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GH and milking frequency act differently on mammary cells

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ABSTRACT

Milk yield can be affected by treatment with growth hormone (GH) and/or changes in milking frequency. Our study provides evidence that the mechanisms underlying these changes involved the modulation of mammary cell numbers in goats. Milking thrice daily (3X) compared with once daily (1X) for 23 days significantly affected milk yield, udder weight, the number of mammary epithelial cells and the level of the Bcl-2/Bax mRNA ratio, suggesting that 3X limits apoptosis when compared with 1X. GH treatment acted only on udder weight. These results demonstrate that milking frequency and GH act differently on epithelial cell number inside the alveoli.

KEY WORDS: milk yield, mammary gland, cell number, GH, milking frequency, goat

INTRODUCTION

Milk yield can be affected by treatments with growth hormone (GH) and/or changes in milking frequency. Some evidence exists to support the hypothesis that the mechanisms underlying these changes are involved in modulating mammary cell numbers. In order to understand the effects of GH and milking frequency on the mammary gland, we measured indicators for changes in mammary cell numbers and for cell death in the mammary gland of goats.

MATERIAL AND METHODS

After adaptation to twice-daily milking, six Saanen goats were submitted to a differential milking frequency: one half-udder was milked once a day (1X) and

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the controlateral half-udder milked thrice a day (3X). Concomitantly, three of the six goats received an injection of 5 mg recombinant bovine GH per day. At day 24, the goats were slaughtered by exsanguination and each half-udder was removed, weighed, rapidly frozen in liquid nitrogen, and stored at -20°C until DNA and RNA determination. The DNA concentration was measured in the mammary gland by a fluorometric method and the RNA was analysed by real time RT-PCR. Mammary tissue preparation was also performed for immunohistochemical analysis. After the counterstaining of the mammary gland sections with Hoechst, the number of epithelial cells per alveolus was counted and the diameter of the alveoli determined under the microscope. We calculated the percentage of apoptotic cells using the TUNEL assay, as well as the levels of Bcl-2 and Bax gene expression (anti- and pro-apoptotic genes, respectively).

RESULTS

Our results indicate that after 23 days of treatment, milking frequency significantly affected milk yield (+76% for 3X compared to 1X, P<0.01; Table 1) and the number of mammary cells (+37% for 3X compared to 1X, P<0.01; Table 1). Considering the number of goats in this study, it was not possible to establish a

Table 1. Milk yield and mammary gland characteristics of udder-halves milked once (1×) vs thrice (3×) daily in control vs GH-treated goats after 23 days of treatment. Least squares means ± SEM

	Control		GH		SEM[1]	Effect[2]		
	1X	3X	1X	3X		M	GH	MG
Milk yield, mL/d	816[a]	1.333[b]	776[a]	1.471[b]	77	0.01	NS	NS
Milk yield, % of the pretreatment week	-26	+8	-31	+19				
Udder weight, g	531[a]	602[b]	613[b]	747[c]	15	0.01	0.05	0.09
DNA, mg/g of tissue	4.25[a]	5.46[c]	4.46[b]	4.74[ab]	0.20	0.02	NS	0.08
Total DNA, mg/half-udder	2.260[a]	3.300[b]	2.710[ab]	3.520[b]	170	0.01	0.14	NS
Cell number per alveolus	22.6[a]	28.4[c]	22.8[a]	24.4[ab]	0.5	0.001	NS	0.02
Alveolar diameter, μm	93.6[a]	108.7[b]	91.9[a]	104.6[b]	3.0	0.02	NS	NS
Apoptotic cells, %	0.30	0.44	0.43	0.29	0.11	NS	NS	NS

[a,b,c] within a row means without a common superscript letter differ (P<0.05)

[1] SEM - standard error of the mean

[2] analysis of variance tested for effects of milking frequency (M), GH treatment (GH), and interaction between milking frequency and GH treatment (MG)

significant effect of GH on milk yield. Mammary glands were heavier in the frequently milked udder-halves (+18% for 3X compared to 1X, P<0.01; Table 1), and in GH-treated goats (+20% for GH compared to controls, P<0.05; Table 1). Based on histological and DNA analyses of mammary tissues, it was shown that only milking frequency clearly affected epithelial cell numbers in the

alveolus (+16% for 3X compared to 1X; $P<0.01$; Table 1) and alveolar diameter (+15% for 3X compared to 1X; $P<0.02$; Table 1), whereas GH induced only a tendency towards increasing and/or maintaining the mammary cell numbers (i.e. total DNA, $P=0.1$; Table 1) without affecting the alveolar component. Using the TUNEL assay, no variations were seen in the number of apoptotic cells in the half-udders (Table 1), and we concluded that this assay was insufficiently sensitive to demonstrate the effects of milking frequency. RT-PCR demonstrated that variations in the Bcl-2/Bax mRNA ratio tended to be higher in the more frequently milked half-udder ($P=0.1$), suggesting that milking thrice a day limited apoptosis compared to milking once a day. We did not observe any effect of GH on this measure.

DISCUSSION

Milking frequency modified the number of epithelial cells in the mammary gland and especially inside the alveolus. This effect could be due to a survival effect induced by increased milking frequency. It has already been shown that a reduced milking frequency induces apoptosis in the goat mammary gland (Li et al., 1999), while increasing the milking frequency stimulates mammary proliferation (Wilde et al., 1987). Our results showed that these phenomena probably occurred inside the alveolus. In contrast, GH had no effect on the number of epithelial cells in the alveolus, even though GH tended to increase the total number of mammary cells. It has been demonstrated elsewhere that GH is able to maintain the number of the mammary cells throughout the lactation period (Knight et al., 1990). This effect may have resulted from a stimulation of proliferation, as this has been demonstrated in the cow (Capuco et al., 2001). In any case, GH did not affect mammary cell apoptosis after 23 days of treatment, as it has also been observed in late lactating goats (Baldi et al., 2002). Furthermore, it has been shown that GH induced a higher number of alveoli in the mammary gland, and especially lactating alveoli (Baldi et al., 2002). This could suggest that GH does not control the modulation of cell number inside the alveolus, but instead the total number of alveoli.

CONCLUSIONS

Thus GH and milking frequency act differently on mammary cell numbers. Milking frequency affects the number of epithelial cells inside the alveolus by limiting apoptosis, whereas GH may act on the total number of alveoli.

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