-R1 and α -LA mRNA in the mammary tissue of cows milked 2 times daily with (2x + PRL) or without (2x) supplemental bovine PRL or 4 times daily (4x) for the first 3 wk of lactation. Mammary biopsies were taken at 7 \pm 2 DIM (n = 5 cows/treatment). Each bar represents the least squares mean \pm pooled SE relative expression normalized to β -actin. Expression of PRL-R1 was not affected by frequent milking, but was reduced in 2x + PRL cows compared with 2x ($P < 0.02$) or 4x ($P < 0.04$) cows. Compared with 2x cows, α -LA expression tended ($P < 0.10$) to be increased by frequent milking or was increased ($P < 0.04$) by exogenous PRL. ^{a,b} $P \leq 0.10$.

distinct effects of these factors on mammary growth and expression of PRL-responsive genes during early lactation in dairy cows.

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