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# Early mammary gland metabolic and immune responses during natural-like and forceful drying-off in high-yielding dairy cows

**Nissim Silanikove,* Uzi Merin,† Fira Shapiro,* and Gabriel Leitner‡**
*Biology of Lactation Laboratory, Institute of Animal Science, ARO, the Volcani Center, PO Box 6, Bet Dagan 50250, Israel
†Department of Food Quality and Safety, Postharvest and Food Sciences, ARO, the Volcani Center, PO Box 6, Bet Dagan 50250, Israel
‡National Mastitis Center, Kimron Veterinary Institute, PO Box 12, Bet Dagan 50250, Israel

## ABSTRACT

The present work compared metabolic and immune responses in genetically high-producing cows that produced a low amount of milk before expected involution and in cows with the same genetic potential that produced copious amounts of milk before their scheduled drying-off. Ten multiparous lactating Israeli Holstein cows producing approximately 10,500 L in the current lactation, without bacterial infection and scheduled for drying-off approximately 60 d before their expected parturition, were studied. Five of the cows that exhibited a sharp, spontaneous reduction in milk yield at the end of their lactation and produced less than ~14 L/d were defined as cows approaching natural involution (ANI), and 5 cows that produced between 25 and 35 L/d were defined as cows approaching forced involution (AFI). Three days before scheduled drying-off, milking was stopped and milk samples were collected from each quarter. After milking cessation, only modest swelling was observed in the udders of the ANI cows. In the ANI cows, lactose and fat concentrations decreased and the fat:lactose concentration ratio indicated that on d 1 and 2 fat concentrations decreased faster than lactose concentration, whereas on d 3, the rate of reduction was about the same for lactose and fat. In contrast, in AFI cows, fat concentrations increased on d 1 and the fat:lactose ratio indicated that changes in fat secretion were minor compared with those of lactose secretion. Rennet clotting time of milk after drying-off in the ANI cows increased, whereas curd firmness decreased rapidly, such that mammary secretions did not coagulate on d 3. In the AFI cows, such significant changes were observed only on d 3. The inflammatory response increased in both groups, but at each stage the increase was greater in ANI cows than in AFI cows. On d 1, the increase in leukocyte numbers in the ANI cows was made up of mononuclear cells (i.e., T lymphocytes and macrophages). In contrast, in the AFI cows, we observed a marked increase in leukocyte numbers, mainly in the form of polymorphonuclear cells. Our data indicate that the abrupt mammary involution induced in AFI cows provoked signs of distress, which were associated with neutrophilia in milk. In contrast, in the ANI cows, cessation of milking occurred without evidence of engorgement of the udder. Physiological differences in ANI and AFI cows are distinct and are reflected in the differences in the leukocyte populations in milk.
**Key words:** involution, mammary gland, adaptation, acute response

## INTRODUCTION

Most of the development of the mammary glands occurs at the end of pregnancy and to a much lesser extent after parturition. After weaning, the glands regress to their prelactating state (Atabai et al., 2007). The first stage of involution (stage I), which comprises the events occurring between drying-off (induction of milk stasis) and extensive degradation of the secretory tissues, is associated with widespread apoptosis of the alveolar epithelial cells (Lund et al., 1996).

Involution of the mammary gland in most mammals under natural conditions begins when their offspring either stop suckling or reduce the frequency of suckling. However, most of the relevant research in bovine and mice was carried out by induction of abrupt involution (Capuco and Akers, 1999; Clarkson et al., 2004), probably because this procedure is quite easy to manipulate and carry out under controlled conditions. However, there is no evidence to indicate that results from abrupt cessation of milking necessarily reflect changes occurring during natural involution. Recent evidence in mice suggests that abruptly induced involution resembles the course of wound healing (Clarkson et al., 2004).

The classical view, however, is that involution of the mammary glands is an orderly, programmed physiological process (Monks et al., 2002; Nilsen-Hamilton et al., 2003). In mice and bovines, natural mammary

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[1]Corresponding author: nsilanik@agri.huji.ac.il

gland involution is associated with immunomodulatory responses that are essential for clearing the apoptotic cells and preventing new infection (Capuco and Akers, 1999). Thus, although there is no doubt that mammary gland involution is associated with activation of the immune system, the question remains whether it is an orderly tissue-remodeling process or a process that includes elements of acute (e.g., wound healing) inflammation during gradual or natural-like involution.

Most cows in modern dairy herds are separated from their offspring soon after giving birth and inseminated during the next 80 to 100 d. Consequently, unlike most wildlife and many farm animal species, pregnancy and lactation occur simultaneously in cows, while lactation is preserved by milking. Despite these changes in management, previous reports led us to hypothesize that when cows with a high genetic potential for milk production produce less milk (<15 L/d) toward the end of lactation, it might reflect a preserved metabolic adaptation toward the expected involution of the mammary gland that typically occurs at the end of the lactation cycle. The intensive selection of modern cows for high milk yield and persistency allows induction of abrupt involution at the end of lactation, whereas, in some cows, this procedure is less acute due to metabolic adaptation (Silanikove et al., 2005; Leitner et al., 2011, 2012). This unique situation in dairy cows allows comparison between abrupt and natural-like involution at the same stage of lactation and with low within-group variability in terms of milk yield.

The aims of the present experiment were to test the following hypotheses: (1) that involution of the mammary glands in cows undergoing natural-like involution (sudden cessation of milking in late-lactating cows with high genetic potential for milk yield but producing low amounts of milk toward drying-off) is preceded by metabolic and immunological adaptations; and (2) that the involution induced in nonadapted cows (sudden cessation of milking in high-producing cows) is distressing and distinctly different from that in cows undergoing natural-like involution.

## MATERIALS AND METHODS

### *Animals, Management, and Experimental Setup*

The experiment was carried out with 10 multiparous lactating Israeli Holstein cows (in their second and third lactations) at 301 ± 6 d in lactation (i.e., ~60 d before expected parturition) that were scheduled for drying-off treatment (cessation of milking to induce involution). The experiment was carried out during December with average maximal ambient temperature of 20 ± 4°C and average minimal temperatures of 7 ± 3°C. Throughout their lactation, cows were fed a typical Israeli TMR that comprised 65% concentrates and 35% forage and contained 17% protein. The estimated $NE_L$ was 1.56 Mcal/kg and it was based on the estimated TDN from feed analysis (provided by the feed supplier) at 3× maintenance intake using NRC (2001) prediction equations.

At entering drying-off, feed changed to 35% concentrates and 65% forage, and contained 13.5% protein. The cows were milked thrice daily, at 0500, 1300, and 2000 h, and their milk yield was automatically recorded (Leitner et al., 2006).

In the present lactation, the milk yield of all enrolled cows was 10,490 ± 650 L, which was very similar to the average farm yield of 10,520 ± 520 L over 305 d. The mammary glands of all enrolled cows were ascertained as being free of bacterial infection, as previously described (Leitner et al., 2006). The health history and lactation curves of the enrolled cows were closely monitored to ensure that their milk yield at the start of the experiment was not influenced by health problems such as acute mastitis and lameness.

Five of the cows exhibited a sharp, spontaneous reduction in milk yield at the end of their lactation and produced <14 L/d and were defined as cows approaching natural-like involution (**ANI**), and 5 cows that produced between 25 and 35 L/d were defined as cows approaching forced involution (**AFI**).

Three days before scheduled drying-off, milking was stopped and milk samples were collected from each quarter of each enrolled cow by evacuating the whole gland on d 0 (before last milking) and 1, 2, and 3 d thereafter by hand milking. Following the last sampling, all cows were dried off and received antibiotic treatment (cefquinome, 150 mg; Intervet, Boxmeer, the Netherlands), as practiced by the herd veterinarian (Leitner et al., 2006). In addition, milk from 3 cows in mid lactation (156 ± 55 d) was used to obtain values on the antibacterial properties of normal (mid lactation) and bacteria-free milk. These cows had SCC of 75 ± 7 × $10^3$ cells/mL and were free of bacterial infection.

All protocols were approved by the Institutional Animal Care Committee of the Agricultural Research Organization (Bet Dagan, Israel), which is the legitimate body for such authorizations in Israel.

### *Sampling and Laboratory Procedures*

Representative samples (~100 mL) of mammary gland secretions were taken from the whole yield of each gland (i.e., 5 cows × 4 glands/cow × 4 samples taken on d 0, 1, 2, and 3 = 80 glands per treatment) during the morning milking. Analytical procedures that used antibodies were performed in duplicate, whereas all the