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Short Communication: Short-Day Photoperiod During the Dry Period Decreases Expression of Suppressors of Cytokine Signaling in Mammary Gland of Dairy Cows

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ABSTRACT

Suppressors of cytokine signaling (SOCS) are induced by prolactin and act through negative feedback to inhibit cytokine signaling. We hypothesized that lower prolactin concentrations in cows exposed to short-day photoperiod (SD; 8 h light:16 h dark) lead to decreased expression of SOCS, which mediate the effects of SD on mammary proliferation during the dry period. Multiparous Holstein cows were dried off 60 d before expected calving and were assigned to long-day photoperiod (LD; 16 h light:8 h dark) or SD during the dry period. Mammary biopsies were obtained at −40, −20, −10, and +10 d relative to expected calving, and expression of SOCS-1, SOCS-2, SOCS-3, and cytokine-inducible SH2-containing protein (CIS) mRNA was assessed by real-time, quantitative, reverse transcription-PCR. Expression of all SOCS increased over time and expression of SOCS-3, SOCS-2, and CIS mRNA was lower in mammary gland of SD cows. These data suggest that lower SOCS expression in cows exposed to SD during the dry period may enhance prolactin signaling to the mammary gland, thereby augmenting mammary development during pregnancy and milk production in the subsequent lactation. Changes in SOCS expression during pregnancy and lactation imply that SOCS may regulate mammary gland development and function in dairy cows.
(**Key words:** photoperiod, suppressors of cytokine signaling, prolactin, mammary gland)

Abbreviation key: CIS = cytokine-inducible SH2-containing protein, **LD** = long-day photoperiod, **PRL** = prolactin, **PRL-R** = prolactin receptor, **SD** = short-day photoperiod, **SOCS** = suppressors of cytokine signaling.

Recently, Miller et al. (2000) reported that cows exposed to short-day photoperiod (**SD**) during the dry period produced 3.5 kg/d more milk in the subsequent lactation than cows exposed to long-day photoperiod (**LD**). Because exposure to LD during the dry period results in increased concentrations of prolactin (**PRL**) in circulation (Miller et al., 2000; Auchtung et al., 2005), and PRL concentrations are negatively correlated with PRL-receptor (**PRL-R**) expression in various tissues (Barash et al., 1983; Di Carlo et al., 1995; Auchtung et al., 2003), altered PRL signaling could play a role in mediating photoperiodic effects. In addition, it has been demonstrated in mice that PRL signaling in the mammary gland depends on a threshold of PRL-R expression (Hennighausen et al., 1997; Ormandy et al., 2003). We recently reported that exposure to SD during the dry period enhances mammary cell proliferation in dairy cows (Wall et al., 2005), and that altered PRL signaling to the mammary gland may mediate these photoperiodic effects.

Sensitivity of target tissues to PRL is regulated by the inverse relationship between concentrations of PRL in circulation and PRL-R expression (Hennighausen et al., 1997) as well as the recently discovered suppressors of cytokine signaling (**SOCS**). Suppressors of cytokine signaling are induced by the action of cytokines including PRL that use the Janus kinase/signal transducers and activators of transcription pathway (Aman and Leonard, 1997), and act through negative feedback to modulate cytokine signaling. To date, 8 members of the SOCS family have been identified: SOCS-1 through 7, and cytokine-inducible SH2-containing protein (**CIS**) (Larsen and Ropke, 2002).

The function of SOCS in the mammary gland has only recently been investigated. Overexpression of CIS results in impaired mammary gland development in pregnant mice, whereas $CIS^{-/-}$ mice have no obvious phenotype (Liu et al., 1998). Gene deletion studies demonstrated that SOCS-1 plays a critical role in the mouse mammary gland. For example, $PRL\text{-}R^{-/-}$ mice fail to lactate due to impaired mammary development, and this defect is resolved by deletion of a single SOCS-1

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Table 1. Primer sequences for bovine suppressors of cytokine signaling (SOCS)-1, SOCS-2, SOCS-3, cytokine-inducible SH2-containing protein (CIS), and β-actin.[1]

Target	Genbank #	Primer	Sequence, 5′ to 3′
SOCS-1	CB460055	Forward	CACAGCAGAAAAATAAAGCCAGAGA
		Reverse	CTCGTACCTCCTACCTCTTCATGTT
		Probe	TCCCCAACCCCTGGTTTGTGCAA
SOCS-2	AY183452	Forward	GGGACTGCCTTTACCAACAA
		Reverse	GTGCTGGGACCTTTCACCTA
SOCS-3	NM_174466	Forward	GGCCACTCTCCAACATCTCTGT
		Reverse	TCCAGGAACTCCCGAATGG
		Probe	CGTCAACGGCCACCTGGACTCCTA
CIS	CB431273	Forward	AAGCTCTGCTGGGTGCTAAC
		Reverse	GACCAAACTAGGGGACAGCA
β-Actin	AY141970	Forward	GCTCTCTTCCAGCCTTCCTT
		Reverse	GGACTCATCGTACTCCTGCTT

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

allele (Lindeman et al., 2001). In addition, SOCS-1$^{-/-}$ mice undergo precocious lactation, indicating that SOCS-1 is required for the prevention of lactation before parturition (Lindeman et al., 2001).

There is also evidence that photoperiod affects SOCS expression. Exposure of Siberian hamsters to LD increased SOCS-3 mRNA expression in the hypothalamic arcuate nucleus relative to hamsters exposed to SD (Tups et al., 2004). The increase in SOCS-3 led to resistance to leptin, a cytokine hormone that, like PRL, utilizes the Janus kinase/signal transducers and activators of transcription signaling pathway (Tups et al., 2004). In addition, leptin is thought to have a functional relationship with PRL in the mammary gland and may stimulate mammary development by increasing PRL concentrations (Motta et al., 2004). Therefore, it is plausible that SOCS may be involved in mediating effects of photoperiod on mammary gland development. We hypothesized that exposure to SD during the dry period would reduce SOCS expression in the mammary gland of dairy cows. Our objectives were to compare expression of SOCS mRNA in mammary tissue of cows exposed to either LD or SD during the dry period and to characterize the expression of SOCS-1, SOCS-2, SOCS-3, and CIS mRNA in bovine mammary gland during pregnancy and early lactation.

Cows used in this study were a subset (n = 6 cows per treatment) of animals from a larger study (Auchtung et al., 2005). Details on animal management and treatments were described previously (Wall et al., 2005). The University of Vermont and University of Illinois Institutional Animal Care and Use Committees approved all animal use.

Primers for all target genes are presented in Table 1. Primer design and PCR for SOCS-2 and CIS were performed as previously described (Wall et al., 2005). For SOCS-1 and SOCS-3, primers and probes were designed using Primer Express (version 1.5; Applied Biosystems, Foster City, CA). The PCR reaction included 1 μL of diluted cDNA and 12.5 μL of TaqMan 2× PCR Master Mix (Applied Biosystems). The PCR reaction started with a 2-min uracil glycosylase step at 50°C followed by a 10-min *Taq* activation step at 95°C. Samples were then subjected to 40 cycles of 15-s denaturation at 95°C and 1-min annealing and extension at 60°C. Expression of the reference genes β-actin and β2-microglobulin was measured and found to be stable over time and between treatments. Therefore, all gene expression values were normalized to that of β-actin in the same sample. Statistical analyses were performed as previously described (Wall et al., 2005).

Auchtung et al. (2005) reported milk yield, blood PRL concentrations, and PRL-R expression for all 39 cows on the study. Briefly, cows exposed to SD during the dry period produced ~3 kg/d more milk than LD cows over the first 16 wk of lactation. Concentrations of plasma PRL were lower in cows exposed to SD, whereas expression of both forms of PRL-R was greater in mammary tissue and lymphocytes of these cows. Similar responses were observed in the subset of 12 cows reported herein.

Mammary expression of SOCS-1 and SOCS-3 mRNA increased late in the dry period and in lactation ($P < 0.002$; Figure 1a, 1b). Although mammary expression of SOCS-1 mRNA was not affected by photoperiod ($P = 0.2$; Figure 1a), expression of SOCS-3 was lower overall in SD cows relative to LD cows ($P = 0.03$; Figure 1b). We also observed a treatment by time interaction in SOCS-3 expression ($P = 0.03$). The photoperiod response that we observed is in agreement with Tups et al. (2004), who reported lower expression of SOCS-3

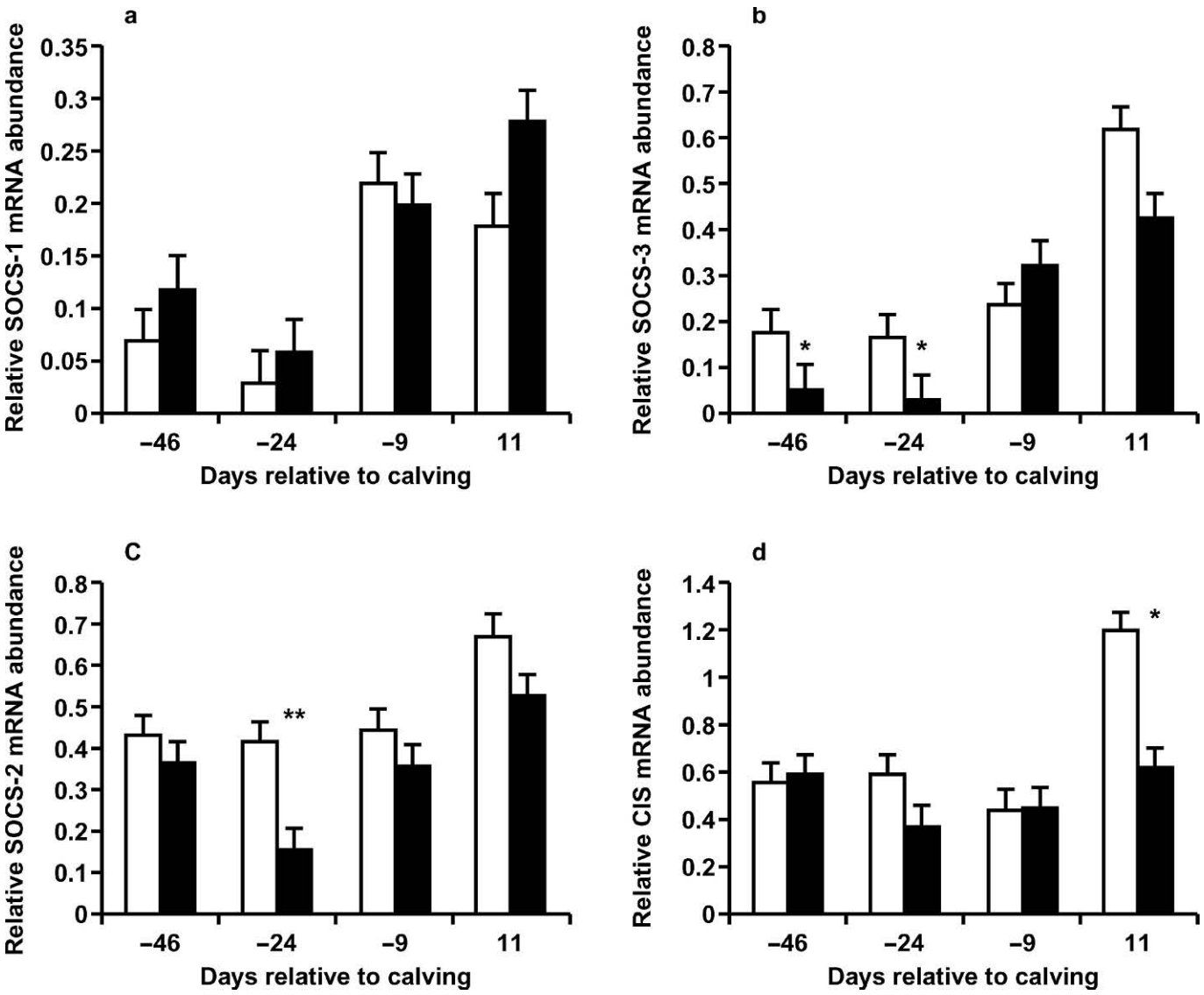

Figure 1. Effects of days relative to parturition and photoperiod on expression of a) suppressors of cytokine signaling (SOCS)-1, b) SOCS-3, c) SOCS-2, and d) cytokine-inducible SH2-containing protein (CIS) mRNA in the mammary gland of cows exposed to long-day (white bars) or short-day (black bars) photoperiod during the dry period. Mammary biopsies were taken at –46, –24, –9, and +11 d relative to parturition, and relative mRNA abundance was detected by real time, quantitative, reverse transcription-PCR. Each bar represents mean ± SEM relative mRNA abundance normalized to β-actin for cows in each photoperiod group at each time point (n = 6 cows/treatment). * = $P < 0.05$, LD vs. SD; ** = $P < 0.01$, LD vs. SD.

mRNA in the brain of hamsters exposed to SD compared with those on LD.

Overall, expression of SOCS-2 and CIS mRNA increased over time ($P = 0.01$; Figure 1c, 1d). Mammary expression of SOCS-2 was lower overall in SD cows than LD cows ($P = 0.05$; Figure 1c), whereas expression of CIS mRNA was lower in SD cows than in LD cows only at d 11 of lactation ($P = 0.02$; Figure 1d).

The temporal changes in SOCS expression observed in the present study imply that SOCS may play a role in regulating or responding to mammary gland development and function during pregnancy and lactation in dairy cows. It has been shown that SOCS-1 is required for the prevention of lactation before parturition in mice (Lindeman et al., 2001), and it may have a similar role in the bovine mammary gland. Expression of SOCS-3 mRNA in the mammary gland of rats is regulated in part by filling of the mammary gland with milk (Tam et al., 2001). Thus, it is possible that the marked increase we observed in SOCS-1 and SOCS-3 expression during late gestation may be due to engorgement of the gland with mammary secretion. Treatment differences

in expression of SOCS during the dry period and during lactation may have contributed to differences in mammary proliferation (Wall et al., 2005) and milk yield (Auchtung et al., 2005) in these cows.

We conclude that SOCS genes are expressed in bovine mammary gland. The pattern of SOCS expression during pregnancy and lactation indicates that they play a role in regulating bovine mammary gland development and lactogenesis. Relative to LD, exposure to SD during the dry period resulted in decreased expression of SOCS-2, SOCS-3, and CIS mRNA in bovine mammary gland. These data suggest that lower SOCS expression in cows exposed to SD during the dry period may enhance PRL signaling to the mammary gland, thereby augmenting mammary development during pregnancy and milk production in the subsequent lactation.

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