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Review Article

Anatomical Defence Mechanisms of The Mammary Gland in Domestic Animal

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Introduction:

The mammary gland of ruminants holds immense economic and biological significance, as it serves as the primary source of milk for a large segment of the global human population. Beyond milk production, this specialized organ plays a vital role in neonatal survival by supplying passive immunity through colostrum and milk, and by possessing intrinsic defense mechanisms that protect it from microbial invasion. However, the functional efficiency of the mammary gland is frequently compromised by mastitis, one of the most prevalent and economically devastating diseases affecting dairy animals. Mastitis is a multifactorial and complex condition that not only reduces milk yield and quality but also deprives society of milk, often regarded as “liquid gold” for its nutritional value.

Conventional mastitis control programs rely heavily on the use of antibiotics. Although antimicrobial therapy has been effective in reducing clinical cases, the indiscriminate and prolonged use of antibiotics has led to the emergence of resistant bacterial strains, posing serious concerns for both animal and public health. In addition, antibiotic residues in milk and the widespread use of chemical disinfectants for teat and equipment sanitation raise potential risks to consumers and the environment. These challenges highlight the limitations of current treatment- and prevention-based strategies.

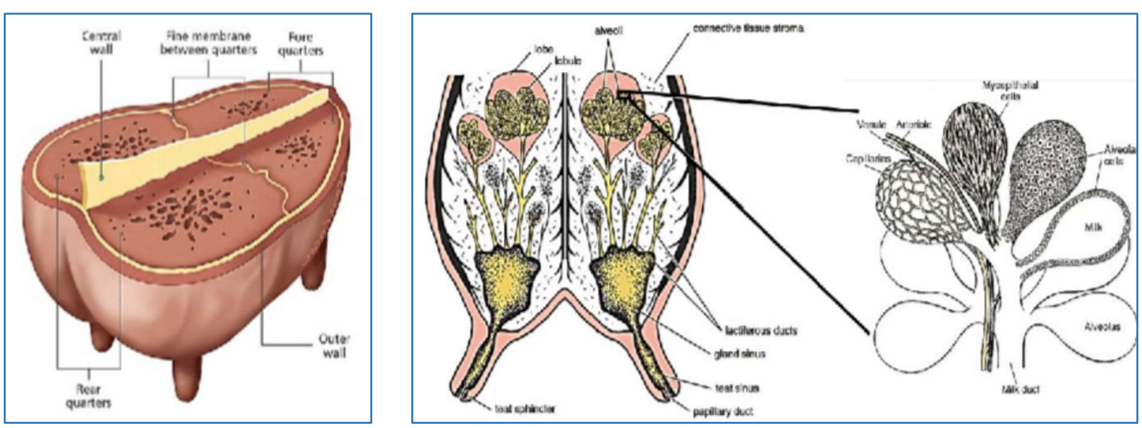
In recent years, emphasis has shifted toward enhancing the innate and adaptive defense mechanisms of the mammary gland as a sustainable alternative for mastitis control. The ability of the mammary gland to mount an effective immune response against invading pathogens is a critical determinant of udder health. Unfortunately, vaccination strategies have shown limited success due to the wide diversity of mastitis-causing organisms and their variable antigenic characteristics. Consequently, strengthening the natural resistance of the mammary gland has emerged as a promising approach to reduce the incidence and severity of mastitis.

Resistance to mastitis is governed by a complex interplay of anatomical barriers, cellular defenses, histochemical properties, and biochemical factors within the mammary tissue. Despite its importance, our understanding of these defense systems and their interactions remains incomplete. A comprehensive insight into the natural immune mechanisms of the mammary gland is essential for developing alternative strategies aimed at improving udder health, reducing dependence on antibiotics, and ensuring sustainable dairy production.

Mammary Gland – Anatomy:

A mammary gland is an exocrine gland that produces milk to feed young offspring It is specific type of apocrine gland, situated in the inguinal region. Mammary gland made up of connective tissue, fat, and tissue that contains the glands that can make milk. The cow has four mammary glands grouped into a structure called an udder. A strong udder suspensory system provides proper attachments of the gland with the body.

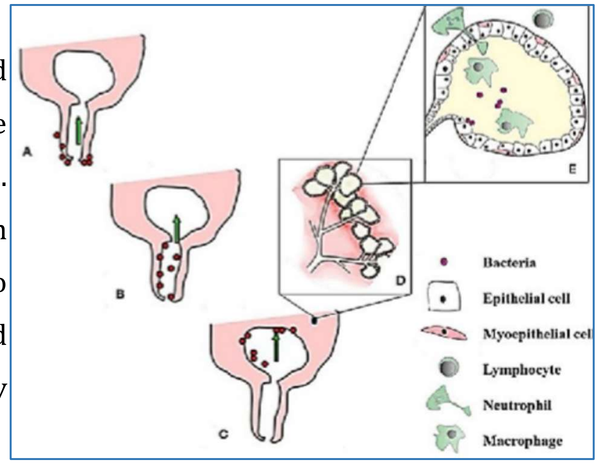
The milk is synthesized in the secretory cells, which are arranged as a single layer on a basal membrane in a spherical structure called alveolus. An alveolus is the discrete milk producing unit and the diameter of each alveolus is about 50-250 mm. The lumen of the alveolus is lined by a single layer of secretory epithelial cells. Several alveoli together form a lobule and each lobule contains 150-220 microscopic alveoli. Groups of lobules are surrounded by a connective tissue sheath and form a structure called lobe. The milk which is continuously synthesized in the alveolar area, is stored in the alveoli, milk ducts, udder and teat cistern between two milking’s. A large proportion of ducts that are the tubing are presents in the mammary gland. These ducts allow the milk moves from the alveoli to the teat for milk removal. In addition, between the teat and the large ducts are open areas called teat cisterns. A teat cistern is a cavity where milk can collect between two milkings.



Anatomy of Mammary Gland

The gland cisterns or sinus lactiferous, also called the udder cistern, opens directly into the teat cistern. The gland cistern and teat cistern are separated by the annular fold. The gland cistern function for milk storage (holds 100-400 ml). The gland cistern varies greatly in size and shape. There are often pockets formed in the cistern at the end of the larger ducts (Akers & Nickerson, 2011)

Process of Udder Infection:



Organisms invade the udder through teat canal and migrate up the teat canal and colonize all parts where the milk gets and finally setup the infection in mammary gland. After getting bacterial infection, cellular defense mechanism becomes active and phagocytic cells (from blood) effort to engulf and kill the bacteria, phagocytosis by products and release of bacterial toxins damage to the secretory mammary epithelial cells (Zigo *et al.*, 2021).

Mammary Gland Immunity:

The mammary gland is protected by a variety of defense mechanisms, which can be separated into two distinct categories: innate immunity and specific immunity.

Innate immunity, also known as nonspecific responsiveness, is the predominant defense during the early stages of infection. Nonspecific responses are present or are activated quickly at the site of infection by numerous stimuli; however, they are not augmented by repeated exposure to the same insult. Nonspecific or innate responses of the mammary gland are mediated by the anatomical barrier of the mammary gland, macrophages, neutrophil, natural killer (NK)- like cells, and by certain soluble factors (Sordillo *et al.*, 1997).

The anatomical factors related to the mastitis resistance are teat sphincter muscle, teat keratin, teat cistern lining, teat shape, teat length, teat diameter, furstenberg's rosette, teat pigmentation, teat skin, size of alveoli etc. Cellular factors responsible for mammary gland protection are epithelial lining and immunological cell population like various leukocytes.

The histochemical and biochemical substances related to the protective immunity of the mammary gland against mastitis are protective proteins such as immunoglobulin, lactoferrin etc. and trace minerals namely copper, zinc, iron, manganese etc.

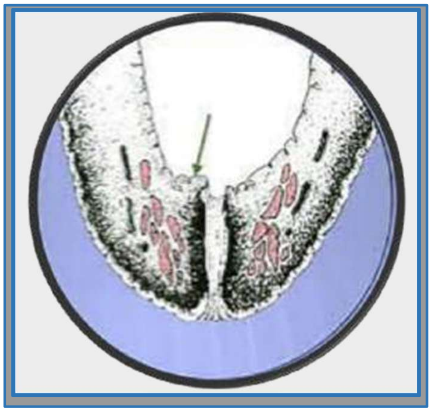
Conversely, the specific or acquired immune system recognizes specific determinants of a pathogen that facilitate selective elimination. Recognition of pathogenic factors is mediated by antibody molecules, macrophages, and several lymphoid populations. Because of the "memory" of certain lymphocytes, specific immune responses can be augmented by repeated exposure to a pathogen. In the mammary gland, both innate and acquired protective factors are coordinated to provide optimal protection from disease (Sordillo *et al.*, 1997).

Anatomical Defense of Mammary Gland:

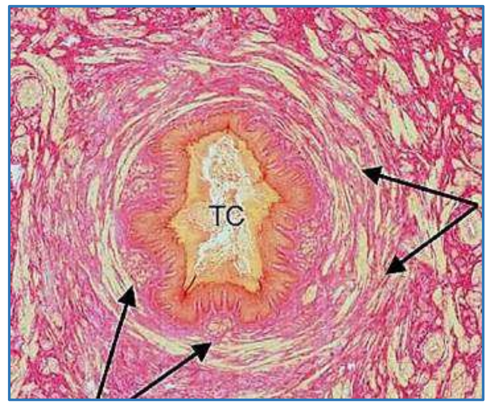
Teat Sphincter Muscle:

Mastitis occurs when bacteria gain entrance to the mammary gland via the teat canal. For this reason, the teat end is considered to be the first line of defence against invading pathogens. The teat end contains sphincter muscles that maintain tight closure between milkings and hinder bacterial penetration. Increased patency of these muscles is directly related to increased incidence of mastitis (Sordillo *et al.*, 1997).

Teat closure after milking, effected by the local teat musculature, is paramount for inhibiting bacterial entrance. However, some closure is achieved 20 to 30 minutes after completion of milking; during that period animals should be prevented from lying down, as this predisposes to increased exposure of their teats to bacteria (DeVries *et al.*, 2010).



Teat sphincter muscle



Transverse section of the bovine teat

Teat Keratin Plague:

The teat canal is lined with keratin, which is crucial to the maintenance of the barrier function of the teat end, and removal of the keratin has been correlated to increased susceptibility to bacterial invasion and colonization (Capuco *et al.*, 1992). Teat keratin is a waxy material that is derived from stratified squamous epithelium. The keratin structure enables trapping of invading bacteria, thus hindering their migration into the gland cistern (Hibbitt *et al.*, 1969). Antimicrobial agents have been identified within the keratin lining. The esterified and nonesterified fatty acids present in teat keratin, such as myristic acid, palmitoleic acid, and linoleic acid, are bacteriostatic (Treece *et al.*, 1966). Additionally, cationic protein in the canal can bind electrostatically to mastitis pathogens, which alters the bacterial cell wall, thus rendering them more susceptible to osmotic pressure. The inability to maintain osmolarity causes lysis and death of the invading pathogens (Sordillo *et al.*, 1997).

During the dry-period, accumulation of keratin at the teat orifice seals the teat preventing bacterial entrance (Katsafadou *et al.,* 2019).

Keratohyaline granules of stratum granulosum of teat canal contributed in formation of large amount of keratin in lumen of streak canal. Keratin formed a mesh like substance at the lumen of streak canal, thus partially occluded lumen and inhibits bacterial penetration by providing physical barrier. (Nickerson, 1985)

Streak canal & Lining of keratine

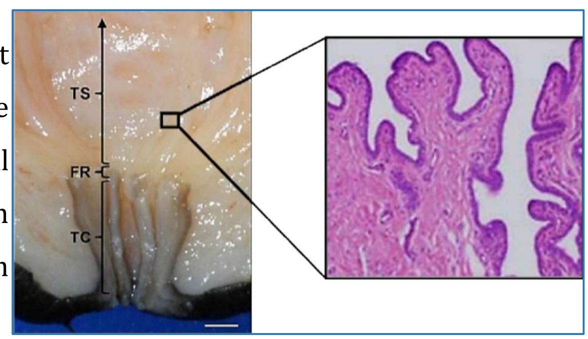
Cross section of teat canal of Madras Red Sheep

The steak canal was lined by stratified squamous keratinized epithelium both in cows and buffaloes. The epithelium was thicker in buffaloes than in cattle, which provided an extra resistance against penetration of pathogen through epithelium. The stratum granulosum contained higher amount of keratohyaline granules in buffaloes than in cows. (Kumar, 1988)

Lipid components of keratin contain antibacterial fatty acid components that have both bacteriostatic and bactericidal activities. The long chain fatty acids of keratin disrupt bacterial lipid membranes, resulting in bacterial cell perforation. Keratin also contains calcium-binding proteins that also may have some antimicrobial activity (Sordillo, 2018).

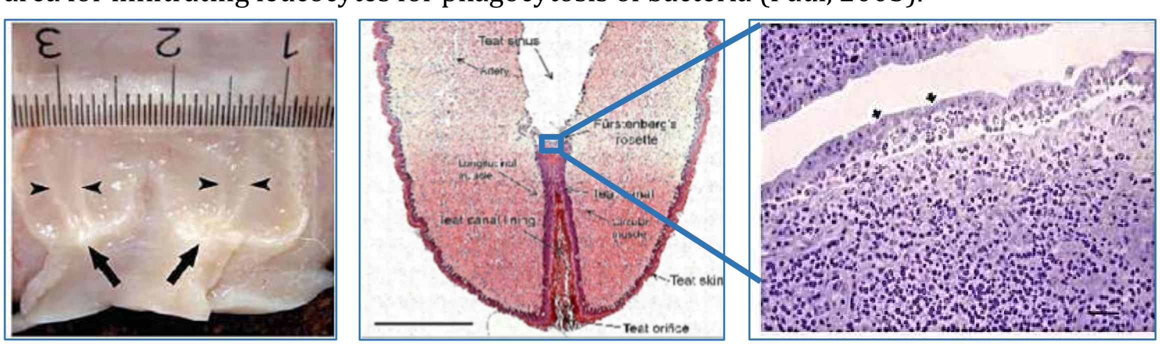
Teat Cistern Lining:

The teat cistern is lined with cuboidal epithelium, that is, a double layer of 'block' cells. In the normal cow these are held tightly together however, in response to bacterial invasion, they have the ability to move slightly apart, which allows the entry of infection-fighting white blood cells from the small blood vessels beneath (Sordillo, 2018).



Furstenberg's rosette:

The Furstenberg's rosette is located in the internal streak canal of the teat. It radiates upward into the teat cistern. It often is considered a barrier for pathogens, yet it offers little resistance to milk leaving the teat. the larger surface area provided by connective tissue folds of Furstenberg's rosette provided more surface area for infiltrating leucocytes for phagocytosis of bacteria (Paul, 2005).



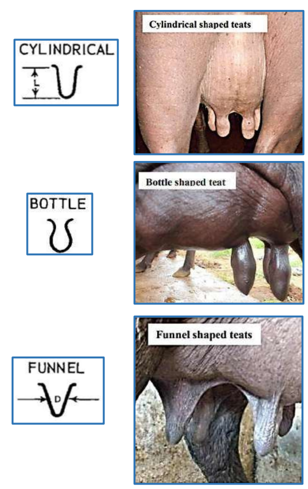
Lymphocytes show considerable numerical increase while gone to Furstenberg's rosette region from teat sinus and reach the highest density in Furstenberg's rosette region. Structures resembling to lymphoid follicles were seen in addition to lymphocyte infiltrations and these may be source of immune response in Furstenberg's rosette region. Solitary and aggregate lymphoid follicles including centrum germinativum, lymphocyte infiltrations were seen in Furstenberg's rosette region. Mucosal lymphoid tissues consist of solitary or aggregate lymphoid follicles and specialized epithelium called as FAE or lympho-epithelium which covers these follicles. It is stated that M cells existing among FAE. M cells (membranous cells) uptake intra luminal antigens and pass them from their narrow cytoplasm. M cells are the specialized cells for uptake of antigen, in Furstenberg's rosette region (Asti *et al.,* 2011).

Teat Length & Diameter:

Cows with teats of smaller diameter milked more completely. incidence of mastitis increased proportionally to teat diameter (Hickman, 1964). Animal with Medium-to-short teats has lowest incidence of mastitis. The shortened teats are less prone to physical trauma, whereas long teats increase the risk of accidental trauma and these lesions constitutes potential source of microorganism which increase the probability of udder infection (Awale *et al*., 2012). The risk of subclinical mastitis was highest for cows with long and thick teats (Uzmay *et al.,* 2003).

Teat Shape:

Funnel-shaped teats offered greater resistance to being drawn into the teat cup and appeared to milk out more completely. On cylindrical and bottle-shaped teats, the teat cups caused occlusion of the orifice between the gland sinus and teat sinus, causing a decrease and then complete stoppage of milk flow. This may cause traumatization of the teat-end orifice. (Seykora & McDaniel 1985). There exists for cylindrical rear teats a significantly greater incidence of mastitis than for funnel- and bottle-shaped rear teats. (Hickman, 1964). The risk of subclinical mastitis for cows with funnel shaped teats was found to be lower than cows with cylindrical teats (Uzmay et al., 2003).



Teat Pigmentation:

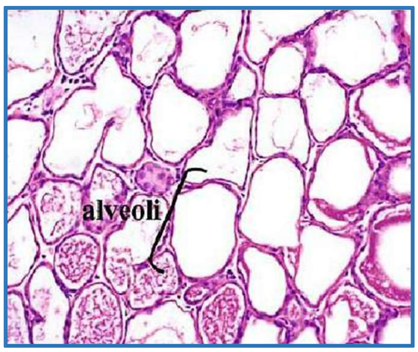
Cows with lighter skin pigmentation about the udders and teats appeared to be more susceptible to irritation of teat skin from harsh environment, whereas animal with dark pigmented skin on teat and udder have ability to withstand against harsh environment (Foust, 1941).

Teat Skin:

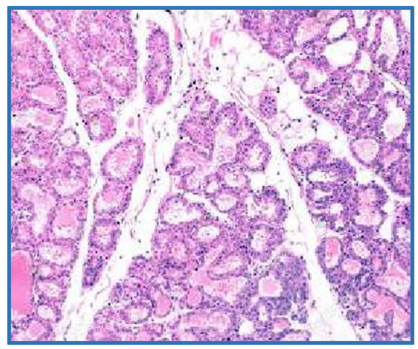
Fatty acids present on the teat skin have bacteriostatic properties and can thus limit bacterial numbers around the teat orifice and on the teat surface (Paul, 2005).

Size of Alveoli / Thickness of Septa:

Udder with small size alveoli have more amount of interalveolar connective tissue, whereas udder with larger size alveoli have very less amount of interalveolar connective tissue. The amount of interalveolar connective tissue was found to be more in the mammary gland of non-de script cow in comparison to that of crossbred cow. Thickness of the interalveolar barrier which included the alveolar epithelial cells of two adjacent alveoli and the connective tissue element in between them was about more in nondescript cow than in crossbred cow. This thickness was taken into consideration for the probable propagation of the infective agents into the surrounding alveoli on the occasion of the entry of mastitis causing organisms into the mammary gland. It may therefore be concluded that the chances of interalveolar spreading of infection in the mammary gland of non-descript cow is far less in comparison to that of crossbred cow (Paul, 2005).



Histological sections of the mammary gland of crossbred cow


Histological sections of the mammary gland of ND cow

Udder Attachment:

The tighter udder attachment indicated lesser chances of mastitis (Rupp and Boichard, 1999). Therefore, higher incidence of tight udder attachment in non-de script and crossbred cows might be one of the causes of lower frequency of mastitis. The udder attachment is heritable character (Rupp & Boichard, 1999) and heritability of udder traits is moderate to high (Seykora & McDaniel, 1985), therefore, indirect selection for tight udder attachment may be considered to reduce the chances of mastitis (Paul, 2005).

Udder Cleft:

The deeper udder cleft showed lesser chances of mastitis (Rogers *et al.*, 1997). Hence higher incidence of deeper udder cleft was one of the causes of lower incidence of mastitis in non-descript cows in comparison to crossbred cows. So deep udder cleft or crease may be taken in to consideration as indirect selection parameter during the formulation of breeding programme to reduce the chances of mastitis (Paul, 2005).

Udder Quarter:

The higher frequency of equal quarter might be one of the factors for lesser incidence of mastitis in non-descript cow. Therefore, this trait may be considered as indirect parameter during selection for crossbreeding programme to get more resistance to mastitis (Paul, 2005).

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