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## BRIEF COMMUNICATION

## Increased litter size elicits cell proliferation in the lactating murine mammary gland

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Milk quality of dairy cows decreases both as lactation progresses and as milk yield declines (Lukey, 1996). Declining milk yield has been associated with a diminishing population of secretory epithelial cells (Knight and Wilde, 1993). One approach to enhancing the persistency of lactation is to stimulate a wave of proliferation of epithelial cells within the gland. A new generation of epithelial cells could increase milk yield and might also improve the overall quality of the milk.

The aim of the present experiment was to compare several treatments designed to stimulate mammary epithelial cell proliferation during lactation.

Sixty primiparous Swiss mice were randomly allocated to one of four treatments to be imposed at one of three stages of lactation (early, mid or late being day 4, 11 or 18). Control mice suckled litters of 5 or 10 pups (T5, T10). Challenged mice suckled litters of 5 or 10 pups and had 5 extra pups added for the last 3 days of lactation (T5+5, T10+5). One hour prior to death, mice were separated from their litters and injected with [<sup>3</sup>H]-thymidine. After death, the inguinal glands were weighed and later analysed for content of DNA and [<sup>3</sup>H]-thymidine incorporation into DNA. Litter growth rate was also recorded. Statistical analyses were performed using the general linear models procedure of SAS (SAS, 1989).

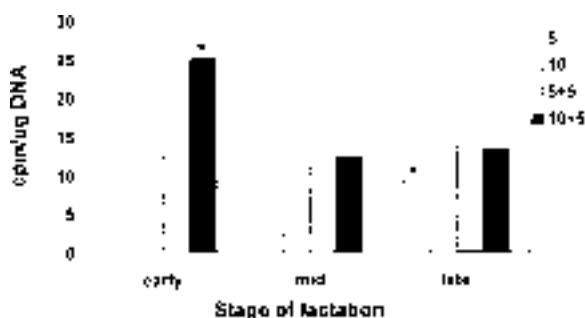
Litter growth rate, which is indicative of milk production, varied in the order T10+5>T5+5>T10>T5 (4.8<sup>a</sup>, 4.0<sup>b</sup>, 3.7<sup>c</sup>, 2.4<sup>d</sup> g/day; means across all three stages of lactation; P<0.01). Litter growth rate was lowest in early lactation (P<0.01) but did not differ between mid and late lactation (P>0.05). A strong interaction between treatment and stage of lactation (P<0.01) was caused by the lower growth rate of the T10+5 litters in early lactation.

Mammary gland size was estimated by two variables; gland weight and DNA content of the mammary gland. Mammary gland DNA content was lowest in early lactation, peaked in mid lactation and declined in late lactation (P<0.05). This is in agreement with previous data in rats (Tucker, 1966) and mice (Knight and Peaker, 1982). The gland DNA content was lowest for T5 while the remaining treatments did not differ (1.43<sup>a</sup>, 1.89<sup>b</sup>, 1.71<sup>b</sup>, 1.75<sup>b</sup>; ug DNA/gland for T5, T10, T5+5 and T10+5 respectively, P<0.05).

Mammary gland weight followed similar patterns of treatment and stage of lactation effects as the DNA content of the gland.

Incorporation of [<sup>3</sup>H]-thymidine into DNA was used as a measure of cell proliferation. Incorporation was highest in early lactation but did not differ between mid and late lactation (18.1<sup>a</sup>, 13.5<sup>b</sup>, 12.4<sup>b</sup> cpm/ug DNA for early, mid and late lactation respectively, P<0.01; Figure 1). Proliferation in T10+5 was greater than in the other treatments (13.2<sup>a</sup>, 13.6<sup>a</sup>, 15.0<sup>a</sup>, 17.0<sup>b</sup> cpm/ugDNA for T5, T10, T5+5 and T10+5 respectively, P<0.05). There was a strong interaction between stage of lactation and treatment (P<0.01) due to two reasons. The first reason was the low rate of proliferation in mice suckling 5 pups in late lactation (treatment T5). This suggests that the sucking stimulus of 5 pups failed to maintain the average basal proliferation rate of ~14 cpm/ug DNA. The second reason for the interaction was the high proliferation rate measured in glands of mice in treatment T10+5 in early lactation. Mice in the same treatment but in mid or late lactation had a lower cell proliferation suggesting a window of responsiveness in early lactation. This result is in agreement with previous work in mice (Knight and Peaker, 1982) and cows (Barpeled et al., 1997). The high proliferation rate measured in glands of mice with 10+5 pups, together with the corresponding low litter growth rate is consistent with the theory of Knight and McLelland (1988) that when proliferation occurs there is a temporary suppression in milk production.

**FIGURE 1:** [<sup>3</sup>H]-thymidine incorporation into mammary gland DNA of mice with litters of T5, T10, T5+5 and T10+5 pups versus stage of lactation. Asterisks denote significant difference at P<0.05.



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In summary, this study revealed three main points of interest: i) the sucking stimulus of only 5 pups in late lactation was insufficient to maintain the basal proliferation rate seen in other treatments, ii) the gland weight, gland DNA content and cell proliferation of glands of mice in treatment group T5+5 were not different to those in the treatment group T10 indicating the gland responded rapidly to 5 extra pups over a period of 3 days at any stage of lactation, and iii) the glands of mice in early lactation with 10+5 pups had the highest cell proliferation (treatment T10+5). The 10+5 treatment in early lactation is the most promising model of cell proliferation and will be part of a future study of the mechanisms proliferation.

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