Physiological Aspects of Bovine Mammary Involution: a Biochemical and Morphological Investigation.

Lorraine Marie Sordillo

Louisiana State University and Agricultural & Mechanical College

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Sordillo, Lorraine Marie, Ph.D.

The Louisiana State University and Agricultural and Mechanical Col., 1987
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PHYSIOLOGICAL ASPECTS OF BOVINE MAMMARY INVOLUTION:
A BIOCHEMICAL AND MORPHOLOGICAL INVESTIGATION

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Dairy Science

by

Lorraine M. Sordillo
B.S., University of Massachusetts, 1981
M.S., University of Massachusetts, 1984
August 1987
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<td>Changes in percent epithelial cell area composed of secretory vesicles from bovine mammary tissue by week of involution and infection status</td>
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ABSTRACT

Quarter milk secretion samples and blood serum for compositional analysis were collected weekly from 29 cows beginning at drying off and continuing until 2 wk postpartum. Quarter biopsies were taken from 5 additional animals at weekly intervals beginning at drying off through parturition. Histological and cytological parameters of tissues were correlated with biochemical characteristics of secretions. Increased tight junction permeability and decreased synthetic ability of secretory epithelium became evident by changes in mammary secretion composition and tissue morphology during the first 2 wk of involution. Somatic cell counts, serum albumin, lactoferrin, immunoglobulin G, pH, total protein, and serum concentrations of α-lactalbumin increased while fat, citrate, and the citrate to lactoferrin molar ratio decreased. Morphometric analysis of tissue demonstrated increases in stroma and nonactive secretory epithelium with decreases in epithelium, lumen, and fully active secretory epithelium during the first 2 wk of involution. Decreases in organelles associated with milk synthesis and secretion were observed also. These biochemical and structural changes reversed beginning 2 wk prepartum, and by parturition, cell function and structure was typical of lactating glands. Infected quarters had significantly higher somatic cell counts, pH, and percent polymorphonuclear leukocytes, but lower concentrations of lactoferrin and percent lymphocytes compared to uninfected quarters. Tissue from infected quarters also had less synthetic and
secretory ability with higher percentages of stroma and nonactive cells, but lower percentages of lumen compared to uninfected quarters. Plasma cell populations in bovine mammary tissue increased gradually from drying off, reached peak concentrations 2 wk prepartum, and dropped significantly during the last wk of gestation. Immunoglobulins G\textsubscript{1} and G\textsubscript{2} were the most numerous isotypes followed by immunoglobulins A and M. Immunoglobulin M cells were more numerous in tissue infected with minor pathogens than uninfected quarters. Ultrastructural examination revealed rough endoplasmic reticulum cisternae engorged with flocculent material indicative of antibody synthesis. Exposure to minor bacterial pathogens apparently elicited an immune response in nonlactating mammary tissue. These data provide information concerning the quantitation and distribution of components involved in the mammary immune system which may be manipulated to enhance the natural defense mechanisms of the involuted bovine mammary gland.
CHAPTER I

LITERATURE REVIEW

Introduction

Functional transitions of the mammary gland

Functional activity of the mammary gland varies from a dormant phase in nonlactating animals to a vigorous level during lactation. During successive reproductive cycles, transformation of mammary cells from an involuted to a secretory state is highly dependent on hormonal (20), nutritional (41,104), and neurohormonal (60,123) influences. Likewise, cessation of copious milk secretion following peak milk yield is under similar control. Involution in the rat results, in part, by a reduction in total secretory cell numbers as determined by mammary gland deoxyribonucleic acid (DNA) (84). Previous studies have reported considerable decreases in both cell size and number resulting from lysosomal digestion during rat mammary involution (31,33). Gradual reduction in milk yield following peak lactation in the cow may be attributed also to reductions in synthetic and secretory capabilities of remaining cells (63). Relatively little information is available regarding the demise of existing mammary secretory cells with each functional transition. Autoradiography of rat mammary glands suggests as much
as a 75% carry-over of secretory cells from one lactation to
the next (93).

The extent to which alveoli persist through involution into the
subsequent lactation in ruminant mammary glands is unknown.
Adequate proliferation and differentiation of mammary secretory
epithelium during the nonlactating period was shown to be essential
for optimal synthetic and secretory function in the ensuing
lactation of both cows and goats (73,115,116). Mechanisms which
regulate cellular differentiation and the onset of lactation need to
be further defined, and a greater understanding of these processes
may provide new approaches for increasing milk production in dairy
cattle.

Mastitis and the dry period

Although the dynamics of lactogenesis and involution are not
understood clearly, susceptibility of the bovine mammary gland to
bacterial infection is greatest during these functional transitions.
Neave and coworkers (67) found the incidence of new intramammary
infection (IMI) during the first 3 wk of involution to be 7 to 10
times greater than during lactation. Moreover, the new infection
rate during the dry period was thought to account for the level of
mastitis in subsequent lactations (106). Studies demonstrated that
unmilked quarters were more susceptible to IMI than milked quarters
as a result of cessation of the flushing action associated with the
milking process and fluid accumulation (69). Cows producing large
quantities of milk at drying off were found to be more susceptible
to new IMI during the early dry period (79). The high rate of IMI
during periods of mammary transition has been associated also with lower levels of natural protective factors in mammary secretion \( (81) \). The early dry period and the periparturient period are logical points of attack for the control of mastitis. Unfortunately, histochemical changes occurring within the udder during involution, and the interaction of IMI with involution are not understood. Further details concerning changes in both mammary morphology and biochemistry with respect to immunological function are warranted and may lead to innovative approaches for mastitis control.

**Physiology of Mammary Gland Involution**

**Cessation of milk synthesis and secretion**

Bovine mammary gland involution can be characterized as progressing through three distinct phases: a) gradual involution; b) initiated involution; and c) senile involution \( (45) \). Gradual involution occurs during the course of a normal lactation following peak milk yield. This is manifested by a decrease in milk yield which results presumably from a gradual reduction in total secretory cell numbers \( (65) \) and/or depressed synthetic activity of remaining cells as observed in mice \( (121) \). Initiated involution has a more drastic effect on the "drying off" process than does naturally-occurring involution following peak lactation. Abrupt cessation of milking in the goat was found to reduce mammary secretion rate up to 80% by day 3 of the dry period \( (25) \). Mammary
distension, with a concomitant increase in intramammary pressure, is thought to be responsible for arresting milk secretion in the goat (91). In addition, infiltration of phagocytic cells has been shown also to expedite the involution process in the rat (31,100). Gradual reduction in milk yield with advancing age or lactation number is referred to as senile involution. Changes in milk production are thought to be due to mammary gland deterioration and reduction in secretory tissue brought about by increased incidence of mastitis in older animals (45).

**Merits of a dry period**

A customary procedure implemented by dairy farmers is to initiate involution during the seventh to eighth month of pregnancy. Previous studies indicated that duration of the nonlactating period is related critically to secretory activity during the ensuing lactation (17,107,117). Dairy cows which averaged 10 to 40 days dry produced less milk in the following lactation than cows having a dry period of 40 to 60 days (17). Moreover, cows which were milked continually throughout pregnancy produced 33% less milk during the subsequent lactation compared to their twins with a 2 mo dry period (117). Benefits derived from a dry period involve more than improvements in the cow's nutritional status for the forthcoming lactation. Favorable effects of involution on subsequent milk yield result from regeneration and/or reactivation of secretory epithelium before the next lactation begins.

Biosynthetic activity of secretory cells and the total population of such cells play decisive roles in determining milk
yield. During lactation, total mammary DNA declines following peak lactation and continues to decrease as lactation progresses in mice (42). Because mitotic activity is absent in secretory cells during established lactation in mice (42), loss in cell numbers based on decreased DNA content cannot be replenished during lactation. The majority of cellular proliferation in ruminants, guinea pigs, and hamsters occurs in nonlactating mammary glands during pregnancy (2,4,105). Previous studies have found the greatest increases in mammary DNA content of goats (3), guinea pigs (4), and heifers (118) to occur in the last trimester of pregnancy and continuing occasionally to day 5 of lactation.

The plant alkaloid, colchicine, has been used extensively to examine factors which regulate milk production and mammary gland development. Mode of colchicine action is disruption of intracellular microtubular integrity necessary for mitosis (53) and exocytotic mechanisms (74,94). Prepartum intramammary infusion of colchicine in heifers altered secretion composition and lowered milk production in the subsequent lactation (1). Histological and cytological evidence in both bovine and caprine mammary glands supported the concept that prepartum colchicine treatment suppressed irreversibly differentiation of mammary epithelia (73,115). In a recent study, colchicine infusion during the last trimester of pregnancy in goats interfered apparently with mitosis during a period of active mammogenesis. Lower milk yields were observed with colchicine-treated udder halves, resulting presumably from a reduction in total secretory cell numbers (116). These findings support the contention that an adequate dry period is
essential for cellular proliferation, epithelial development, and optimal milk production.

**Functional morphology during involution**

Helminen and Ericsson (30,31,32,33) studied the histological and ultrastructural changes of rat mammary glands during involution. They found that once milk cessation occurred, the rat mammary gland became distended with milk, and alterations in milk producing cells became visible within approximately 24 h. Accumulation of milk in alveoli and ducts increased intramammary pressure, and caused degeneration of secretory cells with subsequent disruption of alveolar and lobular structures. Milk stasis became evident with an accumulation of fat droplets and secretory vesicles, and a reduction in size of the rough endoplasmic reticulum (RER) (31,32,99). As involution progressed, secretory and synthetic organelles became reduced substantially in size and number (31,99). Nuclei appeared pyknotic and the cytoplasm became vacuolated extensively with a concomitant increase in cytosomes. Macrophage-like cells containing numerous fat droplets appeared. By 48 h, an increase in autophagocytosis by lysosomes within the epithelium was found, accompanied by leukocytic infiltration and notable reduction in cell volume (33). After 72 h into involution, macrophages were observed often between epithelial cells and ingesting fragments of cellular debris (31). Degenerative cells were shed into alveolar lumens within 48 to 72 h after weaning, leaving only basement membranes intact (31,32). However, the rat mammary gland did not regress
entirely as many alveoli persisted. Many myoepithelial cells remained while secretory cells were eliminated, and appeared to play an important role in bridging gaps where necrotic epithelial cells had sloughed, thereby preventing total loss of organized structure (31).

In the fully involuted caprine mammary gland, total area of secretory tissue decreased proportionately to increased amounts of intralobular and interlobular connective tissue (115). In contrast to what was observed in the fully involuted rat mammary gland, sloughing of epithelial cells into alveolar lumina was not apparent in the goat. Instead, alveoli exhibited small lumina filled with electron-dense proteinaceous material, and alveolar epithelial cells were in an undifferentiated state.

Functional morphology during lactogenesis

Histological and cytological evidence showed lobulo-alveolar growth increased rapidly in cows between days 110 and 140 of gestation (125). Cowie (19) observed limited changes in the structure of primigravid goat mammary glands during the first half of pregnancy. However, a period of advanced alveolar growth occurred between 60 and 120 days of gestation (19). The periparturient period in both mice and goats was associated with intense mammary growth and rapid differentiation of secretory parenchyma (39,42,115). Prepartum goat mammary tissue exhibited characteristics indicative of copious milk synthesis and secretion, and as parturition approached, total area of stroma decreased with synchronous increases in luminal and epithelial areas (115).
Cytological examination indicated gradual differentiation of mammary epithelium during the last trimester of pregnancy with an increased cytoplasmic to nuclear ratio, a higher degree of cellular polarity, and more apically located secretory vesicles.

Association of corpora amylacea with nonlactating tissue

Corpora amylacea are spherical, lamellated inclusion bodies observed frequently in bovine mammary tissues. Notice of their appearance in bovine mammary glands dates back to the early 1900's (82). Biochemical analysis found bovine corpora amylacea to be composed of dicalcium and monocalcium phosphates (28), alkaline and acid phosphatases, proteins, and lipids (52). Early morphological studies of bovine corpora amylacea indicated complex heterogeneous structures composed of a number of distinct concentric layers (59). Ultrastructurally, corpora amylacea appeared in 2 basic morphological forms. Dense bodies were deeply basophilic and displayed often several lamellated striations. Centrally located, casein-like material appeared to be deposited among fibrillar components of these amyloid bodies. These structures comprised 70% of the total amyloid population. Fibrillar bodies (30%) were less basophilic than dense forms and appeared to contain only amyloid fibrils. Fibrils were arranged in parallel arrays measuring approximately 10 nm in diameter and displayed often a filamentous network (10,76).

Occurrence of bovine corpora amylacea throughout the lactation cycle was reviewed extensively by Nickerson et al. (76). Prevalence
of corpora amylacea increased gradually from early to late lactation and ultimately peaked during early involution. As involution progressed, numbers of corpora gradually decreased toward lactogenesis.

Although the origin and demise of corpora amylacea are not understood clearly, previous research suggests they are derived from aggregation of casein micelles in alveolar milk. It has been postulated that mechanisms of aggregation in the initial stages of amylaceum formation involve co-precipitation of casein with calcium phosphate (10). This concept is consistent with theories of others (62,76) who suggested corpora amylacea developed from deposits of synthetic and secretory processes. Recent studies on growth patterns of bovine corpora amylacea suggest the development of these structures is not restricted to a particular stage of lactation, although nucleation appears to occur within alveolar lumens. Gradual increases in size and number of corpora from parturition to late lactation indicate that development of the structures accelerates as lactation progresses (114).

Amyloid concentrations found within the bovine mammary gland during lactation have been implicated in milk stasis and the onset of involution by filling luminal spaces and clogging small ducts (76). Accumulation of corpora during late lactation may interfere with mechanisms of milk synthesis and secretion, resulting in reduced milk yield up to 30% (14). It has been theorized that corpora diminish throughout the dry period by the phagocytic action of macrophages and multinucleated giant cells (MGC) (75). Phagocytosis appears to be instrumental in reducing concentration of
amyloid prior to the subsequent lactation and preventing accumulation throughout the productive lifetime of the animal.

Secretion composition of the nonlactating gland

Several biochemical changes in secretion composition occur following cessation of lactation. Synthesis and secretion of major milk constituents (casein, lactose, and fat) decrease considerably by the fourth day of involution in the cow (29,131). In the rabbit mammary gland, a substantial decrease in both casein messenger ribonucleic acid (mRNA) and its transcription can also be detected during earlier stages of initiated involution (120). Conversely, concentrations of immunoglobulins (Ig), sodium, chloride, bicarbonate, and bovine serum albumin (BSA) increase, and pH increases with cessation of lactation in cows (29) and goats (25,91). These compositional changes are correlated closely to the breakdown of secretory epithelium and reduced metabolic activity of remaining cells during involution.

In cows and goats, gradual increases in serum protein and ion concentrations in milk are synchronous with the period of mammary fluid volume reduction between the third and seventh day of involution (77,91,112) resulting in the concentration of the 2 components. Previous research suggested also that these changes may result from a loss of alveolar cell integrity allowing entry of plasma constituents and ions into alveolar lumina (25,40). Linzell and Peaker (49,51) examined changes in colostrum composition in the goat at about the time of parturition. They found that lactose and
potassium concentrations in the prelactating gland decreased when tight junctions between adjacent secretory cells became "leaky". In the lactating gland, tight junctions became impermeable, enforcing polarized transport of serum-derived components via a transcellular pathway, and resulted in increased lactose and potassium concentrations following parturition.

A continuous increase in concentration of milk constituents derived from de novo synthesis during the initial stages of involution suggests that the involutionary process also involves a change in alveolar cell activity as opposed to complete cellular dissolution. The iron-binding protein, lactoferrin (Lf), is a major whey protein in secretion of fully involuted bovine mammary glands (108). Lactoferrin is thought to be synthesized by secretory epithelium and, to a lesser extent, by polymorphonuclear neutrophilic leukocytes (PMN) (54,55). Lactoferrin concentrations increase by the fourth day of involution and remain elevated as involution progresses (129). Conversely, levels of citrate decrease gradually as the involutionary process continues. Milk citrate is formed from acetyl CoA and oxaloacetate within mitochondria, and secreted by vesicles derived from Golgi components (24,133). Citrate concentrations do not decrease until approximately 7 days after the onset of involution (81). This indicates that metabolic activity in secretory epithelial cells continues past the third, and up to the seventh day after drying off. However, the citrate to Lf molar ratio decreases gradually from drying off and continues to decline as the gland involutes (81).
Lactogenesis is initiated hormonally near the end of pregnancy, and is characterized by biochemical and morphological changes in the mammary gland which have been defined loosely in 2 stages (26). The first comprises the prepartum period during which cytological and enzymatic differentiation of alveolar cells is accompanied by appearance of precolostral fluid. Stage 2 begins just before parturition and is noted by the onset of copious colostral secretion (26,29). As parturition approaches, major changes in the composition of colostrum were demonstrated in cows (29), goats (51), and rats (13). However, only in goats (50,115) and rats (13) were biochemical changes correlated with structural differentiation of mammary cells. In prelactating glands, "leaky" tight junctions readily allow passage of sucrose, lactose, Ig, and sodium and chloride ions from blood to milk and vice versa. At parturition, junctions become less permeable and block paracellular movement of serum proteins and ions into milk (50). Instead, all transport is via the transcellular route resulting in decreased levels of sodium, chloride, and Ig, with increased levels of potassium, α-lactalbumin, and lactose (49,51). Recently, results have demonstrated changes in concentration of α-lactalbumin in dry cow secretions through parturition (35). The mammary gland synthesizes locally α-lactalbumin, and transient increases observed prepartum may reflect cellular redevelopment of mammary tissue as well as the onset of milk synthesis and secretion (35). These findings support the contention that changes in secretion composition following drying off and during lactogenesis result from changes in both the integrity of the blood-milk barrier and metabolic activity of
existing alveolar cells.

Susceptibility to Mastitis During the Dry Period

Economics of mastitis control

Mastitis is a general term which refers to an inflammation of the mammary gland. Most mastitis results from presence of living microorganisms within the gland. In the bovine, it has been estimated that approximately one half of the dairy cows world-wide have some form of mastitis (57). Apart from the debilitating effects of the disease on the animal, production losses have proven to be exorbitant. Dollar losses occur from costs of veterinary services and drugs, increased culling rate of chronically-infected animals, and discarding mastitic or antibiotic-contaminated milk (21). However, the greatest loss occurs from reduced milk production caused by subclinical mastitis. In fact, it has been shown that subclinically-infected quarters produce up to 45% less milk than uninfected quarters (92).

Factors affecting bovine mastitis

Current control programs consist of correct use of functionally adequate milking systems, disinfection of teats immediately after milking, prompt treatment of clinical cases, antibiotic treatment of all quarters at drying off, and culling of chronically-infected cows. Although these procedures have proven to be highly effective in lactating animals, most offer little protection against new IMI
during the dry period. Further progress in mastitis control procedures are clearly needed not only to reduce new infection rate, but also to eliminate existing infections.

Intramammary infections occur when microorganisms gain entrance to the gland via the streak canal and colonize the duct system and alveoli. Several vectors have been identified which facilitate penetrability of these organisms, i.e., environment, milking equipment, and milking hygiene (36). The internal environment of the gland is often favorable to survival and multiplication of invading pathogenic bacteria. Byproducts of bacterial growth and metabolism cause irritation to delicate secretory parenchyma, resulting in an inflammatory reaction. Changes in secretion composition include increases in leukocyte, Ig, ion (sodium and chloride), and trace mineral concentrations, with concomitant decreases in the concentrations of lactose, total protein, solids-not-fat, total solids, calcium, phosphorus, and potassium (71).

Research has indicated that susceptibility of the bovine mammary gland to bacterial pathogens is related critically to functional transitions that occur during involution and lactogenesis (22,67). While establishment of new IMI is greatest during the first 3 days following drying off (110), the lowest incidence of new infection occurs in the fully involuted gland approximately 3 to 5 wk following drying off (67). Moreover, the mammary gland is highly susceptible to Gram-positive pathogens during the early stages of the dry period (103,122) and highly resistant to Gram-negative pathogens in the fully involuted gland (6,23).
Reasons for the high IMI rate at drying off, and changes in susceptibility throughout the dry period are poorly understood. However, several studies have shown that unmilked quarters are markedly more susceptible to new infection than those milked at regular intervals (69,122). Because new infection rates during the dry period exceeded those of lactation, it was suggested that pathogens in the streak canal were not flushed out in the absence of regular milking. At drying off, teat sanitation is discontinued which may also have an effect on susceptibility to mastitis (36). This, however, does not explain the high rate of new infection during colostrogenesis. Susceptibility to infection may also be related to the size and shape of the streak canal. It has been suggested that heightened susceptibility in the early dry period and during colostrogenesis is due to the relative ease with which bacterial pathogens penetrate the streak canal. Temporary increases in intramammary pressure following cessation of milking may cause shortening and dilation of the streak canal, thus allowing penetration of bacterial pathogens (79). Bacteria inoculated into streak canals immediately after drying off multiplied and often penetrated the teat cistern, but were restricted to the site of inoculation or eliminated entirely from cows dry 28 days or more (18). It was postulated that changes within the streak canal, such as development of bacterial inhibitors, may make penetration more difficult in later stages of involution.

Progressive changes in the composition of mammary secretions during involution may also influence establishment of infection after bacterial penetration of the streak canal. Involved bovine
mammary glands appeared to be more susceptible to *Escherichia coli* and *Klebsiella pneumonia* (6,23) just prior to parturition as opposed to the early or mid dry period. This phenomenon was related to fluctuating citrate to Lf molar ratios during involution (5). Lactoferrin sequesters iron from the environment which is required by these bacteria for normal growth. Enteric bacteria also possess an iron-sequestering system involving citrate (111). The degree of growth inhibition when both citrate and Lf are present is related to their molar ratio. Therefore, as the citrate to Lf molar ratio decreased in late lactation and through involution, there appeared to be an increase in growth inhibition of coliform test strains *in vitro*. Conversely, a decrease in the citrate to Lf molar ratio, as encountered just prior to parturition, resulted in a subsequent increase in coliform growth.

Dry cow therapy is currently recommended for prevention and treatment of IMI during involution. However, because of the complex nature of mastitis and the diversity in organisms that cause infection, dry cow therapy products are not always effective in preventing new infections or eliminating those already in existence. Consequently, mastitis remains widespread in most dairy herds.

Manipulation of mammary physiology in an attempt to enhance natural protective systems associated with mammary tissue and secretion may provide an alternative to less effective mastitis control procedures. The natural defense mechanism of the mammary gland is a complex system which includes nonspecific resistance, antibody production, and cell-mediated immunity (CMI) (72,128). The efficiency of this defense system is a major factor which governs
establishment of mastitis. A better understanding of the immunological potential of the mammary gland could evolve into an effective, economical, and practical nonantibiotic mastitis control program. Widespread use of antibiotics and germicides for treatment and prevention of bovine mastitis has raised public concern. Improper use of mastitis treatment may cause residues in animal products and present serious health problems to consumers. Alternative methods of mastitis control which rely less on antibiotics would be advantageous from a public health standpoint.

Defense Systems of the Mammary Gland

Anatomical defense mechanisms

Nonspecific protective factors of the mammary gland include anatomic structures, phagocytic cells, and antibacterial proteins. The streak canal provides the primary line of defense against infection (64). Mastitis-causing organisms first must traverse teat end tissues to establish infection within the gland. It follows that susceptibility to new infection is influenced greatly by factors which increase survivability or penetrability of bacterial pathogens within the streak canal. This structure is surrounded by smooth muscle fibers which function in maintaining tight closure of the canal. The ability of certain bacterial pathogens to penetrate the mammary gland is related to the tonus of the sphincter muscles surrounding the streak canal (64). Cows with patent streak canals are more susceptible to mastitis (69).
Ultrastructural observations of the bovine streak canal revealed the mesh-like character of the keratin lining. This material fills the lumen of the canal and provides an effective barrier against pathogenic bacteria. It was demonstrated in cows inoculated experimentally with *Staphylococcus aureus* that the nature of keratin may inhibit progressive movement of cocci from the streak canal to the gland cistern (12). The keratin lining streak canals of susceptible quarters was found to be much thinner and less dense compared to resistant quarters (56). Moreover, removal of keratin from the streak canal of bovine mammary glands was found to increase susceptibility to *Streptococcus agalactiae* infection (64).

New IMI rates also appear to be related to bacterial populations to which teats of the dry udder are exposed. Neave and Oliver (68) demonstrated a positive correlation between the numbers of *S. aureus* applied to teats of dry cows and occurrence of new IMI. They also found that in the absence of repeated exposure during milking, *S. aureus* numbers on teat skin diminished greatly. Others have shown that cessation of milking favored penetration of Gram-positive cocci into the teat cistern (98). Lactating and early involuted glands appeared susceptible to *S. aureus*, but more resistant to *Streptococcus uberis*. However, isolations of *S. uberis* from teat skin and orifices increased greatly after 21 days into the dry period.

Reasons for the high rate of new IMI and changes in bacterial flora of teat ends during the dry period remain unclear. Findings suggest that Gram-positive bacteria adhere more readily to the ductal epithelium of bovine mammary glands (27). *S. aureus* and *S.
agalactiae colonize better on ductal epithelium than do Streptococcus faecalis, E. coli, or Corynebacterium bovis. Those organisms shown to adhere better are those which cause mastitis most often during the early dry period. Since unmilked quarters are more susceptible to new infection than those which are milked regularly, it follows that pathogens adhere more readily to the streak canal when the flushing action of the milking process ceases.

Cellular aspects of mammary immunity

Once bacteria breach the streak canal, they are attacked by a population of leukocytes within the mammary gland referred to as somatic cells. Electron microscopic studies have shown that mammary somatic cells include PMN, macrophages, lymphocytes, and a small percentage of epithelial cells (48). The concentration of cells in milk from uninfected glands is generally 1.0 x 10^5 to 3.0 x 10^5 cells/ml (72). In infected glands, bacterial products and factors released from affected tissues evoke inflammation resulting in migration of leukocytes from blood to milk with levels as high as millions/ml.

Phagocytosis of invading pathogens is considered the second line of defense against mastitis (89). Polymorphonuclear leukocytes and macrophages are the principal phagocytic cells and comprise 80 to 90% of the cells in uninfected bovine milk (48). It has been demonstrated that more than 5.0 x 10^5 leukocytes/ml of foremilk are required to protect against IMI (102). During the inflammatory process, PMN accumulate in mammary tissue and milk through the
process of chemotaxis. Breakdown products of epithelium, leukocytes, and bacteria serve as chemotactic agents to increase the influx of PMN. Leukocyte levels increase only after microbial populations have increased, causing tissue irritation and damage. A substantial time delay occurs between initiation of irritation in the mammary gland and appearance of PMN in milk (103). Although milk leukocytes are essential for defense against microbial invasion, the time lapse allows a sufficient period for bacteria to become established. Previous studies have also demonstrated deficiencies in the ability of milk PMN to phagocytose mammary gland pathogens (66, 101). Lower phagocytic and bactericidal properties of milk PMN, compared to blood PMN, have been attributed to: a) 38% reduction in milk PMN glucose (66); b) deficiencies in opsonins and complement in milk (132); c) binding of casein to PMN surfaces (101); d) loss of PMN pseudopods due to fat ingestion (130); and e) depletion of hydrolytic enzymes within PMN following fat and casein ingestion (95).

Macrophages may also play an important role in the phagocytosis and intracellular killing of invading microorganisms. These phagocytes are believed to be the first leukocyte type that bacteria encounter in previously uninfected quarters upon breaching the streak canal. Although PMN are most numerous in milk from infected glands, colostrum, and secretion during early involution (47), macrophages are the predominant cell type of uninfected lactating and nonlactating glands (38, 45). In the involuted mammary gland, macrophages actively ingest fat globules and appear often as large foamy cells (48). Bovine mammary macrophages bear Fc receptors for
IgG₁ and IgG₂ which promote ingestion and killing of bacterial pathogens as well (34). Although the major role of these cells appears to be removal of foreign material and cell debris, they also may play an important role in antigen processing, and in regulating the magnitude of lymphocyte response in the bovine (83). During involution, macrophages may make initial contact with, and present bacterial antigens to, lymphoid cells (126). Moreover, macrophages have been shown to enhance the transformation of blood lymphocytes in response to phytohemagglutinin in vitro (83).

Migratory lymphocyte populations constitute the cellular basis of mammary gland immunity. Data from CMI studies demonstrated that 73% T-lymphocytes and 27% B-lymphocytes comprise the total bovine peripheral blood lymphocyte population. Percentages of T- and B-lymphocytes found in normal bovine milk during lactation were approximately 50% and 20%, respectively (16). B-lymphocytes respond to antigenic stimulation by multiplication and differentiation into 2 morphologically and functionally discrete populations: plasma cells and memory cells. Antigens bind to specific surface Ig, leading to proliferation of sensitized B-lymphocyte clones. Some cells acquire RER and eventually develop into antibody-secreting plasma cells. The other population of cells derived from stimulated antigen-sensitive B-lymphocytes possesses Ig receptors of the same specificity as their parent. These are long-lived memory cells which have the ability to initiate a heightened response to a second dose of antigen (124).

T-lymphocytes react to specific antigen in a similar fashion by differentiation into 2 cell populations. Like the B-lymphocytes,
memory cells maintain sensitivity over an extended period of time and will respond to subsequent antigen exposure. However, instead of synthesizing antibody as B-lymphocytes, the other population of T-lymphocytes takes on an effector function and acts as both suppressors and helpers. Activated T-lymphocytes release lymphokines that enhance recruitment, activation, and immobilization of macrophages and PMN in infected tissue areas (119). T-helper cells release substances which stimulate B-lymphocyte response to antigenic stimulation.

**Humoral immune system**

Immunoglobulin concentrations of mammary secretion may play an important role in local immunity to infection. The origin of antibody in milk varies with Ig class. Both IgG_1_ and IgG_2_ are serum derived while IgA and IgM are of local origin (70). Immunoglobulin concentration in mammary secretion varies considerably throughout the lactation cycle and is dependent on the degree of vascular permeability of milk secreting tissue (72). Levels of IgG, IgA, and IgM are lowest during lactation (1 mg/ml) and increase gradually during involution and colostrogenesis (50 to 150 mg/ml) (8,43). Evidence suggests antibodies pass into secretion through mammary cells in small vesicles originating at the basal border. During inflammation, however, Ig levels are elevated due to increased tight junction permeability and passage of serum components into milk (128).

Mammary gland antibodies IgG_1_, IgG_2_, and IgM function by opsonizing bacterial antigens and facilitating phagocytosis by PMN.
Antigen-antibody immune complexes, either alone or with complement, can bind to Fc and C3b receptors on phagocytic cell surfaces (34). Activation of complement pathways can lyse and destroy pathogenic organisms. Bactericidal consequences of antibody-complement complexes are a function of bacterial cell wall thickness and are ineffective against Gram-positive bacteria. Although IgA does not function as an opsonin, it has been implicated in toxin neutralization, bacterial agglutination, and preventing bacterial adherence to cell membranes (78).

Chemical defense mechanisms

Nonspecific bacteriostatic proteins of mammary secretion include Lf, lysozyme, and the lactoperoxidase/thiocyanate/hydrogen peroxide (LP) system. Lactoferrin is a major whey protein in secretions of fully involuted bovine mammary glands (109). Lactoferrin concentrations become elevated by day 4 of involution and continue to increase linearly as involution progresses. Citrate to Lf molar ratio decreases gradually from drying off to day 7 of involution (81). Lactoferrin is bacteriostatic for a variety of bacteria because of its iron-chelating ability which makes iron unavailable for bacterial growth. Gram-negative bacteria have high iron requirements and are consequently more influenced by Lf concentrations than Gram-positive organisms (97).

Lysozyme hydrolyzes the 1-4,β-linkage between muramic acid and N-acetylglucosamine of bacterial cell wall peptidoglycan (127).
Concentrations of lysozyme are extremely low in both bovine milk and PMN; production is thought to occur via local synthesis or diffusion from blood. Although lysozyme levels are too low to be effective, it has been shown to lyse bacteria in the presence of complement, and after lysis, to stimulate opsonic activity of IgM, and increase the bactericidal activity of IgM plus complement (96).

The LP system in bovine lacteal secretion was shown to be bactericidal for Gram-positive and some Gram-negative bacteria (96). Lactoperoxidase is synthesized locally in mammary tissue, whereas thiocyanate (SCN⁻) is derived from serum as a result of glycoside hydrolysis. Hydrogen peroxide (H₂O₂) is not present in milk, but is produced metabolically by streptococci (96,97). The LP system inhibits bacterial growth when lactoperoxidase combines with H₂O₂ to oxidize SCN⁻. The resulting intermediary oxidation product then modifies the sulphydryl groups of bacterial cell membranes, which are necessary for glucose transport. Since Gram-negative organisms are catalase positive, exogenous H₂O₂ is required before the LP system can protect against IMI with E. coli.

Enhancement of mammary defenses

Although considerable information is available regarding natural defense systems of the mammary gland, a practical method of enhancing resistance to infection has not been elucidated. Several techniques for stimulating local immune mechanisms are currently being developed. Considering the importance of PMN in bacterial killing, attempts have been made to induce leukocytosis in the mammary gland to establish a protective PMN barrier. Infusion of
small amounts of *E. coli* endotoxin induced a PMN response which prevented subsequent establishment of experimental *S. agalactiae* infection (11). Other studies have established subclinical infection with *C. bovis* or *Staphylococcus epidermidis* which appeared to stimulate PMN influx upon subsequent exposure to more pathogenic bacteria (7) and provide resistance to infection by *S. aureus* (90).

Research efforts have been directed also toward eliciting local PMN migration within the gland cistern using an intramammary polyethylene device (IMD). The IMD is a sterile plastic loop which is inserted through the streak canal into the gland cistern where it remains for several lactations (86). The IMD provokes chronic increases in leukocyte numbers in foremilk samples to protective levels, e.g., > 900,000 cells/ml (87). Reports indicate that milk quality of IMD-fitted quarters is not affected adversely, and any loss in milk production is offset by preventing losses that would have resulted from infection (86,88). However, a more recent study reports significant decreases in milk yield for IMD-fitted glands (37). Further studies examining adverse side effects of IMD implantation are necessary before the devices can be used for mastitis control.

Attempts have been made also to increase nonspecific components of immunity during early involution when new infections are prevalent (81). The fully involuted mammary gland is highly resistant to IMI due to distinct changes in secretion composition. Mammary secretion from nonlactating cows contains elevated natural protective factors including phagocytes, lymphocytes, Ig, and bacteriostatic proteins such as Lf (45,112), and lower
concentrations of casein, lactose, and citrate which can be utilized for bacterial growth and colonization. These changes enhance resistance to mastitis. Intramammary injection of colchicine, plant lectins (concanavalin A and phytohemagglutinin), and endotoxin during the early dry period in cows have been shown to inhibit bacterial growth by accelerating involution, a process which occurs normally over several wk. These treatments resulted in increased levels of natural protective factors in mammary secretion during the period when the gland is most susceptible to new IMI (9,81). In a more recent study, pathogenesis of S. uberis infection in the mouse was modified effectively by intramammary injections of pokeweed mitogen (PWM) prior to experimental challenge (113). Immunostimulation of mammary glands with PWM at drying off accelerated mammary involution, enhanced antimicrobial defenses, and facilitated a marked cellular response which reduced the severity of experimental S. uberis infection (113).

Considerable effort has been directed toward development of a suitable vaccine against bovine mastitis. Unfortunately, several problems of vaccination during lactation are associated with mammary immunology: a) milk contains relatively few components of immune defense compared to secretion from the fully involuted gland; b) milk fat and casein have inhibitory effects on mammary PMN phagocytic efficiency; c) milk is an excellent growth medium for most bacteria; d) heterogeneity of microorganisms which cause mastitis; and e) the extensive surface area of secretory epithelium requiring immunological surveillance (15). Although several successful attempts at vaccination have been reported under
experimental conditions (15,44,46,58), a better understanding of the immune response of mammary tissue to bacterial infection is needed to overcome the limitations of vaccines against bovine mastitis.

Numerous studies have demonstrated that the bovine mammary gland is most susceptible to invasion by mastitis pathogens during early involution (36,69,79,80,81,106). However, efforts to control the disease have concentrated primarily on lactating animals. Progress in developing an effective mastitis control program for nonlactating dairy cows has been limited by an inadequate understanding of the mammary immune system. The mammary gland has a natural ability to prevent invasion by pathogenic bacteria; however, physiological transition to, or from, a state of active milk synthesis and secretion has been shown to inhibit this defense capability. An understanding of the involutionary process and the interrelationship of IMI with involution is necessary for development of new mastitis control procedures which will be effective in nonlactating glands.

The objectives of this study were: a) to describe the biochemical changes in mammary secretion composition from involution through lactogenesis and compare these with morphological changes in mammary parenchyma; b) to compare infection status with changes in mammary secretion and tissue morphology during involution and lactogenesis; and c) to identify and quantitate the Ig classes of plasma cells associated with nonlactating mammary tissue and compare with infection status.
References


79. Oliver, J., F. H. Dodd, and F. K. Neave. 1956. Udder infections in the dry period. IV. The relationship between the new infection rate in the early dry period and the daily milk yield at drying-off when lactation was ended by either intermittent or abrupt cessation of milking. J. Dairy Res. 23:204.


CHAPTER II

Running head: Bovine Mammary Secretion Composition

Key words: Involution, Lactogenesis, Secretion Composition, Mastitis, Mammary, Bovine

SECRETION COMPOSITION DURING BOVINE MAMMARY INVOLUTION AND THE RELATIONSHIP WITH MASTITIS

L. M. SORDILLO, S. C. NICKERSON, R. M. AKERS1, and S. P. OLIVER.2

Louisiana Agricultural Experiment Station, Louisiana State University Agricultural Center, Hill Farm Research Station, Homer 71040

1Department of Dairy Science, VPI & SU., Blacksburg 24061.

2Department of Animal Science, University of Tennessee, Knoxville 37901.

Correspondence sent to: Lorraine M. Sordillo-Gandy
Department of Animal Science
University of Tennessee
P.O. Box 1071
Knoxville, Tennessee 37901-1071

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Quarter mammary secretion samples for bacteriological and compositional analyses were collected from 29 dairy cows from drying off through early lactation. Bacteriological analysis revealed that 30% contained coagulase-negative staphylococci, *Staphylococcus aureus*, *Corynebacterium bovis*, or streptococci. As involution progressed, somatic cell counts, percent protein, pH, and concentrations of serum albumin, lactoferrin, and immunoglobulin G increased while percent fat, concentrations of citrate, and the citrate to lactoferrin molar ratio decreased. Compared to uninfected quarters, those which were infected had significantly higher somatic cell counts especially during colostrogenesis and early lactation. Differences in numbers of macrophages were not detected between quarters. However, uninfected quarters had significantly higher percentages of lymphocytes but lower percentages of polymorphonuclear leukocytes compared to infected quarters. Percentages of fat and protein and concentrations of serum albumin and citrate were similar between all quarters during most of the sampling period. However, mammary secretion from infected quarters contained significantly lower percentages of fat and higher pH compared to uninfected quarters during the first 2 wk of lactation. Infected quarters also contained lower concentrations of lactoferrin during the nonlactating period compared to uninfected quarters. Results suggest that udders involute by 14 to 21 days post drying off. Intramammary infection altered normal secretion composition during lactogenesis.
Introduction

The bovine mammary gland is most susceptible to invasion by bacterial pathogens during functional transitions from lactation to involution and from involution to lactogenesis (14,18). Incidence of new intramammary infection (IMI) during the first 3 wk of the dry period may be 7 times greater than during lactation (14). The fully involuted mammary gland appears to be highly resistant to new IMI, but as parturition approaches, susceptibility to IMI increases.

Secretions of fully involuted udders contain high concentrations of leukocytes, immunoglobulins (Ig), and bacteriostatic whey proteins that have been implicated as resistance factors to IMI (10,21). Predominant leukocyte types present in dry secretion are macrophages and polymorphonuclear leukocytes (PMN) that function by phagocytosing invading pathogens (7). Immunoglobulin in mammary secretion is of humoral or local origin (9). In the bovine, IgG_{1}, IgG_{2}, and IgM function as opsonins and prepare bacteria for phagocytosis by macrophages and PMN. Immunoglobulin A in milk may play a role in preventing bacterial colonization of epithelial surfaces, inhibiting bacterial multiplication, neutralizing toxins, and agglutinating bacteria (15,22). Lactoferrin (Lf) is a major whey protein of dry secretion that inhibits bacterial growth in presence of bicarbonate by sequestering iron (2,24). The iron-binding activity of Lf in vitro is diminished by citrate (2); consequently, the citrate to Lf molar ratio plays a role in udder defense.
Susceptibility or resistance to mastitis is related to changes in antibacterial components of secretions during the lactation cycle (3,17). The rate of change in these components during early involution apparently is not sufficient to prevent new IMI. Other biochemical components that undergo changes during this time include protein, fat, pH, bovine serum albumin (BSA), and α-lactalbumin which can be measured to monitor secretory activity and glandular permeability. Because new IMI occurring during involution are thought to account for the level of infection in subsequent lactations (23), the nonlactating period is a logical point of attack for mastitis control. The purpose of this study was to examine effects of IMI on lacteal secretions during the nonlactating and early lactating periods to determine if presence of bacteria affected the processes of involution and lactogenesis.
**Materials and Methods**

**Experimental design**

Twenty-nine Jersey cows from the Hill Farm Research Station were used. At drying off, animals were treated with intramammary infusion products containing 1,000,000 units procaine penicillin G and 1 g dihydrostreptomycin (Quartermaster, Upjohn Co., Kalamazoo, MI). Mammary quarter secretion samples were collected at drying off; 7, 14, 21, and 28 days after drying off; 14 and 7 days prepartum; at parturition; and at 7 and 14 days postpartum. Duplicate foremilk samples (10 ml) were used to determine infection status, and to quantitate concentrations of Ig, Lf, citrate, and BSA. Total somatic and differential cell counts, percentages of total protein and fat, pH, and the citrate to Lf molar ratio were determined also. Blood samples were collected at the above times by venipuncture, to quantitate concentration of α-lactalbumin in sera. Data were analyzed by least square analysis of variance using the general linear model procedure to determine effects of wk during involution and infection status on biochemical composition of bovine mammary secretion. The statistical analysis included effect of cow, wk during involution, infection status, and interaction of wk during involution with infection status. The following model was used:

\[ Y_{ijk} = \mu + \tau_i + \beta_j + \gamma_{ij} + \epsilon_{ijk} \]

Where:
- \( \mu \) = effect common to all cows,
- \( \tau_i \) = fixed effect of ith wk during involution,
- \( \beta_j \) = fixed effect of jth infection status,
$$t_{ij} = \text{fixed effect due to interaction between } i\text{th wk during involution and } j\text{th infection status},$$

$$\epsilon_{ijk} = \text{random error associated with each observation.}$$

The effect of cow was absorbed. Preplanned comparisons of least square means from the overall model were made by pairwise T-test. Means were contrasted between infection status group within a time period and between time period within infection status group. No other comparisons were made.

Microbiological procedures

Samples were processed, examined for microbial growth as in (4), and identified to species level. Staphylococcal isolates were identified using the API Staph-Ident System (Analytab Products, Plainview, NY) and streptococcal isolates were identified by the API 20S System (Analytab Products). Microorganisms were classified as minor (coagulase-negative staphylococci and Corynebacterium bovis) and major (streptococci and Staphylococcus aureus) pathogens.

Milk somatic cells

Somatic cell counts (SCC) were determined using a Fossomatic Cell Counter (Foss Electric Ltd., Hillerod, Denmark), and differential cell counts were determined on dried milk film stained with GS-Wright's stain (General Scientific, Richmond, VA). For each smear, 200 cells were differentiated microscopically at 1000 X and expressed as percent of total cells counted. Cells were classified as macrophages, lymphocytes, and PMN.
Total protein and butterfat

A Fossomatic Milk Analyzer (Foss Electric Ltd.) was used to determine total protein and butterfat. Assays were performed on 10 ml-milk samples preserved with potassium dichromate ($K_2Cr_2O_7$) at the Dairy Herd Improvement Association Laboratory, Baton Rouge, LA.

Compositional analyses

Skim milk and whey fractions were prepared as in (19). Immunoglobulin, Lf, and BSA were quantitated in wheys by electroimmunodiffusion (EID) on cellulose acetate plates (17). Rabbit anti-IgG (Fab)$_2$ and the IgG$_1$ standard were purchased from Pel-Freez Biologicals, Rogers, AR, and diluted with a 1:4 dilution of high resolution buffer, pH 8.8 (Gelman Instrument Co., Ann Arbor, MI) in distilled water. Antiserum to bovine Lf and bovine Lf standards were prepared from purified bovine Lf (24), and diluted with .0125 M sodium phosphate buffer, pH 7.4. Rabbit anti-BSA and BSA standards were purchased from Miles Laboratory, Kankakee, IL. Quantification of citrate in skim milk was by a modification of (25).

Blood samples were centrifuged at 1,240 X g for 25 min and sera frozen and stored at -20°C until needed. Concentration of α-lactalbumin in whey samples was determined by radioimmunoassay (1) using a Beckman L-4000 gamma counter.
Results

Frequencies of bacterial isolates throughout the nonlactating and early lactating periods are in Table 1. Percent uninfected quarters increased markedly from D-0 to D+7 and D+14. At D+21, percent uninfected quarters began to decrease gradually through C+14 to levels observed at D-0. Isolations of C. bovis decreased markedly from D-0 to early lactation. Coagulase-negative staphylococci (CNS) included Staphylococcus epidermidis, Staphylococcus simulans, Staphylococcus hyicus, and Staphylococcus warneri. Coagulase-negative staphylococci were the most frequent isolates throughout the sampling period. Percent quarters containing CNS and S. aureus decreased from D-0 to early involution (D+7 and D+14), but increased gradually beginning at D+21 through early lactation to percentages observed at D-0. Isolation of streptococci was most frequent from D-0 to D+28 but remained low from C-14 through C+14.

Somatic cell counts from uninfected quarters increased from D-0 to D+7, then decreased steadily to minimum levels at C+14 (Figure 1). Although infected quarters were more variable over time, they had significantly higher SCC compared to uninfected quarters (Table 2). Somatic cell counts in quarters infected with major pathogens were elevated significantly over uninfected quarters from C-7 to C+7 (Figure 1).

Percentages of macrophages and lymphocytes for all quarters increased from D-0 (33.14 ± .96 and 26.88 ± 1.03) to peak concentrations at D+28 (38.64 ± 1.55 and 37.38 ± 1.51). Beginning
at C-14, percentages of these cell types decreased, reaching lowest levels at C+14 (30.48 ± 1.62 and 27.10 ± 1.74) (Appendix Figures 1a and 2a). There was no significant effect of infection status on macrophage concentration (Table 2, Appendix Tables 1a and 2a). However, uninfected quarters had significantly higher total percentages of lymphocytes, and significantly lower percentages of PMN compared to infected quarters. The proportion of PMN in all quarters decreased as involution progressed, with lowest levels at D+28 (22.56 ± 1.97). Polymorphonuclear leukocyte numbers increased significantly at C-14 (27.61 ± 2.09), continuing through the pre- and postpartum periods, and peaking at C+14 (41.46 ± 2.05, Appendix Figure 3a).

Lactoferrin concentrations in uninfected quarters increased significantly from D-0 to D+7, and remained high through the nonlactating period (Figure 2, Appendix Figure 4a). At C-0, Lf levels dropped significantly and continued to decrease through the early lactating sampling periods. Compared to uninfected quarters (10.87 ± .38 mg/ml) and those infected with minor pathogens (10.09 ± 1.17 mg/ml), quarters infected with major pathogens had significantly lower total concentrations of Lf (7.78 ± 1.04 mg/ml) (Appendix Tables 3a and 4a).

Citrate values and the citrate to Lf molar ratio in uninfected quarters decreased markedly from D-0 to D+7 and remained low during the nonlactating period (Figure 2, Appendix Figure 5a). At C-0, citrate concentrations and the citrate to Lf molar ratio increased markedly and remained high during the early lactating sampling periods. There was no significant effect of infection status on
citrate concentrations.

Changes in percent fat and protein were observed with respect to sampling period (Figure 3, Appendix Figures 6a and 7a). Percent fat from both uninfected and infected quarters decreased gradually as involution progressed from D-0 through C-7. At C-0, the percent fat increased markedly and continued to increase through C+14 where it reached stable values in uninfected quarters. The protein content of mammary secretions from uninfected quarters increased gradually as involution continued up through C-7, then decreased following parturition, reaching lowest percentages at C+14. Infected quarters had lower percentages of fat and protein throughout most of the sampling period. However, infected quarters had significantly higher percentages of protein at C-0 compared with uninfected quarters (Appendix Tables 5a and 6a).

The pH of mammary secretions from both uninfected and infected quarters increased markedly from D-0 to D+7, and remained high until D+28 (Figure 4). At C-14, pH decreased reaching lowest levels at C-0. Slight increases in pH were observed for uninfected and infected quarters at C+7 and C+14. Secretion from quarters infected with major pathogens had significantly higher pH values from C-0 to C+14 compared to uninfected quarters (Appendix Tables 9a and 10a).

Concentration of BSA in all quarters increased significantly from D-0 to D+7 (Figure 5, Appendix Figure 8a). As involution continued, BSA content remained high through the C-7 sampling period. At C-0, BSA concentrations decreased significantly to levels observed at D-0 and continued to decrease as lactation
No differences in BSA concentration were detected between uninfected and infected quarters (Appendix Tables 9a and 10a).

Concentrations of IgG in mammary secretions of both uninfected and infected quarters increased from D-0 to D+7 and continued to increase reaching peak concentrations at C-14 and C-7 (Figure 6). Compared to uninfected quarters (17.86 ± 1.23), secretion from infected quarters (16.15 ± 1.75) had less IgG concentration throughout most of the sampling period. However, concentrations of IgG were higher at C-14 in quarters infected with major pathogens and higher at C-0 in quarters infected with minor pathogens compared to uninfected quarters (Appendix Table 8a).

Concentration of α-lactalbumin in sera of cows over the nonlactating period increased significantly from D-0 to D+7 (Figure 7). At D+14, the α-lactalbumin content of serum decreased significantly and levels remained low through C-14. At C-0, α-lactalbumin levels increased significantly reaching peak concentrations. A marked decrease was observed following C-0 and concentrations continued to decline through C+14. Additional information on α-lactalbumin content in mammary secretion is found in the Appendix on Tables 9a and 10a and on Figure 9a.
Discussion

At D+7, numbers of infected quarters were much lower compared with D-0. Decreased numbers of infected quarters during the early nonlactating period in this study was most likely due to administration of antibiotics at D-0. Percent quarters infected with *C. bovis* remained low throughout the dry period and into lactation. Although infections with CNS and *S. aureus* were lower at D+7 and remained lower throughout C-14, frequency of isolation at C-0 and during early lactation (43%) was higher than that observed at D-0 (32%). These findings suggest that the bovine udder is highly susceptible to IMI by CNS and *S. aureus* during the peripartum period.

Susceptibility to mastitis is associated with the physiological transitions of the mammary gland either to or from a state of active milk synthesis and secretion (13,19). Although several studies have characterized antibacterial components of mammary secretion during the dry period (17,21), our understanding of the changes in composition with respect to susceptibility to mastitis is vague. Major changes in biochemical composition and antibacterial properties of mammary secretion were found during the early and late stages of involution. Increases were observed within the first 7 days of involution in numbers of SCC; percentages of macrophages, lymphocytes, and protein; pH; and concentrations of Lf, BSA, and IgG reaching peak or stable values by D+14 to D+21. As parturition approached (from C-7 to C-0) concentrations of these parameters decreased and remained low during the early lactating period.
Conversely, percentages of PMN and fat, concentration of citrate, and the citrate to Lf molar ratio declined within the first wk of involution while progressively increasing from C-7 to C+14.

Levels of SCC, percent protein, pH, and concentrations of BSA and IgG in bovine mammary secretion during involution reflect degree of cellular integrity and permeability of the blood-milk barrier (8). In this study, transient changes in SCC were similar to those reported in previous studies. It was hypothesized that the influx of cells during the early dry period was a function of the cessation of milk removal and fluid resorption (7,10). Elevated cell populations from D+7 to D+14 may have resulted from an increased migration of leukocytes from the circulatory system and/or a concentration effect resulting from fluid resorption. The subsequent decrease in leukocyte population following parturition may be due to a dilution effect and/or removal of cells from the gland during the milking process. Differential cell counts of mammary secretion revealed approximately equal numbers of macrophages and PMN during the first 14 days of involution. Macrophages and lymphocytes became the major cell types in secretions from the fully involuted gland and PMN were prevalent in colostrum and milk during early lactation. These data are consistent with the findings of others (7,11,12).

Changes in concentrations of Lf, citrate, and the citrate to Lf molar ratio are indicative of the functional transition of the mammary gland (19,24). Increased Lf concentration in the nonlactating mammary gland is a marker for involution and decreased synthetic and secretory ability of mammary epithelial cells (19,24).
Results of this study demonstrated that Lf levels peaked on D+14, suggesting the udder reaches a fully involuted state between D+14 and D+28 when Lf reaches maximum concentrations.

Changes in citrate concentration were monitored to follow mammary secretory activity, which decreased significantly by D+7. The subsequent increase in citrate concentration, and the citrate to Lf molar ratio during the last 7 days of gestation were attributed to the onset of milk synthesis and secretion.

Changes in serum concentrations of α-lactalbumin were determined and used as an indicator of mammary cell synthetic activity during the nonlactating period (1,6). By D+7, concentrations of α-lactalbumin increased significantly and as involution progressed, concentrations decreased through D+28 which suggests alteration in tight junctions between mammary secretory cells. Consequently, α-lactalbumin appears to follow a paracellular route from milk to blood. Decreased serum concentrations of α-lactalbumin after D+14 indicates reduced synthetic capability of remaining cells. During the last wk of gestation, α-lactalbumin concentrations in serum rose dramatically reaching peak levels at parturition as reported in (6). Transient increases in serum concentrations prior to parturition indicate structural differenion of mammary cells, the onset of milk synthesis and secretion, and fluid accumulation resulting in leakage of milk to blood.

Compositional changes in mastitic milk during lactation have been reviewed (8). Mastitis generally results in a decrease in milk yield, reductions in concentrations of lactose and fat, and
increases in SCC, pH, BSA, Ig, and Lf (8). Such alterations have been attributed to inflammatory damage of mammary secretory tissue and disruption of the blood-milk barrier (8). During the early lactating period, significantly lower percentages of fat and higher pH in secretion from infected quarters compared to uninfected quarters reflect this loss in cellular integrity. In secretions from infected quarters, SCC were also higher during involution and early lactation compared to uninfected quarters. Elevated leukocyte numbers may be indicative of an inflammatory response of the mammary gland to bacterial toxins. In infected quarters, PMN were the most numerous cell type during the dry and early lactating periods. A previous study also found PMN to be the predominant cell type in secretion from infected quarters at all stages of the dry period (7). Neutrophilia during the early dry and periparturient periods in uninfected quarters appears ineffective in reducing the high rates of new IMI during this critical time. Paape and Guidry (20) have shown that phagocytosis and intracellular bacteriolysis by PMN are inhibited by the indiscriminant ingestion of fat and casein in mammary secretion. Results of this study support the concept that phagocytosis of bacteria by mammary PMN and macrophages is less effective during periods of functional transition.

Immunoglobulin concentration in lacteal secretions vary with respect to stage of lactation and presence of IMI. Most IgG is serum-derived and selectively transferred into mammary secretions (15). In the lactating gland, concentrations of IgG increased dramatically during acute mastitis (8). Inflammation of the mammary gland ruptures the blood-milk permeability barrier allowing IgG to
passively enter lacteal secretions from serum. In this study, nonlactating uninfected quarters had slightly higher IgG concentrations when compared to infected quarters during most of the sampling period. This may be explained by compromised lymphocyte and Ig-producing plasma cell function as reported previously in quarters infected with chronic staphylococcal mastitis (16). Elevated concentrations of IgG during colostrogenesis is likely due to selective transport via receptors on mammary secretory cells (5). However, significantly higher concentrations of IgG in infected quarters observed at C-14 and C-0 compared to uninfected quarters may be due to rupture of the blood-milk barrier.

Fully involuted mammary glands exhibit increased resistance to IMI (3,19,24). Secretions from involuted mammary glands contain elevated natural protective factors including phagocytes and Lf, but lower concentrations of casein, lactose, and citrate which can be utilized by invading bacteria for colonization and growth (21). Previous studies demonstrated the inhibitory action of secretion from nonlactating mammary glands on mastitis-causing organisms in vitro (3,17). A decline in the citrate to Lf molar ratio, and elevation of Lf and IgG concentrations have been shown to contribute to the antimicrobial action against environmental organisms. Lactoferrin interferes with the ability of these bacteria to sequester iron from mammary secretions. In this study, infected quarters had lower Lf concentrations compared to uninfected quarters. Lower levels of this antibacterial component may have reduced the natural defense potential of the gland, allowing colonization by minor and major pathogens during the peripartum period.
References


TABLE 1. Frequency of bacterial isolates in bovine mammary secretion from drying off through early lactation.

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<th>Bacteria&lt;sup&gt;2&lt;/sup&gt;</th>
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<th>D+14</th>
<th>D+21</th>
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<td>79</td>
<td>95</td>
<td>91</td>
<td>95</td>
<td>886</td>
</tr>
</tbody>
</table>

<sup>1</sup>Days relative to drying off (D) or calving (C).

<sup>2</sup>Pathogens isolated at time of sampling; 0, uninfected; CB, Corynmbacterium bovis; CNS, coagulase-negative staphylococci; SA, Staphylococcus aureus; and STP, streptococci.

<sup>3</sup>Total number of quarter samples.
TABLE 2. Effect of bacterial infection on numbers of total somatic cell and differential cell counts in mammary secretion of nonlactating cows.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>O</th>
<th>INF</th>
<th>MINOR</th>
<th>MAJOR</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCC (10^6)</td>
<td>2.28b ± .12</td>
<td>2.99a ± .22</td>
<td>2.50b ± .38</td>
<td>3.83a ± .34</td>
</tr>
<tr>
<td>MAC (%)</td>
<td>34.61a ± .51</td>
<td>33.51a ± .88</td>
<td>32.36a ± 1.51</td>
<td>34.61a ± 1.26</td>
</tr>
<tr>
<td>LYM (%)</td>
<td>34.26a ± .55</td>
<td>30.91b ± .95</td>
<td>31.99b ± 1.62</td>
<td>29.15b ± 1.35</td>
</tr>
<tr>
<td>PMN (%)</td>
<td>29.92b ± .64</td>
<td>34.33a ± 1.11</td>
<td>34.41a ± 1.91</td>
<td>34.87a ± 1.59</td>
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</tbody>
</table>

Pathogens isolated at time of sampling; O, uninfected; INF, bacteriologically positive; MINOR, Corynebacterium bovis and coagulase-negative staphylococci; MAJOR, Staphylococcus aureus and streptococci.

Means between infection status with different superscripts differ (P<.05).
Figure 1. Changes in total somatic cell counts in bovine mammary secretion from drying off through the first 14 days of lactation. Means within sampling period with different letters (a, b) differ (P < .05).
Figure 2. Changes in concentration of lactoferrin and citrate and the citrate to lactoferrin molar ratio in bovine mammary secretion in uninfected quarters from drying off through the first 14 days of lactation. Means with different letters (a,b,c,d) differ (P<.05). *The citrate to lactoferrin molar ratio was calculated from citrate and lactoferrin least square means at each sampling period.
Figure 3. Effect of infection status on percent fat and total protein in bovine mammary secretion from drying off through the first 14 days of lactation. Means within sampling period with different letters (a,b) differ (P<0.05).
Figure 4. Effect of infection status on pH of bovine mammary secretion from drying off through the first 14 days of lactation. Means within sampling period with different letters (a, b) differ (P<.05).
Figure 5. Changes in concentrations of bovine serum albumin in mammary secretion from drying off through the first 14 days of lactation. Means with different letters (a, b, c, d) differ (P<.05).
Figure 6. Effect of infection status on concentrations of immunoglobulin G in bovine mammary secretion from drying off through the first 7 days of lactation. Means with different letters (a,b) differ (P<.05).
Figure 7. Changes in concentration of α-lactalbumin in bovine serum from drying off through the first 14 days of lactation. Means with different letters (a, b, c) differ (P<.05).
\( \alpha - \text{LACTALBUMIN} \) (mg/ml)

**SAMPLING PERIOD**

- D-0
- D-7
- D-14
- D-21
- D-28
- C-14
- C-7
- C-0
- C-7
- C-14
CHAPTER III

Running head: Morphology of Bovine Mammary Involution

Key words: Bovine, Mammary Ultrastructure, Involution, Lactogenesis.

MORPHOLOGICAL CHANGES IN THE BOVINE MAMMARY GLAND DURING INVOLUTION AND LACTOGENESIS.

L. M. SORDILLO and S. C. NICKERSON

Louisiana Agricultural Experiment Station, Louisiana State University Agricultural Center, Hill Farm Research Station, Homer, 71040.

Correspondence sent to: L. M. Sordillo-Gandy
Department of Animal Science
University of Tennessee
P.O. Box 1071
Knoxville, Tennessee 37901-1071

Approved for publication by the Director of the Louisiana Agricultural Experiment Station as manuscript number 87-80-1143.
Morphological changes occurring during bovine mammary involution were examined. Quarter biopsies were taken weekly from 5 cows beginning at drying off through parturition. Light and electron microscopic examination of mammary tissue indicated a gradual reduction in synthetic and secretory activity of alveolar epithelium as involution progressed. Light microscopic morphologic analysis demonstrated increases in stroma and nonactive secretory epithelium with concomitant decreases in epithelium, lumen, and fully active secretory epithelium during the first 2 wk of involution. Electron microscopic analysis of alveolar epithelium demonstrated a decreased number of organelles associated with milk synthesis and secretion during this time. These changes reversed gradually beginning 2 wk prepartum, and by the time of calving, cell structure exhibited morphology typical of lactating glands. Tissue from infected quarters had less synthetic and secretory ability as indicated by significantly higher percentages of stroma and nonactive cells, but lower percentages of lumen and moderately active cells compared to uninfected quarters. Infected quarters also had more leukocytes infiltrating the epithelium, lumen, and stroma compared to uninfected quarters. Microscopic examination of macrophages and polymorphonuclear leukocytes suggested these cells removed milk components and cellular debris during involution. Large numbers of plasma cells, exhibiting distended cisternae of rough endoplasmic reticulum, suggested local antibody production during the periparturient period.
Introduction

Morphological changes occurring during mammary gland involution and lactogenesis have been reported previously in laboratory animals (3,4,7,11). However, relatively little information is available describing these processes following the cessation of milking in the bovine mammary gland. Research has demonstrated the importance of a nonlactating period of adequate duration on milk yield of dairy cows (2). Dairy cows having a dry period of 40 to 60 days have been shown to produce more milk in subsequent lactations than those with a less than 40 day dry period. It has been hypothesized that mammary cells lost following peak milk production during lactation are replaced with new, more efficient cells during the nonlactating period (7). The nonlactating period is also a time of increased susceptibility to mastitis, and the effect of bacterial infection on mammary tissue during this period has not been determined. An understanding of the extent to which the bovine mammary gland regresses during involution, the nature of secretory cell growth and redevelopment in the prepartum period, and the relationship of intramammary infection with the involutionary process may lead to new approaches to maximize milk production in subsequent lactations and possibly shorten the nonlactating period. The purpose of this study was to examine the structural changes occurring in the bovine mammary gland during involution and lactogenesis, and to determine the effect of infection on changes in tissue morphology.
Materials and Methods

Experimental design

Five Jersey cows from the Hill Farm Research Station were used. Foremilk samples were collected aseptically prior to tissue collection and used to evaluate infection status of each mammary gland quarter. Mammary tissue samples were obtained by needle biopsy technique from each quarter at drying off; 7 and 14 days post drying off; and 14 and 7 days prepartum. At parturition, animals were slaughtered and approximately 1 cm³ of tissue was obtained from each quarter. All tissue samples were prepared for microscopic examination. Data were analyzed by least squares analysis of variance using the general linear model procedure to determine effects of wk during involution and infection status on morphological parameters. The statistical analysis included effect of cow, wk during involution, infection status, and interaction of wk during involution x infection status. The following model was used:

\[ Y_{ijk} = \mu + \tau_i + \beta_j + \tau\beta_{ij} + e_{ijk} \]

Where:

\( \mu \) = effect common to all cows,

\( \tau_i \) = fixed effect of ith wk during involution,

\( \beta_j \) = fixed effect of jth infection status,

\( \tau\beta_{ij} \) = fixed effect due to interaction between ith wk during involution and jth infection status,

\( e_{ijk} \) = random error associated with each observation.
The effect of cow was absorbed. Preplanned comparisons of least squares means from the overall model were made by pairwise T-test. Means were contrasted between infection status group within a time period and between time period within infection status group. No other comparisons were made.

**Tissue preparation**

Cows were anesthetized locally prior to biopsy with 2 ml of 2% Lid-o-cain (Butler Co., Columbus, OH). A small puncture was made through the skin with a scalpel blade above the gland cistern, and approximately 3 cm x 2 mm of tissue was removed using a disposable biopsy needle (Travenol, Dallas, TX), followed by topical application of antibiotics. Biopsy samples and tissue samples obtained at slaughter were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.0 at 37°C) for 2 h. Tissue was then post-fixed in 0.1 M cacodylate-buffered osmium tetroxide (pH 7.0 at 5°C) for 1.5 h, dehydrated in a graded series of ethanol, and embedded in epoxy resins. Thick sections (0.5 to 1.0 μm) were obtained on a Porter Blum MT-5000 microtome and stained with toluidine blue for light microscopy. Ultrathin sections approximately 60 nm thick were stained with 5.0% uranyl acetate in 50% methanol for 20 min followed by 0.4% lead citrate for 10 min and examined using a Philips EM 300 electron microscope at 60 kV.

**Morphometric analysis**

Quantitative morphologic analysis was used to determine percentage mammary tissue area composed of interalveolar stroma,
epithelium, and alveolar lumen. For each tissue sample, 10 replications of 100 contact points were counted per slide at a magnification of X 630 using a Zeiss Standard 18 research microscope. A reference grid in the microscope ocular provided fixed points used in the counting process. Alveolar epithelium was characterized further as (1) nonactive, (2) moderately active, or (3) fully active.

Tissue specimens of mammary parenchyma were also examined for the presence of corpora amylacea, macrophages, lymphocytes, polymorphonuclear leukocytes (PMN), mast cells, and plasma cells. Prevalence of these cell populations and corpora amylacea were quantitated with respect to tissue location (i.e. within the epithelial lining, lumen, or stroma) in 10 randomly selected microscopic fields per tissue sample at X 630.

**Ultrastructural examination**

Based on light microscopic observations, tissue areas from all quarters were selected to evaluate secretory cell activity. Morphometric analysis of secretory epithelial cells sectioned through the approximate midportion of the nucleus and showing apical, basal, and lateral cell membranes was performed using 12 x 20 cm micrographs at a magnification of X 9,500. A modification of the Solari method (19) was used to determine percentages of cell area occupied by nucleus, membrane-free cytoplasm, rough endoplasmic reticulum (RER), Golgi apparatus, mitochondria, fat droplets, secretory vesicles, and milk stasis vacuoles. The cytoplasmic to nuclear ratio was determined also.
Microbiological procedures

Prior to tissue collection, duplicate foremilk samples were collected for microbiological analysis. Presumptive identification of bacterial isolates was determined by methods described by Brown et al. (1). Intramammary infection was defined as isolation of the same microorganism from duplicate foremilk samples.
Results

Frequencies of bacterial isolates during the sampling period are in Table 3. Because of the low number of isolates, quarters were classified as uninfected or infected. Quarters were further classified as infected with minor (Corynebacterium bovis or coagulase negative staphylococci), or major (Staphylococcus aureus, Streptococcus uberis, or Nocardia) pathogens.

Percentages of tissue area composed of epithelium and lumen from uninfected and infected quarters decreased gradually as involution progressed reaching lowest levels at D+1A while stromal area peaked (Table 4, Appendix Figures 10a-12a). At C-14, percentages of epithelium and lumen began to increase as stroma decreased, continuing through C-0, reaching values observed at D-0. Compare Figures 8 and 9.

Percent of epithelial area composed of nonactive cells from uninfected and infected quarters increased significantly from D-0 to D+7 peaking at D+14 (Table 5, Appendix Figure 13a). At C-14, percent nonactive epithelium began to decrease, reaching a significantly lower percentage at C-0 compared to D+14. Conversely, percent epithelial area composed of fully active cells decreased significantly from D-0 to D+7 reaching a minimum at D+14 (Appendix Figure 14a). At C-14, percent fully active epithelium began to increase, reaching a significantly higher percentage at C-0. Percent of epithelial area composed of moderately active cells
fluctuated very little from D-0 to C-14, but was significantly higher at C-7 compared to D-0.

Percent tissue areas composed of stroma and nonactive epithelia were significantly higher from infected quarters, regardless of pathogen, compared to uninfected quarters (Tables 6 and 7). Likewise, infected quarters had significantly lower percentages of lumen and moderately active epithelium irrespective of sampling time. No significant differences were observed between quarters infected with minor or major pathogens in histological or cytological analyses.

Ultrastructural analysis demonstrated that at D-0, epithelial cells exhibited abundant parallel RER cisternae, supranuclear Golgi dictyosomal components, numerous mitochondria scattered about the cytoplasm, several apically located secretory vesicles containing casein micelles, apical fat droplets, and a large cytoplasmic to nuclear ratio. Microvilli were present on the apical surfaces, tight junctions between cells were intact, and nuclei were oval and basally located (Figure 10).

At D+7, considerable changes in fine structure were observed compared to D-0 (Table 8). Cytoplasm exhibited significantly fewer RER cisternae, Golgi dictyosomal components, and mitochondria. Large fat droplets occupied most of the cytoplasm, nuclei were irregular in shape, and there was a significant decrease in the cytoplasmic to nuclear ratio. Stasis vacuoles increased with a concomitant significant decrease in secretory vesicles (Appendix Figures 16a-23a).
At D+14 and C-14, percent cell area composed of nuclei continued to increase significantly, while percentages of RER, Golgi, and mitochondria decreased (Table 8, Figure 11). Epithelial cells exhibited increases in numbers of stasis vacuoles, as well as a high percentage of cytoplasmic area composed of fat. Lysosomes were observed within the cytoplasm.

At C-7, percentages of cell area occupied by nuclei, unoccupied cytoplasm, fat, and stasis vacuoles decreased, while percentages of RER, Golgi, mitochondria, and secretory vesicles increased significantly in comparison with cells obtained at C-14. The cytoplasmic to nuclear ratio also increased. Epithelial cells began to exhibit secretory activity with appearance of small fat droplets, and vesicles containing casein micelles that accumulated under the apical plasma membrane. The nuclei of some epithelial cells took on a smooth ovoid shape and microvilli became evident along the apical surface. The alveolar lumen was filled with fat and protein granules. The outline of the alveolar structure appeared rounded as the secretory material accumulated in lumina and epithelial cells became cuboidal and flattened (Figure 12).

At C-0, ultrastructural examination showed that, in comparison with C-7, percentages of Golgi and secretory vesicles increased while percent fat and nuclei decreased. Epithelial cells appeared polarized and exhibited abundant and parallel RER in the basal region, supranuclear Golgi apparatus with secretory vesicles containing casein micelles, and numerous mitochondria throughout the cytoplasm. Secretory vesicles and fat droplets accumulated in the apical cytoplasm. Microvilli on the apical surface were shown to
protrude into the alveolar lumen, nuclei were oval and basally located, and there was a larger cytoplasmic to nuclear ratio (Figure 10).

Comparison of tissue from uninfected and infected quarters revealed no differences in percent Golgi apparatus, mitochondria, fat, or stasis vacuoles (Table 9). However, infected quarters had significantly higher percentages of nuclei and organelle-free cytoplasm, but significantly lower percentages of RER and secretory vesicles compared to uninfected tissues (Appendix Tables 24a and 25a). For the most part, no significant differences in ultrastructural analysis were observed between quarters infected with minor and major pathogens. However, quarters infected with major pathogens had significantly lower percentages of RER compared to uninfected quarters or those infected with minor pathogens.

Corpora amylacea were observed most frequently during the first 14 days of the dry period. The majority of corpora were seen in alveolar lumina where they often completely filled the luminal space and caused flattening of the epithelium (Figure 13). Electron microscopic observations demonstrated the dense granular material abutting the apical cytoplasm of degenerating epithelial cells. Corpora amylacea were observed to a lesser extent in the alveolar epithelial lining and in the interalveolar stroma. Infection status had no effect on the prevalence of corpora amylacea (Appendix Tables 22a and 23a).

Lymphocytes were the most prevalent cell type in the epithelial lining, followed by macrophages and PMN. Macrophages increased significantly from D-0 to D+7 and continued to increase through C-0.
Lymphocyte populations fluctuated throughout the sampling period as numbers increased significantly from D-0 to D+14, but then decreased significantly at C-14 and C-0 compared to concentrations at C-7. No significant changes in numbers of PMN were observed throughout the sampling period. Comparison of cell numbers within the epithelial lining demonstrated no differences in numbers of lymphocytes and macrophages between uninfected and infected quarters (Appendix Tables 12a and 13a). However, compared to uninfected quarters, those which were infected had significantly higher numbers of PMN (1.22 ± .78 vs 6.05 ± .77).

Macrophages and PMN were the most prevalent cell types in alveolar lumina. Many exhibited cytoplasmic vacuoles containing fat, casein, and cellular debris (Figure 14). Lymphocytes were observed less frequently throughout the sampling period. Numbers of macrophages increased gradually throughout the sampling period reaching significantly higher concentrations at C-0 compared to D-0. Lymphocyte populations were highest at D+14 and C-0, but were significantly lower at C-7 in comparison. No significant changes in PMN numbers were observed throughout the sampling period. Comparison of total cell numbers found within the alveolar lumen demonstrated no differences between uninfected and infected quarters in numbers of lymphocytes. Conversely, when compared to uninfected quarters, those which were infected had significantly higher numbers of macrophages (5.76 ± 1.26 vs 12.33 ± 1.25) and PMN (3.47 ± 1.65 vs 13.86 ± 1.63) (Appendix Tables 14a and 15a).
Subepithelial stromal areas were the most common site of leukocyte infiltration. Macrophages were the most prevalent cell type followed by lymphocytes, plasma cells, mast cells, and PMN (Table 10). Plasma cells, macrophages, and lymphocytes increased gradually from D-0 through C-7 where they reached peak concentrations that were significantly higher than at D-0. At parturition, numbers of plasma cells and macrophages remained high while lymphocyte numbers decreased significantly. Mast cell numbers decreased gradually from D-0 reaching significantly lower concentrations at C-0 compared to all other sampling times. No significant differences in PMN numbers were observed throughout the sampling period. Comparison of total cell numbers indicated no differences in levels of macrophages, lymphocytes, mast cells, or plasma cells within the stroma of uninfected and infected quarters. However, numbers of PMN were significantly higher in infected quarters (10.10 ± 1.26) compared to uninfected quarters (1.53 ± 1.27) throughout the sampling period (Appendix Tables 16a and 17a).
Discussion

Although considerable information is available in rats and mice regarding alterations in mammary structure during involution, morphological changes accompanying involution and the onset of lactation in the bovine have not been elucidated. The rate and extent to which milk producing tissues regress following cessation of milking, and redevelopment during lactogenesis varies greatly with species (7). Consequently, data available in laboratory animals cannot be extrapolated effectively to the processes of involution and lactogenesis in the bovine. This study examined morphological changes in bovine mammary tissue during the nonlactating period.

Morphological changes during involution indicated marked shifts in the secretory activity of the gland during the first wk of the nonlactating period followed by more gradual changes as calving approached. Following the first wk of involution, secretory activity of mammary epithelium decreased as evidenced by a reduction in alveolar luminal area with a concomitant increase in stromal area. Luminal spaces shrunk when secretion no longer displaced the alveolar area. Once milk synthesis ceased and mammary fluid was resorbed, stromal areas expanded proportionately to compensate for the reduced alveolar luminal area. Cytological analysis of mammary epithelium also revealed an increase in prevalence of nonactive cells with a concurrent decrease in fully active cells through D+14. Lee and Lascelles (8) reported similar changes in the ewe mammary gland. They found as regression of the parenchyma progressed, there
was a corresponding increase in stroma within 16 days of weaning (8).

Ultrastructure of bovine alveolar cells at D-0 resembled closely cells described previously from lactating glands (5,12). The cell cytoplasm contained abundant RER with polarized and parallel cisternae, well developed Golgi apparatus, and numerous mitochondria, all of which are indicative of synthetic activity.

On D+7, regressive changes became evident at the ultrastructural level which indicated reduction in secretory activity. The pronounced accumulation of large fat droplets in the cytoplasm and reduced luminal area were changes observed also in rat mammary glands 3 days following weaning (16). Early changes in bovine alveoli also included alterations in numbers and organization of cytoplasmic organelles. Rough endoplasmic reticulum cisternae were broken up into irregular strands, Golgi dictyosomes were reduced considerably in size, and numbers of mitochondria were decreased. Such reductions in synthetic and secretory organelles observed after 1 wk dry closely resembled changes reported in rat (3,16) and mouse (19) mammary glands on the second to third day of induced involution.

Stasis vacuoles were first observed in epithelial cells at D+7, but increased in number to D+14 and C-14. The build up of intramammary pressure associated with initial stages of involution may trigger the formation of stasis vacuoles due to intracellular stasis and failure of the fusion and release mechanism (19,23).
Studies of mammary gland involution in the mouse suggest these intracellular protein granules and other secretory products may be removed during involution through digestion in lysosomal vacuoles (19). Many epithelial cells in this study exhibited vacuolation of the cytoplasm with electron-dense granules. Previous studies suggested these electron dense granules were lysosomes (3).

The mammary gland of ewes involutes completely by 32 days postweaning. At the light microscopic level, degenerative cells disappeared, and the alveolar remnants were lined with a few layers of closely packed epithelial cells (8). The present study suggested involution of mammary glands from pregnant cows did not regress to the same extent as noted in other species. Morphological data indicated bovine mammary glands regressed markedly between D+14 and C-14 although not to the degree as previously noted in sheep. Histological examination of mammary tissue found luminal spaces still discernible, but filled with a deeply basophilic, proteinaceous fluid. In a companion study (22), changes in mammary secretion composition from nonlactating cows revealed significant increases in total protein and concentrations of bovine serum albumin and lactoferrin during this same stage of involution. Although a portion of the proteinaceous fluid occupying luminal areas is of serum origin (bovine serum albumin), elevated levels of locally produced whey proteins (lactoferrin) suggests some synthetic capabilities in the bovine mammary gland during this period of maximum regression.
Ultrastructural examination of mammary tissue from D+14 and C-14 found the cytoplasmic to nuclear ratio was smallest at this point and the minimal amount of cytoplasm was occupied by numerous stasis vacuoles and fat droplets. Although epithelial cells were dedifferentiating and appeared less capable of synthetic activity, the general lobulo-alveolar framework remained intact. Bovine alveoli apparently did not involute to the same degree as observed in other species. In the rat, no alveoli were observed 15 days after weaning, and only small ducts passing through dense connective tissue remained (16). Epithelial cells were found to slough into alveolar lumens, leaving only the basement membrane intact. Previous studies suggested that myoepithelial cells survived the destructive processes and functioned as a framework to prevent total loss of organized structure in the fully involuted rat mammary gland (15).

Corpora amylacea were most numerous during the first 14 days of the dry period. Previous studies have noted the prevalence of corpora amylacea during the later stages of lactation and early involution (13). Morphological relationships between these amyloid bodies and mammary parenchyma suggest they may suppress milk secretion by engorging luminal spaces and obstructing small ducts.

Changes in histological structure of rat and mouse mammary glands showed a marked rise in alveolar diameter and a corresponding fall in alveoli per unit area at parturition (11). In the present study, similar changes were observed at the light microscopic level in prepartum bovine mammary tissue. During
the last 2 wk of gestation, increased synthetic and secretory activity was apparent by higher percentages of epithelium and lumen accompanied by lower percentages of stroma. As mammary fluid accumulated, luminal spaces became engorged. Expansion of alveolar lumina area resulted in a concomitant compression of surrounding stromal area. As parturition approached, fully active cells also became more numerous while nonactive cells decreased. At C-7, the epithelial cell cytoplasm enlarged significantly and contained numerous fat droplets at the basal region and accumulated secretory vesicles at the apical region, showing the typical morphology indicative of milk synthesis and secretion (12). In the mouse, similar lobulo-alveolar development did not become complete until the third or second day prepartum (5), indicating marked species variation.

Infection status influenced normal changes in mammary structure during involution and lactogenesis. Quarters infected with minor and major pathogens had less synthetic and secretory activity as indicated by significantly lower percentages of lumen, RER, and secretory vesicles, but significantly higher percentages of stroma and nonactive epithelium compared to uninfected quarters. Quarters infected with major pathogens had also significantly higher percentages of membrane-free cytoplasm and lower percentages of RER which further substantiate impaired synthetic ability. The presence of bacteria within the gland during the nonlactating period appeared to have a deleterious effect on development of secretory cells. These findings support the contention that infections during the dry period may interfere with normal mammary secretory cell
differentiation and decrease milk yields in subsequent lactations.

Jensen and Eberhart (6) found that macrophages were the most numerous cell type during most of the dry period. PMN were prevalent initially, decreased as the dry period progressed, and increased as parturition approached. The proportion of lymphocytes increased during involution and then decreased near parturition. Previous studies have demonstrated the presence of leukocytes infiltrating the epithelium, lumen, and underlying connective tissue in nonlactating rat and mouse mammary glands (3,17). In this study, quantification of infiltrating leukocytes in nonlactating bovine mammary tissue revealed an increase in all cell types during the first wk of involution. Numbers of macrophages and PMN were elevated at parturition also. Large numbers of foamy cells observed within the alveolar lumen were similar morphologically to the fat-laden macrophages described by others in involuted mouse mammary glands (8,17). The presence of fat, casein, and cellular debris observed in the cytoplasm of both PMN and macrophages supports the findings of others that these cells play an important role in the resorption of milk components and facilitating the removal of degenerated epithelial cells from the involuting mammary gland (9).

Mast cells were most numerous in the connective tissue during the first 2 wk of the dry period, but were observed less frequently as parturition approached. A similar pattern in mast cell numbers was observed in involuted ewe mammary glands (8). Mast cells in connective tissue of other organ systems have been reported to release heparin and histamine under pathological conditions, resulting in increased vascular permeability (18). Increased
concentrations of these cells during involution has been attributed to the increasing prominence of stroma as a consequence of alveolar degeneration (8). However, mast cell numbers could have decreased at C-0 from degranulation as a result of the inflammation associated with parturition.

Macrophages have been shown to be the predominant cell type in secretions from noninfected involuted glands (10). However, during mammary infection, PMN may accumulate to over $50 \times 10^6$ cells per ml (14). In this study, infection status of quarters influenced greatly the mean number of leukocytes infiltrating the mammary parenchymal tissue. Compared to uninfected quarters, those infected with major pathogens had consistently higher numbers of leukocytes throughout the nonlactating period. Numbers of macrophages and PMN within the epithelial lining, lumen, and stroma of infected quarters were especially high during the first and last wk of involution. Presence of bacteria within the gland may have evoked an inflammation resulting in amplified migration of leukocytes from blood to milk, and exceeding levels normally associated with involution. Plasma cell numbers of infected tissue were especially elevated at C-0. Ultrastructural examination of these cells revealed grossly distended RER cisternae engorged with flocculent material. In a recent study (21), immunocytochemical techniques were used to demonstrate immunoglobulin secretory activity by these plasma cells. Exposure to bacterial pathogens may have enhanced plasma cell proliferation and local antibody production in response to antigenic stimulation.
Results of this study suggest the bovine mammary gland involutes gradually over a 2 wk period. Bovine epithelial cells do not regress to the same extent observed in rat mammary glands and appear to maintain some synthetic and secretory activity during the nonlactating period. The presence of intramammary infection altered normal mammary structure during involution and lactogenesis which may decrease milk yields in subsequent lactations.
References


TABLE 3. Frequency of bacterial isolates from bovine mammary foremilk samples from drying off through lactogenesis.

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<th>Organism</th>
<th>Sampling Period^1</th>
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<th>C-14</th>
<th>C-7</th>
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</table>

^Days relative to drying off (D) or calving (C).

%: Percentage of total quarters within each sampling period.

N: Number of quarters within each sampling period.
TABLE 4. Histological analysis\(^1\) of bovine mammary tissue from drying off through lactogenesis.

<table>
<thead>
<tr>
<th>Tissue classification</th>
<th>Sampling Period(^2)</th>
<th>D-0</th>
<th>D+7</th>
<th>D+14</th>
<th>C-14</th>
<th>C-7</th>
<th>C-0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epithelium</td>
<td>( \bar{X} )</td>
<td>45.36(^a)</td>
<td>41.14(^ab)</td>
<td>38.59(^b)</td>
<td>41.89(^a)</td>
<td>43.73(^a)</td>
<td>45.82(^a)</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>1.93</td>
<td>1.32</td>
<td>1.18</td>
<td>1.36</td>
<td>1.51</td>
<td>1.93</td>
</tr>
<tr>
<td>Lumen</td>
<td>( \bar{X} )</td>
<td>16.87(^ab)</td>
<td>13.59(^b)</td>
<td>13.38(^b)</td>
<td>15.48(^ab)</td>
<td>20.49(^a)</td>
<td>20.88(^a)</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>2.92</td>
<td>1.99</td>
<td>1.79</td>
<td>2.06</td>
<td>2.28</td>
<td>2.92</td>
</tr>
<tr>
<td>Stroma</td>
<td>( \bar{X} )</td>
<td>37.66(^cd)</td>
<td>45.61(^ab)</td>
<td>48.21(^a)</td>
<td>42.70(^bcd)</td>
<td>35.98(^d)</td>
<td>33.53(^d)</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>2.68</td>
<td>1.83</td>
<td>1.64</td>
<td>1.89</td>
<td>2.09</td>
<td>2.67</td>
</tr>
</tbody>
</table>

\(^1\)Data are expressed as mean percent of tissue area.

\(^2\)Days relative to drying off (D) or calving (C).

\(a, b, c, d\) Means between sampling periods with different superscripts differ (\(P<.05\)).
TABLE 5. Cytological analysis\(^1\) of bovine mammary epithelium from drying off through lactogenesis.

<table>
<thead>
<tr>
<th>Epithelial Classification</th>
<th>Sampling Period(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D-0</td>
</tr>
<tr>
<td>Nonactive</td>
<td>(\bar{X})</td>
</tr>
<tr>
<td></td>
<td>SE</td>
</tr>
<tr>
<td>Moderately active</td>
<td>(\bar{X})</td>
</tr>
<tr>
<td></td>
<td>SE</td>
</tr>
<tr>
<td>Fully active</td>
<td>(\bar{X})</td>
</tr>
<tr>
<td></td>
<td>SE</td>
</tr>
</tbody>
</table>

\(^1\)Data are expressed as mean percent of tissue area.

\(^2\)Days relative to drying off (D) or calving (C).

\(^a,b,c\)Means between sampling periods with different superscripts differ (P<.05).
TABLE 6. Effect of infection status on histological analysis\(^1\) of nonlactating bovine mammary tissue.

<table>
<thead>
<tr>
<th>Tissue Classification</th>
<th>Infection Status(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Epithelium</td>
<td></td>
</tr>
<tr>
<td>(\bar{X})</td>
<td>41.95(^a)</td>
</tr>
<tr>
<td>SE</td>
<td>.73</td>
</tr>
<tr>
<td>Lumen</td>
<td></td>
</tr>
<tr>
<td>(\bar{X})</td>
<td>21.25(^a)</td>
</tr>
<tr>
<td>SE</td>
<td>1.10</td>
</tr>
<tr>
<td>Stroma</td>
<td></td>
</tr>
<tr>
<td>(\bar{X})</td>
<td>36.60(^b)</td>
</tr>
<tr>
<td>SE</td>
<td>1.01</td>
</tr>
</tbody>
</table>

\(^1\)Data are expressed as mean percent of tissue area.

\(^2\)Infection status at time of sampling: 0, uninfected; INF, infected; MINOR, *Corynebacterium bovis* and coagulase negative staphylococci; MAJOR, *Staphylococcus aureus*, *Streptococcus uberis*, and Nocardia.

\(^ab\)Means between infection status with different superscripts differ (P<.05).
TABLE 7. Effect of infection status on cytological analysis\(^1\) of nonlactating bovine mammary epithelium.

<table>
<thead>
<tr>
<th>Classification</th>
<th>0</th>
<th>INF</th>
<th>MINOR</th>
<th>MAJOR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\overline{x}$</td>
<td>SE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonactive</td>
<td>21.99(b)</td>
<td>2.29</td>
<td>32.26(a)</td>
<td>35.09(a)</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td></td>
<td>2.27</td>
<td>5.40</td>
</tr>
<tr>
<td>Moderately active</td>
<td>52.66(a)</td>
<td>2.52</td>
<td>44.37(b)</td>
<td>48.14(ab)</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td></td>
<td>2.50</td>
<td>5.96</td>
</tr>
<tr>
<td>Fully active</td>
<td>25.39(a)</td>
<td>2.62</td>
<td>23.33(a)</td>
<td>16.42(a)</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td></td>
<td>2.57</td>
<td>6.18</td>
</tr>
</tbody>
</table>

\(^1\)Data are expressed as mean percent of tissue area.

\(^2\)Infection status at time of sampling: 0, uninfected; INF, infected; MINOR, Corynebacterium bovis and coagulase negative staphylococci; MAJOR, Staphylococcus aureus, Streptococcus uberis, and Nocardia.

\(a, b\) Means between infection status with different superscripts differ (\(P < .05\)).
TABLE 8. Ultrastructural analysis\(^1\) of alveolar cells in bovine mammary tissue from drying off through lactogenesis.

<table>
<thead>
<tr>
<th>Parameter(^3)</th>
<th>D-0</th>
<th>D+7</th>
<th>D+14</th>
<th>C-14</th>
<th>C-7</th>
<th>C-0</th>
</tr>
</thead>
<tbody>
<tr>
<td>NUC</td>
<td>25.95(^c)±2.29</td>
<td>32.19(^b)±1.57</td>
<td>43.85(^a)±1.40</td>
<td>41.27(^a)±1.52</td>
<td>28.20(^c)±1.79</td>
<td>22.27(^c)±2.29</td>
</tr>
<tr>
<td>CYT</td>
<td>18.45(^b)±2.15</td>
<td>20.14(^b)±1.46</td>
<td>22.49(^b)±1.31</td>
<td>27.75(^a)±1.42</td>
<td>18.17(^b)±1.68</td>
<td>17.91(^b)±2.15</td>
</tr>
<tr>
<td>RER</td>
<td>19.15(^a)±1.84</td>
<td>12.03(^b)±1.26</td>
<td>4.68(^c)±1.24</td>
<td>5.46(^c)±1.21</td>
<td>18.00(^a)±1.44</td>
<td>17.84(^a)±1.83</td>
</tr>
<tr>
<td>GOL</td>
<td>11.44(^b)±1.23</td>
<td>8.34(^c)±.84</td>
<td>2.74(^d)±.75</td>
<td>3.45(^d)±.81</td>
<td>11.11(^b)±.96</td>
<td>18.68(^b)±1.22</td>
</tr>
<tr>
<td>MIT</td>
<td>13.42(^a)±1.21</td>
<td>5.99(^c)±.83</td>
<td>3.63(^d)±.75</td>
<td>3.84(^d)±.80</td>
<td>9.91(^b)±.95</td>
<td>8.68(^b)±1.22</td>
</tr>
<tr>
<td>FAT</td>
<td>6.45(^b)±2.00</td>
<td>17.00(^a)±1.37</td>
<td>16.69(^a)±1.23</td>
<td>14.69(^ab)±1.32</td>
<td>12.08(^b)±1.57</td>
<td>8.23(^b)±1.99</td>
</tr>
<tr>
<td>SEC</td>
<td>4.50(^a)±1.23</td>
<td>2.05(^b)±.77</td>
<td>.02(^c)±.69</td>
<td>.18(^c)±.75</td>
<td>2.73(^ab)±.88</td>
<td>6.04(^a)±1.12</td>
</tr>
<tr>
<td>STA</td>
<td>.11(^a)±1.11</td>
<td>.93(^a)±.76</td>
<td>2.44(^a)±.68</td>
<td>1.07(^a)±.73</td>
<td>.21(^a)±.87</td>
<td>.50(^a)±1.11</td>
</tr>
<tr>
<td>CYT:NUC</td>
<td>2.85(^a)±.71</td>
<td>2.11(^b)±.32</td>
<td>1.28(^c)±.49</td>
<td>1.42(^c)±.63</td>
<td>2.55(^ab)±.71</td>
<td>3.49(^a)±.82</td>
</tr>
</tbody>
</table>

\(^1\)Data are expressed as mean percentage contribution ± S.E. to a total cell area.

\(^2\)Days relative to drying off (D) or calving (C).

\(^3\)Parameters measured: NUC, nucleus; CYT, unoccupied cytoplasm; RER, rough endoplasmic reticulum; GOL, Golgi apparatus; MIT, mitochondria; FAT, fat droplets; SEC, secretory vesicles; STA, stasis vacuoles; and CYT:NUC, cytoplasmic to nuclear ratio.

\(^a,b,c,d\)Means between sampling periods with different superscripts differ (P<.05).
TABLE 9. Effect of infection status on ultrastructural analysis\(^1\) of alveolar cells in bovine mammary tissue.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>O</th>
<th>INF</th>
<th>MINOR</th>
<th>MAJOR</th>
</tr>
</thead>
<tbody>
<tr>
<td>NUC</td>
<td>(\bar{x})</td>
<td>31.12(^b)</td>
<td>34.30(^a)</td>
<td>30.69(^{ab})</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>.85</td>
<td>.86</td>
<td>2.06</td>
</tr>
<tr>
<td>CYT</td>
<td>(\bar{x})</td>
<td>19.46(^b)</td>
<td>22.09(^a)</td>
<td>20.56(^{ab})</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>.79</td>
<td>.79</td>
<td>1.92</td>
</tr>
<tr>
<td>RER</td>
<td>(\bar{x})</td>
<td>13.66(^a)</td>
<td>11.39(^b)</td>
<td>14.49(^a)</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>.68</td>
<td>.69</td>
<td>1.64</td>
</tr>
<tr>
<td>GOL</td>
<td>(\bar{x})</td>
<td>9.58(^a)</td>
<td>8.68(^a)</td>
<td>10.03(^a)</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>.48</td>
<td>.48</td>
<td>1.09</td>
</tr>
<tr>
<td>MIT</td>
<td>(\bar{x})</td>
<td>8.28(^a)</td>
<td>7.23(^a)</td>
<td>7.16(^a)</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>.45</td>
<td>.45</td>
<td>1.09</td>
</tr>
<tr>
<td>FAT</td>
<td>(\bar{x})</td>
<td>12.13(^a)</td>
<td>12.10(^a)</td>
<td>13.72(^a)</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>.72</td>
<td>.73</td>
<td>1.79</td>
</tr>
<tr>
<td>SEC</td>
<td>(\bar{x})</td>
<td>3.79(^a)</td>
<td>2.27(^b)</td>
<td>1.41(^b)</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>.41</td>
<td>.41</td>
<td>1.01</td>
</tr>
<tr>
<td>STA</td>
<td>(\bar{x})</td>
<td>1.09(^a)</td>
<td>.86(^a)</td>
<td>.38(^a)</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>.40</td>
<td>.41</td>
<td>.99</td>
</tr>
</tbody>
</table>

\(^{1}\)Data are expressed as mean percentage contribution to a total cell area.

\(^{2}\)Infection status at time of sampling: 0, uninfective; INF, infected; MINOR, Corynebacterium bovis and coagulase negative staphylococci; and MAJOR, Staphylococcus aureus, Streptococcus uberis, and Nocardia.

\(^a, b\)Means between infection status with different superscripts differ (\(P < .05\)).
TABLE 10. Cytological comparison of infiltrating cells in uninfected mammary tissue from drying off through lactogenesis.

<table>
<thead>
<tr>
<th>Cell Types</th>
<th>Sampling Period 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D-0</td>
</tr>
<tr>
<td>epithelium</td>
<td>MAC</td>
</tr>
<tr>
<td></td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>SE</td>
</tr>
<tr>
<td>MAC</td>
<td>10.93c</td>
</tr>
<tr>
<td>SE</td>
<td>2.57</td>
</tr>
<tr>
<td>LYM</td>
<td>42.41b</td>
</tr>
<tr>
<td>SE</td>
<td>7.16</td>
</tr>
<tr>
<td>PMN</td>
<td>.15b</td>
</tr>
<tr>
<td>SE</td>
<td>.71</td>
</tr>
<tr>
<td>lumen</td>
<td>MAC</td>
</tr>
<tr>
<td></td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>SE</td>
</tr>
<tr>
<td>MAC</td>
<td>.62b</td>
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<tr>
<td>SE</td>
<td>2.77</td>
</tr>
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<td>LYM</td>
<td>.37ab</td>
</tr>
<tr>
<td>SE</td>
<td>.28</td>
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<tr>
<td>PMN</td>
<td>.78b</td>
</tr>
<tr>
<td>SE</td>
<td>3.60</td>
</tr>
<tr>
<td>stroma</td>
<td>MAC</td>
</tr>
<tr>
<td></td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>SE</td>
</tr>
<tr>
<td>MAC</td>
<td>32.96b</td>
</tr>
<tr>
<td>SE</td>
<td>6.76</td>
</tr>
<tr>
<td>LYM</td>
<td>23.19b</td>
</tr>
<tr>
<td>SE</td>
<td>3.22</td>
</tr>
<tr>
<td>PMN</td>
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<tr>
<td>MAST</td>
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<tr>
<td>SE</td>
<td>1.96</td>
</tr>
<tr>
<td>PLM</td>
<td>16.10b</td>
</tr>
<tr>
<td>SE</td>
<td>5.31</td>
</tr>
</tbody>
</table>

1 Data are expressed as mean number of cells enumerated per unit tissue area (6 x 10^4 μm^2).

2 Days relative to drying off (D) and calving (C).

3 Cell types are: MAC, macrophages; LYM, lymphocytes; PMN, polymorphonuclear leukocyte; MAST, mast cells; and PLM, plasma cells.

a, b Means between sampling period with different superscripts differ (P<.05).
Fig. 8. Mammary tissue typically obtained at drying off and calving exhibiting minimal stromal area (S) with larger proportions of epithelium (E) and distended lumina (L) occupying the tissue area. Fully active epithelial cells were characterized by the basally located nuclei (N), large cytoplasmic to nuclear ratio, and presence of numerous secretory vesicles (SV) in the apical cytoplasm X 935.

Fig. 9. Mammary tissue obtained at 14 days after drying off appeared nonactive with a large proportion of stromal area (S) with minimal luminal area (L). The shrunken alveoli (A) were characterized by a layer of closely packed cells, and the limited luminal areas stained deeply basophilic X 935.

Fig. 10. Portion of an alveolus typically obtained at drying off and calving characterized by polarized cells with a basal nuclei (N) and supranuclear Golgi (G). Abundant rough endoplasmic reticulum (R), mitochondria (M), and apically situated fat (F) and secretory vesicles (SV) occupied the cytoplasmic area with microvilli protruding from the apical surface. L, lumen X 6,075.

Fig. 11. Nonactive epithelial cells 14 days after drying off characterized by a small cytoplasmic to nuclear ratio and irregularly shaped nuclei (N). The cytoplasm consisted only of Golgi dictyosomal membranes (G) and scattered mitochondria, but no rough endoplasmic reticulum cisternae. The apical surface (arrows) lacked extensive microvilli and the alveolar lumen (L) contained an accumulation of electron-dense material X 6,075.
Fig. 12. Portion of an alveolus obtained at 7 days prior to calving demonstrating fluid accumulation. F, fat; G, Golgi apparatus; L, lumen; N, nucleus; S, stroma; and SV, secretory vesicle X 7,560.
Fig. 13. Corpora amylacea (CA) were most frequently observed filling the alveolar lumen. E, epithelium and S, stroma X 650.

Fig. 14. Polymorphonuclear leukocytes (P) exhibited phagocytic vacuoles containing mammary secretion components (arrows), and macrophages (M) with internalized cellular debris (arrowheads) X 5,625.
CHAPTER IV

Running head: Plasma Cell Isotypes in Mammary Tissue

Key words: Involution, Lactogenesis, Plasma Cells, Mastitis, Mammary Biopsy, Immunohistochemistry, Immunoglobulin.

QUANTIFICATION AND IMMUNOGLOBULIN CLASSIFICATION OF PLASMA CELLS IN NONLACTATING BOVINE MAMMARY TISSUE.

L. M. SORDILLO and S. C. NICKERSON

Louisiana Agricultural Experiment Station, Louisiana State University Agricultural Center, Hill Farm Research Station, Homer, 71040

Correspondence sent to: L. M. Sordillo-Gandy
Department of Animal Science
University of Tennessee
P.O. Box 1071
Knoxville, Tennessee 37901-1071

Approved for publication by the Director of the Louisiana Agricultural Experiment Station as manuscript number 87-80-1148.
Abstract

Plasma cell populations in bovine mammary tissue were examined during involution using electron microscopic and immunohistochemical techniques. Biopsies were taken from each quarter of 5 Jersey cows at weekly intervals beginning at drying off through parturition. Plasma cells were observed proximal to alveolar epithelial cells. This association may facilitate transport of antibody through epithelium and into milk. Immunoglobulin-producing plasma cell numbers increased gradually from drying off, reached peak concentrations 2 wk prepartum, and dropped significantly during the last wk of gestation. Immunoglobulin G₁- and G₂-producing plasma cells were the most numerous isotypes observed during the nonlactating period followed by immunoglobulin M- and immunoglobulin A-producing plasma cells. Immunoglobulin M-producing plasma cells were more numerous in tissue infected with minor pathogens than uninfected quarters. Ultrastructural examination of these cells revealed rough endoplasmic reticulum cisternae engorged with flocculent material, indicative of antibody synthesis. Exposure to minor pathogens may have enhanced plasma cell proliferation and local antibody production in response to antigenic stimulation. Results of plasma cell distribution over the nonlactating period in bovine mammary tissue indicate times when local immunostimulation may be most effective in enhancing immunity to intramammary infection.
Introduction

Immunoglobulins are an important soluble component of mammary immunity. Immunoglobulin (Ig) isotypes G\textsubscript{1}, G\textsubscript{2}, and M opsonize bacteria for phagocytosis by leukocytes (10,16). Immunoglobulin A does not function as an opsonin, but has been implicated in toxin neutralization, bacterial agglutination, and preventing bacterial adhesion to cell membranes (15). Immunoglobulins in mammary secretion are derived from blood, or produced locally by plasma cells in the subepithelial connective tissue. Most IgG is serum-derived, whereas IgA and IgM are primarily of local origin (3, 7).

In ruminants, IgG\textsubscript{1} is the predominant isotype in mammary secretions for all stages of lactation and is transported selectively from blood to milk in the absence of udder inflammation (3). Production of IgA and IgM by plasma cells creates a concentration gradient adjacent to the basement membrane of alveoli and these Igs are transported in pinocytotic vesicles across mammary epithelia and released into milk (5,16).

Systemic immunization of ruminants has been shown to reduce the severity of mastitis, but does not prevent occurrence of new intramammary infection (IMI) (14,17). Failure has been attributed to the blood-milk barrier which may prevent most circulating Ig from reaching milk (7). Local immunization to elevate Ig concentrations in lacteal secretions has proven to be a more effective means of enhancing udder defense mechanisms (10,17). Previous studies demonstrated the role of plasma cells in antibody production in
ruminant mammary glands (12,18). This locally synthesized Ig may function in udder defense against infection (9), but basic information is needed concerning the prevalence and Ig isotypes of plasma cells in mammary tissues. Immunocytochemical methods were used previously to demonstrate Ig-producing cells in normal mammary glands of cows (19), and during udder inflammation (11,18). However, no data are available describing changes in plasma cell isotype concentrations in the bovine mammary gland during the nonlactating period and in response to bacterial infection during this time. Because locally-synthesized Ig may provide a form of specialized immunological protection of bovine mammary tissue, this study examined changes in plasma cell populations during involution and through parturition to develop a basis for future attempts at local immunostimulation and enhancing Ig production.
Materials and Methods

Experimental design

Five Jersey cows from the Hill Farm Research Station were used. Approximately 5 ml of foremilk were collected aseptically prior to tissue collection and used to evaluate infection status. Mammary tissue samples were obtained by needle biopsy from each quarter at drying off, 7 and 14 days after drying off, and 14 and 7 days prior to parturition. At parturition, animals were slaughtered and approximately 1 cm$^3$ of tissue was obtained from each quarter. All tissue samples were processed for immunohistochemical and ultrastructural examination. Data were analyzed by least squares analysis of variance using the general linear model procedure to determine effects of wk during involution and infection status on each of the 4 Ig-producing plasma cell isotypes. The statistical analysis included effect of cow, wk during involution, infection status, and interaction of wk during involution by infection status. Dependent variables were Ig$_G$- $^-$, Ig$_G$- $^-$, Ig$^A$-, and Ig$^M$-producing plasma cells enumerated per unit tissue area. The following model was used:

$$Y_{ijk} = \mu + \tau_i + \beta_j + \tau\beta_{ij} + \epsilon_{ijk}$$

where:

$\mu$ = effect common to all cows,

$\tau_i$ = fixed effect of ith wk,

$\beta_j$ = fixed effect of jth infection status,

$\tau\beta_{ij}$ = fixed effect due to interaction between ith wk and jth infection status,
\( e_{ijk} \) = random error associated with each observation. The effect of cow was absorbed. Preplanned comparisons of least square means from the overall model were made by pairwise T-test. Means were contrasted between status group within a time period and between time period within a status group. No other comparisons were made.

**Microbiological procedures**

Quarter secretion samples were cultured, and presumptive identification of bacterial isolates was as described by Brown (2). Intramammary infection was defined as isolation of the same microorganism from duplicated foremilk samples.

**Tissue preparation**

Mammary tissues for immunohistochemical and ultrastructural examination were obtained and prepared for microscopy. Cows were anesthetized locally with 2 ml of 2% Lid-o-cane (Butler, Co., Columbus, OH). A small puncture was made through the skin with a scalpel blade above the gland cistern and approximately 3 cm x 2 mm of tissue was removed using a disposable biopsy needle (Travenol, Dallas, TX). Antibiotics were administered topically at the biopsy site. For light microscopy, biopsy samples and tissue samples obtained at slaughter were fixed for 48 h in 10% formalin buffered
to pH 7.4 with .025 M phosphate. Mammary tissues were prepared for sectioning by infiltration and embedding in Paraplast (American Scientific Products, McGaw Park, IL). For electron microscopy, biopsy samples and tissue sample obtained at slaughter were fixed in .1 M cacodylate buffered 2.5% glutaraldehyde, followed by .1 M cacodylate buffered osmium tetroxide, dehydrated in a graded series of ethanol, washed in propylene oxide, and embedded in epoxy resin. Tissue blocks were sectioned on an MT-5000 ultramicrotome, and 60 nm thick sections stained with 5.0% uranyl acetate in 50% methanol for 20 min followed by .4% lead citrate for 10 min.

Staining procedure for histochemical analysis

Sections (2μm thick) were stained using a peroxidase-antiperoxidase (PAP) staining kit (Miles Scientific, Naperville, IL). Following deparaffinization, sections were treated with a 3% hydrogen peroxide solution to destroy endogenous peroxidase activity, and incubated at room temperature with normal serum to suppress nonspecific protein binding. A drop of 20% primary antibody (rabbit antibovine-IgG, IgG₂, IgA, or IgM), provided by Dr. A. Guidry (USDA, Beltsville, MD), was placed on the section to react with the antigen in a humidity chamber for 30 min. Free antibody was washed off with .05 M Tris buffer (pH 7.6), and the link antibody (porcine antirabbit IgG) was added in excess to ensure one free binding site. Sections were washed again in .05 M Tris buffer, incubated for 20 min in PAP complex, and unbound PAP reagent was removed by washing in Tris buffer. Sections were then incubated for 40 min in a substrate solution (.3% hydrogen peroxide
in water and amino-ethyl-carbazole) forming a reddish precipitate at antigen sites. Sections were rinsed, counterstained in Mayer's hematoxylin, and coverslipped with glycerol-gelatin for microscopic examination.

Morphometric analysis

Quantitative morphometric analysis was used to enumerate Ig-producing plasma cells. Sections were observed with a Zeiss Standard 18 research microscope at X 1000 and plasma cells were counted in 10 randomly selected microscopic fields (6.0 x 10⁴ μm²/section). Tissue specimens were examined also for the location of plasma cells with respect to mammary epithelium and ultrastructural characteristics.
Results

Frequencies of bacterial isolates during the sampling period are in Table 11. Because of the low numbers of isolates, quarters were classified as uninfected, infected (bacteriologically positive), infected with minor pathogens (coagulase negative staphylococci or Corynebacterium bovis), or infected with major pathogens (Staphylococcus aureus, Streptococcus uberis, or Nocardia).

Plasma cell numbers/unit area for IgG\(_1\), IgA, and IgM isotypes across uninfected and infected quarters increased significantly from D-0 to D+7, while IgG\(_2\)-producing plasma cells increased significantly at D+14 (Table 12, Appendix Figures 24a-27a). Immunoglobulin G\(_1\) cells remained elevated through C-0. At C-7, numbers of IgG\(_2\)-, IgA-, and IgM-producing plasma cells decreased significantly and remained relatively lower through C-0. Immunoglobulin G\(_1\)- and IgG\(_2\)-producing plasma cells were the predominant isotypes observed throughout the sampling period, while IgA- and IgM-producing plasma cells were less abundant.

No significant differences in numbers of IgG\(_1\)-, IgG\(_2\)-, or IgA-producing plasma cells were detected between uninfected and infected quarters (Appendix Tables 26a and 27a). Mean number of IgM-producing plasma cells were significantly higher in quarters infected with minor pathogens compared to all other quarters (Table 13). Compared to uninfected quarters, those infected with minor pathogens had higher numbers of all plasma cell isotypes while those infected with major pathogens had lower numbers of these cells.
Examination of immunohistochemically stained tissues demonstrated that the majority of plasma cells were located in the subepithelial stroma proximal to, but not directly associated with the alveolar epithelium (Figures 15 and 16). Some plasma cells were observed lodged between the basal portions of epithelial cells lining alveoli (Figure 15). Electron microscopic examination of these cells revealed typical ultrastructure exhibited by antibody-producing plasma cells. The nuclei exhibited peripherally condensed chromatin and prominent nucleoli. The cytoplasm was filled with abundant, parallel arrays of rough endoplasmic reticulum (RER) which contained an electron-lucent substance (Figure 17).
Discussion

This study demonstrated that the bovine mammary gland has the cellular machinery available for Ig synthesis during the dry period. Plasma cells were observed most frequently proximal to basal surfaces of epithelial cells lining alveolar lumina of nonlactating tissue. Ultrastructural examination of these cells exhibited the typical morphology of antibody-producing cells observed by others (12) with abundant and dilated RER cisternae containing granular or flocculent material previously identified as immunoglobulin. Others have noted the proximity of parenchymal plasma cells with epithelia (8,12) and suggested direct access for antibody transport through alveolar cells and into milk with minimal diffusion into surrounding connective tissue. Mean number of plasma cells for all Ig classes increased significantly from D-0 to D+7 and D+14, reaching peak concentrations at C-14. Increasing numbers of Ig-producing plasma cells with advancing pregnancy was shown also in the mammary glands of rats (8). Elevated concentrations of Ig in bovine mammary secretions were reported during the periparturient period (4). More recently, total IgG in mammary secretion from nonlactating cows was shown to also reach peak concentrations 2 wk prepartum (L. M. Sordillo et al., 1986, unpublished data). Increased numbers of Ig-producing cells at C-14 reported in this study supports the contention that the bovine mammary gland produces Ig locally as well as accumulates Ig from serum. Enhancing Ig concentrations during the last 2 wk of gestation by either stimulating plasma cell proliferation or increasing productivity of existing cells may help
to protect the gland when susceptibility to IMI is increased. Alternatively, because the bovine mammary gland is most susceptible to IMI during the first 3 wk of involution, local immunostimulation of mammary glands at drying off may result in elevated plasma cell populations when numbers are normally low.

Yurchak et al. (19) used a fluorescent antibody technique to localize Ig-producing plasma cells in lactating bovine mammary tissue. He found that mammary tissue had mainly IgG-producing plasma cells while the other isotypes were present only in small numbers. In this study, IgG$_1$ and IgG$_2$ cells were also the predominant isotypes throughout the entire nonlactating period. Numbers of Ig-producing plasma cells in nonlactating bovine mammary tissue during colostrogenesis apparently reflect the concentrations of Ig in secretions of cows during this time. During the periparturient period, colostrum normally contains up to 150 mg Ig/ml of which IgG is about 85%, IgM about 7%, and IgA about 5% (3,4). Although most IgG is derived from blood, high concentrations of IgG cells in the underlying connective tissue may contribute to total concentrations in mammary secretion. Because most IgA and IgM are produced locally (3,5), lower levels of these Ig in mammary secretion may be explained by fewer IgA and IgM cells in the underlying connective tissue observed in this study.

Prevalence of some cells may have resulted from local antigenic stimulation via the teat canal and/or following blast transformation induced in peripheral lymphoid tissue. No differences in numbers of IgG$_1$-, IgG$_2$-, or IgA-producing plasma cells were detected between uninfected and infected quarters. These findings are consistent
with those of Nickerson and Heald (11) who found no significant
difference in numbers of IgG- and IgA-staining plasma cells between
*S. aureus* infected and control quarters. However, in quarters
infected with minor pathogens, numbers of IgM-producing plasma cells
were higher at most sampling times compared to all other quarters.
Minor pathogens are isolated frequently from the bovine mammary
gland and may produce elevated somatic cell counts. However,
infections caused by minor pathogens go unnoticed often as they only
produce a mild or subclinical form of mastitis. Boddie et al. (1)
have shown a marked plasma cell response in tissues from quarters
infected with coagulase negative staphylococci. Data from this
study suggests colonization of the streak canal by these minor
pathogens in previous lactations may have resulted in sensitization
of mammary lymphocytes and subsequent proliferation into
Ig-producing plasma cells. Conversely, lower numbers of
Ig-producing plasma cells in quarters infected with major pathogens
suggest depressed lymphocyte proliferation or compromised plasma
cell function. Similar findings have been reported previously by
Nonnecke and Harp (13). They found depressed lymphocyte response to
mitogenic stimulation in quarters with chronic staphylococcal
mastitis.

Studies have shown that local immunization of bovine mammary
glands with staphylococcal vaccines provided a substantial degree of
protection against experimental staphylococcal challenge (17).
Intramammary infusion of antigens during the dry period in sheep
induced local production of Ig which persisted into the ensuing
lactation (9). Most locally-produced Ig following antigenic
stimulation in ruminants was IgA and to a less extent, IgM (6,7,9). This study demonstrated higher IgM-producing plasma cell numbers in quarters infected with minor pathogens possibly due to longer periods of antigenic stimulation from teat canal colonization. Although relatively fewer numbers of IgA and IgM cells were demonstrated in nonlactating bovine mammary glands, numbers of these Ig-producing plasma cell isotypes peaked along with IgG1 and IgG2 cells approximately 2 wk prior to parturition.

Importance of locally synthesized Ig in mammary defense against infection has been demonstrated (7). Low concentrations of Ig-producing plasma cells at D-0 may render the udder more susceptible to IMI at this time. Alternatively, high concentrations of Ig-producing plasma cells in the bovine mammary tissue 2 wk prepartum should provide a source of immunity during lactogenesis when incidence of new IMI is high. Local antigenic stimulation of nonlactating bovine mammary glands at D-0 and C-14 may stimulate and amplify, respectively, the local antibody response to mastitis pathogens and help protect against new IMI during the nonlactating and early lactating periods.
References


### TABLE 11. Frequency of bacterial isolates from bovine mammary foremilk samples from drying off through lactogenesis.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Sampling Period</th>
<th>D-0</th>
<th>D+7</th>
<th>D+14</th>
<th>C-14</th>
<th>C-7</th>
<th>C-0</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td>55</td>
<td>50</td>
<td>45</td>
<td>55</td>
<td>55</td>
<td>50</td>
</tr>
<tr>
<td>coagulase (-) staphylococci</td>
<td></td>
<td>0</td>
<td>15</td>
<td>20</td>
<td>10</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>C. bovis</td>
<td></td>
<td>5</td>
<td>0</td>
<td>5</td>
<td>10</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>S. aureus</td>
<td></td>
<td>35</td>
<td>30</td>
<td>25</td>
<td>20</td>
<td>25</td>
<td>30</td>
</tr>
<tr>
<td>S. uberis</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Nocardia</td>
<td></td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

1 Days relative to drying off (D) or calving (C).

%, Percentage of total quarters within each sampling period.

N, Number of quarters within each sampling period.
TABLE 12. Enumeration of specific plasma cell classes (mean number of cells/6 x 10^4 μm^2 tissue area) in the bovine mammary gland from drying off through lactogenesis in infected and uninfected quarters.

<table>
<thead>
<tr>
<th>Immunoglobulin Class</th>
<th>Sampling Period</th>
<th>D-0</th>
<th>D+7</th>
<th>D+14</th>
<th>C-14</th>
<th>C-7</th>
<th>C-0</th>
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<tbody>
<tr>
<td>IgG₁</td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>X</td>
<td></td>
<td>7.39&lt;sup&gt;c&lt;/sup&gt;</td>
<td>26.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>41.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>35.72&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>28.68&lt;sup&gt;b&lt;/sup&gt;</td>
<td>23.51&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>S.E.</td>
<td></td>
<td>6.31</td>
<td>4.34</td>
<td>3.87</td>
<td>4.45</td>
<td>4.94</td>
<td>6.30</td>
</tr>
<tr>
<td>IgG₂</td>
<td></td>
<td>19.31&lt;sup&gt;b&lt;/sup&gt;</td>
<td>24.54&lt;sup&gt;b&lt;/sup&gt;</td>
<td>32.89&lt;sup&gt;a&lt;/sup&gt;</td>
<td>32.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28.71&lt;sup&gt;b&lt;/sup&gt;</td>
<td>28.11&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>X</td>
<td></td>
<td>4.70</td>
<td>3.24</td>
<td>2.88</td>
<td>3.32</td>
<td>3.68</td>
<td>4.69</td>
</tr>
<tr>
<td>IgA</td>
<td></td>
<td>2.05&lt;sup&gt;c&lt;/sup&gt;</td>
<td>21.46&lt;sup&gt;b&lt;/sup&gt;</td>
<td>23.46&lt;sup&gt;b&lt;/sup&gt;</td>
<td>31.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>21.99&lt;sup&gt;b&lt;/sup&gt;</td>
<td>20.06&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>X</td>
<td></td>
<td>3.31</td>
<td>2.28</td>
<td>2.03</td>
<td>2.34</td>
<td>2.59</td>
<td>3.30</td>
</tr>
<tr>
<td>IgM</td>
<td></td>
<td>12.26&lt;sup&gt;d&lt;/sup&gt;</td>
<td>20.89&lt;sup&gt;c&lt;/sup&gt;</td>
<td>29.18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>37.43&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28.92&lt;sup&gt;b&lt;/sup&gt;</td>
<td>26.25&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>X</td>
<td></td>
<td>3.46</td>
<td>2.51</td>
<td>2.23</td>
<td>2.57</td>
<td>2.85</td>
<td>3.64</td>
</tr>
</tbody>
</table>

<sup>1</sup>Days relative to drying off (D) and calving (C).

<sup>a,b,c,d</sup>Means between sample period with different superscripts differ (P<.05).
TABLE 13. Effect of infection status on distribution (mean number of cells/6 x 10^4 μm^2 tissue area) of specific plasma cell classes in the bovine mammary gland from drying off through lactogenesis.

<table>
<thead>
<tr>
<th>Immunoglobulin Class</th>
<th>O</th>
<th>INF</th>
<th>MINOR</th>
<th>MAJOR</th>
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</thead>
<tbody>
<tr>
<td>IgG₁</td>
<td>28.71ᵃ</td>
<td>26.84ᵃ</td>
<td>28.47ᵃ</td>
<td>24.14ᵃ</td>
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<tr>
<td>S.E.</td>
<td>2.42</td>
<td>2.36</td>
<td>5.67</td>
<td>2.85</td>
</tr>
<tr>
<td>IgG₂</td>
<td>28.14ᵃ</td>
<td>26.65ᵃ</td>
<td>29.69ᵃ</td>
<td>24.75ᵃ</td>
</tr>
<tr>
<td>S.E.</td>
<td>1.80</td>
<td>1.75</td>
<td>4.22</td>
<td>2.12</td>
</tr>
<tr>
<td>IgA</td>
<td>21.63ᵃ</td>
<td>19.62ᵃ</td>
<td>20.38ᵃ</td>
<td>18.01ᵃ</td>
</tr>
<tr>
<td>S.E.</td>
<td>1.27</td>
<td>1.26</td>
<td>2.97</td>
<td>1.50</td>
</tr>
<tr>
<td>IgM</td>
<td>23.47ᵇ</td>
<td>23.37ᵇ</td>
<td>34.62ᵃ</td>
<td>19.39ᵇ</td>
</tr>
<tr>
<td>S.E.</td>
<td>1.40</td>
<td>1.48</td>
<td>3.27</td>
<td>1.65</td>
</tr>
</tbody>
</table>

¹Pathogens isolated at time of sampling: O, uninfected; INF, infected; MINOR, Corynebacterium bovis and coagulase negative staphylococci; MAJOR, Staphylococcus aureus and streptococci.

ᵃ,ᵇMeans within immunoglobulin class with different superscripts differ (P<.05).

(15) Positive staining of plasma cells (arrows) located in the subepithelial stroma (S) and within the alveolar epithelial (E) lining of mammary tissue involuted for 7 days. L, lumen X 1,460.

(16) Clusters of positive staining plasma cells (arrows) within the stromal (S) area of mammary parenchyma obtained at parturition. E, epithelium X 650.
FIGURE 17. Clusters of plasma cells exhibiting abundant parallel lamellae of rough endoplasmic reticulum containing granulated, electron-lucent material (arrowheads). N, nucleus and S, stroma X 6,320.
CHAPTER V

SUMMARY AND CONCLUSIONS

Biochemical and morphological examination of bovine mammary glands from D-0 through lactogenesis clarified the physiological events which occur during transition to or from a state of active milk synthesis and secretion. Quarter foremilk samples were collected throughout the sampling period and bacteriologically analyzed to determine infection status. Mammary secretion composition was analyzed for changes in SCC, differential cell counts, percentages of fat and protein, pH, concentrations of BSA, Lf, citrate, and IgG, and the citrate to Lf molar ratio. Concentrations of α-lactalbumin in blood serum was determined also. Quarter biopsies were taken at weekly intervals from 5 additional cows beginning at D-0 through C-0. Histological and cytological parameters evaluated were the characterization of parenchymal tissue area; classification and ultrastructural changes of mammary epithelium; frequency and location of infiltrating leukocytes; and immunocytochemical quantification and classification of Ig-producing plasma cells.

Prior to this study, no data were available which correlated biochemical changes in secretion and serum composition with structural changes in mammary tissue during bovine mammary involution and lactogenesis. Changes in the biochemical
composition of mammary secretion during the first 2 wk of involution included decreased concentrations of normal milk components (fat, citrate, and the citrate to Lf molar ratio), which indicate decreased synthetic ability of mammary epithelial cells as involution progressed. The concomitant increase in parameters which indicate the loss of tight junction permeability (SCC, BSA, Lf, IgG, pH, and total protein) reflect the loss of cellular integrity and the influx of serum derived components through the blood-milk barrier. As C-0 approached, reverse trends in secretion and serum composition were observed. At about the last wk of gestation, decreases in SCC, total protein, BSA, Lf, and IgG with a concomitant increase in fat, citrate, the citrate to Lf molar ratio, and serum levels of α-lactalbumin reflected the cellular redevelopment of mammary tissue and the onset of milk synthesis and secretion.

Changes in mammary structure as involution progressed coincided with altered secretion composition and serum α-lactalbumin concentration associated with the cessation of lactation. Histological and ultrastructural changes in mammary parenchymal tissue during the first wk of involution indicated a gradual reduction in synthetic and secretory activity of alveolar epithelium also. Changes at the light microscopic level included an increase in stroma and nonactive epithelium with a concomitant decrease in secretory epithelium, lumen, and fully active cells. A progressive regression of epithelial cells also was evident by the decreased numbers of cytoplasmic organelles and increased prevalence of stasis vacuoles at the electron microscopic level.
Beginning approximately 2 wk prepartum, cell structure gradually assumed the morphology typically found in lactating bovine mammary glands with increased epithelium, lumen, and fully active secretory cells, but accompanied by decreased amounts of stroma and nonactive secretory cells. A progressive differentiation of epithelial cells also was apparent by the increased numbers of cytoplasmic organelles and secretory vesicles. These transient changes in secretion composition and tissue morphology throughout the non-lactating period provide an accurate indicator of secretory cell involution and subsequent development during bovine lactogenesis. Results of this study support the contention that functional transition from lactation to involution and vice versa occurs during the first and last 2 wk of the nonlactating period, respectively.

The non-lactating period has been shown to be critically related to the incidence of new intramammary infection (2,4,6,7,12). Most new infections occur during the first 2 wk of involution and from 1 wk prior to through 1 wk after parturition (2,6,20). Factors affecting susceptibility and resistance to mastitis during the dry period have not been studied extensively. Observations made in this study has allowed some speculation as to the affect of mastitis pathogens on the process of mammary involution as well as identifying specific factors which may be responsible for decreased resistance to infection during this critical time.

The presence of mastitis-causing organisms in nonlactating and early lactating glands was associated with alterations in secretion composition and tissue morphology when compared to normal uninfected
glands. Total SCC in secretion and mean number of leukocytes infiltrating mammary parenchymal tissues were consistently higher from mastitic quarters compared to uninfected quarters. Elevated leukocyte numbers, especially PMN, are indicative of an inflammatory reaction, which may have resulted from release of bacterial toxins (9,14,16,17). Differential cell counts of mammary secretion and morphometric analysis of mammary tissue revealed significantly higher numbers of PMN in infected glands compared to uninfected glands. An important function of mammary PMN is to engulf and digest bacteria and thereby reduce bacterial numbers (14). The ability of PMN to phagocytize mastitis causing organisms may be compromised in the nonlactating gland due to the indiscriminate ingestion of accumulated fat and casein (13,22); consequently, they lose pseudopodia and become degranulated. In this study, PMN frequently exhibited cytoplasmic vacuoles containing fat, casein, and cellular debris. Although PMN were prevalent in both secretions and tissues of infected quarters, their role in fluid resorption during involution may have rendered them less efficient in defending the gland against bacterial infection.

Immunoglobulins present in mammary secretions defend the mammary gland through opsonization and formation of antibody-antigen complexes in conjunction with complement (3,9). Neutralization of toxins and promotion of inflammation are other important biological functions of mammary Ig (1,9,11). Immunoglobulins in lacteal secretions are either of humoral origin or are produced locally by plasma cells in the sub-mucosa (8). This study described changes in specific plasma cell isotypes in mammary tissue during the
nonlactating period and in response to bacterial infection. Concentrations of IgG in secretion were often lower in infected quarters compared to uninfected quarters. Numbers of Ig-producing plasma cells in mammary tissue were also lower in quarters infected with major pathogens compared to uninfected quarters. These findings suggest that mastitis caused by major pathogens may depress lymphocyte proliferation or compromise plasma cell function as reported previously by others (10). Lower concentrations of opsonizing Ig in secretions from quarters infected with major pathogens may also be responsible for inferior performance of PMN in mastitic secretion and tissue during the nonlactating period.

Recently, the ability of IgG₂, IgM, and IgA to enhance in vitro phagocytosis by mammary PMN were evaluated (5). Results demonstrated that IgM was the most important opsonin. In this study, quarters infected with minor pathogens had significantly higher numbers of IgM-producing plasma cells at most sampling times compared to all other quarters. Prolonged colonization of the streak canal by minor pathogens in previous lactations may have resulted in sensitization of mammary lymphocytes and subsequent proliferation into Ig-producing plasma cells. Because IgM antibodies are important opsonins, perhaps progress in mastitis control can be made by exploiting the mammary gland's ability to synthesize IgM via local stimulation. Mean number of plasma cells in mammary tissue for all Ig classes reached peak concentrations at C-14. Stimulating plasma cell productivity at this time could elevate opsonizing levels of Ig in secretion and enhance protection of the gland during times of increased susceptibility to IMI.
Lactoferrin is a nonspecific antimicrobial protein found in wheys of bovine mammary secretion (19). Bacterial growth is restricted by the ability of bacteria to compete with Lf for protein-bound iron in mammary secretion (15). Concentrations of Lf increase up to 100-fold in secretions of fully involuted glands and at times, concentrations may equal or exceed that of IgG (19). It has been shown also that Lf, in conjunction with specific antibody, provided a powerful antimicrobial environment (18,21). In this study, Lf concentrations in mammary secretion from infected quarters were significantly lower compared to uninfected quarters. Infected quarters often had lower concentrations of IgG compared to uninfected quarters. Lower levels of these antibacterial components in secretion may have reduced the natural defense potential of the gland, allowing colonization of mastitis-causing organisms.

These data provide information regarding the quantification and distribution of essential components of the mammary immune system during the nonlactating period. Although the nonlactating mammary gland is protected by a complex system of primary and secondary defense mechanisms, the presence of bacterial infection altered levels of these components. Additional research which could manipulate the defense potential of the gland in response to pathogenic bacteria may lead to an effective mastitis control program focused on the nonlactating dairy herd.
References


APPENDIX
TABLE 1a. Sources of variation, degrees of freedom, and mean squares for numbers of total somatic cells and differential cell counts in bovine mammary secretion from uninfected quarters and those infected with minor and major pathogens.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df</th>
<th>SCC</th>
<th>MAC</th>
<th>LYM</th>
<th>PMN</th>
</tr>
</thead>
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<tr>
<td>Week</td>
<td>9</td>
<td>67.37***</td>
<td>157.03</td>
<td>711.60***</td>
<td>1393.00***</td>
</tr>
<tr>
<td>Status</td>
<td>2</td>
<td>74.03***</td>
<td>104.42</td>
<td>774.70***</td>
<td>972.91***</td>
</tr>
<tr>
<td>Week*Status</td>
<td>18</td>
<td>35.17***</td>
<td>73.59</td>
<td>120.46</td>
<td>218.13</td>
</tr>
<tr>
<td>Error (df)</td>
<td>8.04(870)</td>
<td>100.96(681)</td>
<td>116.27(681)</td>
<td>160.46(681)</td>
<td></td>
</tr>
</tbody>
</table>

Variables were: SCC, total somatic cell counts; MAC, macrophages; LYM, lymphocytes; and PMN, polymorphonuclear leukocytes.

Variations were: Week, week of the sampling period; Status, quarter infection status; and week * status, interaction between week of sampling period and quarter infections status.

Degrees of freedom.

***P<.001.
<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df</th>
<th>SCC</th>
<th>MAC</th>
<th>LYM</th>
<th>PMN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week</td>
<td>9</td>
<td>72.72***</td>
<td>238.96**</td>
<td>1074.93***</td>
<td>2013.01***</td>
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<td>Status</td>
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<td>63.26**</td>
<td>120.92</td>
<td>1114.64**</td>
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<td>Week*Status</td>
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<td>39.59***</td>
<td>45.60</td>
<td>130.11</td>
<td>216.85</td>
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<td>Error (df)</td>
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<td>8.36(823)</td>
<td>101.11(691)</td>
<td>116.43(691)</td>
<td>160.99(692)</td>
</tr>
</tbody>
</table>

1Variables were: SCC, total somatic cell counts; MAC, macrophages; LYM, lymphocytes; and PMN, polymorphonuclear leukocytes.

2Variables were: Week, week of the sampling period; Status, quarter infection status; and week * status, interaction between week of sampling period and quarter infections status.

3Degrees of freedom.

**P<.01.

***P<.001.
TABLE 3a. Sources of variation, degrees of freedom, and mean squares for concentrations of lactoferrin and citrate in bovine mammary secretion from uninfected quarters and those infected with minor and major pathogens.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Variable</th>
<th>df³</th>
<th>LF</th>
<th>CIT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week</td>
<td></td>
<td>9</td>
<td>2750.3***</td>
<td>8.31***</td>
</tr>
<tr>
<td>Status</td>
<td></td>
<td>2</td>
<td>281.59*</td>
<td>0.01</td>
</tr>
<tr>
<td>Week*Status</td>
<td></td>
<td>18</td>
<td>100.31</td>
<td>0.26</td>
</tr>
<tr>
<td>Error (df)</td>
<td></td>
<td></td>
<td>72.37(791)</td>
<td>0.11(801)</td>
</tr>
</tbody>
</table>

1Variables were: LF, lactoferrin and CIT, citrate.

2Variations were: Week, week of the sampling period; Status, quarter infection status; and week * status, interaction between week of sampling period and quarter infections status.

3Degrees of freedom.

*P<.05.

***P<.001.
TABLE 4a. Sources of variation, degrees of freedom, and mean squares for concentrations of lactoferrin and citrate in bovine mammary secretion from uninfected and infected quarters.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df</th>
<th>Variable Mean Square</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>LF</td>
</tr>
<tr>
<td>Week</td>
<td>9</td>
<td>4175.83***</td>
</tr>
<tr>
<td>Status</td>
<td>1</td>
<td>303.19*</td>
</tr>
<tr>
<td>Week*Status</td>
<td>9</td>
<td>74.55</td>
</tr>
<tr>
<td>Error (df)</td>
<td>73</td>
<td>73.02(801)</td>
</tr>
</tbody>
</table>

Variables were: Lf, lactoferrin and CIT, citrate.

Variations were: Week, week of the sampling period; Status, quarter infection status; and week * status, interaction between week of sampling period and quarter infections status.

Degrees of freedom.

*P < .05.

***P < .001
TABLE 5a. Sources of variation, degrees of freedom, and mean squares for percent fat and total protein in bovine mammary secretion from uninfected quarters and those infected with minor and major pathogens.

<table>
<thead>
<tr>
<th>Source of Variation(^1)</th>
<th>df(^2)</th>
<th>Variable</th>
<th>Mean Square</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Fat</td>
<td>Protein</td>
</tr>
<tr>
<td>Week</td>
<td>9</td>
<td>71.50***</td>
<td>243.04***</td>
</tr>
<tr>
<td>Status</td>
<td>2</td>
<td>3.69</td>
<td>49.02***</td>
</tr>
<tr>
<td>Week*Status</td>
<td>18</td>
<td>9.63*</td>
<td>24.58***</td>
</tr>
<tr>
<td>Error (df)</td>
<td>5.94(485)</td>
<td>5.13(483)</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\)Variations were: Week, week of the sampling period; Status, quarter infection status; and week * status, interaction between week of sampling period and quarter infections status.

\(^2\)Degrees of freedom.

*P < .05.

***P < .001.
TABLE 6a. Sources of variation, degrees of freedom, and mean squares for percent fat and total protein in bovine mammary secretion from uninfected and infected quarters.

<table>
<thead>
<tr>
<th>Source of Variation&lt;sup&gt;1&lt;/sup&gt;</th>
<th>df&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Variable Mean Square</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Fat</td>
<td>Protein</td>
<td></td>
</tr>
<tr>
<td>Week</td>
<td>9</td>
<td>85.92***</td>
<td>311.32***</td>
<td></td>
</tr>
<tr>
<td>Status</td>
<td>1</td>
<td>2.00</td>
<td>18.39</td>
<td></td>
</tr>
<tr>
<td>Week*Status</td>
<td>9</td>
<td>11.88*</td>
<td>38.85***</td>
<td></td>
</tr>
<tr>
<td>Error (df)</td>
<td></td>
<td>5.99(493)</td>
<td>5.40(491)</td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup>Variations were: Week, week of the sampling period; Status, quarter infection status; and week * status, interaction between week of sampling period and quarter infections status.

<sup>2</sup>Degrees of freedom.

*P<.05.

***P<.001.
TABLE 7a. Sources of variation, degrees of freedom, and mean squares for concentration of immunoglobulin G in bovine mammary secretion from uninfected quarters and those infected with minor and major pathogens.

<table>
<thead>
<tr>
<th>Source of Variation(^1)</th>
<th>df(^2)</th>
<th>Mean Square</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week</td>
<td>8</td>
<td>5368.42***</td>
</tr>
<tr>
<td>Status</td>
<td>2</td>
<td>59.07</td>
</tr>
<tr>
<td>Week*Status</td>
<td>16</td>
<td>1191.35***</td>
</tr>
<tr>
<td>Error</td>
<td>407</td>
<td>241.04</td>
</tr>
</tbody>
</table>

\(^1\)Variations were: Week, week of the sampling period; Status, quarter infection status; and week * status, interaction between week of sampling period and quarter infections status.

\(^2\)Degrees of freedom.

***p<.001
TABLE 8a. Sources of variation, degrees of freedom, and mean squares for concentration of immunoglobulin G in bovine mammary secretion from uninfected and infected quarters.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df</th>
<th>Mean Square</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week</td>
<td>8</td>
<td>6684.88***</td>
</tr>
<tr>
<td>Status</td>
<td>1</td>
<td>165.36</td>
</tr>
<tr>
<td>Week*Status</td>
<td>8</td>
<td>919.67***</td>
</tr>
<tr>
<td>Error</td>
<td>416</td>
<td>264.06</td>
</tr>
</tbody>
</table>

1Variations were: Week, week of the sampling period; Status, quarter infection status; and week * status, interaction between week of sampling period and quarter infections status.

2Degrees of freedom.

***P<.001.
TABLE 9a. Sources of variation, degrees of freedom, and mean squares for pH and concentrations of bovine serum albumin and α-lactalbumin in bovine mammary secretion from uninfected quarters and those infected with minor and major pathogens.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df</th>
<th>Variable Mean Square</th>
<th>pH</th>
<th>BSA</th>
<th>LAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week</td>
<td>9</td>
<td>2.14***</td>
<td>316.57***</td>
<td>764.33***</td>
<td></td>
</tr>
<tr>
<td>Status</td>
<td>2</td>
<td>0.95***</td>
<td>15.75</td>
<td>1003.04***</td>
<td></td>
</tr>
<tr>
<td>Week*Status</td>
<td>18</td>
<td>0.10**</td>
<td>7.23</td>
<td>223.11*</td>
<td></td>
</tr>
<tr>
<td>Error (df)</td>
<td></td>
<td>0.05(810)</td>
<td>9.30(801)</td>
<td>140.64(759)</td>
<td></td>
</tr>
</tbody>
</table>

1Variables were: BSA, bovine serum albumin and LAC, α-lactalbumin.

2Variations were: Week, week of the sampling period; Status, quarter infection status; and week * status, interaction between week of sampling period and quarter infections status.

3Degrees of freedom.

*P<.05.

**P<.01.

***P<.001.
TABLE 10a. Sources of variation, degrees of freedom, and mean squares for pH and concentrations of bovine serum albumin and α-lactalbumin in bovine mammary secretion from uninfected and infected quarters.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df</th>
<th>Variable Mean Square</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>pH</td>
</tr>
<tr>
<td>Week</td>
<td>9</td>
<td>4.22***</td>
</tr>
<tr>
<td>Status</td>
<td>1</td>
<td>1.50***</td>
</tr>
<tr>
<td>Week*Status</td>
<td>9</td>
<td>0.11*</td>
</tr>
<tr>
<td>Error (df)</td>
<td>0.05(820)</td>
<td>9.30(811)</td>
</tr>
</tbody>
</table>

1Variables were: BSA, bovine serum albumin and LAC, α-lactalbumin.

2Variations were: Week, week of the sampling period; Status, quarter infection status; and week * status, interaction between week of sampling period and quarter infections status.

3Degrees of freedom.

*P<.05.

***P<.001.
TABLE 11a. Sources of variation, degrees of freedom, and mean squares for concentrations of α-lactalbumin in bovine blood sera.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df</th>
<th>Mean Square</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal</td>
<td>27</td>
<td>382066.93</td>
</tr>
<tr>
<td>Week</td>
<td>9</td>
<td>2562106.10***</td>
</tr>
<tr>
<td>Error</td>
<td>149</td>
<td>518536.98</td>
</tr>
</tbody>
</table>

Variations were: Week, week of the sampling period; Status, quarter infection status; and week * status, interaction between week of sampling period and quarter infections status.

Degrees of freedom.

***P<.001.
TABLE 12a. Sources of variation, degrees of freedom, and mean squares for numbers of infiltrating leukocytes within the epithelial lining of bovine mammary tissue from uninfected quarters and those infected with minor and major pathogens.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Variable</th>
<th>df</th>
<th>MAC</th>
<th>LYM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week</td>
<td></td>
<td>5</td>
<td>121.44</td>
<td>1150.53</td>
</tr>
<tr>
<td>Status</td>
<td></td>
<td>2</td>
<td>46.47</td>
<td>977.14</td>
</tr>
<tr>
<td>Week*Status</td>
<td></td>
<td>10</td>
<td>101.16</td>
<td>457.95</td>
</tr>
<tr>
<td>Error</td>
<td></td>
<td>94</td>
<td>69.34</td>
<td>524.47</td>
</tr>
</tbody>
</table>

1Variables were: MAC, macrophages; LYM, lymphocytes; and PMN, polymorphonuclear leukocytes.

2Variations were: Week, week of the sampling period; Status, quarter infection status; and week * status, interaction between week of sampling period and quarter infections status.

3Degrees of freedom.

*P<.05.

**P<.01.

***P<.001.
TABLE 13a. Sources of variation, degrees of freedom, and mean squares for numbers of infiltrating leukocytes within the epithelial lining of bovine mammary tissue from uninfected and infected quarters.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df</th>
<th>Variable ( ^1 ) Mean Square</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MAC</td>
</tr>
<tr>
<td>Week</td>
<td>5</td>
<td>494.09***</td>
</tr>
<tr>
<td>Status</td>
<td>1</td>
<td>26.08</td>
</tr>
<tr>
<td>Week*Status</td>
<td>5</td>
<td>122.62</td>
</tr>
<tr>
<td>Error</td>
<td>100</td>
<td>69.33</td>
</tr>
</tbody>
</table>

1 Variables were: MAC, macrophages; LYM, lymphocytes; and PMN, polymorphonuclear leukocytes.

2 Variations were: Week, week of the sampling period; Status, quarter infection status; and week * status, interaction between week of sampling period and quarter infections status.

3 Degrees of freedom.

* \( P < .05 \).

** \( P < .01 \).

*** \( P < .001 \).
TABLE 14a. Sources of variation, degrees of freedom, and mean squares for numbers of infiltrating leukocytes within alveolar lumina of bovine mammary tissue from uninfected quarters and those infected with minor and major pathogens.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df^3</th>
<th>Variable^1 Mean Square</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MAC</td>
</tr>
<tr>
<td>Week</td>
<td>5</td>
<td>168.56*</td>
</tr>
<tr>
<td>Status</td>
<td>2</td>
<td>790.95***</td>
</tr>
<tr>
<td>Week*Status</td>
<td>10</td>
<td>167.16**</td>
</tr>
<tr>
<td>Error</td>
<td>94</td>
<td>67.85</td>
</tr>
</tbody>
</table>

1Variables were: MAC, macrophages; LYM, lymphocytes; and PMN, polymorphonuclear leukocytes.

2Variations were: Week, week of the sampling period; Status, quarter infection status; and week * status, interaction between week of sampling period and quarter infections status.

3Degrees of freedom.

*P<.05.

**P<.01.

***P<.001.
TABLE 15a. Sources of variation, degrees of freedom, and mean squares for numbers of infiltrating leukocytes within alveolar lumina of bovine mammary tissue from uninfected and infected quarters.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df</th>
<th>MAC</th>
<th>LYM</th>
<th>PMN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week</td>
<td>5</td>
<td>583.40***</td>
<td>0.30***</td>
<td>962.59***</td>
</tr>
<tr>
<td>Status</td>
<td>1</td>
<td>995.36***</td>
<td>0.64</td>
<td>2486.67***</td>
</tr>
<tr>
<td>Week*Status</td>
<td>5</td>
<td>124.35</td>
<td>0.71</td>
<td>546.42***</td>
</tr>
<tr>
<td>Error</td>
<td>100</td>
<td>80.76</td>
<td>0.83</td>
<td>136.20</td>
</tr>
</tbody>
</table>

1Variables were: MAC, macrophages; LYM, lymphocytes; and PMN, polymorphonuclear leukocytes.

2Variations were: Week, week of the sampling period; Status, quarter infection status; and week * status, interaction between week of sampling period and quarter infections status.

3Degrees of freedom.

***P<.001.
TABLE 16a. Sources of variation, degrees of freedom, and mean squares for numbers of infiltrating leukocytes within the subepithelial stroma of bovine mammary tissue from uninfected quarters and those infected with minor and major pathogens.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df</th>
<th>MAST</th>
<th>PLMS</th>
<th>MAC</th>
<th>LYM</th>
<th>PMN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week</td>
<td>5</td>
<td>141.71**</td>
<td>1422.21***</td>
<td>839.99</td>
<td>450.08***</td>
<td>139.32</td>
</tr>
<tr>
<td>Status</td>
<td>2</td>
<td>44.68</td>
<td>284.43</td>
<td>1864.48**</td>
<td>21.51</td>
<td>889.39***</td>
</tr>
<tr>
<td>Week*Status</td>
<td>10</td>
<td>16.48</td>
<td>506.55</td>
<td>696.82</td>
<td>198.39*</td>
<td>238.37**</td>
</tr>
<tr>
<td>Error</td>
<td>94</td>
<td>41.06</td>
<td>284.82</td>
<td>438.98</td>
<td>103.07</td>
<td>78.01</td>
</tr>
</tbody>
</table>

1Variables were: MAST, mast cells; PLMS, plasma cells; MAC, macrophages; LYM, lymphocytes; and PMN, polymorphonuclear leukocytes.

2Variations were: Week, week of the sampling period; Status, quarter infection status; and week * status, interaction between week of sampling period and quarter infections status.

3Degrees of freedom.

*P<.05.

**P<.01.

***P<.001.
TABLE 17a. Sources of variation, degrees of freedom, and mean squares for numbers of infiltrating leukocytes within the subepithelial stroma of bovine mammary tissue from uninfected and infected quarters.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df</th>
<th>MAST</th>
<th>PLMS</th>
<th>MAC</th>
<th>LYM</th>
<th>PMN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week</td>
<td>5</td>
<td>231.89***</td>
<td>1654.16***</td>
<td>1757.46**</td>
<td>765.04***</td>
<td>354.88***</td>
</tr>
<tr>
<td>Status</td>
<td>1</td>
<td>69.26</td>
<td>440.26</td>
<td>416.29</td>
<td>12.00</td>
<td>1690.03***</td>
</tr>
<tr>
<td>Week*Status</td>
<td>5</td>
<td>7.25</td>
<td>530.31</td>
<td>561.62</td>
<td>164.25</td>
<td>1690.03***</td>
</tr>
<tr>
<td>Error</td>
<td>100</td>
<td>39.98</td>
<td>294.90</td>
<td>479.14</td>
<td>108.72</td>
<td>364.53***</td>
</tr>
</tbody>
</table>

1Variables were: MAST, mast cells; PLMS, plasma cells; MAC, macrophages; LYM, lymphocytes; and PMN, polymorphonuclear leukocytes.

2Variations were: Week, week of the sampling period; Status, quarter infection status; and week * status, interaction between week of sampling period and quarter infections status.

3Degrees of freedom.

**P<.01.

***P<.001.
TABLE 18a. Sources of variation, degrees of freedom, and mean squares for percent tissue area composed of epithelium, lumen, and stroma in bovine mammary glands from uninfected quarters and those infected with minor and major pathogens.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df²</th>
<th>Variable Mean Square</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Epithelium</td>
<td>Lumen</td>
<td>Stroma</td>
<td></td>
</tr>
<tr>
<td>Week</td>
<td>5</td>
<td>8904.60**</td>
<td>12556.84</td>
<td>3942.19***</td>
<td></td>
</tr>
<tr>
<td>Status</td>
<td>2</td>
<td>5047.43</td>
<td>36772.58**</td>
<td>37304.16***</td>
<td></td>
</tr>
<tr>
<td>Week*Status</td>
<td>10</td>
<td>4592.32</td>
<td>5846.47</td>
<td>2984.80</td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>94</td>
<td>2674.46</td>
<td>6134.37</td>
<td>5139.48</td>
<td></td>
</tr>
</tbody>
</table>

¹Variations were: Week, week of the sampling period; Status, quarter infection status; and week * status, interaction between week of sampling period and quarter infections status.

²Degrees of freedom.

**P<.01.

***P<.001.
TABLE 19a. Sources of variation, degrees of freedom, and mean squares for percent tissue area composed of epithelium, lumen, and stroma in uninfected and infected bovine mammary glands.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df</th>
<th>Variable Mean Square</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Epithelium</td>
</tr>
<tr>
<td>Week</td>
<td>5</td>
<td>4057.46</td>
</tr>
<tr>
<td>Status</td>
<td>1</td>
<td>169.56</td>
</tr>
<tr>
<td>Week*Status</td>
<td>5</td>
<td>5731.9</td>
</tr>
<tr>
<td>Error</td>
<td>100</td>
<td>2716.22</td>
</tr>
</tbody>
</table>

1Variations were: Week, week of the sampling period; Status, quarter infection status; and week * status, interaction between week of sampling period and quarter infections status.

2Degrees of freedom.

**P<.01.

***P<.001.
TABLE 20a. Sources of variations, degrees of freedom, and mean squares for percent tissue area composed of nonactive, moderately active, and fully active secretory epithelium in bovine mammary glands from uninfected quarters and those infected with minor and major pathogens.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df ³</th>
<th>NON</th>
<th>MOD</th>
<th>FULLY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week</td>
<td>5</td>
<td>15.21***</td>
<td>4.91</td>
<td>18.73***</td>
</tr>
<tr>
<td>Status</td>
<td>2</td>
<td>16.32**</td>
<td>12.05*</td>
<td>2.90</td>
</tr>
<tr>
<td>Week*Status</td>
<td>10</td>
<td>2.85</td>
<td>1.74</td>
<td>2.95</td>
</tr>
<tr>
<td>Error</td>
<td>94</td>
<td>2.61</td>
<td>3.18</td>
<td>3.42</td>
</tr>
</tbody>
</table>

1Variables were: NON, nonactive secretory epithelium; MOD, moderately active secretory epithelium; and FULLY, fully active secretory epithelium.

2Variations were: Week, week of the sampling period; Status, quarter infection status; and week * status, interaction between week of sampling period and quarter infections status.

³Degrees of freedom.

*P<.05.

**P<.01.

***P<.001.
TABLE 21a. Sources of variation, degrees of freedom, and mean squares for percent tissue area composed of nonactive, moderately active, and fully active secretory epithelium in bovine mammary glands from uninfected and infected quarters.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df³</th>
<th>Variable Mean Square¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>NON</td>
</tr>
<tr>
<td>Week</td>
<td>5</td>
<td>35.24***</td>
</tr>
<tr>
<td>Status</td>
<td>1</td>
<td>23.08***</td>
</tr>
<tr>
<td>Week*Status</td>
<td>5</td>
<td>1.72</td>
</tr>
<tr>
<td>Error</td>
<td>100</td>
<td>2.66</td>
</tr>
</tbody>
</table>

¹Variables were: NON, nonactive secretory epithelium; MOD, moderately active secretory epithelium; and FULLY, fully active secretory epithelium.

²Variations were: Week, week of the sampling period; Status, quarter infection status; and week * status, interaction between week of sampling period and quarter infections status.

³Degrees of freedom.

*P<.05.

***p<.001.
TABLE 22a. Sources of variation, degrees of freedom, and mean squares for numbers of corpora amylacea found within the lumen, epithelium, and stroma of bovine mammary tissue from uninfected quarters and those infected with minor and major pathogens.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df</th>
<th>Epithelium</th>
<th>Lumen</th>
<th>Stroma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week</td>
<td>5</td>
<td>10.05**</td>
<td>.15</td>
<td>.09</td>
</tr>
<tr>
<td>Status</td>
<td>2</td>
<td>7.00</td>
<td>.39</td>
<td>.05</td>
</tr>
<tr>
<td>Week*Status</td>
<td>10</td>
<td>2.64</td>
<td>.10</td>
<td>.26</td>
</tr>
<tr>
<td>Error</td>
<td>94</td>
<td>2.56</td>
<td>.15</td>
<td>.56</td>
</tr>
</tbody>
</table>

1Variations were: Week, week of the sampling period; Status, quarter infection status; and week * status, interaction between week of sampling period and quarter infections status.

2Degrees of freedom.

**p<.01.
TABLE 23a. Sources of variation, degrees of freedom, and mean squares for numbers of corpora amylacea found within the lumen, epithelium, and stroma of bovine mammary tissue from uninfected and infected quarters.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df^2</th>
<th>Variable Mean Square</th>
<th>Epithelium</th>
<th>Lumen</th>
<th>Stroma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week</td>
<td>5</td>
<td>12.33***</td>
<td>.09</td>
<td>.33</td>
<td></td>
</tr>
<tr>
<td>Status</td>
<td>1</td>
<td>10.49*</td>
<td>.10</td>
<td>.01</td>
<td></td>
</tr>
<tr>
<td>Week*Status</td>
<td>5</td>
<td>2.37</td>
<td>.13</td>
<td>.49</td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>100</td>
<td>2.56</td>
<td>.15</td>
<td>.53</td>
<td></td>
</tr>
</tbody>
</table>

1Variations were: Week, week of the sampling period; Status, quarter infection status; and week * status, interaction between week of sampling period and quarter infections status.

2Degrees of freedom.

*P<.05.

***P<.001.
TABLE 24a. Sources of variation, degrees of freedom, and mean squares for percent alveolar cell area composed of nucleus, cytoplasm, rough endoplasmic reticulum, Golgi, mitochondria, fat, secretory vesicles, and stasis vacuoles in bovine mammary tissue from uninfected quarters and those infected with minor and major pathogens.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df 3</th>
<th>NUC</th>
<th>CYT</th>
<th>RER</th>
<th>GOL</th>
<th>MIT</th>
<th>FAT</th>
<th>SEC</th>
<th>STA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week</td>
<td>5</td>
<td>890.63***</td>
<td>191.86***</td>
<td>520.40***</td>
<td>373.98***</td>
<td>154.16***</td>
<td>191.20***</td>
<td>58.07***</td>
<td>10.69</td>
</tr>
<tr>
<td>Status</td>
<td>2</td>
<td>161.08*</td>
<td>75.68</td>
<td>117.37**</td>
<td>17.90</td>
<td>10.44</td>
<td>13.52</td>
<td>27.22</td>
<td>2.42</td>
</tr>
<tr>
<td>Week*Status</td>
<td>10</td>
<td>35.62</td>
<td>30.33</td>
<td>29.04</td>
<td>26.35*</td>
<td>9.91</td>
<td>29.72</td>
<td>7.52</td>
<td>6.99</td>
</tr>
<tr>
<td>Error</td>
<td>98</td>
<td>37.69</td>
<td>33.19</td>
<td>24.22</td>
<td>10.78</td>
<td>10.67</td>
<td>28.79</td>
<td>9.13</td>
<td>8.90</td>
</tr>
</tbody>
</table>

1Variables were: NUC, nucleus; CYT, cytoplasm; RER, rough endoplasmic reticulum; GOL, Golgi; MIT, mitochondria; FAT, fat droplets; SEC, secretory vesicles; and STA, stasis vacuoles.

2Variations were: Week, week of the sampling period; Status, quarter infection status; and week * status, interaction between week of sampling period and quarter infections status.

3Degrees of freedom.

*p < .05.

**p < .01.

***p < .001.
TABLE 25a. Sources of variation, degrees of freedom, and mean squares for percent alveolar cell area composed of nucleus, cytoplasm, rough endoplasmic reticulum, Golgi, mitochondria, fat, secretory vesicles, and stasis vacuoles in bovine mammary tissue from uninfected and infected quarters.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df</th>
<th>NUC</th>
<th>CYT</th>
<th>RER</th>
<th>GOL</th>
<th>MIT</th>
<th>FAT</th>
<th>SEC</th>
<th>STA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week</td>
<td>5</td>
<td>1320.44***</td>
<td>210.54***</td>
<td>734.66***</td>
<td>571.02***</td>
<td>262.95***</td>
<td>475.49***</td>
<td>141.34***</td>
<td>25.26*</td>
</tr>
<tr>
<td>Status</td>
<td>1</td>
<td>235.01*</td>
<td>160.68*</td>
<td>119.65*</td>
<td>19.25</td>
<td>25.70</td>
<td>.02</td>
<td>54.26*</td>
<td>1.23</td>
</tr>
<tr>
<td>Week*Status</td>
<td>5</td>
<td>41.73</td>
<td>33.59</td>
<td>39.10</td>
<td>12.42</td>
<td>10.14</td>
<td>53.77</td>
<td>9.86</td>
<td>13.40</td>
</tr>
<tr>
<td>Error</td>
<td>104</td>
<td>38.48</td>
<td>32.73</td>
<td>24.84</td>
<td>12.29</td>
<td>10.53</td>
<td>27.64</td>
<td>8.90</td>
<td>8.54</td>
</tr>
</tbody>
</table>

Variables were: NUC, nucleus; CYT, cytoplasm; RER, rough endoplasmic reticulum; GOL, Golgi; MIT, mitochondria; FAT, fat droplets; SEC, secretory vesicles; and STA, stasis vacuoles.

Variations were: Week, week of the sampling period; Status, quarter infection status; and week * status, interaction between week of sampling period and quarter infections status.

Degrees of freedom.

*P<.05.

***P<.001.
TABLE 26a. Sources of variation, degrees of freedom, and mean squares for percent tissue area composed of immunoglobulin G1, G2, A, and M plasma cells in bovine mammary tissue from uninfected quarters and those infected with minor and major pathogens.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df²</th>
<th>IgG1</th>
<th>IgG2</th>
<th>IgA</th>
<th>IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week</td>
<td>5</td>
<td>1413.41***</td>
<td>282.50</td>
<td>825.21***</td>
<td>775.71***</td>
</tr>
<tr>
<td>Status</td>
<td>2</td>
<td>216.07</td>
<td>152.27</td>
<td>125.69</td>
<td>846.59***</td>
</tr>
<tr>
<td>Week*Status</td>
<td>10</td>
<td>354.78</td>
<td>132.94</td>
<td>125.59</td>
<td>123.63</td>
</tr>
<tr>
<td>Error</td>
<td>93</td>
<td>285.96</td>
<td>158.69</td>
<td>78.69</td>
<td>95.35</td>
</tr>
</tbody>
</table>

1Variations were: Week, week of the sampling period; Status, quarter infection status; and week * status, interaction between week of sampling period and quarter infections status.

2Degrees of freedom.

***P<.001.
TABLE 27a. Sources of variation, degrees of freedom, and mean squares for percent tissue area composed of immunoglobulin G<sub>1</sub>, G<sub>2</sub>, A, and M plasma cells in bovine mammary tissue from uninfected and infected quarters.

<table>
<thead>
<tr>
<th>Source of Variation&lt;sup&gt;1&lt;/sup&gt;</th>
<th>df&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Variable Mean Square</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IgG&lt;sub&gt;1&lt;/sub&gt;</td>
<td>IgG&lt;sub&gt;2&lt;/sub&gt;</td>
</tr>
<tr>
<td>Week</td>
<td>5</td>
<td>2512.55***</td>
</tr>
<tr>
<td>Status</td>
<td>1</td>
<td>61.29</td>
</tr>
<tr>
<td>Week*Status</td>
<td>5</td>
<td>450.07</td>
</tr>
<tr>
<td>Error</td>
<td>99</td>
<td>286.14</td>
</tr>
</tbody>
</table>

<sup>1</sup>Variations were: Week, week of the sampling period; Status, quarter infection status; and week * status, interaction between week of sampling period and quarter infections status.

<sup>2</sup>Degrees of freedom.

***P<.001.
FIGURE 1a. Changes in percent monocytes in bovine mammary secretion by week of involution and infection status.
FIGURE 2a. Changes in percent lymphocytes in bovine mammary secretion by week of involution and infection status.
FIGURE 3a. Changes in percent polymorphonuclear leukocytes in bovine mammary secretion by week of involution and infection status.
FIGURE 4a. Changes in concentrations of lactoferrin in bovine mammary secretion by week of involution and infection status.
FIGURE 5a. Changes in concentrations of citrate in bovine mammary secretion by week of involution and infection status.
FIGURE 6a. Changes in percent fat in bovine mammary secretion by week of involution and infection status.
FIGURE 7a. Changes in percent protein in bovine mammary secretion by week of involution and infection status.
FIGURE 8a. Changes in concentration of bovine serum albumin in bovine mammary secretion by week of involution and infection status.
FIGURE 9a. Changes in concentration of α-lactalbumin in bovine mammary secretion by week of involution and infection status.
FIGURE 10a. Changes in percent tissue area composed of epithelium from bovine mammary glands by week of involution and infection status.
FIGURE 11a. Changes in percent tissue area composed of lumen from bovine mammary glands by week of involution and infection status.
FIGURE 12a. Changes in percent tissue area composed of stroma from bovine mammary gland by week of involution and infection status.
FIGURE 13a. Changes in percent alveolar area composed of nonactive secretory cells from bovine mammary glands by week of involution and infection status.
FIGURE 14a. Changes in percent alveolar area composed of moderately active secretory cells from bovine mammary glands by week of involution and infection status.
FIGURE 15a. Changes in percent alveolar area composed of fully active secretory cells from bovine mammary glands by week of involution and infection status.
FIGURE 16a. Changes in percent epithelial cell area composed of nucleus from bovine mammary tissue by week of involution and infection status.
FIGURE 17a. Changes in percent epithelial cell area composed of unoccupied cytoplasm from bovine mammary tissue by week of involution and infection status.
FIGURE 18a. Changes in percent epithelial cell area composed of rough endoplasmic reticulum from bovine mammary tissue by week of involution and infection status.
FIGURE 19a. Changes in percent epithelial cell area composed of Golgi apparatus from bovine mammary tissue by week of involution and infection status.
FIGURE 20a. Changes in percent epithelial cell area composed of mitochondria from bovine mammary tissue by week of involution and infection status.
FIGURE 21a. Changes in percent epithelial cell area composed of fat from bovine mammary tissue by week of involution and infection status.
FIGURE 22a. Changes in percent epithelial cell area composed of secretory vesicles from bovine mammary tissue by week of involution and infection status.
FIGURE 23a. Changes in percent epithelial cell area composed of milk stasis vacuoles from bovine mammary tissue by week of involution and infection status.
FIGURE 24a. Changes in numbers of immunoglobulin G1-producing plasma cells in bovine mammary tissue by week of involution and infection status.
FIGURE 25a. Changes in numbers of immunoglobulin G₂-producing plasma cells in bovine mammary tissue by week of involution and infection status.
FIGURE 26a. Changes in numbers of immunoglobulin A-producing plasma cells in bovine mammary tissue by week of involution and infection status.
FIGURE 27a. Changes in numbers of immunoglobulin M-producing plasma cells in bovine mammary tissue by week of involution and infection status.
VITA

NAME: Lorraine Marie Sordillo

BIRTH: November 5, 1959, Winchester, Massachusetts

EDUCATION:

1977 Graduated from Malden High School
Malden, Massachusetts

1981 B.S., University of Massachusetts, Amherst
Major: Animal Science; Minor: Zoology

1984 M.S., University of Massachusetts, Amherst
Major: Animal Science/Lactation Physiology
Thesis: "Caprine Mammary Gland Development and
Initiation of Lactation Following
Intramammary Colchicine Infusion."

EMPLOYMENT:

1984-1986 Graduate Research Assistant
Mastitis Research Laboratory
Hill Farm Research Station
Louisiana State University

1981-1983 Graduate Teaching Assistant
Department of Veterinary and Animal Sciences
University of Massachusetts

1980-1981 Research Laboratory Assistant
Department of Veterinary and Animal Sciences
University of Massachusetts

MEMBERSHIPS:

(A) Alpha Zeta Fraternity
(B) American Dairy Science Association
(C) Louisiana Society for Electron Microscopy
(D) Mastitis Research Workers Conference, 1983-present
(E) Society for Experimental Biology and Medicine

HONORS AND AWARDS:

Arceneaux Memorial Award for Outstanding Student Research in
Electron Microscopy. Louisiana Society for Electron

American Dairy Science Association Annual Award for Outstanding
PUBLICATIONS:

Articles in Refereed Journals:


PUBLICATIONS: (continued)

Articles in Refereed Journals: (continued)


Research Papers Presented at Annual Meetings:


Other Publications:


DOCTORAL EXAMINATION AND DISSERTATION REPORT

Candidate: Lorraine M. Sordillo

Major Field: Dairy Science

Title of Dissertation: Physiological Aspects of Bovine Mammary Involution: A Biochemical and Morphological Investigation

Approved:

S. C. Nickerson (Chairman)
Major Professor and Chairman

Dean of the Graduate School

EXAMINING COMMITTEE:

S. C. Nickerson (Chairman)
R. W. Adkinson
E. Chandler
Gerald T. Haldiman
L. C. Kappel
William A. Philpot
R. J. Siebeling

Date of Examination:
September 11, 1986