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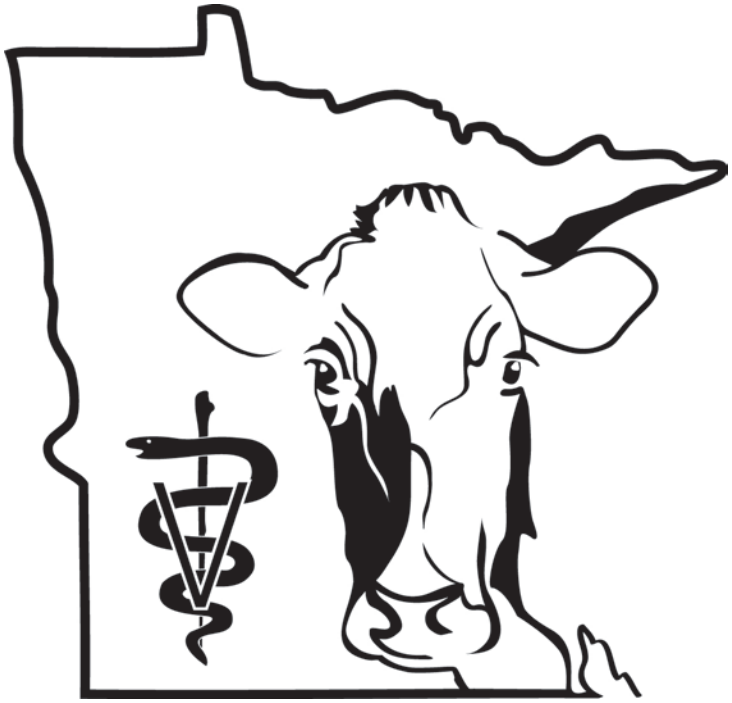

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Immunological dysfunction in periparturient cows - is this why periparturient cows get sick?.

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Annual cash receipts in the United States for livestock sales have run between $91 and $93 billion between 1996 and this year's March projection by the USDA's Economic Research Service. Among livestock sales, dairy is the second largest commodity (behind beef and ahead of poultry). Milk sales alone account for 23-25% of all cash receipts for livestock in the U.S over the past 3 years. These economic figures emphasize the need for maintaining healthy dairy cows for providing our Nation's milk supply. Expansion of our dairy export markets will require U.S. dairy farmers to produce a high quality milk supply at somatic cell count standards set by our export customers. Those of us serving the livestock health industry must therefore be prepared to provide the best quality advice and care in managing the Nation's dairy herd. The most important factor in providing a low somatic cell count milk supply is keeping cows free from mastitis. Mastitis is anything causing inflammation of the mammary gland and infectious mastitis is caused by a plethora of microbial agents.[1] Nearly half of the Nation's herd of 9.5 million dairy cows will experience at least one episode of mastitis during each lactation. Research in the past 15-20 years has already resulted in genetic selection for cows with lower somatic cell counts by the incorporation of this trait into the A.I. sire summary ranking indices. This approach will mainly serve to reduce the normal increase in mastitis incidence that occurs as milk production go up.

Our research at the National Animal Disease Center in Ames, IA has taken a two-fold approach for managing healthier dairy cows - immunomodulation and identification of cows with genetically superior immune systems. Here we will present how immunomodulation will help provide dairy farmers with a new tool to prevent infectious disease in their cows. We will not discuss antibiotic treatment of cows with mastitis. Antibiotics play only a limited role in mastitis treatment and control programs, and efficacious antibiotic therapy is largely restricted to dry cow intramammary therapy and lactating cow therapy for streptococcal mastitis.[2] The traditional techniques of antibiotic therapy of all 4 quarters of all cows at dry off and teat-dipping with an FDA-approved germicidal teat dip, were for years, the only proven methods to treat and prevent mastitis. Much of the current mastitis control and prevention programs for the dairy industry depends on antibiotic and germicidal chemical usage. Problems related to drug residues and perceived health dangers to people focus our need to reduce the industry's dependency on these compounds.

It is important to note that coliforms are the single most common etiologic agent isolated from clinically severe mastitis cases on well managed dairy farms.[3,4] Clinical trials and experimental studies have demonstrated repeatedly *no benefits* of antibiotic therapy in cattle with clinical or subclinical coliform mastitis.[5-7] The advent of the *Escherichia coli* J-5 and other endotoxin core mutant vaccines in veterinary medicine have now provided us new tool to reduce the incidence and severity of clinical coliform mastitis.[8-10] However, there still is an urgent need to seek new ways to prevent or treat coliform mastitis.

Immunosuppression in the pathogenesis of mastitis

More than 15 years ago we embarked on a decidedly different approach to deal with mastitis on dairy farms. Our research was driven by the observation that most clinical mastitis occurs in dairy cows in early lactation and our view that bovine mastitis is caused by opportunistic pathogens and therefore these cows must be immunosuppressed. What evidence supported our hypothesis of periparturient immunosuppression?

First of all, there is an extremely high incidence of clinical disease in periparturient cows with nearly 25% of all clinical mastitis occurring during the first 2 weeks after calving. Clinical mastitis caused by virtually all pathogens (but especially coliform bacteria and streptococci other than *Streptococcus agalactiae*) has a very high incidence in early lactation. Cows must first become infected and then develop clinical mastitis. The rates of new intramammary infections (IMI) caused by environmental pathogens are highest during the first and last 2 weeks of a 60-day, nonlactating period of dairy cows.[4, 11-13] The rate of new IMI during these periods of peak susceptibility is 2 to 12 times higher than any other time in the production cycle of the cow. Most coliform and environmental streptococcal infections established in the nonlactating period and that are present at parturition result in clinical mastitis soon afterward.[11, 14] The proportion of all cases of clinical coliform mastitis that develop during the first 2, 4, and 8 weeks of lactation has been reported to be 25, 45 and 60%, respectively.[15, 16]

The second piece of evidence supporting the notion of immunosuppression in the pathogenesis of mastitis was that we are traditionally taught that opportunistic infections are associated with severe compromises of host defense mechanisms. These two points lead us to conduct experiments evaluating how functional a cow's immune system is around calving time. Over the past decade, an overwhelming amount of evidence of immunological dysfunction of lymphocytes and neutrophils has been generated in several research institutes around the world.[17-30] Today we believe that as the immune system becomes progressively more compromised at the end of gestation, cows become more readily infected in the mammary gland, then as the immune system "bottoms out" the first week or two after calving, these subclinical infections begin to win the battle with the cow's immune system and clinical mastitis results. This can also be extended to infectious diseases of virtually any system of the postpartum cow (gastrointestinal, respiratory and reproductive tracts all have increased disease incidence in postpartum cows).

What causes periparturient immunosuppression?

Many neuroendocrine changes develop in cows during the periparturient period. Periparturient hormone fluxes may adversely affect immune cell function. Surprisingly, there is no effect of estrogen on bovine neutrophil function either during the follicular phase of the estrous cycle in cows or after administration of high doses of estradiol to steers.[31, 32] However, supraphysiologic concentrations of estradiol have been reported to suppress neutrophil function.[33, 34] These high concentrations of estrogens may be germane to immunosuppression and the high new IMI rates prior to calving. Before calving, total plasma estrogen concentrations increase in the cow (at least 10 times greater than during estrus).[35]

During normal pregnancy, the progesterone binding capacity of human lymphocytes is increased (perhaps as a result of increasing estrogen levels) and the concentration of progesterone in serum during pregnancy are sufficient to reduce lymphocyte functions.[36, 37] This raises the possibility that hormone sensitivities of immune cells during gestation may be altered and result in functional changes in immune cells. Very high concentrations of both estrogens and progesterone are reached during the final days of gestation in cows.[35] This may be germane to the onset of impaired lymphocyte function in the prepartum cow whose lymphocyte hormone binding capacity may be higher than that in barren cows.

Many of the hormonal and metabolic changes that prepare the mammary gland for lactation take place during the 3 weeks preceding parturition. Lymphocyte and neutrophil function could be affected by prepartal increases in estrogen, prolactin, growth hormone, and/or insulin.[35, 38-40] During this critical period, the dairy cow's metabolism shifts from the demands of pregnancy to include those of lactation, with increased demands for energy and protein. Negative energy and protein balances that exist during early lactation may also contribute to impaired neutrophil function and, thus, account for a portion of the periparturient immunosuppression observed.

The specific physiological factors contributing to periparturient immunosuppression and increased incidence of clinical disease have not been fully elucidated. We do know, however, that there is a very broad-based suppression of immune function in cows the first week or two after calving. Normally, neutrophils can move rapidly from the blood into the mammary gland in response to an infection.[41] Phagocytosis and killing of bacteria by neutrophils in conjunction with humoral factors are critical defense mechanisms of the mammary gland.[42, 43] We have demonstrated a wide range of leukocyte functional activities between dairy cows and during different production stages of dairy cows (around calving time).[18-22, 29, 44, 45] These findings have been validated in other labs around the world.[23, 24, 26, 27, 46-53] Most importantly, associations between neutrophil dysfunction and periparturient disorders in cows have been reported.[28, 30] Periparturient immunosuppression is not limited to cattle, investigations of immunosuppression and coliform mastitis in sows revealed depressed neutrophil function to be associated with the susceptibility to postpartum mastitis caused by *Escherichia coli*.[54]

Defects in lymphocyte function also contribute to the dairy cow's increased susceptibility to mastitis during the periparturient period. In addition to antibody production, other roles for lymphocytes in bovine mammary gland immunity include production of various hormone messengers of the immune system called cytokines. These cytokines can activate bovine neutrophils to have enhanced activity against pathogens.[55] Suppression of critical lymphocyte functions after calving have been reported.[25, 29, 48, 56-58]

In summary, a multitude of the components of the immune system of a dairy cow begin to become impaired as early as 2-3 weeks before she actually gives birth (long before the elevation of endogenous cortisol which occurs 36 hours before and after calving). The cow's immune system then bottoms out and is seriously impaired for 1-2 weeks after calving. We call this effect on the cow's immune system, periparturient immunosuppression. Regardless of its causation, it is our contention that periparturient immunosuppression makes the dairy cow quite susceptible to the establishment of new infections (particularly in the mammary gland) and the subsequent development of these new subclinical infections into clinical disease (mastitis, metritis, and postpartum outbreaks of intestinal diseases such as salmonellosis, just to name a few).

What are the prospects for immunomodulation to prevent disease?

In an effort to study methods to prevent immunosuppression, we have evaluated injections of various cytokines that are part of the cow's normal immune system. One of these cytokines, granulocyte-colony stimulating factor (G-CSF) has proven to be quite safe to administer[18, 22, 59, 60] and will reduce the incidence of clinical coliform mastitis by 50% during the first week of lactation following experimental challenge.[61] It has also been shown useful with *Staphylococcus aureus* and *Klebsiella pneumoniae* challenge models.[62, 63] It is crucial to understand that immunomodulators often work best in immunocompromised hosts, hence the periparturient period is an excellent time for such compounds to be given to animals. It is also important that the immumodulators have no adverse side effects. A major pharmaceutical firm has recently initiated obtaining fast track approval through the FDA for bringing this cytokine, *granulocyte-colony stimulating factor* or **G-CSF** into the market place for veterinary medicine. The human equivalent of G-CSF (sold under the name Neupogen®) has been successfully used for several years as an adjunct therapy for cancer patients undergoing chemotherapy. It is extremely helpful in reducing the incidence of infectious disease (particularly pneumonia) in these cancer patients.

What can we do until G-CSF is available?

Production of milk from mastitis-free cows is quite simple - keep them in clean, dry and unstressful environments and feed your cows right. Unfortunately, knowing keeping her dry and unstressed, and knowing what it means to feed her right, is not simple. Keeping cows in very clean environments is only achieved through common sense and lots of elbow grease. Minimizing

stress is also common sense but also very much out of our control when it comes to environmental factors. We emphasize feeding cows optimal rations because of several indications of the influence of inadequate nutrition on immunity. For example, the activities of the neutrophil in combating microbial infection are complex and all involve expenditure of cellular energy. The average cow has ~3500 neutrophils per µl of blood, this translates into ~1.4 x 10^{11} neutrophils in an 1800 lb Holstein cow. The circulating half-life of neutrophils is about 6 hours, so the cow is replacing half of those cells every 6 hours from bone marrow stores. Clearly, a significant component of the dietary energy and protein consumption for maintenance is spent on replenishment of immune cells. The negative energy and protein balance of dairy cows during the periparturient period and up to peak lactation undoubtedly influence immune function. Recent studies at the NADC have shown that mastectomized cows recover from periparturient immunosuppression within one week after calving, whereas intact lactating cows were immunosuppressed for 2-3 weeks postpartum.[64]

Giesecke suggested that lactating dairy cows are unique in their response to stress, since ruminant metabolism is dependent on glycogenesis/glycogenolysis and lipogenesis/lipolysis for energy-efficient and glucose-sparing feed conversion.[65] The lactational ability of dairy cows, combined with ruminant metabolism, may be a metabolically-demanding phenomenon unique to dairy cows. The ensuing negative energy and protein balances in early lactation may limit the immune system of cows. It is unlikely that periparturient immunosuppression is the result of a single physiological factor; more likely, it will be found that several entities act in concert with profound effects on the function of many organ systems of the dairy cow. The most clinically evident effect of periparturient stressors on dairy cows may be immunosuppression, thus explaining the high incidence of clinical mastitis. The best we can do today is to give transition or periparturient cows the best possible hygeine conditions and appropriate diets.

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