Short Communication: Suppression of Estrous Cycles in Lactating Cows Has No Effect on Milk Production¹

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ABSTRACT

The decline in milk yield observed after peak production in dairy animals results from apoptotic death of mammary epithelial cells. In cows, this decrease in milk yield can be accelerated by injection of 17 β -estradiol, thus evoking a possible role of estrogens in the regulation of bovine mammary gland involution. In nonpregnant cows, mammary involution could be induced or enhanced by the return of estrous cycles and the accompanying cyclic peaks of estrogen concentration in the serum of lactating animals. To test this hypothesis, we inserted implants of a GnRH agonist, deslorelin, in an ear of each cow ($n = 10$) on d 10 and 100 of lactation, to temporarily suppress the return of ovarian cycles. Cows were studied from calving to d 210 of lactation. Deslorelin had no impact on feed intake or animal health. Deslorelin significantly reduced serum concentrations of 17 β -estradiol and progesterone as compared with untreated cows ($n = 10$). Deslorelin had no effect on milk fat and protein, whereas milk lactose content was lower in treated cows than in control cows on d 100 of lactation. Finally, there was no difference in milk production between the 2 groups of cows. These results are consistent with previous observations that showed that delaying estrous cycles after calving had no effect on milk yield and they extend those observations to late lactation. Based on milk production data, the estrogen profiles associated with recurring estrous cycles apparently do not cause bovine mammary tissue to undergo gradual involution.

Key words: estrogen, progesterone, milk yield, involution

It is now well established that the decline in lactation that follows peak production in dairy animals is the consequence of gradual, apoptotic death of secretory cells in the mammary gland (Knight and Wilde, 1993;

Wilde et al., 1997a,b; Capuco et al., 2001). Results obtained with pregnant cows have identified estrogens as factors possibly involved in the decline in lactation (Bachman et al., 1988; Bormann et al., 2002), a hypothesis supported by several experimental data. However, Roche (2003) noted that milk yields of pregnant vs. nonpregnant twin cows were similar until after 250 d of lactation or 168 d of gestation. Administration of 17 β -estradiol (**E2**) and progesterone to lactating cows causes mammary gland regression and a decrease in milk production (Mollett et al., 1976). Furthermore, E2 administered to cows at the end of lactation accelerates involution of mammary tissue (Athie et al., 1996). We have recently confirmed this inhibitory effect of E2 on milk production in dairy cows and have suggested that this E2 effect could be mediated in the mammary gland by the hormone stanniocalcin (Delbecchi et al., 2005). The question then is, “Do estrogens contribute to the post-peak decline in physiological conditions?” A simple hypothesis would be that with the return of ovarian function after calving, the resultant cyclic variations in blood estrogen levels of lactating cows could eventually exert a negative influence on the activity or the number of mammary epithelial cells. This hypothesis was tested by evaluating the effect that suppression of the return of estrous cycles had on the milk production of lactating nonpregnant cows. An agonist of GnRH was used to inhibit the release of ovarian hormones.

Milk production of nonpregnant Holstein cows ($n = 20$) was measured from calving to d 210 of lactation. An implant containing 5 mg of deslorelin (Peptech Animal Health, North Ryde, NSW, Australia) was placed subcutaneously in the right ear of 10 cows (treated cows) on d 10 of lactation. At 100 DIM, a second implant was inserted adjacent to the first implant to ensure that estrous cycles remained suppressed throughout the experiment. Deslorelin is an agonist of GnRH that has been used in beef heifers to block estrous cycles for a defined interval (D’Occhio et al., 1996). Its action results in an inhibition of FSH and LH release by the pituitary gland, which, in turn, leads to a suppression of estrogen and progesterone release by the ovaries. Control cows ($n = 10$) received no implant. Primiparous cows ($n = 12$) and multiparous cows ($n = 8$) were equally

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distributed between the control and treated groups. The distribution of multiparous cows between the 2 groups was done according to their previous milk yield to have comparable total amounts of milk production for each group. Experimental cows were housed in a tie-stall barn at the Dairy and Swine Research and Development Centre (Lennoxville, QC, Canada).

Milk production and feed intake were recorded daily from calving. All dates of calving occurred within 78 d. Milk samples were taken on d 10, 100, and 200 for evaluation of milk composition and SCC by the Program d'Analyse des Troupeaux Laitiers du Québec. Cows had free access to water and were fed 6×/d. They were milked daily at 0600 and 1800 h. Blood (25 mL) was taken thrice weekly from d 10 to 210, twice from the tail vein and once from the jugular vein alternately, using sterile tubes with no inhibitor of coagulation (Vacutainer, Becton Dickinson and Co., Rutherford, NJ). Blood was centrifuged ($5,000 \times g$, 20 min, 4°C), and aliquots of the serum were stored at -20°C. Progesterone concentration was measured once each week on a fresh serum sample using the Ovucheck plasma kit from Biovet (St-Hyacinthe, QC, Canada) to detect the return of ovarian cycles. Return of estrus was also monitored by daily observation of the cows by barn personnel. No indication of a diminution of deslorelin efficacy was apparent until the end of the experiment (d 210), except for one cow that was removed from the experiment. Concentration of E2 in the stored serum samples was measured using the ImmunoChem Double Antibody 17β -estradiol I^{125} radioimmunoassay (RIA) kit from ICN Biomedicals (Costa Mesa, CA). The manufacturer's protocol was followed, except that an ethyl ether extraction was performed on samples before analysis. This extraction had 2 purposes: 1) to remove proteins from the samples and thus avoid interference from BSA, as the RIA kit was originally designed for humans, and 2) to concentrate the samples. Thawed serum samples were vigorously shaken with 4 volumes of ethyl ether and then frozen at -80°C for 30 min. Then, the organic phase was collected and evaporated in a Savant SpeedVac concentrator. The extracted material was dissolved in the sample buffer included in the RIA kit (0.1 of the starting volume) before being assayed for E2 content.

Data were analyzed as repeated measurements using the MIXED procedure of SAS (2002). The covariance structure used was the spatial power. Individual lactation curves were fitted to milk production data using the following function (Wilink, 1987):

$$y_t = a + bt + ce^{-0.05t}$$

where y_t represents an average test-day production per animal, t is the number of days of lactation, e is the

Figure 1. Concentration of progesterone in the serum of control (◇; $n = 10$) or treated (■; $n = 9$) cows. Blood samples were taken once weekly from d 10 to 210 of lactation. Progesterone concentrations are given as means of experimental values $\pm$ standard errors. Arrows indicate when the GnRH agonist, deslorelin, implants were placed in an ear of each treated cow.

base of the natural log, and a , b , and c are parameters describing the lactation curve. Parameters a and c represent the level of production (at $t = 0$), c is the rate of increase in production to the peak, and b is the rate of decline thereafter. The factor 0.05 is related to the time of peak production, which is about 50 d. Mean values and standard errors of parameters a , b , and c were calculated for each group of cows, and a Student's t test was used to compare those respective parameters between control and treated animals.

Deslorelin prevented the resumption of estrous cycles as evidenced by the lack of signs of estrus and by the E2 and progesterone profiles. Indeed, deslorelin significantly reduced ($P < 0.0001$) E2 and progesterone levels in the serum of cows. Progesterone concentrations averaged over the experimental period were 3.05 ± 0.19 and 1.34 ± 0.21 ng/mL for control and treated cows, respectively. One of the treated cows had serum progesterone >4 ng/mL throughout the experiment. She was not pregnant and could have had a persistent corpus luteum (D'Occhio et al., 1996). When she was excluded, the mean progesterone concentration for treated cows was 0.86 ± 0.18 ng/mL, and none had typical cyclic patterns. For E2, average concentrations over the same period were 1.98 ± 0.07 and 0.81 ± 0.06 pg/mL for control and treated cows, respectively. The effects of deslorelin on progesterone and E2 profiles in the 2 groups of cows are shown in Figures 1 and 2, respectively. Although not shown, both progesterone and E2 profiles in individual control cows were typical of cyclic cows. As illustrated in Figure 1, insertion of the first deslorelin implant was followed by an increase in the progesterone concentration of treated cows. Occurrence of this luteotropic effect might depend on the stage of the estrous cycle at the time of treatment (D'Occhio et al., 1996).

Figure 2. Concentration of 17β -estradiol in the serum of control ($\diamond$; $n = 7$) or treated ($\blacksquare$; $n = 9$) cows. Blood samples were taken thrice weekly from d 10 to 210 of lactation. Estradiol concentrations are given as means of experimental values $\pm$ standard errors. Arrows indicate when the GnRH agonist, deslorelin, implants were placed in an ear of each treated cow.

Deslorelin implants had no effect on feed intake (not shown), and they did not affect general behavior or health. Milk fat and protein were not affected by deslorelin, whereas lactose percentage was lower ($P < 0.05$) for treated cows (4.55 ± 0.05) than for controls (4.72 ± 0.04) on d 100 of lactation. It is unclear what caused the reduction in milk lactose, although subclinical mastitis is unlikely because SCC was numerically lower in treated cows than in control cows (50,200 vs. 263,400 SCC/mL; $P > 0.1$) on d 100 of lactation. Also, there were no differences in the SCC between the 2 groups of cows on d 10 and 200 either.

No difference in the milk production of control and treated cows was detected (Figure 3; $P > 0.1$). The flatness of the curves, in part, can be attributed to the high proportion of first-parity cows ($n = 12$) in the experiment

Figure 3. Milk yield of control ($\diamond$; $n = 10$) or treated ($\blacksquare$; $n = 9$) cows. Weekly milk yields are given as means of experimental values $\pm$ standard errors. Arrows indicate when the GnRH agonist, deslorelin, implants were placed in an ear of each treated cow. Treatment blocked estrous cycles and lowered progesterone and estradiol concentrations in those cows had no effect on milk production.

Table 1. Comparison of parameters a, b and c between control and treated (DES; deslorelin) cows

Parameter ¹	Control ²	DES ²	P ³
a	36.656 $\pm$ 2.215	37.529 $\pm$ 2.607	0.800
b	-0.072 $\pm$ 0.011	-0.080 $\pm$ 0.010	0.606
c	-17.268 $\pm$ 1.284	-17.951 $\pm$ 1.992	0.772

¹In the Wilink's function, a and c represent the level of production (at $t = 0$), c is the rate of increase of production to the peak, and b is the rate of decline thereafter.

²Mean values $\pm$ standard errors are given for each group. For control, $n = 10$; for DES, $n = 9$.

³Probability associated with the Student's t test.

and to the nonpregnant status of all of the cows. Individual lactation curves were fitted to our data using the model of Wilink (1987). Means of parameters a, b, and c were calculated for each group of cows and compared using a Student's t test. No difference could be seen for these parameters between the 2 groups of cows (Table 1), which showed that the deslorelin treatment had no effect on several important features of the lactation curve, including the prepeak rise, the postpeak decline (persistence), the total lactation yield, the peak yield, and days to peak. Hence, suppressing the periodic peaks of estrogen in the serum of dairy cows over a long period did not improve milk yield, even slightly. This result was not totally unexpected. Blood estrogen levels in cycling cows were pulsatile with narrow peaks that reached rather modest maxima (Figure 2). This estrogen profile differs from the continuous increase in serum estrogen observed in pregnant cows as gestation progresses (Monk et al., 1975) and differs from the values attained peripartum (Smith et al., 1973; Robertson, 1974) and, in nonpregnant cows, upon repeated E2 injections that can affect milk production (our unpublished data; Mollett et al., 1976). Our data, in fact, extend the observations of Mattos et al. (2001) to late lactation. Their use of an implant containing 2.1 mg of deslorelin had no effect on milk yield of dairy cows over the first 100 d of lactation. We obtained the same results with implants containing 5 mg of deslorelin. We also showed that a second implant inserted 100 d after calving had no effect on milk production from d 100 to 210 when lactation was in its normal decline phase.

The postpeak declines in the amount of milk produced by mammary epithelial cells were similar in nonpregnant dairy cows that had estrous cycles and those in which the estrous cycles were suppressed via deslorelin implants. This similarity suggests that the magnitude and duration of the E2 peaks associated with recurring estrous cycles are not sufficient to affect the number or activity of differentiated mammary epithelial cells.

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