The Relationship of Immunity and Reproduction in Dairy Cows

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Take Home Messages

- Both innate and adaptive immunity have a significant impact on reproductive performance in dairy cows.
- Bacterial colonization of the lower reproductive tract in cows after delivery is normal and drives the action of neutrophils and macrophages to direct epithelial regeneration of the uterine lining.
- IgA in the female reproductive tract appears to arise locally and to function in control of acute colonization by microbes, but IgG in the female reproductive tract is from the circulation and represents systemic response. IgG is associated with ongoing, occult colonization and damage in the reproductive tract.
- Prostaglandins are crossover products that function both in the inflammatory response and reproduction in cows.
- Leukocytes play critical roles in ovarian function and in implantation; activated leukocytes also produce aromatase that alters estrogen:progesterone balance.

Introduction

Reproduction and immunity are complex processes that involve interactive physiological process 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 resolving 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

WCDS Advances in Dairy Technology (2015) Volume 27: 129-141

amounts of calories, fats and protein, and elements that properly stimulate the gut-associated immune tissues has 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 with mastitis or in establishment of pregnancy are leading reasons for culling cows from the milking herd. Inflammation associated with mastitis has been associated with failure to establish pregnancy (Hansen 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

Physical barriers are important innate protective features. The body is covered with skin and hair. The mucus-covered surfaces that form the interface to the outside world for the eyes, respiratory tract, gastrointestinal tract and reproductive tract of animals form another 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, on mucosal surfaces, including the female reproductive tract, antimicrobial peptides and 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 to modulate the growth of invaders. 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.

The next level of innate immune function was fueled by 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. Medzhitov and Janeway (2000) provide a review of this topic. These receptors sense dangerous,

foreign invaders, and initiate a set of core, common signaling pathways involved in activation of the innate immune system.

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. The innate immune system uses a fixed array of sensors for dangerous and foreign invaders and when the invaders are encountered enables the action arm of the response to become 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 uses a family of sensor elements that are highly conserved. 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 dangerous and foreign invaders. 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 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 (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 are called chaperones. It is their job to move other proteins around inside cells and aid in their proper folding. Chaperones function in internal membrane arrays and should never be seen outside of host cells. This is a sure signal of damage when they are found outside of cells.

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 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 these cells have invaders or damaged components inside the cell, in walled off compartments, they 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 use many of the same sensors to initiate and regulate the local responses to invaders and damage. (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. Many molecules 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, C3b and C3a, when it encounters the invader. 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. C3a recruits the cells of the innate immune system from the circulation, particularly those that become macrophages and neutrophils. C3a also activates these cells. The MBL recognizes the common pattern of sugar addition to the surface components of invaders. The MBL 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.

The body also uses 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.

Innate immunity plays one more critical role in the ecology of disease. It provides important connections to the adaptive immune response that are 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 used for processed extra-cellular invaders is called major histocompatibility complex (MHC) protein class II, or MHC II for short. The protein used when the invader is attacking from within host cells is called MHC class I, or MHC I for short. One of these 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. 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

Dairy cows 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).

The adaptive immune system is capable of being modulated to become faster, better and stronger with repeated exposure to a specific invader. 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.

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.

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 one unit antibody structure. It is often represented by several subclasses (such as IgG1 and IgG2,) 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, how well it activates cells (like macrophages and neutrophils) to function, and how well it interacts with complement. IgM is the first antibody made after activation of “first timer” B cells. It is composed of 5 unit antibodies and 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, which is composed of 2 unit antibody structures joined end to end, and IgE, which has an extra constant domain on the function-determining end that is associated with fighting parasites and allergies.

IgA is very important at blocking the entry of invaders on mucosal surfaces. 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 4 antigen molecules at once and functions primarily by binding up invaders so they do not get into the body. IgA is generally produced by clusters of lymphocytes and antigen presenting cells associated with mucosal tissue and is directly secreted onto mucosal surfaces. In contrast, antigen sampled in the reproductive tract is transported by APC to the secondary lymphoid tissue for production of IgG and activation of T cells. IgG is produced by plasma cells in the secondary lymphoid tissues and released into the circulation. IgG enters the female reproductive tract from the circulation.

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 one specific MHC protein is required for recognition of antigen. Further, T cells demand proof of a dangerous context. When an antigen presenting cell encounters evidence of invaders, the cells becomes 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.

T cells come in several major types. One type functions to manage adaptive immunity by making the right combination of cytokines and surface proteins to ensure that expansion of B and T cells is sufficient 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”. Another 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. Two more types are also commonly recognized: Treg cells that block lymphocyte, neutrophil and macrophage function once activated, and Th17 cells that recruit, arm and activate neutrophils to work with antibody and complement in tissues.

Because adaptive immune responses are so diverse and complex, the body cannot process them 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 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. 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 are 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. 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.

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 make the animal better able to deal with the natural invader later.

■ Mucosal Immunity

The classic view of mucosal immunity is what we have learned from the GI tract and how it manages constant contact with invaders from the outside. Microbial invaders are samples by specialized structures formed from the epithelial layer that feed microbial antigen to APC. These APC interact with organized foci of lymphocytes in the sub-epithelial space and drive local and regional responses. In the lymphoid foci, B and T cells that recognize the specific molecular structures on the samples microbes are activated. These activated cells divide several times. Some of the B cells that divide differentiate to produce and export IgA across the epithelial cell barrier. Some of the T cells that divide move to the sub-epithelial space and produce IL-17, IL-22, and Treg cytokines (IL-10 and TFG-beta) to regulate the immune and inflammatory responses in the epithelial tissues. Lymphocytes and APC also traffic by the lymphatics to the regional lymph nodes and provide expanded support for IgG production and killer and delayed hypersensitivity primed T cells that return via the circulation.

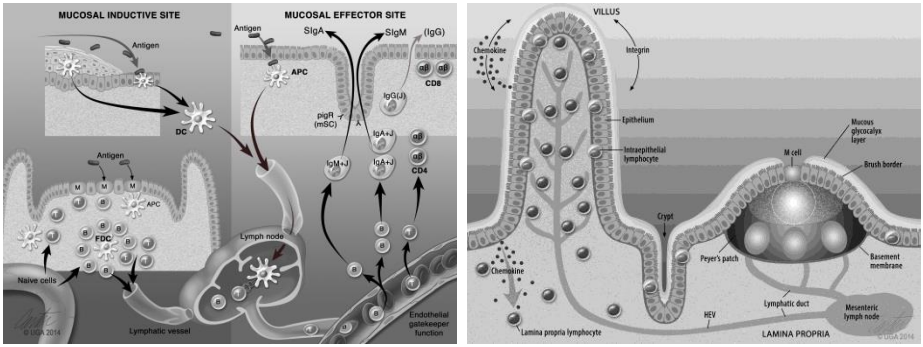

Figure 1. The Classical view of how mucosal immunity works

The first panel shows the inductive and effector processes at mucosal surfaces and the second panel shows the differential processes on villi and domes with M cell sampling (Kip Carter, UGA)

The female reproductive tract also develops mucosal responses with local production of IgA and IL-17 and 22, but on a much more restricted and smaller scale. In the female reproductive tract the mucosal response has two domains, upper and lower, that reflect the organization of the epithelial cells involved and frequency of exposure to invading organisms. Local responses are primarily in response to acute colonization.

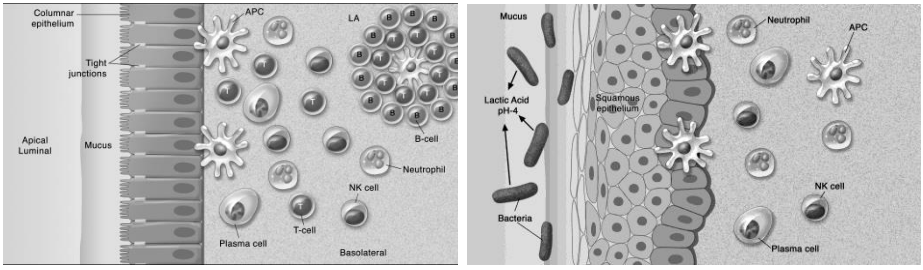

Figure 2. Mucosal immunity in the female reproductive tract

The first panel shows the immune organization of the upper female reproductive tract, and the second panel shows the immune organization of the lower female reproductive tract. Local immune responses occur in lymphoid aggregates (LA) of APC, T and B cells. (Kip Carter, UGA)

Systemic innate cell recruitment, IgG transfer, and armed T cell transfer provide immunity to ongoing colonization and tissue damage.

■ Common Elements of Immune and Reproductive Function

The establishment and success of pregnancy is a complex process. In many species the 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 follicule 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 follicule, and the 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. (Shirasuna et al., 2012)

Alterations of critical 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.

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 were colonized in the uterus based on recovery of bacteria from uterine flush fluid.

Inflammation also releases mediators in the form of cytokines, prostaglandins and chemokines that function to up and down regulate inflammatory processes. Several of these mediators play important roles in regulating the response of reproductive cells to luteinizing hormone (LH), in steroidogenesis 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 of leukocytes regulating the production of estradiol and estrone (Shippen and West, 2004). 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 (Cutolo et al., 2004).

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).

Further, systemic inflammation associated with acute disease elsewhere in the cow, such as the mammary gland, has 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 to the problems we see in the dairy cow. 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 dairy cattle, inflammation problems are fueled by problems in nutrition, housing, and management. These problems 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 practices 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 or increase milk production in the U.S. and world-wide. As subfertility is a complex, multi-gene and multi-factorial condition, understanding and mitigation of its causes require 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 2 weeks before to 3 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.

Colonization of the lower reproductive tract in cows shortly after delivery is essentially universal. This colonization triggers the actions of neutrophils and macrophages to drive restoration of the uterine epithelial layer and prepare the cow for the next pregnancy. The consequences of peripartum inflammatory disruption on clearance of the bacteria that colonize the uterus may be a significant problem in dairy cows.

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