

IMPORTANCE OF FUNCTIONAL MAMMARY GLAND IMMUNITY DURING TIMES OF STRESS

Marcus E. Kehrli, Jr.
Pfizer Global Research & Development
Terre Haute, Indiana

Periparturient cows experience an elevated incidence of infectious and metabolic diseases. Mastitis is one of these diseases and is typically an infectious disease characterized by inflammation of the mammary gland and abnormal lacteal secretions regardless of the cause. Mastitis is a major source of economic loss to dairy industries around the world, affecting both milk production and milk quality. The extreme difficulty in controlling mastitis has almost resulted in the dairy farmer's and veterinarian's acceptance of it as a constant problem on even well managed dairy farms. Many organisms that cause bovine mastitis are opportunistic pathogens. Opportunistic bacterial infections typically occur when the integrity of native host defenses is breached and are often indicative of predisposing immunosuppression in the host animal. In the 1980's at the National Animal Disease Center-USDA-ARS we embarked on a decidedly different research approach to understand the pathogenesis of mastitis on dairy farms. Our research was driven by the observation that most clinical mastitis occurs in dairy cows in early lactation. Because bovine mastitis is caused by opportunistic pathogens we hypothesize that these cows must be immunosuppressed. This review will consider the experimental evidence supporting our hypothesis of periparturient immunosuppression.

First of all, there is an extremely high incidence of clinical mastitis in early lactation caused by opportunistic environmental pathogens (mostly coliform bacteria and streptococci other than *Streptococcus agalactiae*). Cows must first become infected and then develop clinical mastitis. Rates of new intramammary infections (IMI) caused by environmental pathogens are highest during the first and last 2 weeks of the dry period (20, 51, 64, 65). The rate of IMI during these periods of peak susceptibility is 2 to 12 times higher than at any other time (64). Most coliform and environmental streptococcal infections that are established in the nonlactating period and that are still present at calving time result in clinical mastitis soon afterward (46, 64). 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 (26, 44). The majority of initial cases of clinical mastitis occur early in lactation and the risk of clinical mastitis increases with age (56).

The second piece of evidence supporting the notion of immunosuppression in the pathogenesis of mastitis was that medicine traditionally teaches that opportunistic infections are associated with severe compromises of host defense mechanisms. These two points led us and others to evaluate the functional capacity of the bovine immune system around parturition. An overwhelming amount of evidence of immunological dysfunction of lymphocytes and neutrophils, as well as reduced levels of humoral immunity factors in the transition cow has now been generated (6, 13, 23, 24, 25, 31, 32, 33, 34, 35, 47, 48, 71, 72). From a practical viewpoint, combining periparturient immunosuppression with poor hygiene in dry cow lots, results in transition cows

being more readily infected in the mammary gland and/or uterus. As the immune system reaches its functional low-point the first week or two after calving, these subclinical infections often progress into clinical mastitis. This reduced immune function is likely contributing to infectious diseases of virtually any system of the postpartum cow (enteric, respiratory and reproductive tracts all have increased disease incidence in postpartum cows).

Immunosuppression In The Pathogenesis of Mastitis

To understand the role of immunosuppression in the pathogenesis of mastitis, it is important to understand the cellular immune defense mechanisms operative in the mammary gland. The essential role of functional neutrophils in preventing microbes that have invaded the mammary gland from gaining an edge and causing mastitis has long been recognized (27, 58, 59, 60, 61). A key step in host defense is leukocyte recruitment into tissues following infection by microorganisms. Leukocyte trafficking through tissues is crucial for effective immune surveillance for infectious agents. Trafficking enables rapid neutrophil (innate immunity) accumulation at sites of infection or tissue injury and subsequent movement of lymphocytes (adaptive immunity) through secondary lymphoid tissues for development of memory immune responses against antigens presented in germinal centers (1). The interaction of different populations of circulating leukocytes with postcapillary venule endothelial cells is essential for leukocyte emigration into tissue (1).

Neutrophils are a fairly uniform population of rather unsophisticated white blood cells whose recruitment to sites of inflammation (often recognized as pus formation). Neutrophil migration is a sophisticated process involving the coordinated function of multiple families of adhesion molecules, cytokines, and chemoattractants. A multistep-model for this process includes a transient adhesion between the leukocyte and endothelial cells of the vessel wall mediated by members of the selectin family, followed by triggering of leukocyte activation and subsequent tight adhesion between leukocyte integrins and the intercellular adhesion molecules on endothelial cells. Selectivity in the process of leukocyte recruitment comes from the diversity of molecules capable of mediating each step. Various studies have shown that endocrine factors (e.g., glucocorticoids) released during stress reduce leukocyte trafficking between the blood and other immune compartments as well as their movement into tissues to fight infections (14). When impairment of neutrophil migration or killing functions occurs, we know that cows are made more susceptible to mastitis, as exemplified by conversion of subclinical mastitis into peracute toxic mastitis (5, 27, 61). We believe that periparturient immunosuppression explains why cows infected with mastitis pathogens at calving, almost always experience clinical mastitis in early lactation (64). Studies have also associated retained placenta with impaired neutrophil function and lower levels of an essential chemokine (interleukin-8) in uterine fluids and cotyledon homogenates (36, 38). Optimal recruitment of functional neutrophils into these tissues is likely involved with timely separation of fetal placental membranes from the maternal tissues.

What Causes Periparturient Immunosuppression?

Many factors contributing to the increased susceptibility to IMI and the progression to clinical disease have been fully elucidated over the past 10 years. Neutrophils can move rapidly from the

blood into the mammary gland in response to an irritant (28). Phagocytosis and bacterial killing mechanisms of neutrophils in conjunction with humoral factors are critical defense mechanisms of the mammary gland (27, 61). Conditions that may compromise immune function include physiologic stress of lactation (52), decreased number of circulating neutrophils capable of phagocytosis after parturition (17, 49), delayed inflammatory response in early lactation (18, 19, 41), and impaired neutrophil microbicidal functions (13, 31, 33, 35). Dystocia, ketosis, and milk fever also have been associated with coliform mastitis after calving (10, 77). Investigation of immunosuppression and coliform mastitis in sows has revealed depressed neutrophil functional capacity to be associated with the susceptibility to postpartum mastitis caused by *Escherichia coli* (42).

Defects in lymphocyte function also contribute to the periparturient cow's increased susceptibility to mastitis. 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 macrophages and neutrophils to have enhanced killing activity against pathogens (43). Suppression of various critical lymphocyte functions in periparturient cows have been reported (13, 23, 25, 29, 30, 31, 34, 45, 48, 54, 63, 78, 79).

Many neuroendocrine changes develop in cows during the periparturient period. Hormonal changes during the periparturient period have been reported (55, 57, 66) and likely contribute to immunologic dysfunction. Increased plasma concentration of the endogenous opioids, β -endorphin and met-enkephalin, during the periparturient period in cows may also reduce immune function (3). Plasma concentration of these opioids peaks at parturition and cows experiencing dystocia have significantly elevated concentrations of β -endorphin several hours postpartum compared to normal cows.

Pregnancy has been postulated to result in suppression of Th1-type immunity (i.e., cell-mediated immunity) and enhancement of Th2-type immune responses (i.e., humoral immunity). As pregnancy progresses, the ability of the immune system to produce Th1 cytokines [interferon- γ (IFN- γ) and interleukin-2 (IL-2)] decreases and Th2 cytokine (IL-4, IL-5, IL-6 and IL-10) production increases. (12, 76) Estrogen and progesterone play roles as suppressers of cell-mediated immune responses and enhancers of humoral responses in mice (62). Corticosteroids are also known to suppress cell-mediated immune responses and enhance humoral responses by suppressing the production of Th1 cytokines such as IL-2 and IFN- γ (11, 75). Similar findings have been reported with cattle (15, 50). Cytokine production by bovine leukocytes is disturbed during the periparturient period in that IFN- γ and IL-2 production are dramatically reduced, suggesting suppression of Th1 immune responses (25, 63, 71). It has been postulated that these changes in cytokine production might contribute to the increased incidence of disease in periparturient cows (67, 69, 70) and would certainly alter the host response to vaccination. We believe the decline in IFN- γ production is particularly damaging to phagocytic cell function. This is supported by findings that IFN- γ is capable of restoring suppressed neutrophil function (7, 53). Moreover, impairment of the capacity of B cells from periparturient cows to secrete IgM has been reported (13); this likely stems from impaired IFN- γ and IL-2 production.

A wide array of immunologic disturbances in cellular and humoral components of immune responses have been documented in cattle during the periparturient period and related to the

marked reduction in the ability of dairy cattle to respond to invasive microorganisms (6, 13, 31, 33, 34, 47, 48, 54, 68, 69, 70, 71). Before calving, total plasma estrogen concentration increase in the cow (at least 10 times greater than during estrus) (8). Supraphysiologic concentrations of estradiol as seen at the end of gestation have been reported to suppress neutrophil function (4, 40) and may be germane to immunosuppression and the high new IMI rates prior to calving.

Elevations in estrogen at the end of gestation may make immune cells more sensitive to the immunosuppressive effects of an already elevated level of progesterone, thus explaining the systemic immune suppression in the periparturient cow. How does this work? 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 (73, 74). 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 (8).

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 (2, 8, 9, 22). 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 immune function and, thus, account for a portion of the periparturient immunosuppression observed. For example, the activities of neutrophils in combating infection are complex and involve expenditure of cellular energy. The average cow has ~3,500 neutrophils per μl of blood, this translates into $\sim 1.4 \times 10^{11}$ neutrophils in an 1800 lb. Holstein cow. The circulating half-life of neutrophils is about 6 hours, so a cow is replacing half of her neutrophils 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. Conclusive evidence that the demands of lactation contribute to postpartum immune suppression derives from recent studies at the NADC. In studies with mastectomized cows it was found that they recover from periparturient immunosuppression within one week after calving, whereas intact lactating cows can be immunosuppressed for 2-3 weeks postpartum (39).

What Are The Prospects For Immunomodulation To Prevent Disease?

Can we find compounds to restore immune function to the periparturient animal? We would like to believe this is possible -- but it won't be simple. It is crucial to understand that immunomodulators work best in immune-compromised hosts, hence the periparturient period is an excellent time for such compounds to be given to cows. Immunomodulators must have no adverse side effects. A goal for an immune modulator for the periparturient dairy cow would be to restore innate immune function without interfering with the teleological basis of periparturient immunosuppression.

What Can We Do Until An Immune Restorative Is Available?

Production of milk from mastitis-free cows is based on keeping cows in clean, dry, stress-free environments and proper nutrition. 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 extremes of environmental conditions. We emphasize optimal nutrition because of several indications of the influence of nutrition (21) and the stress of lactation on immunity (37).

Conclusion

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 (16). Negative energy and protein balances in early lactation may limit the immune system. Periparturient immunosuppression is likely the result of several factors working in concert with profound effects on the function of many systems of the cow. The most economically important effects of periparturient immunosuppression, are the clinical infections of the mammary gland and uterus. The best we can do today is to give periparturient cows the best possible hygiene conditions, appropriate diets and as few additional stress events as possible. Early detection and intervention of health problems in transition cows is paramount to maintaining her as an economically viable production unit in a herd.

Bibliography

- 1 Adams, D. H., and S. Shaw. 1994. Leukocyte-endothelial interactions and regulation of leukocyte migration. *The Lancet* 343:831.
- 2 Akers, R. M. 1985. Lactogenic hormones: binding sites, mammary growth, secretory cell differentiation, and milk biosynthesis in ruminants. *J. Dairy Sci.* 68:501.
- 3 Aurich, J. E., I. Dobrinski, H.-O. Hoppen, and E. Grunert. 1990. β -endorphin and met-enkephalin in plasma of cattle during pregnancy, parturition and the neonatal period. *J. Repro. Fert.* 89:605.
- 4 Bodel, P., G. M. Dillard, Jr., S. S. Kaplan, and S. E. Malawista. 1972. Anti-inflammatory effects of estradiol on human blood leukocytes. *J. Lab. Clin. Med.* 80:373.
- 5 Burton, J. L., and M. E. Kehrli, Jr. 1995. Regulation of neutrophil adhesion molecules and shedding of *Staphylococcus aureus* in milk of cortisol- and dexamethasone-treated cows. *Am. J. Vet. Res.* 56:997.
- 6 Cai, T.-Q., P. G. Weston, L. A. Lund, B. Brodie, D. J. McKenna, and W. C. Wagner. 1994. Association between neutrophil functions and periparturient disorders in cows. *Am. J. Vet. Res.* 55:934.
- 7 Canning, P. C., and J. A. Roth. 1989. Effects of in vitro and in vivo administration of recombinant bovine interferon- γ on bovine neutrophil responses to *Brucella abortus*. *Vet. Immunol. Immunopathol.* 20:119.

- 8 Comline, R. S., L. W. Hall, R. B. Lavelle, P. W. Nathanielsz, and M. Silver. 1974. Parturition in the cow: endocrine changes in animals with chronically implanted catheters in the foetal and maternal circulations. *J. Endocrinol.* 63:451.
- 9 Convey, E. M. 1974. Serum hormone concentrations in ruminants during mammary growth, lactogenesis, and lactation: a review. *J. Dairy Sci.* 57:905.
- 10 Curtis, C. R., H. N. Erb, C. J. Sniffen, R. D. Smith, P. A. Powers, M. C. Smith, M. E. White, R. B. Hillman, and E. J. Pearson. 1983. Association of parturient hypocalcemia with eight periparturient disorders in Holstein cows. *J. Am. Vet. Med. Assoc.* 183:559.
- 11 De Rijk, R. H., and F. Berkenbosch. 1994. Suppressive and permissive actions of glucocorticoids: a way to control innate immunity and to facilitate specificity of adaptive immunity? Page 73 *in* *Endocrinology and Metabolism: Bilateral communication between the endocrine and immune systems.* Springer-Verlag, New York.
- 12 Delassus, S., G. C. Coutinho, C. Saucier, S. Darche, and P. Kourilsky. 1994. Differential cytokine expression in maternal blood and placenta during murine gestation. *J. Immunol.* 152:2411.
- 13 Detilleux, J. C., M. E. Kehrli, Jr., J. R. Stabel, A. E. Freeman, and D. H. Kelley. 1995. Study of immunological dysfunction in periparturient Holstein cattle selected for high and average milk production. *Vet. Immunol. Immunopathol.* 44:251.
- 14 Dhabhar, F. S., A. H. Miller, B. S. McEwen, and R. L. Spencer. 1995. Effects of stress on immune cell distribution: dynamics and hormonal mechanisms. *J. Immunol.* 154:5511.
- 15 Doherty, M. L., H. F. Bassett, P. J. Quinn, W. C. Davis, and M. L. Monaghan. 1995. Effects of dexamethasone on cell-mediated immune responses in cattle sensitized to *Mycobacterium bovis*. *Am. J. Vet. Res.* 56:1300.
- 16 Giesecke, W. H. 1985. The effect of stress on udder health of dairy cows. *Onderstepoort J. Vet. Res.* 52:175.
- 17 Guidry, A. J., M. J. Paape, and R. E. Pearson. 1976. Effects of parturition and lactation on blood and milk cell concentrations, corticosteroids and neutrophil phagocytosis in the cow. *Am. J. Vet. Res.* 37:1195.
- 18 Hill, A. W. 1981. Factors influencing the outcome of *Escherichia coli* mastitis in the dairy cow. *Res. Vet. Sci.* 31:107.
- 19 Hill, A. W., A. L. Shears, and K. G. Hibbitt. 1979. The pathogenesis of experimental *Escherichia coli* mastitis in newly calved dairy cows. *Res. Vet. Sci.* 26:97.
- 20 Hogan, J. S., K. L. Smith, K. H. Hoblet, P. S. Schoenberger, D. A. Todhunter, W. D. Hueston, D. E. Pritchard, G. L. Bowman, L. E. Heider, B. L. Brockett, and H. R. Conard. 1989. Field survey of clinical mastitis in low somatic cell count herds. *J. Dairy Sci.* 72:1547.
- 21 Hogan, J. S., W. P. Weiss, D. A. Todhunter, K. L. Smith, and P. S. Schoenberger. 1992. Bovine neutrophil responses to parenteral vitamin E. *J. Dairy Sci.* 75:399.
- 22 Houdebine, L.-M., J. Djiane, I. Dusanter-Fourt, P. Martel, P. A. Kelly, E. Devinoy, and J.-L. Servely. 1985. Hormonal action controlling mammary activity. *J. Dairy Sci.* 68:489.
- 23 Ishikawa, H. 1987. Observation of lymphocyte function in perinatal cows and neonatal calves. *Jpn. J. Vet. Sci.* 49:469.
- 24 Ishikawa, H., and T. Shimizu. 1983. Depression of B-lymphocytes by mastitis and treatment with levamisole. *J. Dairy Sci.* 66:556.

- 25 Ishikawa, H., T. Shirahata, and K. Hasegawa. 1994. Interferon- γ production of mitogen stimulated peripheral lymphocytes in perinatal cows. *J Vet Med Sci* 56:735.
- 26 Jackson, E., and J. Bramley. 1983. Coliform mastitis. *In Practice* 5:135.
- 27 Jain, N. C., O. W. Schalm, E. J. Carroll, and J. Lasmanis. 1968. Experimental mastitis in leukopenic cows: Immunologically induced neutropenia and response to intramammary inoculation of *Aerobacter aerogenes*. *Am. J. Vet. Res.* 29:2089.
- 28 Jain, N. C., O. W. Schalm, and J. Lasmanis. 1978. Neutrophil kinetics in endotoxin-induced mastitis. *Am. J. Vet. Res.* 39:1662.
- 29 Kashiwazaki, Y. 1984. Lymphocyte activities in dairy cows with special reference to outbreaks of mastitis in pre- and post-partus. *Jpn. J. Vet. Res.* 32:101.
- 30 Kashiwazaki, Y., Y. Maede, and S. Namioka. 1985. Transformation of bovine peripheral blood lymphocytes in the perinatal period. *Jpn. J. Vet. Sci.* 47:337.
- 31 Kehrli, M. E., Jr., and J. P. Goff. 1989. Periparturient hypocalcemia in cows: effects on peripheral blood neutrophil and lymphocyte function. *J. Dairy Sci.* 72:1188.
- 32 Kehrli, M. E., Jr., J. P. Goff, M. G. Stevens, and T. C. Boone. 1991. Effects of granulocyte colony-stimulating factor administration to periparturient cows on neutrophils and bacterial shedding. *J. Dairy Sci.* 74:2448.
- 33 Kehrli, M. E., Jr., B. J. Nonnecke, and J. A. Roth. 1989. Alterations in bovine neutrophil function during the periparturient period. *Am. J. Vet. Res.* 50:207.
- 34 Kehrli, M. E., Jr., B. J. Nonnecke, and J. A. Roth. 1989. Alterations in bovine lymphocyte function during the periparturient period. *Am. J. Vet. Res.* 50:215.
- 35 Kelm, S. C., J. C. Detilleux, A. E. Freeman, M. E. Kehrli, Jr., A. B. Dietz, L. K. Fox, J. E. Butler, I. Kasckovics, and D. H. Kelley. 1997. Genetic association between parameters of innate immunity and measures of mastitis in periparturient Holstein cattle. *J. Dairy Sci.* 80:1767.
- 36 Kimura, K., J. Goff, T. A. Reinhardt, and S. Sato. 2001. Association between retained placenta and blood interleukin-8 concentration and lack of association of retained placenta with energy and calcium metabolic profiles. Page 151 *in Proc. J. Dairy Sci.*, Indianapolis, IN.
- 37 Kimura, K., J. P. Goff, and M. E. Kehrli, Jr. 1999. Effects of the presence of the mammary gland on expression of neutrophil adhesion molecules and myeloperoxidase activity in periparturient dairy cows. *J. Dairy Sci.* 82:2385.
- 38 Kimura, K., J. P. Goff, and M. E. Kehrli, Jr. 2001. Decreased Neutrophil Function as a Cause of Retained Placenta in Dairy Cattle. *J. Dairy Sci.* (In Press)
- 39 Kimura, K., J. P. Goff, M. E. Kehrli, Jr., J. A. Harp, and B. J. Nonnecke. 1997. Effect of mastectomy on phenotype and function of leukocytes in periparturient dairy cows. *J. Dairy Sci.* 80:264.
- 40 Klebanoff, S. J. 1979. Effect of estrogens on the myeloperoxidase-mediated antimicrobial system. *Infect. Immun.* 25:153.
- 41 Lee, E.-K., and M. E. Kehrli, Jr. 1998. Expression of adhesion molecules on neutrophils of periparturient cows and neonatal calves. *Am. J. Vet. Res.* 59:37.
- 42 Löfstedt, J., J. A. Roth, R. F. Ross, and W. C. Wagner. 1983. Depression of polymorphonuclear leukocyte function associated with experimentally induced *Escherichia coli* mastitis in sows. *Am. J. Vet. Res.* 44:1224.

- 43 Lukacs, K., J. A. Roth, and M. L. Kaeberle. 1985. Activation of neutrophils by antigen-induced lymphokine, with emphasis on antibody-independent cytotoxicity. *J. Leukocyte Biol.* 38:557.
- 44 Malinowski, E., J. Krzyzanowski, W. Wawron, J. Slawomirski, and J. Gluszak. 1983. Analysis of cases of *Escherichia coli* mastitis in cows. *Med. Weter.* 39:608.
- 45 Manak, R. C. 1982. Mitogenic responses of peripheral blood lymphocytes from pregnant and ovariectomized heifers and their modulation by serum. *J. Reprod. Immunol.* 4:263.
- 46 McDonald, J. S., and A. J. Anderson. 1981. Experimental intramammary infection of the dairy cow with *Escherichia coli* during the nonlactating period. *Am. J. Vet. Res.* 42:229.
- 47 Nagahata, H., S. Makino, S. Takeda, H. Takahashi, and H. Noda. 1988. Assessment of neutrophil function in the dairy cow during the perinatal period. *J. Vet. Med. B* 35:747.
- 48 Nagahata, H., A. Ogawa, Y. Sanada, H. Noda, and S. Yamamoto. 1992. Peripartum changes in antibody producing capability of lymphocytes from dairy cows. *Vet. Q.* 14:39.
- 49 Newbould, F. H. S. 1976. Phagocytic activity of bovine leukocytes during pregnancy. *Can. J. Comp. Med.* 40:111.
- 50 Nonnecke, B. J., J. L. Burton, and M. E. Kehrl, Jr. 1997. Associations between function and composition of blood mononuclear leukocyte populations from Holstein bulls treated with dexamethasone. *J. Dairy Sci.* 80:2403.
- 51 Oliver, S. P., and B. A. Mitchell. 1983. Susceptibility of bovine mammary gland to infections during the dry period. *J. Dairy Sci.* 66:1162.
- 52 Paape, M. J., F. C. Gwazdauskas, A. J. Guidry, and B. T. Weinland. 1981. Concentrations of corticosteroids, leukocytes and immunoglobulins in blood and milk after administration of ACTH to lactating dairy cattle: Effects on phagocytosis of *Staphylococcus aureus* by polymorphonuclear leukocytes. *Am. J. Vet. Res.* 42:2081.
- 53 Roth, J. A., and D. E. Frank. 1989. Recombinant bovine interferon- γ as an immunomodulator in dexamethasone-treated and nontreated cattle. *J. Interferon Res.* 9:143.
- 54 Saad, A. M., C. Concha, and G. Åström. 1989. Alterations in neutrophil phagocytosis and lymphocyte blastogenesis in dairy cows around parturition. *J. Vet. Med. B* 36:337.
- 55 Saleem, M. A., P. Jha, K. Buckshee, and A. Farooq. 1992. Studies on the immunosuppressive role of steroid hormones during pregnancy. *Immunol. Invest.* 21:1.
- 56 Sargeant, J. M., H. M. Scott, K. E. Leslie, M. J. Ireland, and A. Bashiri. 1998. Clinical mastitis in dairy cattle in Ontario: frequency of occurrence and bacteriological isolates. *Can. Vet. J.* 39:33.
- 57 Sasser, R. G., D. E. Falk, and R. H. Ross. 1979. Estrogen in plasma of parturient paretic and normal cows. *J. Dairy Sci.* 62:551.
- 58 Schalm, O. W., E. J. Carroll, and J. Lasmanis. 1964. The leukocyte barrier and serologic investigations of experimental coliform (*Aerobacter aerogenes*) mastitis in cattle. *Am. J. Vet. Res.* 25:90.
- 59 Schalm, O. W., J. Lasmanis, and E. J. Carroll. 1964. Effects of pre-existing leukocytosis on experimental coliform (*Aerobacter aerogenes*) mastitis in cattle. *Am. J. Vet. Res.* 25:83.
- 60 Schalm, O. W., J. Lasmanis, and E. J. Carroll. 1966. Significance of leukocytic infiltration into the milk in experimental *Streptococcus agalactiae* mastitis in cattle. *Am. J. Vet. Res.* 27:1537.

- 61 Schalm, O. W., J. Lasmanis, and N. C. Jain. 1976. Conversion of chronic staphylococcal mastitis to acute gangrenous mastitis after neutropenia in blood and bone marrow produced by an equine anti-bovine leukocyte serum. *Am. J. Vet. Res.* 37:885.
- 62 Screpanti, I., D. Meco, S. Morrone, A. Gulino, B. J. Mathieson, and L. Frati. 1991. In vivo modulation of the distribution of thymocyte subsets: effects of estrogen on expression of different T cell receptor V β gene families in CD4-, CD8- thymocytes. *Cell. Immunol.* 134:414.
- 63 Shafer-Weaver, K. A., and L. M. Sordillo. 1997. Bovine CD8+ suppressor lymphocytes alter immune responsiveness during the postpartum period. *Vet. Immunol. Immunopathol.* 56:53.
- 64 Smith, K. L., D. A. Todhunter, and P. S. Schoenberger. 1985. Environmental pathogens and intramammary infection during the dry period. *J. Dairy Sci.* 68:402.
- 65 Smith, K. L., D. A. Todhunter, and P. S. Schoenberger. 1985. Environmental mastitis: cause, prevalence, prevention. *J. Dairy Sci.* 68:1531.
- 66 Smith, V. G., L. A. Edgerton, H. D. Hafs, and E. M. Convey. 1973. Bovine serum estrogens, progestins and glucocorticoids during late pregnancy, parturition and early lactation. *J. Anim. Sci.* 36:391.
- 67 Sordillo, L. M., G. Afseth, G. Davies, and L. A. Babiuk. 1992. Effects of recombinant granulocyte-macrophage colony-stimulating factor on bovine peripheral blood and mammary gland neutrophil function *in vitro*. *Can. J. Vet. Res.* 56:16.
- 68 Sordillo, L. M., and L. A. Babiuk. 1991. Controlling acute *Escherichia coli* mastitis during the periparturient period with recombinant bovine interferon gamma. *Vet. Microbiol.* 28:189.
- 69 Sordillo, L. M., and J. E. Peel. 1992. Effect of interferon- γ on the production of tumor necrosis factor during acute *Escherichia coli* mastitis. *J. Dairy Sci.* 75:2119.
- 70 Sordillo, L. M., G. M. Pighetti, and M. R. Davis. 1995. Enhanced production of bovine tumor necrosis factor- α during the periparturient period. *Vet. Immunol. Immunopathol.* 49:263.
- 71 Sordillo, L. M., M. J. Redmond, M. Campos, L. Warren, and L. A. Babiuk. 1991. Cytokine activity in bovine mammary gland secretions during the periparturient period. *Can. J. Vet. Res.* 55:298.
- 72 Stabel, J. R., M. E. Kehrli, Jr., J. R. Thurston, J. P. Goff, and T. C. Boone. 1991. Granulocyte colony-stimulating factor effects on lymphocytes and immunoglobulin concentrations in periparturient cows. *J. Dairy Sci.* 74:3755.
- 73 Szekeres-Bartho, J., V. Csernus, J. Hadnagy, and A. S. Pacsa. 1983. Progesterone-prostaglandin balance influences lymphocyte function in relation to pregnancy. *Am. J. Reprod. Immunol.* 4:139.
- 74 Szekeres-Bartho, J., J. Hadnagy, and A. S. Pacsa. 1985. The suppressive effect of progesterone on lymphocyte cytotoxicity; unique progesterone sensitivity of pregnancy lymphocytes. *J. Reprod. Immunol.* 7:121.
- 75 Vacca, A., M. P. Felli, A. R. Farina, S. Martinotti, M. Maroder, I. Screpanti, D. Meco, E. L. F. Petrangeli, and A. Gulino. 1992. Glucocorticoid receptor mediated suppression of the interleukin-2 gene expression through impairment of the cooperativity between nuclear factor of activated T cells and AP1 enhancer elements. *J. Exp. Med.* 175:637.

- 76 Wegmann, T. G., H. Lin, L. Guilbert, and T. R. Mosmann. 1993. Bidirectional cytokine interactions in the maternal-fetal relationship: is successful pregnancy a TH2 phenomenon? *Immunol. Today* 14:353.
- 77 Weigt, U. 1983. Clinical aspects of coliform mastitis in the bovine. *Vet. Res. Commun.* 7:253.
- 78 Wells, P. W., C. Burrells, and W. B. Martin. 1977. Reduced mitogenic responses in cultures of lymphocytes from newly calved cows. *Clin. Exp. Immunol.* 29:159.
- 79 Yamanaka, H. 1987. B and T lymphocyte activity in perinatal dairy cows. *Jpn. J. Vet. Res.* 35:157.

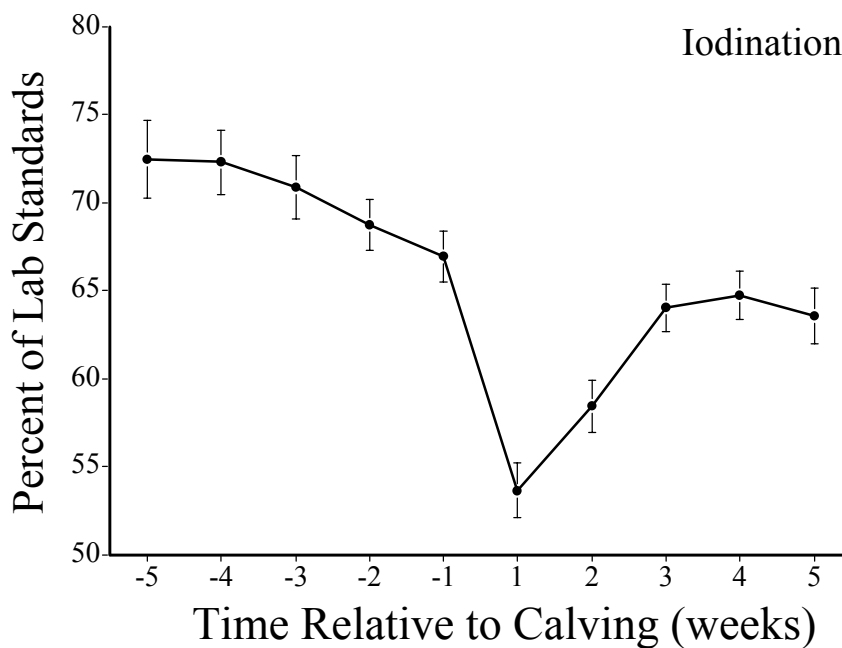


Figure 1. Suppression of neutrophil function as assessed by myeloperoxidase-catalyzed enzymatic activity. Data are from 137 Holstein dairy cows.

PHA-stimulation of lymphocyte IFN γ production (Mean \pm Std Dev)

Ishikawa et al., J Vet Med Sci 56:735, 1994

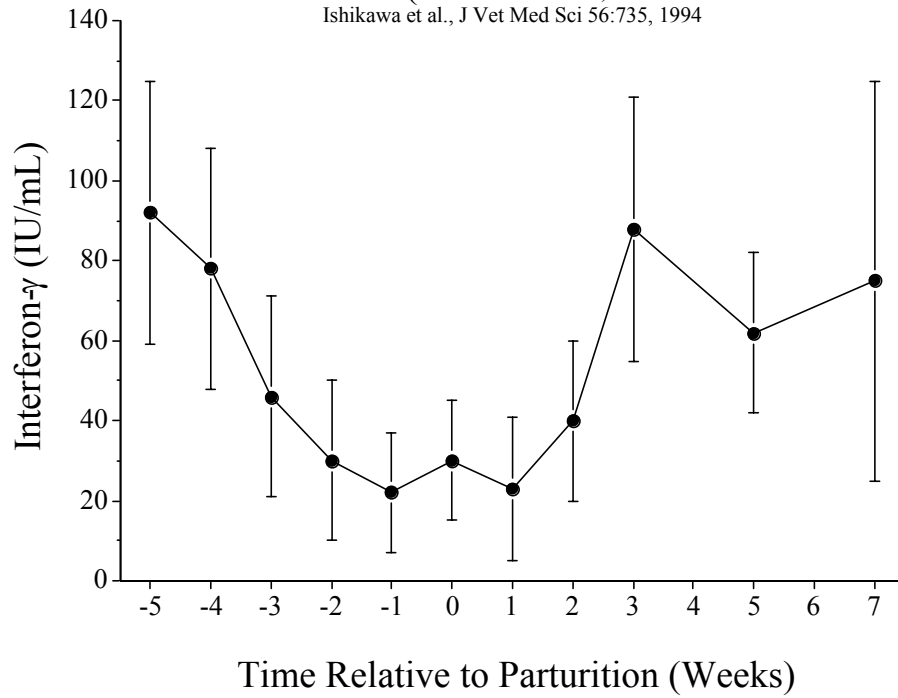


Figure 2. Suppression of lymphocyte interferon- γ (IFN- γ) production in Holstein cows.