DRY YOUR BEST

Physiology of the mammary gland at dry-off: What do we really know?

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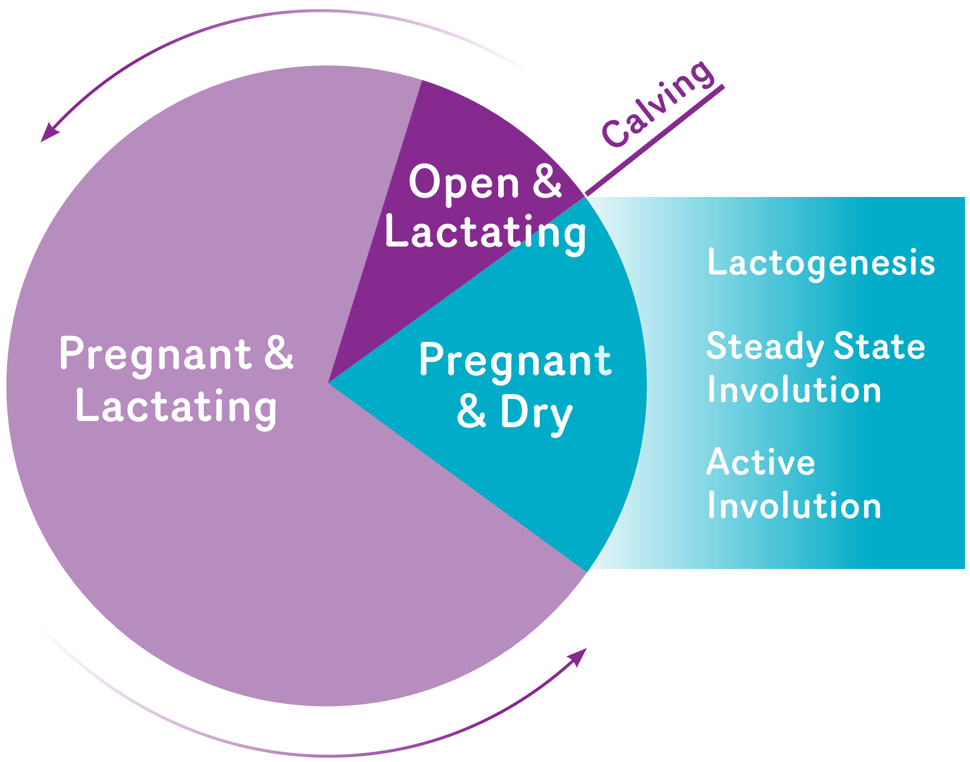
The bovine mammary gland undergoes dramatic changes during the production cycle of the cow. From the drying-off to calving, the mammary gland will be required to stop milk production, rest and just before calving be prepared for the next lactation.

Key messages:

- The mammary gland involution is a complex multistep process that happens during the dry period.
- Any factors, such as shortened dry period (<30 days), that interfere with the involution process will adversely impact milk production in the subsequent lactation.
- From the udder health perspective the dry period is a critical period in terms of risk of intramammary infections
- The mammary gland immunity has several layers of defense: physical barriers (teat end), cellular immune mechanisms and soluble immune factors
- Speed of involution is related to optimal tissue regeneration and efficiency of local defence mechanisms

Functional Transitions of the Mammary Gland

The bovine mammary gland is a dynamic organ that undergoes dramatic changes in functional activity during the production cycle of the cow. The shift of the mammary gland from a relatively dormant phase in non-lactating animals to vigorous milk synthesis and secretion during lactation is highly dependent upon hormonal, nutritional, and neurohormonal influences during successive reproductive cycles [2, 4, 12]. Mammary gland involution can be characterised as a complex multistep process leading to the termination of lactation or the dry period of dairy cows. Many studies have clearly documented the importance of the dry period for optimal milk production in the subsequent lactation. Although the optimal length will vary to some extent depending on numbers of lactation cycles, a dry period of 45-60 days between lactations is generally recommended to prevent milk production losses in a subsequent lactation [5]. Benefits derived from a dry period involve more than improvements in the cow's nutritional status for the forthcoming lactation [4]. Favorable effects of involution on subsequent milk yield results from regeneration and/or reactivation of secretory epithelium before the next lactation begins and optimising host defences against mastitis-causing pathogens.

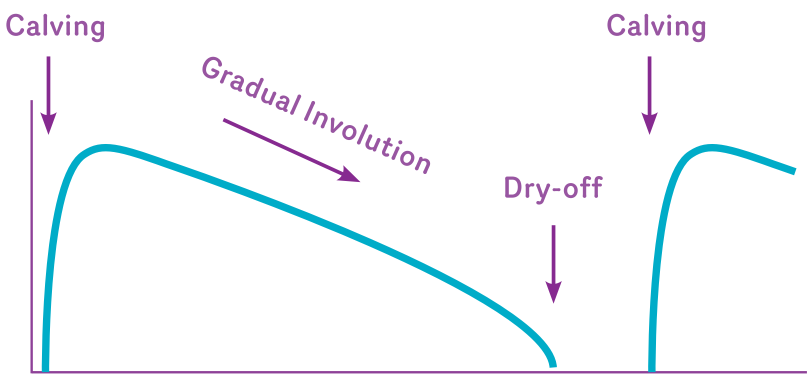


Functional Transitions of the Mammary Gland

Physiology of Involution

The mammary gland is a complex organ composed of a network of cells and supportive stroma that communicates to control the various cycles of involution and lactation. Biosynthetic activity of alveolar epithelial cells and the total population of such cells play decisive roles in determining milk yield. Bovine mammary gland involution progresses through two distinct stages including a gradual decline in milk production following peak lactation and abrupt cessation of the milking. The gradual decline in milk yield following peak lactation is due, in part, to a decrease in mammary cell numbers. The loss of alveolar epithelial cells during the declining phase of lactation is a result of programmed cells death or apoptosis [13]. Concurrent pregnancy can also influence milk yields during the declining phase of lactation. Although the precise mechanisms are not completely understood, placental-derived increased in plasma estrogen around mid-pregnancy may influence persistency of milk yield following peak lactation [2].

Gradual decline in milk yield along the cow productive cycle



The most rapid changes in bovine mammary tissue morphology and function during involution occur at the time of abrupt milk cessation (dry period). Milk stasis and distension of secretory tissues are factors thought to contribute to mammary involution through local chemical feedback by milk constituents, formation of other inhibitory factors in secretions, and mechanical stress to cells can lead to the loss of secretory function [3,7]. The complete transition from a lactating to nonlactating state is thought to be completed 21 to 30 days after the abrupt cessation of lactation.

Mammary Gland changes during the cow productive cycle

Gradual involution

- Gradual decline following peak lactation
- Some decrease in total cell numbers
- Concurrent pregnancy – hormonal influences

During the dry period

- Abrupt cessation of lactation
- Milk stasis and distension of secretory tissues
- Inhibitory factors in secretion – negative feedback
- Essential for maximal milk yields

In the first few days of the dry period the synthesis and secretion of milk constituents such as casein, lactose and fat decreases. Conversely, concentrations of immunoglobulins, lactoferrin, sodium, chloride, bicarbonate and serem albumin increases when lactation ceases. [7,10].

Several morphological changes in bovine mammary tissues also occur within days of milk cessation. In contrast to the extensive tissue degeneration observed in rodents, the bovine mammary epithelial cells appear to maintain some synthetic and secretory activity throughout the nonlactating period. Bovine alveoli exhibit small lumina that contain electron-dense proteinaceous material and undifferentiated epithelial cells [9]. Within the first week of the non-lactating period, diminished secretory activity is evidenced by reduction in alveolar luminal area with a concomitant increase in stromal area. Stromal areas increase proportionately to compensate for reduced alveolar luminal area once milk synthesis decreases and mammary fluid is resorbed [7, 9]. An increase in the prevalence of nonactive secretory epithelial cells can be observed during the first few weeks of the dry period [9].

Indicative of changes in milk synthesis and secretion

Milk Component	Active Involution
Lactose	decreases
Casein	decreases
Milk Fat	decreases
Immunoglobulin	increases
Lactoferrin	increases
Sodium	increases
Chloride	increases
Bicarbonate	increases
Serum Albumin	increases

Oliver & Sordillo, 1989, J Dairy Sci 72 = 1647. Sordillo et al, 1987, Int J Biochem 19:1165.

Thus, changes in bovine mammary gland morphology during the dry period reflect changes in secretory state versus tissue regression associated with fully involuted rodent mammary glands. One explanation for these species differences is related to the degree of tissue remodeling that occurs during the nonlactating period. In mice, the rapid loss of tissue function is due to massive loss of epithelial cells due to widespread apoptosis and autophagy. In dairy cows, however, there is an increase in epithelial cell turnover characterized by an increase in both apoptosis and proliferation [13]. During the early dry period, cell turnover is characterized by a greater rate of apoptosis whereas the perinatal period is characterized as a greater rate of cellular regeneration [13]. Concurrent gestation and active milk secretion in cows are likely to have a pronounced impact on the relative degree of tissue remodeling that occurs during mammary gland involution when compared to other species [3].

Mastitis Susceptibility during the Dry Period

An adequate dry period is necessary for sustainable milk production and any situations that interfere with epithelial cell turnover during the dry period can have detrimental effects on subsequent lactation performance. Ample evidence exists that bovine mammary glands are highly susceptible to new intramammary infections during the physiological transition from lactation to the dry period and from the dry period to early lactation [6]. In contrast, the fully involuted mammary gland in the mid-dry period is highly resistant to new intramammary infections. Many infections that originate during the nonlactating period persist into the subsequent lactation and are a major cause of clinical disease. Moreover, mastitis that occurs during the dry period significantly reduce milk yield after parturition, and the decrease in production appears to be related to the duration of infection [6]. Increased susceptibility to new intramammary infections during the early nonlactating and peripartum periods has been attributed to the relative capability of the mammary gland defense systems [1]. Therefore, it is not surprising that considerable research efforts have been focused on defining how mammary gland defences change as a consequence of lactation cycle and understanding those factors that may contribute to immune-dysfunction during this critical period [1,11].

The mammary gland is protected by a variety of defense mechanisms that can be broadly separated into:

- *physical barriers*
- *immune cell populations*
- *soluble defences* [11].

Several of these defense mechanisms are compromised during the early and later stages of the dry period that can account for the increased susceptibility to mastitis. For example, the teat end is considered the first line of defense against invading bacterial. After the last milking of lactation, at least three important changes to the teat end may occur that may adversely affect resistance to new intramammary infections:

1. Bacteria are no longer flushed from the mammary gland during the milking process;
2. Teat ends are no longer disinfected with antibacterial dips on a daily basis to reduce bacterial exposure;
3. Increased intramammary pressure can cause milk leakage that can facilitate bacterial penetration of the streak canal.

Once bacteria are able to penetrate the teat end barrier, it is then the functional capacity of the mammary gland's cellular and soluble immune components that determine if new intramammary infections occur.
The efficiency of local mammary gland defences is diminished during the functional transition of the mammary gland due in part to endocrine changes, physiological stresses, and energy imbalances [8]. During the early stages of the dry period, for example, impaired host defences can be attributed to milk stasis. There is a dramatic increase in somatic cell counts following milk stasis where neutrophils and macrophages tend to be the dominant cell types.

Approximate changes in cellular composition of milk

Cell Type	Lactation	Involution	Lactogenesis
Neutrophils	25%	30%	25%
Macrophages	35%	35%	40%
Lymphocytes	40%	35%	35%

Studies suggest that the ability of neutrophils and macrophages to engulf and kill bacteria are diminished during the early dry period due to an indiscriminant ingestion of milk components (fat and casein). The concentrations of opsonizing immunoglobulin isotypes and complement are also very low during the early dry period that may further contribute to diminished neutrophil functions. Thus, phagocytosis by neutrophils and macrophages will be more efficient when mammary gland are fully involuted because of increased numbers of these phagocytic cells, lower concentrations of fat and casein that need to be eliminated, and higher concentrations of immunoglobulins and complement that will enhance bactericidal activities [6,7,10].

There is some evidence to suggest that infiltrating neutrophils not only play a role in local mammary gland defence, but also in the remodeling of the mammary gland during the dry period [13]. The higher rates of apoptosis during involution may divert neutrophils to eliminate apoptotic epithelial cells during active cell turnover, thus making them less available to confront invading pathogens.

Lactoferrin is another important defence system of the bovine mammary gland that is directly related to disease susceptibility during the dry period. Lactoferrin concentrations are highest in the fully involuted mammary gland. Lactoferrin is thought to participate in the defence of involuting mammary glands by its ability to bind iron with great affinity and thus, inhibiting bacteria that requires iron for growth [10]. The interaction of citrate and lactoferrin in bovine mammary glands is of considerable importance since both compounds sequester iron [7]. In the case of citrate, however, the citrate-bound iron can be preferentially utilised by bacteria for growth. Citrate and lactoferrin are reciprocally related and the relatively higher concentration of citrate in milk and colostrum appear to diminish the bacteriostatic capabilities of lactoferrin during the early and later stages of the dry period [7,10].

Soluble Defences

Immune Component	Active Involution	Steady State Involution	Lactogenesis	Lactation
Immunoglobulin	increases	high	high	low
Citrate	decreases	low	high	high
Lactoferrin	increases	high	high	low

Sordillo et al, 1987, Int J Biochem 19:1165.

Future Considerations

Current information suggests that the speed and efficiency of involution is directly related to optimal tissue regeneration, efficiency of local mammary gland defences, prevention of mastitis and maximising of milk yields in the ensuing lactation. Although a dry period of 60 days is generally considered ideal, it has become increasingly difficult to achieve this goal as the genetic potential for milk production continues to increase. Depending on the level of milk production, there are several methods used to stop milk production at the end of lactation including the abrupt cessation of milking or to intermittently milk cows on a fixed schedule to gradually lead into a final milking. In both cases, dry cow antibiotic therapy is usually administered immediately after the last milking.

Although there is no single regime to end lactation that has been widely adopted by the dairy industry, strategies that decrease milk production at the time of dry off is essential to the regeneration of mammary tissues and for maximal protection against mastitis-causing bacteria. Thus, protocols that hasten the involution process at the cessation of lactation could greatly improve the quantity and quality of milk in the ensuing lactation.

A more thorough understanding of the physiological and immunological events that occur during the early dry period could provide more effective means of hastening the involution process in high-producing dairy cows.

References

1. Aitken, S.L., C.M. Corl, and L.M. Sordillo, Immunopathology of mastitis: Insights into disease recognition and resolution. Journal of mammary gland biology and neoplasia, 2011. 16(4): p. 291-304.

2. Akers, R.M., Major advances associated with hormone and growth factor regulation of mammary growth and lactation in dairy cows. J Dairy Sci, 2006. 89(4): p. 1222-34.

3. Capuco, A.V. and R.M. Akers, Mammary involution in dairy animals. J Mammary Gland Biol Neoplasia, 1999. 4(2): p. 137-44.

4. Drackley, J.K., S.S. Donkin, and C.K. Reynolds, Major advances in fundamental dairy cattle nutrition. J Dairy Sci, 2006. 89(4): p. 1324-36.

5. Kuhn, M.T., J.L. Hutchison, and H.D. Norman, Dry period length to maximize production across adjacent lactations and lifetime production. J Dairy Sci, 2006. 89(5): p. 1713-22.

6. Oliver, S.P. and L.M. Sordillo, Udder health in the periparturient period. J Dairy Sci, 1988. 71(9): p. 2584-606.

7. Oliver, S.P. and L.M. Sordillo, Approaches to the manipulation of mammary involution. J Dairy Sci, 1989. 72(6): p. 1647-64.

8. Sordillo, L.M. and V. Mavangira, The nexus between nutrient metabolism, oxidative stress and inflammation in transition cows. Animal Production Science, 2014. 54(9): p. 1204-1214.

9. Sordillo, L.M. and S.C. Nickerson, Morphologic changes in the bovine mammary gland during involution and lactogenesis. Am J Vet Res, 1988. 49(7): p. 1112-20.

10. Sordillo, L.M., S.C. Nickerson, R.M. Akers, and S.P. Oliver, Secretion composition during bovine mammary involution and the relationship with mastitis. Int J Biochem, 1987. 19(12): p. 1165-72.

11. Sordillo, L.M. and K.L. Streicher, Mammary gland immunity and mastitis susceptibility. J Mammary Gland Biol Neoplasia, 2002. 7(2): p. 135-46.

12. Svennersten-Sjaunja, K. and K. Olsson, Endocrinology of milk production. Domest Anim Endocrinol, 2005. 29(2): p. 241-58.

13. Zarzynska, J. and T. Motyl, Apoptosis and autophagy in involuting bovine mammary gland. J Physiol Pharmacol, 2008. 59 Suppl 9: p. 275-88.

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