The Relationship of Immunity and Reproduction in Dairy Cows

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Introduction

Reproduction and immunity are complex processes that involve interactive physiological processes and share some common elements. Dairy cow reproduction has several critical checkpoints where interactions with the immune system are necessary and dysregulation of the immune response can lead to reproductive failure. Understanding these processes is one critical component in building a resolution to dairy reproductive problems. Management of dairy cattle affects both reproduction and immunity. Nutrition is a key management tool in optimizing both reproduction and immune function. Feeding sufficient amounts of calories, fats and protein, and elements that properly stimulate the gut-associated immune tissues have been shown to positively impact dairy cow reproduction and health.

Under current management models, most dairy cows spend only two lactations in the herd. Problems in establishing pregnancy or mastitis are leading reasons that cows are culled from the milking herd. Inflammation associated with mastitis has been associated with failure to establish pregnancy (Hanson et al., 2004; Schrick et al., 2001). It appears that inflammatory problems in other tissues or systemically and the reproductive tract may be linked.

Innate Immunity

Barriers that are part of innate immunity have been recognized as critical and nonspecific protection of the internal structures of the body for a very long time. Break or remove the skin, or damage an area of mucus membrane and problems quickly follow. The value of physical barriers in innate defense has never been questioned. As we came to understand that the world is full of microscopic creatures, we recognized that the surface of the physical barriers of the body was coated with chemical compounds that controlled the growth of microbes. The skin is dry and covered with organic acids and specific oils to encourage the growth of some microbes and block the growth of most others. The internal parts of animal bodies that interact with the outside are covered in mucus that traps microbes, and systems (such as cilia or muscular waves) to move the mucus that remove the trapped microbes. These physical barriers are a first line of defense.

The mucus-covered surfaces that form the interface to the outside world for the eyes, respiratory tract, gastrointestinal tract, and reproductive tract of animals also form

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an effective barrier. Mucus is an effective trap for many microbes. Mucus and ciliated cells in the respiratory tract sweep invading microbes up and out of the airway so that they are expelled from the mouth or swallowed. In the gastrointestinal tract, waves of smooth muscle contraction move the contents through the system and carry away many pathogens. Further, antimicrobial peptides, sugar-binding proteins (called lectins) that recognize structures on microbes lead to the aggregation of invaders. Then, enzymes and acids inhibit the growth of these pathogens. Proteins that control critical nutrients and minerals are also secreted into the mucus. In addition, in the gastrointestinal tract the pH changes markedly as materials pass through. This leads to the death of many microbes along the way. Acidic compounds are also secreted into the most exterior portions of the female reproductive tract. These "select" which microbes will grow and which will have a tough time growing.

Together these barrier functions form the truly non-specific portion of innate immunity. The measures are broadly applied to keep microbes from becoming invaders and all comers are treated exactly the same. There is little in the way of "effector activity" in this part of the system (save the enzymes and anti-microbial peptides that are secreted), and none of these elements appear to be secreted as a result of sensing the presence of microbial organisms on the skin or in mucus. However, these barriers do not function alone.

Since the late 1980's, we have been rediscovering other aspects of the power and importance of innate immunity as that part of immunity that can sense and respond to foreign invaders. Much of that research has been focused on the discovery of the receptors that recognize conserved structures of "pathogens" (in reality essentially all microbes) and how they signal within cells to initiate the complex of inflammatory responses in the host. This research has been the subject of many reviews. However, three of these are my favorites. Beutler (2004) is the most complete, Janeway and Medzhitov (2002) has the best coverage of innate immunity across all organisms and how strongly they are conserved, and Medzhitov and Janeway (2000) has the most compact review. These reviews focus primarily on the receptors that sense dangerous, foreign invaders, and set of core, common signaling pathways involved in activation of the innate immune system. They provide far less discussion of the cells and factors that carry out the effector function of removing invading microbes.

The innate immune response can be divided into two major activities. First, common features of microbes from the environment must be recognized as having entered the body of the animal. This is the sensing component of innate immunity. Sensing can occur at the cellular or molecular level. The second component is the action arm that allows for binding, targeting, or killing the microbial invaders. Again, cells or molecules of the innate immune system can carry out these functions of innate immunity. The innate immune system uses a fixed array of sensors for dangerous and foreign invaders. When they are encountered, the effector arm of the response becomes active. These processes are connected by the signaling network that has been recently described in great detail leading to the production of cytokines, lipid

mediators, release of antimicrobial proteins, and activation of effector cells and molecules.

The innate immune system is represented across both the animal and plant kingdom. Most of the sensor elements are highly conserved. These sensor receptors take a similar form across all species. The definition of self and foreign, which has been recognized as the basis for immune protection, is most clearly defined in the innate response. The activities of the innate response are ready at birth, constantly primed to act upon encounter with dangerous and foreign invaders, and approach the problem of these invaders using the same array of sensors and responses repeatedly without significant change. The innate response is biologically programmed. It is based on the presence of a few hundred receptors that signal through a limited family of intracellular and extracellular molecular pathways leading to the activation of a common set of "killing systems" that clear the invaders from the body. In general, these immediate response systems are highly effective and up to the task of keeping the body safely in balance. In mammals, including food animals, this innate immune response is backed up by the slower, but more narrowly focused adaptive immune response. The two are connected in ways that will be discussed.

A number of families of sensor molecules have been uncovered since we started to look closely for them about 20 years ago. The most completely studied is the family of Toll-like receptors (known as the TLR family). This family of foreign invader and cell damage sensing molecules is highly conserved across all animals. In food animals, nine different TLR molecules have been demonstrated. These molecules are distributed on the cell surface or on internal membranes. They recognize foreign invader signature molecules (such as cell wall components, hydrophobic protein like bacterial flagellin, and microbial patterns of DNA and RNA processing) both at the cell surface and internally. Further, they can recognize common patterns of cellular damage in the extra-cellular environment.

In addition to the TLR family, a number of other families of sensor molecules have been described, but are only well characterized in mice or humans. Far less is known about their analogs in cows, goats, sheep, horses, or pigs. We may learn more about these in the next few years and they may add to our ability to detect the pathogenesis of disease.

The families of sensors for microbial components are specifically gram negative and gram positive cell wall structures (TLR4 and TLR2 [with TLR1 or TLR6 as partner], respectively), the hydrophobic domains of bacterial flagella (TLR5), double stranded RNA (TLR3), unmethylated DNA (TLR9), nucleic acid folds (TLR7 and 8), lipid domains (C3 cleavage structures), and glycosylation patterns (MBL) that provide a context for signaling of danger and the initiation of a cascade of inflammatory events. The signaling that follows activation of these receptors initiates one of two core pathways called MyD88 or TRIF. The pathways both lead to activation of gene activating nuclear proteins through the Nf-kB nuclear activator protein. From this gene activation, the cascade of inflammatory events unfolds leading to production of cytokines, chemokines,

prostaglandins, vasoactive mediators, and other processes that lead to the signs of disease.

In addition to recognizing products of invading microbes directly, there are also sensors that detect cellular damage. One of the primary triggers of cell damage recognition is the family of heat shock proteins (referred to as HSP). Members of this family interact with several of the extracellular sensors on the cell surface to initiate the inflammatory cascade or enhance its development. These proteins that move other proteins around inside cells and aid in their proper folding should never be seen outside of host cells. This is a sure signal of damage.

The innate immune system has a wide variety of effector activities. These activities are mediated either by cells that become activated to remove and kill invaders, or by molecules that can either form complexes to "bind up" invaders or kill them directly. These effectors have hard-wired functions. That is they approach all invaders with the same set of tools and attempt to remove and kill the invaders in the same, preprogrammed way without regard to the specific invader encountered.

Cellular effectors of the innate immune system include macrophages, neutrophils, eosinophils, basophils, and mast cells. Once they have invaders or damaged components inside the cell, in walled off compartments, these cells release radicals, enzymes, and other killing molecules into the compartments containing the invader. This effectively breaks down the invader into pieces that are not dangerous to the host. In addition, macrophages and neutrophils may encounter invaders (often groups of invaders attached to a surface) that cannot be readily taken up. At this point, these cells release their killing molecules into the extra-cellular space and attempt to kill the invaders where they are. Often, this release of killing power causes damage to cells of the host and this damage is perceived as part of the symptoms of disease. All the cells of the innate immune system also make and release compounds that change the flow of blood and allow fluid, protein, and often more cells to enter the invaded and damaged tissue. This leads to the swelling, redness, and pain perceived at the site of infection. In addition to the cells associated with innate immune function, epithelial cells also utilize many of the same sensors to initiate and regulate the local responses to invaders and damage (Akira et al., 2006; Schaefer et al., 2005) These changes are often seen as signs of the disease.

The molecular effectors of the innate immune system provide recognition of invaders in the extra-cellular space. There are many molecules that play a role in this recognition, but the proteins of the complement system and the mannose binding lectin (MBL) family of sugar-binding proteins play particularly important roles. The complement protein known as C3 is particularly sensitive to the surface of microbial and enveloped viral invaders. This protein is cleaved into two parts when it encounters the invader, C3b and C3a. The C3b leads to the activation of a cascade of protein activations that can lead to the direct killing of the invader by "boring holes" in the lipid membrane of the invader. The C3a recruits the cells of the innate immune system from the circulation, particularly those that become macrophages and neutrophils. The C3a

also activate these cells. The MLB recognizes the common pattern of sugar addition to the surface components of invaders. The MLB binds to terminal mannose sugars on those structures and leads to C3 breakdown. Again, C3b can lead to direct killing of the invader and C3a to recruitment and activation of cells that provide effector activity for the innate immune system.

Cells that have been activated by invaders and damage also produce a large number of cytokines and chemokines. Cytokines are hormones that trigger the regulated set of inflammatory activation events that follow signaling by TLR receptors (and their relatives) sensing invaders and damage. Chemokines are a subgroup of cytokines that primarily attract effector cells of the innate immune system to the site of invasion and damage. Cytokines have many jobs in fighting invaders. Some of these cytokines, such as tumor necrosis factor alpha (TNF) and interleukin 1 beta (IL-1), travel from the site of invasion to the brain to regulate body temperature. The result is fever, a major sign of disease. In another context, cytokines enhance or suppress cellular metabolism to help fight or starve the invader. The cytokines and chemokines produced after an encounter with an invader will often play a large role in how the symptoms of the disease the invader has caused is recognized in the host. They affect the timing, severity, and localization of symptoms and offer clues to the nature of the disease.

In addition to the cytokine protein messengers, the body also utilizes a family of lipid mediators. The members of this family that receive the most attention in recognizing and treating food animal disease are the prostaglandins and leukotrienes. These mediators are involved in pain and fever. Thus, they play a role in disease processes we can both see and modulate.

The cascade of events that arise from activation of innate immunity caused by an invader is closely linked to the signs of the disease the invader causes. Signs induced by innate immunity include fever, local swelling, redness, warmth, and pain. Pus is also a sign of the infiltration of neutrophils (and macrophages) into tissue that has been invaded. The cytokines produced often lead to the animal going off feed, becoming "depressed", or having a change in social behavior. The signs we count on to recognize infectious diseases generally have their source in the innate immune response to invasion.

Innate immunity plays one more critical role in the ecology of disease. It provides important connections to the adaptive immune response. The next essay will describe the adaptive immune response in more detail, but it is all about recognizing many different unique molecular components of invaders and building a rapid response network to block their invasion in the future. The process is based on sampling of invaders in both the extra-cellular and intra-cellular environment of the host and presenting those samples to the cells of the adaptive immune response under a set of rules that indicate a dangerous threat.

The sampling and presentation of the invaders is done by a class of cells that arise from monocytes entering the tissues and become antigen-presenting cells. The

most potent and common antigen-presenting cells are called dendritic cells. These cells differentiate from monocytes that enter the tissue from the blood and seek evidence of invaders. They sample the components of the invaders and present molecular pieces of the invader on their surface by placing them into the groove of either of two proteins. The protein utilized for processed extra-cellular invaders is called major histocompatibility complex (MHC) protein class II, or MHC II for short. The protein utilized when the invader is attacking from within host cells is called MHC class I, or MHC I for short. One of these quality control proteins containing a sample of invader is a necessary part of getting the host to respond to individual molecules from the invader.

In addition, the host requires that antigen-presenting cells provide signals of danger and damage to allow for activation of adaptive immunity. These signals are produced and expressed on the antigen-presenting cell surface as part of the inflammatory cascade. So, if the invader is causing inflammation and is present in large enough numbers to be effectively sampled, the antigen-presenting cells will become loaded with sample and danger signal, then migrate to where the cells of the adaptive immune system are waiting.

Adaptive Immunity

Mammalian food animals have very well developed and complex adaptive immune systems. These systems are based on the function of a family of specialized cells called lymphocytes. Lymphocytes come in two large families, B cells (derived from bone marrow in mammals) and T cells (named for their source in the Thymus). Each of the families has a number of members that have different activation requirements and functional activities. However, all the lymphocytes develop their specificity by gene rearrangement (reviewed in any immunology textbook if you want to know how). The B cells develop their specificity by rearrangement of the antigen-binding domain of the heavy and light chains of antibody (antibody structure and function will be covered below). The B cells then provide specific effector targeting by exchanging heavy chain "constant" domains to make antibodies of different "classes." The T cells attain their molecular specificity by rearrangement of their T cell antigen receptor (TCR) genes. There are two types of rearranged TCR that are produced, those with alpha and beta chains, and those with gamma and delta chains. Both are well represented in the circulation and tissues of the cow, goat, sheep, and pig, but alpha and beta chain TCR bearing T cells are much more common in the horse, human, and rodent.

The adaptive immune system is capable of being modulated to become faster, better, and stronger in response to a specific invader with repeated exposure. We would like to use the enhanced capacity to help us manage the cost and suffering associated with diseases in food animals. The adaptive immune response is the basis of our programs aimed at biological control of disease, primarily vaccination. Vaccination is an exploitation of the biology of the adaptive immune response to the benefit of the animal and the producer.

The B cells use antibody bound to their surface as their antigen (parts of a foreign invader) receptor. This antibody "sees" antigen in 3-D. Binding of surface

antibody activates the B cell and causes it to divide, so there are more B cells that see that specific molecularly defined antigen, and after several rounds of division, some of the B cells become antibody factories to make more antibody. The B cells can recognize a wide variety of antigens, as a typical mammal is capable of making about 10^{18} different gene rearrangement combinations.

The basic structure that allows antibody to function is the complex of four peptide chains, two "light chains" and two "heavy chains" to form the unit structure. Each light chain is composed of a variable (the gene rearranged part) and a constant portion. The light chain is smaller than the heavy chain (about 25K da vs. 50K da, respectively). The heavy chain is similar in construction, except it has three constant portions in each molecule. The variable part of the light and heavy chain "overlay" each other forming the antigen-binding site of the antibody. The first constant part of the heavy chain and the constant part of the light chain interact to make the complex stable. Finally, the distal two constant domains of the heavy chain interact to form the function-determining portion of the antibody molecule. The heavy and light chains and the two heavy chains are further bound together by the formation of disulfide bonds. The more disulfide bonds in the antibody structure the more rigid the antibody molecule, and the fewer places it can go.

Antibody comes in classes that are associated with where in the body they function and how they interact with cells and molecules that remove invaders. The class of antibody found at the highest concentration in healthy individuals is IgG. It is composed of a one unit antibody structure. It is often represented by several subclasses (such as IgG1 and IgG2, but they can have unique names as they do in the horse) that differ in the placement and number of disulfide bonds. This regulates how effectively the antibody enters the tissue or gets to the mucosal surfaces and how well it activates cells (like macrophages and neutrophils) to function, and how well it interacts with complement. The IgM is the first antibody made after activation of "first timer" B cells. It is composed of five unit antibodies. It is very large and rigid, almost flat. It is found in the circulation, but it is not easy for it to get out of the blood. It is good at activating complement-mediated killing of invaders and very good at encouraging cells to take up invaders from the circulation. Two other types of antibody are often observed, IgA composed of two unit antibody structures joined end to end, and IgE that has an extra constant domain on the function-determining end that is associated with fighting parasites and allergies. The IgA is very important at blocking the entry of invaders on mucosal surfaces. The IgA has a modified chemical structure that allows it to be freely transported across epithelial cell barriers and is found on mucosal surfaces in large quantities. It is capable of binding four antigen molecules at once and functions primarily by binding up invaders so they do not get into the body. The IgE is a special antibody molecule. It generally functions when bound to mast cells or eosinophils. When antigen binds, it triggers those cells to release large quantities of preformed vasoactive compounds and leads to the symptoms you may have experienced as allergy.

T cells have antigen receptors on their surface that recognize a small piece of peptide and the MHC antigen that is wrapped around it on the surface of an antigen-

presenting cell. Both the piece of antigen and the one, specific MHC protein is required for recognition of antigen. Further, T cells demand proof of a dangerous context. When an antigen presenting cells encounters evidence of invaders, the cells become activated. With activation, these cells make new copies of proteins that are expressed on their surfaces that indicate that they have faced "danger." These surface proteins, when in the presence of the right piece of antigen and MHC protein, give "permission" to T cells to become activated and begin to divide. Thus, just like B cells, T cells are selected and expanded to provide a better, stronger, and faster immune response in the body of the host. The typical mammal can make about 10^{21} rearrangements of TCR genes.

The T cells come in two major types. One type functions to manage adaptive immunity by making the right combination of cytokines and surface proteins to assure that enough expansion of B cells and T cells occurs to protect the body, and when the time is right, that antibody is produced and killing activity by T cells is armed. These are called "helper cells." The other type produces less cytokine, but can be armed to kill cells that express the right piece of antigen in the right MHC protein indicating that an invader is active inside that host cell. These are called killer T cells.

Because adaptive immune responses are so diverse and complex, the body cannot control these processes in an ad hoc fashion. Therefore, specific tissues, like lymph nodes and the spleen, are organized as adaptive immunity screening and production facilities. Antigen-presenting cells coming from the tissues of the body home to their friendly, local lymph node (or to the spleen from the blood) to report on the invaders present and the danger found. There they migrate among the waiting B cells and T cells until they find those that recognize their antigen "message" about the invaders in the tissue. As the lymphocytes are packed close together in these organized screening and production centers, the process is pretty efficient.

Once a good match between the antigen and lymphocytes is found, the process of lymphocyte activation and division is started. The division phase of this process is often referred to as clonal expansion. Each lymphocyte that encounters a proper match is encouraged to divide and make identical copies. The level of adaptive immune response and its speed are based on the number of cells that recognize the antigen properly and respond when called. Thus, the effectiveness of B cells and T cells at protecting the body is based on experiencing antigen and expanding the clones. Thus, the larger the number of responding lymphocytes that there are in the lymphoid tissues of the body, the faster and stronger the responses to invasion is mounted. The activation of B cells is a complex process that requires antigen-specific signaling in the B cell and support by cytokines and growth factors from other cells. Similarly, T cell activation is a complex process. The T cell activation requires activation by a piece of antigen in the framework of an MHC molecule, permission to act from an antigen-presenting cell confirming danger, and "help" from other T cells. It shares many processes with B cell activation.

So, a major difference between the innate immune response and the adaptive response is how quickly the response occurs. Innate immunity begins within seconds

and is often apparent in hours. The adaptive response occurs in days (about 7 days until the first antibody is measurable in serum after a first time exposure, and 3 days after a later exposure). The innate immune response deals with the invasion and its immediate consequences, but the adaptive response is responsible for the rigor of the response and assurance that the invader is completely removed and neutralized. Innate immune responses can become chronic. They can be sustained by serial recruitment of innate immune cells and release of inflammatory factors to do damage or change local physiology over a considerable period of time.

Another difference between innate immunity and adaptive immunity is that the innate immune response brings the same tools and players to the challenge of invasion every time. The first time response is no different in character or nature than the 50th. The adaptive immune response gets better at responding with each invasion. The number of responsive cells is increased and the time required to respond decreases each time the body encounters an invader. The process of more focused and rapid response is called immune memory. This enhancement with exposure is what we exploit in the production of vaccines. We provide evidence of invasion without the disease consequences to the host to make it better able to deal with the natural invader later.

Common Elements of Immune and Reproductive Function

The establishment and success of pregnancy is a complex process. It appears from studies performed in many species that local immune and inflammatory processes, which are often reflected by changes in systemic activities that can be monitored in the circulation, have a major impact on "fertility" in the female. Prior to fertilization, inflammatory and immune activities in the reproductive tract alter the interaction between egg and sperm. Changes in the viscosity and physical/chemical composition of uterine mucus can alter sperm penetration and survival. Increased inflammatory cell activation can create a hostile environment for sperm, reducing the duration of their viability, and damaging their membranes so that they are less capable of fertilization. Similarly, the fertilized ovum faces challenges to its survival and in its interaction with the lining of the uterus when a strong pro-inflammatory response is occurring.

Immune cells, proteins controlling lymphocyte interaction and products of inflammatory activation are all part of the development of a functional ovum, organization of the primary follicle and the corpus luteum. The development of an ovum and primary follicle requires the expression of the lymphocyte marker Thy-1 on the surface of specific epithelial cells in the ovary, the interaction of these cells with CD8 positive T cells, CD14 and MHC class II antigen-bearing macrophages and monocytes, a loss of the MHC class I antigen by cells in the developing follicle, and interaction with immunoglobulin molecules (Bukovsky et al., 2005). Further interaction with immune cells and a requirement for the chemokine IL-8 have been documented for the proper development and vascularization of the corpus luteum. Neutrophils must infiltrate the epithelial layer to allow for proper functional development of the bovine corpus luteum

and they appear to be the source of angiogenic factors required for maturation of the corpus luteum.

In contrast, the fertilized ovum once it implants in the wall of the uterus requires that the mother go through three sequential periods of immune interaction. First, a pro-inflammatory phase which causes systemic effects on appetite and homeostasis. In this phase, the placental membranes develop and become vascularized appearing to the body as an open wound. The pro-inflammatory responses provide the cytokine context for the enhanced vascularization and promote the complex matrix of tissue formation to foster the development of the placenta and fetus in close contact with the maternal tissues of the uterus. Next during the second trimester, there is a period of immune neutrality and of rapid fetal growth. The mother suppresses cell-mediated immune activity. The condition is stable and the mother does not recognize a new threat. The systemic inflammatory responses have shifted to an anti-inflammatory profile. Finally, as birth approaches, the mother mounts a stronger and stronger pro-inflammatory response that triggers expulsion of the fetus at term.

Alterations of these necessary immunological relationships by infection or chronic immune disruption yield fertility problems. Occult infections with bacteria lead to a change in the level of inflammatory activity that throws the developing fertilized ovum out of sync with the uterus and leads to failure of implantation (Weiss et al., 2009). Many of these interactions are regulated by innate immune receptors that trigger pro-inflammatory responses, like the Toll-like receptors. This continuing pro-inflammatory state is deliberately mimicked by the function of the intra-uterine device for birth control.

The dairy cow has been selectively bred for the production of milk, but in that process, the efficacy of her reproduction has been largely ignored. The problem is multifaceted. It includes energy partitioning problems involved in supporting the volume of milk produced relative to all other essential physiological functions, the depletion of body stores of minerals that can impact inflammatory regulation, and management programs directed at the narrow aim of milk production per lactation cycle (rather than life-time milk yield).

Each of these management choices has led to potential problems in a critical event in profitable milk production, establishing pregnancy and delivering a calf. It appears that it is the rule, rather than the exception, that more than one service is required to obtain a successful pregnancy in dairy cows. This situation has both practical and economic consequences to the industry.

Inflammatory processes have been recognized as both a necessary component of the development of pregnancy and as significant impediments to the development and success of pregnancy (Weiss et al., 2009). Inflammatory processes secondary to acute and occult infections play a significant role in infertility. In unpublished studies in my lab, we found that 30% of dairy cattle examined had occult colonization of the uterus based on recovery of bacteria from uterine flush fluid.

Inflammation also releases mediators that function to up and down regulate inflammatory processes in the form of cytokines, prostaglandins, and chemokines. Several of these mediators play important roles in regulating the response of reproductive cells to leutinizing hormone (LH), in steriodogenesis and steroid conversion, and in the progression of ovum development and release, implantation, and fetal-placental interactions.

The expression and level of the cytokines, IL-1 beta, TNF alpha, and IL-6 (the classic pro-inflammatory triad) modulate aromatase activity regulating the production of estradiol, progestone, and progesterone. They also impact COX-2 activity and the production of PGE and PGF, which are important to the production and release of the ovum. Local, resident macrophages in the reproductive tract modulate the cytokine environment and favor the production of IL-1 alpha, TGF-beta, and IL-10 that modulate LH activity and promote steroid conversion under control of cells within the reproductive tract. Infiltrating inflammatory cells, monocytes, macrophages, neutrophils, eosinophils, and activated lymphocytes produce high levels of cytokines that disrupt regulation of these processes.

The establishment of pregnancy by implantation of the embryo into the uterine wall and establishment of the placenta requires interaction with immune cells, particularly a specific class of NK cells and resident macrophages. This is a pro-inflammatory process and results in systemic inflammatory symptoms. Relatively quickly, the interaction of the placenta with the developing fetus causes a shift in the immune relationship to a somewhat suppressive environment to protect the fetal graft from rejection. Finally, late in pregnancy, a return to a more pro-inflammatory environment serves to trigger events to expel the fetal graft in the birth process (Mor, 2008).

Modern genetic selection, implemented by artificial insemination and complemented by improved management, has dramatically increased the milk yield per lactation in dairy cattle. Over the last decades, yields have increased from 3000 L/cow per year in the 1940s to a current estimate of 9500 L/cow per year. Over the same period, these increases have allowed for reduction in the total number of animals by 85% while maintaining production of 1.5 fold more total milk (Powell and Norman, 2006). The negative effect that increased milk production had on reproductive success was caused by both genetic and metabolic factors (Gilbert et al., 2005). Large-scale analysis of dairy herd fertility in the United States revealed that conception rates declined by more than 30% in Jersey and Holstein cows between 1975 and the beginning of the current century (Powell and Norman, 2006). As gestation is a prerequisite for lactation, a direct implication of this low fertility rate is the requirement for larger herd numbers to maintain the same number of cows in lactation. This is indicated by a heifer replacement rate of infertile cows that can exceed 35% of the herd per year.

Further, systemic inflammation associated with acute disease elsewhere in the cow, such as the mammary gland, have also been shown to be negatively correlated

with the success of pregnancy (Moore et al., 2005; Chebel et al., 2004; Moore et al., 1991; Hansen et al., 2004). Difficulties in human fertility may have parallels in the problems we see in the dairy cow. A large number of cases of human infertility are related to chronic infection, the development of anti-sperm antibody related to chronic inflammation in the reproductive tract of women, and the effects of the inflammatory process yielding altered levels of pro-inflammatory cytokines (particularly IL-1 beta, IL-6, and TNF-alpha) (Weiss et al., 2009). While inflammation alone is not likely the whole cause of infertility in dairy cows, it may in fact represent an important common "sign" of the problem.

In cattle, inflammation problems are fueled by problems in nutrition, housing, and management. These result in part from the huge partitioning of energy that has been focused intentionally on milk production as a mono-focal goal. This narrow focus leads to the development of an "incubator state" in the confinement dairy resulting in more frequent and substantial exposure to pathogens that initiate sub-acute infections with inflammatory consequences on overall health, including having a likely impact on fertility (Garnsworthy, 2004). Therefore, it appears that inflammation is a consequence and possibly a component in the multi-factorial problem of poor fertility in dairy cows.

It is imperative that remediation of low fertility be addressed as a strategy to decrease the number of dairy cows required to maintain and increase milk production in the US and world-wide. As subfertility is a complex, multi-gene, and multi-factorial condition, understanding and mitigation of its causes requires a multidisciplinary approach (Royal et al., 2002).

Parturition Associated Changes in the Dairy Cow

Very significant changes in the dairy cow occur during the period from two weeks before to three weeks after birth. These changes appear to be fueled by significant negative energy balance and the inability of the cow to eat enough to meet all the energy demands of the growing calf and the initiation of milk production (Goff and Horst, 1997). The changes observed include a reduction in neutrophil function, increased incidence of intra-mammary infections, reduced adaptive immune function, reduction in rumen efficiency, spikes in cortisol levels at calving, a shift in progesterone and estrogen levels, and a reduction in circulating calcium.

These changes are essentially universal in the dairy cow and in the normal cow they are transient. However, failure to rapidly resolve these problems leads to chronic colonization of the mammary gland and new intra-mammary infections with systemic inflammatory effects (Hansen et al., 2004), occult colonization of the reproductive tract with increased indicators of inflammatory mediators (Hurley et al. unpublished), and changes in the normal profile of production of mediators and hormones involved in fertility.

There is some hope that at least some of the inflammatory problems can be modulated in dairy cows based on recent research. Long-term (six months of age to

parturition) or short-term (60 day) feeding of Omnigen-AF$^®$ to dairy heifers offered evidence of better managed and primed inflammatory activity (Ryman et al., 2013, Nace et al., unpublished). In these studies, we observed enhanced phagocytic activity, better controlled radical production, and stabilized expression of CD62L and CD11c. In addition, in the first study we observed fewer IMI and greater milk production.

Conclusions

Fertility in the dairy cow is linked with many functions shared with the immune system. Common mediators play roles in the function of both immunity and the female reproductive function. Further, there is a direct role for cells of the innate immune system in oogenesis, vascular development of the corpus luteum, and in regulation of embryo implantation in the cow. Enhanced inflammation associated with local infections appears to have a negative effect on pregnancy development. This has been demonstrated clearly by a link between mastitis and poor conception. Nutritional challenges appear to play a significant role in the problems associated with parturition. Our research indicates that modulation of innate immune function may be possible by use of oral immune stimulants. It is time to consider and examine the function of oral immune stimulants that strengthen the regulatory activity by priming innate immune function by action in the gut-associated immune tissue and loading competent innate immune cells into the circulation of dairy cows that would otherwise be significantly compromised as a method for control of the role of inflammation in fertility.

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SESSION NOTES