

IMPORTANCE OF IMMUNE FUNCTION FOR OPTIMAL REPRODUCTION OF DAIRY COWS

P.J. Hansen
Dept. of Animal Sciences
University of Florida

INTRODUCTION

One of the most widely-discussed issues regarding reproductive management of dairy cows is the historic decline in fertility for lactating cows that began about 1960. It is important to recognize that this situation is being reversed and there has been a steady increase in reproductive function in the last several years (Figure 1). Genetically, the dairy cow is still less fertile than the cow of 50 years ago: the predicted transmitting ability (PTA) of a Holstein bull for daughter pregnancy rate (DPR) in 1965 was +7.1 vs a PTA of +1.4 in 2015. Nonetheless, genetic merit for fertility has been increasing: the nadir for bull PTA for DPR (-1.1) in Holsteins was experienced in 2002. The improvement in genetic merit has involved increased emphasis on health and reproduction traits in leading selection indices like net merit. Fertility has also improved in recent years because of the incorporation of timed artificial insemination (AI) programs in reproductive management systems. Initially, these programs were designed to avoid problems inherent in estrus detection. Now, however, refinements in timed AI programs to optimize the endocrine environment of the cow mean that fertility to timed AI can exceed that following AI at detected estrus (Santos et al., 2017).

While difficult to document, it is likely that increased management emphasis on cow comfort has also contributed to the increase in reproductive function of lactating cows. Indeed, as will be shown in this paper, health is closely connected to resumption of estrous cycles after calving, pregnancy rate per insemination once estrous cyclicity has resumed and maintenance of a pregnancy after a cow is diagnosed pregnant.

The connection between health, immune function and reproductive function is complex and not fully understood. A schematic diagram illustrating some mechanisms by which inflammation and immune activation cause disruption in reproductive function is shown in Figure 2.

Activation of the inflammatory response by local tissue damage (due to injury, ischemia, exposure to noxious molecules, etc.) or by viral or bacterial infection leads to the release of a variety of biologically-active molecules such as cytokines, chemokines, and prostaglandins that assist in tissue repair and promote activation of immune responses that in turn involve synthesis and release of additional cytokines. Biologically-active products of inflammation and the immune system can act at sites distant from the site of tissue injury. For example, specific cytokines affect the hypothalamus to increase body temperature which is damaging to the oocyte and embryo (Hansen, 2014). Additionally, inflammatory signals can reduce appetite and alter metabolism of

carbohydrates, fat and protein (Gifford et al., 2010). Immune responses also increase energy utilization although the magnitude of this increase in cattle is unclear. Resultant changes in energy balance associated with inflammation make it more difficult for cows to resume estrous cycles after calving because the length of the postpartum anestrus is related to energy status (Crowe, 2008). Other actions of cytokines on the hypothalamic-pituitary axis cause inhibition of release of the gonadotropins necessary for establishment and maintenance of estrous cyclicity. Specific cytokines can disrupt the function of the oocyte and developing embryo (see for example, the negative effects of tumor necrosis factor- α ; Soto et al., 2003). Molecules released by microorganisms, particularly those, like bacterial lipopolysaccharides, that activate toll-like receptors on mammalian cells, can act directly on the ovary to affect follicular development and oocyte function (Bromfield and Sheldon, 2011, 2013) and on the endometrium to increase secretion of prostaglandins, cytokines and chemotactic agents that disrupt uterine function and the local environment of the embryo (MacKintosh et al., 2013). Perturbation of the local environment is particularly likely when bacterial infection is ongoing in the uterus itself (Bromfield et al., 2015).

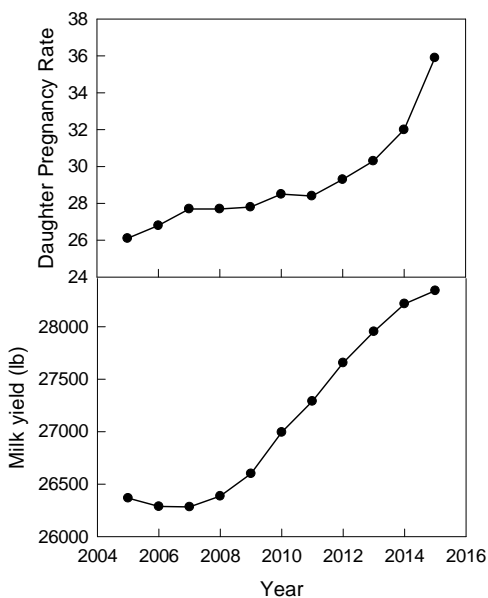


Figure 1. Changes in daughter pregnancy rate and milk yield in US Holsteins from 2005-2015 based on records maintained by the Council on Dairy Cattle Breeding (<https://www.uscdcb.com/eval/summary/trend.cfm>). Note that daughter pregnancy rate is the proportion of a bull's daughters eligible to be pregnant in a 21-day period that are pregnant. It is determined by a combination of the proportion of cows that are bred and the proportion of bred cows that become pregnant.

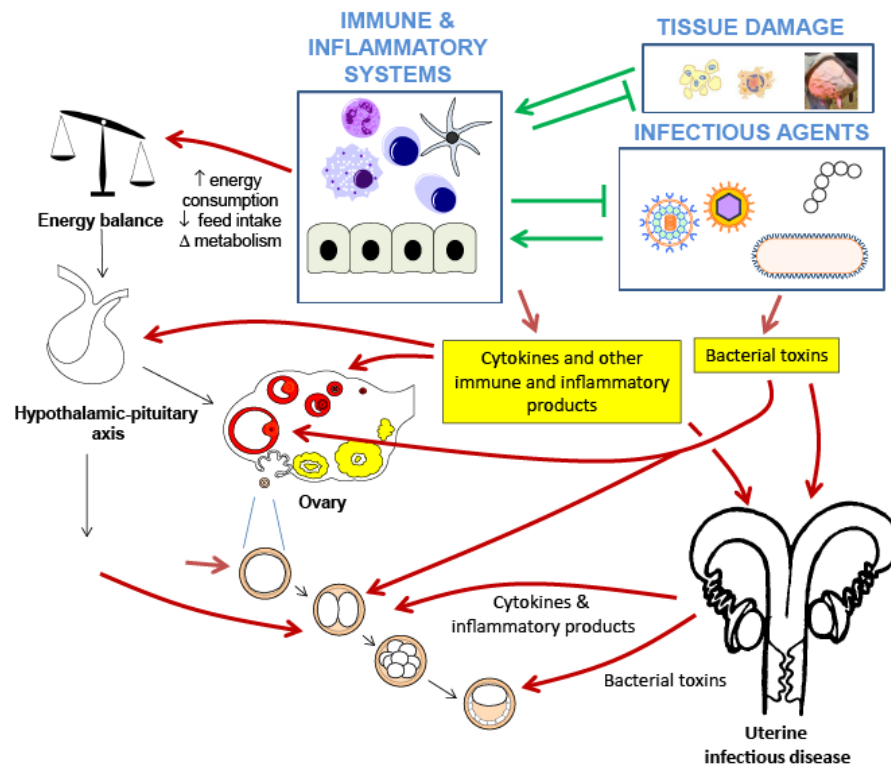


Figure 2. Simplified cartoon to describe some of the mechanisms by which disease and activation of the inflammatory and immune systems can compromise reproductive function in the cow. Red arrows indicate pathways leading to disruption of the reproductive system.

Given the nexus between the immune, inflammatory and reproductive systems, approaches to improve dairy cow health should also result in improved reproductive function. Given this reasoning, there are two goals of the current paper. The first is to show some of the evidence that health and immune function is related to reproductive function. The second goal is to review some recent studies that evaluated prospects for improving cow fertility through regulation of immune function.

EVIDENCE THAT DISEASE IS ASSOCIATED WITH REDUCED REPRODUCTIVE FUNCTION

Probably the best evidence that a host of disease events compromise reproduction in postpartum cows is derived from studies conducted at the University of Florida by José Santos, Edward Ribeiro, and colleagues. The approach was to classify individual cows as to incidence of specific diseases and then compare reproductive function of cows without disease to those experiencing one or more diseases.

Selected results from two of these studies are summarized in Table 1. In the first experiment (Santos et al., 2010), a total of 5,709 cows were examined. Resumption of estrous cycles, as determined by the percent of cows that were cyclic at day 65 postpartum, was not affected by a single occurrence of disease. However, fewer cows experiencing more than one disease were cyclic at day 65 than cows not experiencing

disease. Pregnancy rate at first AI was reduced if cows experienced one disease and was even lower when cows experienced more than one disease. A wide range of diseases were associated with a significant reduction in reproductive function. For example, pregnancy rate at first AI was reduced from 51.4% in cows without disease to 40.3% for cows with calving problems, 37.8% for cows with metritis, 38.7% for cows with clinical endometritis, 39.8% for cows with fever postpartum, 28.8% for cows with clinical ketosis, and 33.3% for cows with lameness.

Table 1. Association of disease incidents in the postpartum period with resumption of cyclicity and fertility at first insemination.

Study	Number and type of cows	Health status	Endpoint	Value	Adjusted odds ratio	P
Santos et al., 2010	5719 cows on 7 dairies	Healthy	% cyclic at day 65 postpartum	84.1	1.00	--
		1 case of disease		80.0	0.97	0.83
		> 1 case of disease		70.7	0.60	0.001
		Healthy	% pregnant at first AI	51.4	1.00	--
		1 case of disease		43.3	0.79	0.001
		> 1 case of disease		34.7	0.57	<0.001
Ribeiro et al., 2013	957 cows on 2 grazing dairies	No disease	% cyclic at day 49 postpartum	91.1	1.00	--
		1 clinical disease		88.3	0.74	0.22
		> 1 clinical disease		77.8	0.34	<0.01
		No disease	% pregnant at first AI	66.9	1.00	--
		1 clinical disease		56.5	0.64	<0.01
		> 1 clinical disease		40.8	0.34	<0.01

Similar findings were obtained in a second study of 957 cows on two seasonal grazing dairies (Ribeiro et al., 2013). Again, more than 1 clinical disease was associated with reduced cyclicity while even a single clinical disease was associated with reduced pregnancy rate at first AI (Table 1). The diseases associated with reduced pregnancy rate at first AI were calving problems, metritis, clinical endometritis, and digestive problems. There was also a tendency ($P \leq 0.10$) for pregnancy rate to be lower for cows with mastitis or lameness.

In a recent study, Ribeiro et al. (2016) used records from 5,085 cows to evaluate whether the impact of uterine disease (metritis and retained placenta) on postpartum reproductive function was greater than the impact of other diseases (including mastitis, digestive problems, pneumonia, and lameness). The reduction in pregnancy rate at day 45 after breeding (via AI or embryo transfer) and calving rate after the first breeding postpartum was similar for cows with uterine disease vs those with other diseases. Cows that had both types of disease were less fertile than cows that experienced one kind of disease only. Both types of disease also increased pregnancy loss after cows were confirmed pregnant at day 45 after breeding. Santos et al. (2010) and Ribeiro et al. (2013) also noted association of disease with increased pregnancy loss.

Ribeiro et al. (2016) found that disease reduced pregnancy rate for both cows that were bred by AI and those that received an embryo. Thus, at least some of the association of disease with pregnancy failure represents disruption of reproductive function after the early period of pregnancy. In another experiment reported in the same paper, the uterus was flushed at Day 5 or 6 after first AI postpartum to evaluate effect of disease on fertilization of the oocyte and development of the early embryo. Occurrence of uterine disease and other, non-uterine types of diseases were associated with reduced cleavage rate and proportion of embryos classified as live or high quality. Effects of disease on elongation of the embryo at Day 15 and 16 of gestation, and accumulation of the antiluteolytic hormone interferon-tau, in the uterus was also assessed. Previous occurrence of either uterine disease or non-uterine disease were associated with smaller conceptuses and lower accumulation of interferon-tau in the uterine lumen. Taken together, results indicate that disease can affect a wide variety of events in early pregnancy that are required for successful gestation.

VARIATION IN IMMUNE FUNCTION IS AN IMPORTANT DETERMINANT OF POSTPARTUM DISEASE

Studies discussed in the previous section provide compelling evidence that a cow that experiences one or more diseases in the postpartum period is at risk for suboptimal reproduction including delayed breeding, reduced pregnancy rate and increased rate of pregnancy loss. Whether or not a cow develops a disease in the postpartum period depends on a variety of factors including the nature of pathogen exposure (number and virulence), function of physiological and nutritional systems important for development of specific diseases like gastrointestinal problems and lameness, and characteristics of the immune system. The importance of the nature of pathogen exposure is evident because while most cows have bacteria present in the uterus after calving, there are differences

in the microbiome of cows whose uterus remains healthy and those that develop metritis or endometritis (Bicalho et al., 2017ab).

Experiments on genetic variation in the function of the immune system have revealed the importance of the immune system for establishment of specific diseases in the postpartum period. In particular, Millard and colleagues at the University of Guelph have used measurements of antibody production and delayed hypersensitivity reactions in the skin to estimate genetic breeding value for antibody-mediated immune function, cell-mediated immune function and overall immune response (based on the first two measurements). For some diseases, including mastitis, displaced abomasum, and retained fetal membranes, cows with low estimated breeding value (EBV) for overall immune response were most likely to experience disease (Table 2).

Table 2. Disease incidence (percent) in cows classified based on estimated breeding value (EBV) for overall immune response (Thompson-Crispi et al., 2012).

Disease	Low EBV for immune response (n=153)	Average EBV for immune response (n=407)	High EBV for immune response (n=139)
Mastitis	25.7 ^{ab}	29.0 ^a	19.4 ^b
Metritis	7.2	4.7	4.3
Ketosis	5.9	5.7	5.8
Displaced abomasum	5.8 ^a	1.7 ^b	3.6 ^{ab}
Retained fetal membranes	13.1 ^a	5.9 ^b	5.0 ^b

^{ab} Means with different superscripts differ ($P < 0.05$).

There are also indications that numbers or function of polymorphonuclear leukocytes (PMN) in the blood are important for adequate resistance to disease. Cows that subsequently developed metritis or endometritis in the postpartum period had less functional neutrophils than cows that did not develop uterine disease (Hammon et al., 2006). Treatment of periparturient cows with the granulocyte colony stimulating factor (G-CSF) to increase PMN numbers and function reduced the incidence of clinical mastitis (Canning et al., 2017; Ruiz et al., 2017). Similarly, supporting PMN function by feeding OmniGen-AF® reduced incidence of udder edema and tended to reduce incidence of mastitis (Nace et al., 2014).

PRELIMINARY STUDIES TO TEST WHETHER ENHANCING IMMUNE FUNCTION CAN IMPROVE REPRODUCTION

Given that postpartum disease is associated with reduced reproductive function and that immune function is one determinant of whether a cow experiences an adverse health effect, it follows that it may be feasible to improve reproductive function in lactating cows by enhancing immune function. There are many potential approaches for doing so including regulating calcium metabolism postpartum (Vieira-Neto et al., 2017), improving energy balance (Lacasse et al., 2017), feeding nutritional supplements that enhance

immune function (Nace et al., 2014) or by provision of biologicals like G-CSF that regulate components of the immune system (Canning et al., 2017; Ruiz et al., 2017).

It is too soon to know whether regulating immune function to improve reproduction will be effective but the idea is worth pursuing because reduction in disease incidence can also have other beneficial effects on the productivity and longevity of the cow. The only large-scale studies on effects of immune stimulants on reproductive function have been performed using G-CSF (Canning et al., 2017, Ruiz et al., 2017). In those studies, treated cows were injected with G-CSF at 7 days before expected calving and again within 24 hours after calving. The effects of this treatment on reproductive function were mixed. In the experiment by Canning et al. (2017), 320 cows per treatment were enrolled (80 cows per treatment at each of 4 dairies). Treatment with G-CSF reduced the incidence of mastitis but not of dystocia, metritis, lameness, pneumonia or a combination of ketosis, left displaced abomasum or peritonitis. A greater percent of cows treated with G-CSF were detected in estrus by day 80 postpartum (95.4% vs 90.6%) but there was no effect of treatment on pregnancy rate at first service (42.6% vs 38.2%). The study by Ruiz et al. (2017) involved 10,238 cows in 17 herds. Incidence of mastitis was reduced by G-CSF but there was no significant effect on incidence of retained placenta. Surprisingly, G-CSF increased incidence of metritis from 8.4 to 9.8%. Cows treated with G-CSF were 5.8% more likely to be inseminated in the first 100 days after calving than control cows.

These studies with G-CSF have not yet made clear whether reproductive function is amenable to change through immune stimulation. It may be that activation of PMN function at other times postpartum or promotion of function of other components of the immune system would be more effective at increasing fertility than that cause by injection of G-CSF or other agents around the time of calving. More studies are warranted.

REFERENCES

- Bicalho MLS, Machado VS, Higgins CH, Lima FS, Bicalho RC. Genetic and functional analysis of the bovine uterine microbiota. Part I: Metritis versus healthy cows. *J Dairy Sci* 2017b;100:3850-3862.
- Bicalho ML, Santin T, Rodrigues MX, Marques CE, Lima SF, Bicalho RC. Dynamics of the microbiota found in the vaginas of dairy cows during the transition period: Associations with uterine diseases and reproductive outcome. *J Dairy Sci* 2017a;100:3043-3058.
- Bromfield JJ, Santos JE, Block J, Williams RS, Sheldon IM. Uterine infection: linking infection and innate immunity with infertility in the high-producing dairy cow. *J Anim Sci* 2015;93:2021-2033.
- Bromfield JJ, Sheldon IM. Lipopolysaccharide initiates inflammation in bovine granulosa cells via the TLR4 pathway and perturbs oocyte meiotic progression in vitro. *Endocrinology* 2011;152:5029-5040.
- Bromfield JJ, Sheldon IM. Lipopolysaccharide reduces the primordial follicle pool in the bovine ovarian cortex ex vivo and in the murine ovary in vivo. *Biol Reprod* 2013;88:98.

- Canning P, Hassfurth R, TerHune T, Rogers K, Abbott S, Kolb D. Efficacy and clinical safety of pegbovigrastim for preventing naturally occurring clinical mastitis in periparturient primiparous and multiparous cows on US commercial dairies. *J Dairy Sci* 2017;100:6504-6515.
- Crowe MA. Resumption of ovarian cyclicity in post-partum beef and dairy cows. Crowe MA. Resumption of ovarian cyclicity in post-partum beef and dairy cows. *Reprod Domest Anim* 2008; 43 Suppl 5:20-28.
- Gifford CA, Holland BP, Mills RL, Maxwell CL, Farney JK, Terrill SJ, Step DL, Richards CJ, Burciaga Robles LO, Krehbiel CR. Impacts of inflammation on cattle growth and carcass merit. *J Anim Sci* 2012;90:1438-1451.
- Hammon DS, Evjen IM, Dhiman TR, Goff JP, Walters JL. Neutrophil function and energy status in Holstein cows with uterine health disorders. *Vet Immunol Immunopathol* 2006;113:21-29.
- Hansen PJ. Genetic variation in resistance of the preimplantation bovine embryo to heat shock. *Reprod Fertil Dev* 2014;27:22-30.
- Lacasse P, Vanacker N, Ollier S, Ster C. Innovative dairy cow management to improve resistance to metabolic and infectious diseases during the transition period. *Res Vet Sci* 2017, in press.
- MacKintosh SB, Schuberth HJ, Healy LL, Sheldon IM. Polarised bovine endometrial epithelial cells vectorially secrete prostaglandins and chemotactic factors under physiological and pathological conditions. *Reproduction* 2013;145:57-72.
- Nace EL, Nickerson SC, Kautz FM, Breidling S, Wochele D, Ely LO, Hurley DJ. Modulation of innate immune function and phenotype in bred dairy heifers during the periparturient period induced by feeding an immunostimulant for 60 days prior to delivery. *Vet Immunol Immunopathol* 2014;161:240-250.
- Ribeiro ES, Lima FS, Greco LF, Bisinotto RS, Monteiro AP, Favoreto M, Ayres H, Marsola RS, Martinez N, Thatcher WW, Santos JE. Prevalence of periparturient diseases and effects on fertility of seasonally calving grazing dairy cows supplemented with concentrates. *J Dairy Sci* 2013;96:5682-5697.
- Ribeiro ES, Gomes G, Greco LF, Cerri RL, Vieira-Neto A, Monteiro PL Jr, Lima FS, Bisinotto RS, Thatcher WW, Santos JE. Carryover effect of postpartum inflammatory diseases on developmental biology and fertility in lactating dairy cows. *J Dairy Sci* 2016;99:2201-2220.
- Ruiz R, Tedeschi LO, Sepúlveda A. Investigation of the effect of pegbovigrastim on some periparturient immune disorders and performance in Mexican dairy herds. *J Dairy Sci* 2017;100:3305-3317.
- Santos JEP, Bisinotto RS, Ribeiro ES, Lima FS, Greco LF, Staples CR, Thatcher WW. Applying nutrition and physiology to improve reproduction in dairy cattle *Soc Reprod Fertil Suppl* 2010; 67: 387-403.
- Santos VG, Carvalho PD, Maia C, Carneiro B, Valenza A, Fricke PM. Fertility of lactating Holstein cows submitted to a Double-Ovsynch protocol and timed artificial insemination versus artificial insemination after synchronization of estrus at a similar day in milk range. *J Dairy Sci* 2017, in press.
- Soto P, Natzke RP, Hansen PJ. Actions of tumor necrosis factor-alpha on oocyte maturation and embryonic development in cattle. *Am J Reprod Immunol* 2003;50:380-388.

- Thompson-Crispi KA, Hine B, Quinton M, Miglior F, Mallard BA. Short communication: Association of disease incidence and adaptive immune response in Holstein dairy cows. *J Dairy Sci.* 2012; 95:3888-3893.
- Vieira-Neto A, Lima IRP, Lopes F Jr, Lopera C, Zimpel R, Sinedino LDP, Jeong KC, Galvão K, Thatcher WW, Nelson CD, Santos JEP. Use of calcitriol to maintain postpartum blood calcium and improve immune function in dairy cows. *J Dairy Sci* 2017;100:5805-5823.