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Cellular Defense Mechanisms in the Udder and Lactation of Goats

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ABSTRACT: Migration of neutrophils into mammary tissue provides the first immunological line of defense against bacteria that penetrate the physical barrier of the teat canal. Evasion of neutrophil defenses provides an opportunity for invading bacteria to become established. Depletion of neutrophils results in a dramatic increase in susceptibility to intramammary infection. Numerous cytoplasmic particles are shed from the apical surface of mammary secretory cells during milk secretion in goats. Only those counting methods that are specific for deoxyribonucleic acid can distinguish cell-like particles from somatic cells and thereby give reliable estimates of somatic cell numbers in goat milk. Unlike in milk from dairy cows, the somatic cell count in goat milk is influenced by the presence of nucleated cytoplasmic particles, stage of lactation, parity, and caprine arthritis-encephalitis. Investigations indicate that a dry period is necessary for optimal milk production in dairy cows but may not be necessary in goats. However, in many other respects regulation of bovine and caprine lactation seems to be quite similar. Studies have demonstrated additive galactopoietic effects of growth hormone and frequent milking in both species and a recently isolated chemical feedback inhibitor of lactation seems effective across both species. Increasing lactational performance has the potential for decreasing milk somatic cell counts in late lactation.

Key Words: Goats, Mastitis, Milk Somatic Cells, Lactation, Somatotropin, Milking Interval


Introduction

The first line of defense against mammary infection is the teat canal. Bacteria that pass this barrier and enter the teat cistern meet the second line of defense: phagocytic leukocytes. Phagocytes, consisting of neutrophils and macrophages, ingest and kill mastitis pathogens. Neutrophils exhibit directed migration toward chemical messengers produced by invading organisms, resulting in accumulation of neutrophils in the milk. This review will explore mechanisms regulating migration of neutrophils into mammary tissue, factors controlling survival and function of neutrophils after they leave the circulation, and the importance of this process for maximizing milk production.

Somatic cell counts in milk from goats are higher than somatic cell counts in milk from cows. Although it is recognized that increased cell counts in cow milk result in decreased milk yield, there is no evidence to indicate that this situation exists in goat milk production. Several factors contribute to this elevated cell count. Milk secretion in the goat is apocrine, compared to merocrine in cows, and results in the shedding of cytoplasmic particles into milk. Cytoplasmic particles in the size range of milk somatic cells commonly found in goat milk can be mistakenly counted as somatic cells. Further, neutrophils make up 50 to 70% of the somatic cell count in milk from goats free of intramammary infection, whereas neutrophils only make up 5 to 20% of the total cell count in bovine milk. Unlike in milk from cows, cell counts in goat milk increase with stage of lactation and parity.

In addition to the importance of leukocyte function to udder health and milk production, this review will consider factors that influence milk production during the lactation cycle of goats. The relative importance of a dry period for cows and goats will be compared and a combination of galactopoietic treatments (e.g., growth hormone administration, increased milking frequency, or manipulation of the activity of feedback inhibitor[s] of lactation) to increase production efficiency and persistency will be discussed.
Neutrophil Defense of the Mammary Gland

Migration (diapedesis) of neutrophils into mammary tissue provides the first immunological line of defense against bacteria that penetrate the physical barrier of the teat canal. After an inflammatory response is initiated, neutrophils are the first cells to be recruited to sites of infection. Evasion of neutrophil defenses provides an opportunity for invading bacteria to become established. Depletion of neutrophils results in a dramatic increase in susceptibility to intramammary infection (Schalm et al., 1976).

The nursing or milking stimulus induces directed migration of fresh neutrophils into mammary tissue (reviewed by Paape et al., 1992). Thus, the normal sterile mammary gland is supplied with a constant source of neutrophils. However, once in the lumen of alveoli, ingestion of fat and casein causes a loss in phagocytic and bactericidal functions and leads to death of neutrophils (Paape and Wergin, 1977). Milking removes compromised neutrophils, which are replaced by healthy neutrophils, thus enhancing defense against bacterial infection. This phenomenon could partially explain the reduced incidence of clinical mastitis for cows milked four times a day compared to cows milked two times a day (Hillerton, 1991).

In healthy animals, production and destruction of neutrophils is tightly regulated, which keeps their number in blood, milk, and tissue constant (Jain, 1986). Neutrophils mature in the bone marrow and are released into the circulation, where they spend approximately 9 h before migrating into tissue (Carlson and Kaneko, 1975). Diapedesis of neutrophils into mammary tissue, where they survive for 1 to 2 d, occurs at a low level for immune surveillance. Senescent neutrophils are thought to undergo apoptosis (programmed cell death) before ingestion by macrophages (Squier et al., 1995). The significance of this process is that this prevents unwanted disintegration in vivo, which would cause release of toxic chemicals and cause mammary tissue damage. Neutrophils rapidly migrate into mammary tissue and milk in response to inflammation. Chemotactic factors released by infectious bacteria and other components of the immune system are the signals for neutrophil recruitment to sites of infection. However, this influx of neutrophils is a double-edged sword. They may cause an inflammatory reaction that results in the elimination of infection but also tissue damage that leads to fibrosis and impaired mammary function (Nickerson and Heald, 1981; Capuco et al., 1986; Akers and Thompson, 1987). Neutrophils promote tissue injury and disturbed mammary function via 1) reactive oxygen metabolite generation (the respiratory burst) and 2) granular enzyme release (degranulation) (Miller et al., 1993; Kehrli and Shuster, 1994). Peroxynitrite, produced during inflammation, has recently been implicated as a major cytotoxic agent. Peroxynitrite (ONOO⁻) is the reaction product of superoxide (O²⁻) and nitric oxide (NO). Pathological conditions such as activation of inflammatory cells induces tissues and phagocytic cells to produce superoxide and nitric oxide, leading to the formation of peroxynitrite (Ischiropoulos et al., 1992).

Ingestion of pathogens by neutrophils is mediated by antibodies (immunoglobulins) and complement that bind to bacteria in a process called opsonization. Neutrophil receptors for immunoglobulins and complement act as bridges between the neutrophil and pathogen. Neutrophils bind immunoglobulins and complement in the circulation. Some bound ligands are removed and new immunoglobulin and complement receptors are expressed during diapedesis (Berning et al., 1991; DiCarlo and Paape, 1992; Worku et al., 1994a; Paape et al., 1996). Also, interferon-gamma, a T-cell-derived cytokine secreted in response to inflammation, induces a 4.5-fold increase in IgG2 receptors (Worku et al., 1994b). Thus, the neutrophil now has more receptors for a more efficient recognition of opsonized bacteria, resulting in a more rapid ingestion and elimination of invading pathogens. During phagocytosis of pathogens, cytosolic granules fuse with the invaginating plasma membrane to form the phagolysosome, into which they release their contents, thereby creating a highly toxic microenvironment (Paape and Wergin, 1977).

Monoclonal antibodies were developed to neutrophil surface antigens that identified four subpopulations of circulating neutrophils (Guidry et al., 1992). The physiological significance of neutrophil heterogeneity is not understood, but inflammation of the mammary gland alters the distributions of subpopulations in the circulation (Jain et al., 1991). Under normal conditions, blood may contain a mixture of normal, primed, activated, and senescent neutrophils. Further, not all of the circulating neutrophils are phagocytically, chemotactically, and oxidatively active, and differences exist among individuals in these neutrophil functions (Paape et al., 1978; Salgar et al., 1991; Berning et al., 1993). This variation might partially explain the difference in susceptibility to intramammary infection that exists among animals within a species.

Lactating dairy goats are exposed to thousands of Gram-negative bacteria and harbor many potential pathogens on their skin and mucosal surfaces. Despite this high rate of exposure, intramammary infections by this class of bacteria is low compared to infections by Gram-positive bacteria. On average, 1% of the mammary glands in a dairy goat population have intramammary infections with Gram-negative bacteria, compared to an infection rate of 15 to 55% with Gram-positive bacteria (Dulin et al., 1983; Poutrel and Lerondelle, 1983; Contreras et al., 1995). It has been estimated that 2,000 to 4,000 milkings are required to observe one new intramammary infection by a Gram-negative organism (Eberhardt, 1977). For Gram-positive organisms this estimated frequency is 1 in 600 milkings (O’Shea, 1985).
Several human cell-surface receptors on leukocytes (CD14, CD18, and carbohydrate) have been identified as crucial in the control of infections by Gram-negative bacteria (Anderson and Springer, 1987; Boner et al., 1989; Maliszewski and Wright, 1991). Lipopolysaccharide (LPS) is a complex glycolipid released by Gram-negative bacteria. Biological effects of LPS include the induction of endotoxin-shock syndrome, nonspecific activation of the immune system, and activation of the complement cascade (Van Miert, 1991). The CD14 receptor is a 53-kDa phosphoinositol (PI)-linked protein known to be present on human monocytes and macrophages and, to a lesser degree, on neutrophils (Jayaram and Hogg, 1989; Landmann et al., 1991). It binds LPS with a 60-kDa serum acute phase protein, LPS-binding protein (LBP) (Shumann et al., 1990). Release of the cytokine tumor necrosis factor (TNF) is due to the binding of the LPS-LBP complex to the CD14 molecule (Shumann et al., 1990; Dentener et al., 1993). Tumor necrosis factor is a potent activator of leukocytes and enhances the phagocytosis and killing of mastitis pathogens by bovine neutrophils (Kabbur and Jain, 1995). Under defined conditions, the CD14 receptor can also mediate phagocytosis of LBP-coated Gram-negative bacteria (Wright et al., 1989).

Recent findings indicate that bovine neutrophils have the capacity to bind antihuman CD14 and CD18 monoclonal antibodies, indicating that CD14 and CD18 on human and bovine neutrophils share a common antigenic determinant (Paape et al., 1996). In this study, it was also shown that a large number of neutrophils in milk of normal mammary glands expressed CD14 and at a higher density than did blood neutrophils. Endotoxin-induced migration did not cause increased expression of CD14 on mammalian neutrophils compared to blood neutrophils. Thus, it seems that factors present in milk of normal mammary glands upregulate expression of CD14. Endotoxin-induced migration also increased the percentage of neutrophils expressing CD18 compared to mammalian neutrophils before injection of endotoxin. Similar to results obtained with CD14, it also seems that factors in milk upregulated expression of CD18. Ability of neutrophils to adhere to and phagocytose Escherichia coli was observed in the absence of opsonins (Paape et al., 1996). Lectin-carbohydrate interactions also seemed to be important in regulating opsonic phagocytosis of E. coli by neutrophils. These recent findings indicate that mammary neutrophil cell surface CD14, CD18, and carbohydrate receptors may be important in controlling infections by Gram-negative bacteria such as E. coli.

### Somatic Cells in Goat Milk

Somatic cells are used as an index of milk quality for cow and goat milk. Milk somatic cell counts for uninfected goats are higher than milk somatic cell counts for uninfected cows (Dulin et al., 1983; Poutrel and Lerondelle, 1983). On average, somatic cell counts in milk from cows free from intramammary infection range from 40 to 80 × 10³/mL. In milk from goats free from intramammary infection counts range from 50 to 400 × 10³/mL. A number of factors have been reported to contribute to the high milk somatic cell counts for goats. Milk secretion in the cow is merocrine, and secretion in the goat is apocrine (Wooding et al., 1970). As a result, cytoplasmic particles are shed into milk from the apical portion of mammary secretory cells (Figure 1). Although the majority of these particles are generally anucleated, some of these particles have been observed to contain nuclear fragments (Figure 2). To what extent these nucleated cytoplasmic particles contribute to the total milk somatic cell count is presently unknown. Because of the presence of anucleate cytoplasmic particles, only cell counting procedures that are specific for DNA (Fossomatic electronic cell counter, direct microscopic cell counts using DNA specific stains) should be used for estimating concentration of somatic cells in goat milk (Table 1). Particle counters such as the Coulter electronic cell counter and direct microscopic cell counts using stains that are not DNA-specific should not be used (Dulin et al., 1982).

Other factors contributing to the high somatic cell count in goat milk are stage of lactation, parity, intramammary infection, and caprine arthritis-encephalitis virus infection (Dulin et al., 1983; Ryan et al., 1993; Zeng and Escobar, 1995). It was recently determined that more than 90% of the variation in milk somatic cell counts in goats was not due to intramammary bacterial infection (Wilson et al., 1995). They reported that increasing days in milk and month of the year were among the most important factors contributing to increased cell count in the absence of intramammary infection. To a lesser extent, parity and reduced milk production also contributed significantly to increased cell count. Interestingly, 75% of the variation in does free of intramammary infection was unexplained. It was hypothesized that this unexplained variation could be due to infections by mycoplasmas, caprine arthritis

### Table 1. Comparison of methods for estimating somatic cells in milk from 24 goat halves

<table>
<thead>
<tr>
<th>Cell counting method</th>
<th>Cells × 10⁵/mL milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMSCC-DNA specific stain</td>
<td>3.03b</td>
</tr>
<tr>
<td>Fosomatic cell counter</td>
<td>3.40b</td>
</tr>
<tr>
<td>Coulter cell counter</td>
<td>6.44c</td>
</tr>
<tr>
<td>DSMCC-nonspecific stain</td>
<td>7.92c</td>
</tr>
<tr>
<td>Standard error</td>
<td>1.13</td>
</tr>
</tbody>
</table>

*Adapted from Dulin et al., 1982.

*Means with same letter are not significantly different (P > .05).
Figure 1. Transmission electron micrograph of lactating goat mammary gland. The alveolar lumen (L) is bounded by epithelial cells whose apex has external microvilli (V) and internal granular accumulations (G) in the endoplasmic reticulum that resemble those found in the “cytoplasmic particles” (CP) of milk. Some of the cytoplasmic particles being shed from the apical surface of the secretory cells contain nuclear material (N). Bar = 10 μm.

and encephalitis virus and anaerobic bacteria. Unlike in milk from goats, somatic cell counts in milk from cows free from intramammary infection are not affected by stage of lactation and parity (Paape et al., 1975). Furthermore, the composition of the somatic cells in milk differs between goats and cows. For animals free of intramammary infection, neutrophils constitute 5 to 20% of the somatic cells in cow milk and 45 to 74% in goat milk (Dulin et al., 1983; Miller et al., 1991). This suggests that leukocyte migration into goat milk proceeds at a faster rate than migration into cow milk and may contribute to a naturally higher milk somatic cell count. Because somatic cell counts in goat milk are influenced by normal physiological factors that do not affect somatic cell counts in cow’s milk, regulating goat dairies based on standards established for cows is not appropriate.

The Lactation Cycle

Milk production of goats and cows typically peaks 3 to 4 wk after parturition and progressively declines thereafter. In contrast to other species, normal management of dairy cows and goats results in an
overlap of lactation and pregnancy. Cows are generally pregnant for 60 to 70% of their lactation, whereas goats, which are seasonal breeders in temperate climates, may be pregnant and lactating for the last 20% of the lactation cycle. Following cessation of milking before parturition, the mammary gland must prepare for the next lactation. Processes of mammary growth and differentiation occur during this nonlactating or "dry" period between successive lactations. Milk production efficiency can be increased by development and use of schemes that increase persistency of lactation and minimize the duration of the dry period.

In cows, a dry period of at least 40 d seems necessary to maximize milk production in the following lactation (Swanson, 1965; Coppock et al., 1974; Sorensen and Enevoldsen, 1991). This may be to permit restoration of body reserves before the next lactation, or to permit necessary growth and differentiation events within the mammary gland during this period. Although the data are not definitive, they strongly suggest that a dry period is necessary for reasons other than nutritional (Swanson, 1965; Smith et al., 1966, 1967). Recently, aspects of mammary growth during the dry period have been investigated in cows at the Beltsville Agricultural Research Center (Capuco et al., 1997). Multiparous Holstein cows were dried off 60 d before expected parturition or were milked twice daily during this prepartum period. Cows were slaughtered at 53, 35, 20, and 7 d prepartum and total mammary DNA and thymidine incorporation into mammary tissue slices was determined (Figure 3). There was no net loss of mammary cells (DNA) during the dry period, and total number of mammary cells increased with advancing stages of the dry period.
Figure 3. Proliferation of mammary cells during the dry period. Cows were dried off (all quarters) 60 d before expected parturition or were milked (all quarters) during the prepartum period. Panel A: Total mammary DNA content of udder-halves from dry (solid bars) and from lactating (cross-hatched bars) cows. DNA content did not differ between dry and lactating cows. Panel B: Incorporation of [3H]thymidine by mammary tissue from dry (solid bars) and from lactating (cross-hatched bars) cows. Incorporation by mammary tissue from dry cows was greater \((P < .05)\) than that from lactating cows.

Figure 4. Parenchymal volume of caprine udder-halves continuously milked during the prepartum period or dried off 24 wk before expected parturition. Volume was determined by magnetic resonance imaging. Adapted from Fowler et al., 1991.

One gland was milked during the prepartum period and the other was dried off 24 wk (170 d) before parturition. In this experiment, magnetic resonance imaging was used to monitor parenchymal volume as an index of mammary growth and involution (Figure 4). Mammary parenchymal volume in both udder halves decreased to the same minimal volume at wk 13. The volume then increased more rapidly in the dry gland than in the lactating gland, so that the dry gland had a significantly greater volume at parturition than the continuously milked gland. Parenchymal volume in the milked gland continued to increase during early lactation when parenchymal volume in the gland that had experienced a dry period declined. There was no difference in milk production between glands. Indeed, at no stage of lactation was milk yield of glands that had experienced a dry period numerically greater than that of continuously milked glands, even though those glands were larger than continuously milked glands during the first few weeks after parturition. These data suggest that a dry period is not necessary for optimizing milk production in goats. Why goats and cows seem to differ in this regard is an important question. However, the effect of a half-udder experimental design should be considered. It is plausible that milking one gland during the prepartum period inhibited the ability of the opposite gland to produce maximal quantities of milk during the subsequent lactation, or that milk production was increased in glands milked continuously when the opposite gland was dried off. When one of an udder’s mammary glands is no longer milked, milk production (Henderson and Peaker, 1983) and mammary growth (Capuco
and Akers, 1990) increase in a compensatory fashion in the lactating gland within that udder. Conversely, involu- 

tion is inhibited in the nonlactating gland (Turner and Reineke, 1936; Akers and Keys, 1984). The potential interaction of glands of differing lactational state within an udder on cell turnover within the glands is unknown. Despite the uncertainties, half-udder experiments with goats suggest that, unlike in cows, a dry period is not important for maximal lactation in goats. Additional study is warranted to examine potential species differences.

There is little information available to indicate whether changes in secretory activity during the course of lactation are the result of changes in cell number or cellular activity. Anderson et al. (1981) demonstrated that the number of mammary cells increases during early lactation in goats. Knight and Peaker (1984) determined udder volume by water displacement and obtained mammary biopsies at various times during first lactation. By extrapolating data from biopsies to the entire gland, they demonstrated that increases in milk production during early lactation are first the result of an increase in cell number followed by an increase in activity per cell. During the declining phase of the lactation curve, decreased cell number was the predominant component responsible for decreased milk yield. However, during late lactation, when the goats were pregnant, cell activity declined. Similar studies have not been performed in dairy cows.

Development of schemes to alter the lactation curve would provide methods for increasing milk production efficiency and profitability. Two potential methods have been investigated in recent years, growth hormone administration and milking frequency.

Growth hormone has been shown to be galactopoietic in cows and goats (Peel et al., 1983; Mepham et al., 1984). Several lines of evidence indicate that exogenous growth hormone does not act directly on mammary tissue (Gertler et al., 1983; Akers, 1985; McDowell et al., 1987). Rather, its galactopoietic effects are likely to be produced by effects on nutrient partitioning (Peel and Bauman, 1987) and through a hormone intermediary, such as the insulin-like growth factors, affecting mammary tissue (Gluckman et al., 1987; Prosser et al., 1994). Data indicate that although growth hormone administration is mammary gland cell activity declined. Similar studies have not been performed in dairy cows.

Growth hormone administration and increased milking frequency are combined, the treatments are additive but not synergistic (Knight et al., 1990, 1992). Data from Knight et al. (1990) are summarized in Figure 5. Beginning during midlactation (wk 19), goats were administered recombinant bovine growth hormone (.15 mg/kg BW) or vehicle. One gland of each goat was milked twice daily and the other three times daily. In addition to the additive effect of the treatments, data indicate that persistence of lactation was increased in glands of growth hormone-treated goats milked three times daily (Figure 5A). Although parenchymal growth occurred in response to increased milking frequency and growth hormone administration, the growth was due to hypertrophy rather than hyperplasia (Figure 5B). In no case did the quantity of DNA differ from that of the mammary gland sampled before treatment. Because glands did not differ with regard to thymidine incorporation rate, the differences in DNA content among glands at wk 22 can be attributed to treatment effects on longevity of existing epithelial cells. The retention of cell number in the glands of growth hormone-treated goats, milked three times daily, is consistent with the increased persistence of milk production in this group.

Increased milking frequency is hypothesized to increase milk production by lessening the accumulation of a feedback inhibition of milk secretion. Because frequent milking of one udder-half has no effect on milk secretion by the other half, it is clear that milk removal and not systemic effects of milk plays an important role in establishing secretion rate (Linzell and Peaker, 1971). By infusing isotonic sucrose into the udder of goats after milking, Henderson and Peaker (1984) demonstrated that physical distension does not cause the observed reduction in milk secretion toward the end of a milking interval. They concluded that accumulation and negative feedback by a local, chemical inhibitor is responsible. Indeed a 7.6-kDa polypeptide that inhibits milk synthesis in vitro and in vivo has been isolated from whey (Wilde et al., 1988, 1995). N-terminal amino acid analysis of the polypeptide yielded a sequence of 12 amino acids with no homology with other characterized milk proteins (Wilde et al., 1995). The putative feedback inhibitor of lactation (FIL) is secreted by mammary epithelial cells (Wilde et al., 1995) and rapidly and reversibly inhibits milk synthesis in vitro (Rennison et al., 1993). When FIL is infused into the mammary gland cistern, milk yield is decreased (Wilde et al., 1988, 1995). However, maximum inhibition of milk production is reached after 2 d and does not return to normal until the 4th day. These data suggest that if FIL is the endogenous feedback inhibitor of lactation,
Figure 5. Separate and additive effects of growth hormone administration and frequent milking on milk production and mammary DNA. Goats were administered .15 mg of bovine growth hormone (GH) per kg BW/d or vehicle. One gland of each goat was milked twice (2\times) and the other three times (3\times) daily. Panel A: Influence of treatment on milk production by the udder-half. Milk production was increased (P < .05) by 3\times milking and by GH treatment and the combined treatment was additive. Panel B: Influence of treatment on mammary DNA. With GH treatment mammary DNA was maintained, and without GH treatment, mammary DNA decreased (P < .05) compared with pretreatment. Adapted from Knight et al., 1990.

more needs to be learned about its metabolism in milk that would permit the rapid and reversible response of the mammary epithelium to changes in milking frequency.

Increased knowledge of mammary epithelial growth and function continue to hold out the promise of regulating the persistence and intensity of milk secretion.

Implications

Efforts are underway to reduce the current goat milk somatic cell count (SCC) standard of 1,000,000/mL to the 750,000/mL standard for cow milk. However, SCC for goats is naturally higher than SCC for cows, and it increases with stage of lactation. Milk secretion in the goat is apocrine, compared to merocrine secretion in cows, and results in the shedding of nucleated cytoplasmic particles into milk, which are included in SCC. Investigations of mammary proliferation, cell death, and differentiation during the dry period and lactation may provide a means to extend lactation and to limit the non-productive dry period. Furthermore, the combination of galactopoietic treatments (e.g., growth hormone administration, increased milking frequency, or manipulation of the activity of feedback inhibitor[s] of lactation) offer fertile areas for research to increase production efficiency and persistency and potentially lower milk somatic cell counts in late lactation.

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