Nutrition, metabolism, and uterine health in postpartum dairy cows

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SUMMARY

- Increasing energy balance and increasing glucose entry rate in early lactation coordinates the homeorhetic mechanisms. These same mechanisms may impact the reproductive systems that are undergoing restoration during the first 30 days postpartum.

- Two essential processes occur during the first 30 days postpartum – the restoration of ovarian cyclicity and uterine involution. These two essential processes may be directly affected by postpartum metabolites and metabolic hormones.

- The tissue damage and infection that occurs in the uterus postpartum lead to a massive inflammatory response; this is particularly true for cows that mount an unsuccessful disease defense and develop metritis. Metritis leads to infertility long-term.

- Some of the pathogens that infect the uterus may come from the pregnancy itself.

- There is an immunological imprint of early postpartum uterine disease on uterine function later postpartum. This imprint may lead to embryonic loss.

Introduction

The period of peak milk production is in early lactation (within 30 to 60 d after calving) when the cow's uterus is involuting and the cow's ovary is returning to cyclicity. The competing processes of milk production, uterine involution, and the restoration of ovarian activity do not work together well during early lactation and will become imbalanced if the cow experiences extreme negative energy balance and (or) metabolic disease during early lactation. A potential end result is that the cow does not become pregnant during the breeding period. Understanding the mechanisms that link the first 60 days of lactation with the subsequent reproductive success or failure is an important area of research for the dairy industry.

The importance adequate glucose supply to postpartum dairy cows

Cows go into negative energy balance postpartum. Maintaining adequate circulating glucose supply during negative energy balance is challenging for the cow. The cow undergoes a series of homeorhetic mechanisms that are aimed toward elevating glucose supply (Bauman and Currie, 1980). In addition to a large increase in hepatic gluconeogenesis shortly after calving, the cow assumes a state of insulin resistance that redirects glucose to the mammary gland. In spite of

these mechanisms, the postpartum cow has chronically low blood glucose concentrations because she fails to meet the glucose requirement.

Glucose may be a mediator of postpartum reproduction because it acts as a substrate for the production of milk and is also essential for innate immune system function and reproduction. Glucose infusion increased blood insulin concentrations (Lucy et al., 2013). There was a marked decrease in both NEFA and beta-hydroxybutyrate (BHB) in response to glucose infusion. In addition to changes in insulin and circulating metabolites, the glucose infusion increased circulating IGF1 concentrations. Insulin may have mediated the stimulatory effects of glucose on IGF1 through its capacity to recouple the somatotropic axis (Butler et al., 2003). The infusion studies demonstrated that a single molecule (glucose) could rapidly reverse the metabolic profile that typifies early lactation (greater NEFA and BHBA with lesser insulin and IGF1). Based on these results, it is possible that increasing energy balance and increasing glucose entry rate in early lactation coordinates the homeorhetic mechanisms. These same mechanisms may be impacting the reproductive systems that are undergoing restoration during the first 30 days postpartum.

Mechanisms that link glucose deficiency with the return to cyclicity and restoration of uterine health postpartum

Negative energy balance and inadequate blood glucose during early lactation theoretically compromises the function of tissues that control reproduction. Metabolites such as NEFA and BHB as well as insulin and IGF1 may also play a role in controlling tissue function. The first 30 days postpartum may be the most-critical in terms of the impact that metabolites and metabolic hormones have on reproduction. Two essential processes occur during the first 30 days postpartum – the restoration of ovarian cyclicity and uterine involution. These two essential processes may be directly affected by glucose supply.

Restoration of ovarian cyclicity postpartum. The bulk of the research performed on metabolites and metabolic hormones has focused on the re-initiation of ovarian cyclicity. Cows that are not cycling are infertile. Furthermore, fertility generally improves with each successive estrous cycle before the breeding period. There has been a traditional focus on understanding the mechanisms that control the timing of the restoration of ovarian activity before the breeding period. A common topic is the positive association between insulin, IGF1, and the day postpartum that the cow begins to cycle (Velazquez et al., 2008).

A variety of metabolites and metabolic signals can act at the level of the hypothalamus to increase GnRH and LH pulsatility (LeRoy et al., 2008). LeRoy et al. (2008) concluded that glucose and insulin were the most-likely molecules to exert an effect on hypothalamic GnRH secretion in the postpartum dairy cow. At the level of the ovary, both insulin and IGF1 promote the proliferation, differentiation, and survival of follicular cells (Lucy, 2008; Lucy, 2011). The most important actions of insulin and IGF1 are observed when either hormone acts synergistically with the gonadotropins (either FSH or LH). Glucose controls insulin secretion in the whole animal and ultimately controls hepatic IGF1 secretion via insulin release. Circulating glucose and the insulin/IGF1 systems, therefore, are functionally linked in the whole animal (Lucy 2011; Kawashima et al., 2012).

The associations between postpartum hormone and metabolites and subsequent reproduction are found early postpartum when the most-extreme homeorhetic states are known to occur. The

early postpartum metabolic profile, therefore, may have the capacity to imprint ovarian tissue either through permanent effects on the genome (epigenetic mechanisms) or by changing the chemical composition of the cells themselves. Perhaps the best-studied example of this metabolic imprint is the relationship between early postpartum NEFA and its effect on the composition of the oocyte and function of follicular cells (Leroy et al., 2011). The possibility that there are permanent epigenetic modifications to the genome during the early postpartum period that affect long-term developmental competence of follicular cells has not been demonstrated at this time.

Uterine health and immune function. The re-initiation of ovarian activity postpartum is a traditional focus of studies of postpartum metabolism. Recently, however, greater emphasis has been placed on uterine health and the central place that uterine immune cell function occupies in determining the reproductive success of the postpartum cow (LeBlanc, 2012; Wathes, 2012). Under normal circumstances, uterine involution is completed during the first month postpartum. During involution, the uterus shrinks in size, reestablishes the luminal epithelium, and immune cells (primarily polymorphonuclear neutrophils or PMN) infiltrate the uterus to clear residual placental tissue as well as infectious microorganisms (LeBlanc et al., 2011). The postpartum cow has a depressed immune system particularly during the first month after calving. With respect to uterine involution and disease, the current theory is that the metabolic environment in postpartum cows suppresses the innate immune system through effects on PMN function (Graugnard et al., 2012; LeBlanc, 2012). In most cases, changes in circulating concentrations of nutrients and metabolites that occur in the postpartum cow are exactly opposite to those that would benefit the function of PMN. There is good agreement between in vitro analyses of PMN function and epidemiological evidence that indicates that an abnormal metabolic profile during the periparturient period predisposes the cow to uterine disease during the early postpartum period and infertility later postpartum (Chapinal et al., 2012).

Glucose is the primary metabolic fuel that PMN use to generate the oxidative burst that leads to killing activity. The glucose is stored as glycogen within the PMN. PMN undergo a brief period (approximately 14 d) of maturation and differentiation from progenitor cells within bone marrow prior to their release. It is during this time that glycogen is stored within the PMN. Glycogen concentrations in PMN within the postpartum cow decrease in a manner that is similar to the decrease in blood glucose postpartum (Galvão et al., 2010). Galvão *et al.* (2010) observed that cows developing uterine disease had lesser glycogen concentration in their PMN. Their conclusion was that the lesser glycogen reserve led to a reduced capacity for oxidative burst in PMN that predisposed the cow to uterine disease.

Most of the available data indicate that metabolic profile of the *prepartum* cow is equally important to that of the postpartum cow for subsequent uterine health and(or) the establishment of pregnancy (Castro et al., 2012). In their work in which an index for physiological imbalance was created, Moyes et al. (2013) concluded that an index that included NEFA, BHB, and glucose was predictive of postpartum uterine disease especially when the *prepartum* index was used. In all likelihood the metabolic profile associated with uterine disease is initiated before or shortly before calving. This is not surprising given the relatively acute nature of the physiological events at the time of calving and the homeorhetic mechanisms at the initiation of lactation. A cow's homeorhetic capacity (i.e., capacity for gluconeogenesis, lipid mobilization, etc.) and her inherent resistance to disease are largely manifested after calving but the underlying biology is theoretically in place before she calves.

New thinking - uterine involution as an inflammatory process

Inflammation is an important process in both healthy and diseased individuals that must be held in check (Buckley et al., 2011). Too little inflammation in response to infection, for example, leads to a failed response to the pathogen, a heightened disease state and possible death. Too much inflammation leads to pain, swelling, and tissue damage (scarring and fibrosis) that may have long-term consequences in terms of tissue function. Both the uterus and the liver are highly inflamed in postpartum dairy cows (Ribeiro et al., 2016). Liver inflammation is caused by the accumulation of triglycerides in hepatocytes and the development of fatty liver (White, 2015). A second site of inflammation is the uterus (LeBlanc, 2014). Detachment of the placenta and the expulsion of both placenta and calf cause hemorrhage and physical trauma. At the same time, the lumen of the uterus is exposed to environmental pathogens that can rapidly cause disease. The tissue damage and infection that occurs postpartum lead to a massive inflammatory response within the uterus; this is particularly true for cows that mount an unsuccessful disease defense and develop metritis (clinical disease of the postpartum uterus).

In their recent review, Karin and Clevers (2016) described how the microbiome within the intestine affects the capacity of the epithelium to regenerate in response to injury or disease. Injury and disease lead to inflammation through the release of pathogen-associated molecular patterns (PAMPs), damage-associated molecular patterns (DAMPs) and reactive oxygen species (ROS). Cytokine release from immune cells then stimulates stem cell proliferation and adult cell differentiation. We have adopted a similar model for the postpartum cow uterus (**Figure 1**).

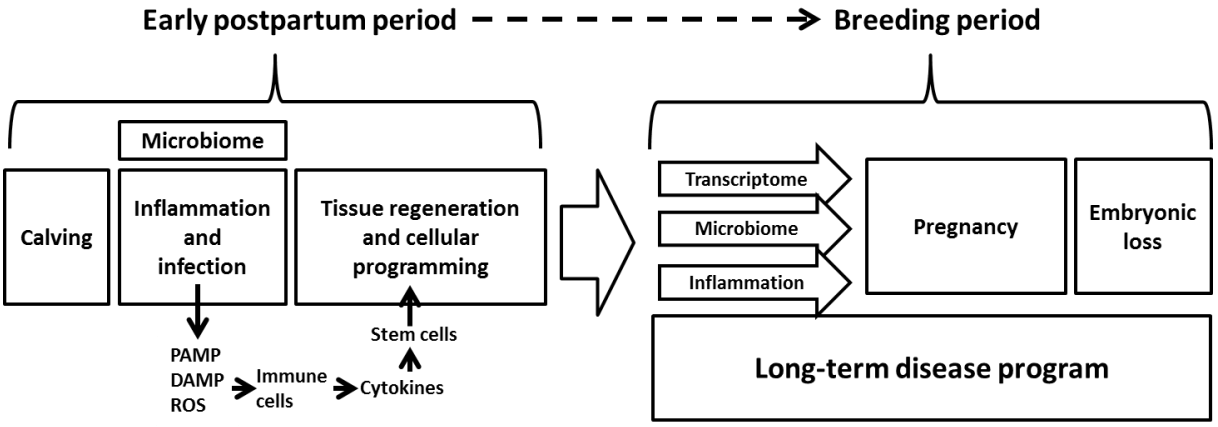


Figure 1. Model for disease programming in the postpartum cow uterus.

The extent of the inflammatory response to the microbiome determines the success of the regenerative process. For example, too little inflammation and the tissue may be lost; too much inflammation and the tissue may become either fibrotic (scarring; Stramer et al., 2007) or cancerous (West et al., 2015). We believe that different populations of clonal cell lines develop within a healthy compared with diseased postpartum uterus and give rise to uniquely imprinted cells within the endometrium. This mechanism gives rise to long-term changes in the transcriptome of the uterus and affects the capacity of the uterus to establish and also maintain a pregnancy. Pregnancy loss occurs in cows with a failed response.

Uterine disease leads to long-term infertility

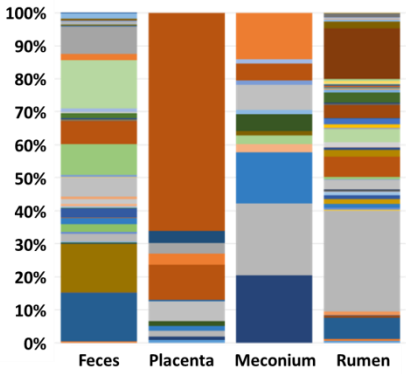
Nearly all cows have pathogenic bacteria in the uterus postpartum. Some cows develop puerperal (acute) metritis which is clinically diagnosed by fever, loss of appetite, fetid discharge, and inflammation of the uterus. Other cows develop clinical metritis (fetid discharge without fever or loss of appetite) or subclinical endometritis (superficial inflammation of the endometrium). One-half to two-thirds of cows remain healthy (no uterine disease). Cows that develop uterine disease suffer from long-term infertility (Brick et al., 2012; Giuliodori et al., 2013; Maquivar et al. 2015; Pinedo et al., 2015).

Dairy cows are immunosuppressed after calving. The depressed immune system is theoretically caused by homeorhetic endocrine and metabolic state that supports early lactation (reviewed by Lucy et al., 2014). Failure to resolve uterine disease leads to inflammation that can damage uterine tissue. Although the symptoms of metritis can be treated with antimicrobials, the literature is equivocal in terms of whether treating a cow for metritis affects fertility postpartum (LeBlanc, 2008; Haimerl and Heuwieser, 2014; LeBlanc, 2014). In a recent review, Haimerl and Heuwieser (2014) noted that none of the 17 studies of the antimicrobial ceftiofur demonstrated improved reproductive performance after treatment. This may be because early postpartum antimicrobial treatment does not target the mechanisms or cells that cause long-term infertility. It may be necessary to develop new treatments that act on the inflammation in the uterine tissue. These treatments could potentially cure the cow of long-term infertility associated with metritis.

Some of the pathogens that infect the uterus may come from the pregnancy itself

The predominant species in metritic cows are *Trueperella pyogenes* (*T. pyogenes*), *Escherichia coli* (*E. coli*), *Fusobacterium necrophorum* (*F. necrophorum*), and *Prevotella melaninogenica* (*P. melaninogenica*) (Carneiro et al., 2016). These bacterial species are found in the environment and are believed to gain access to the uterus from the environment when the cow calves (Bromfield et al., 2015). The bacteria that cause metritis are part of the normal bovine microbiome and are non-pathogenic when found outside of the uterus. For example, *T. pyogenes* is in the nasopharynx; *E. coli* is in the intestine; *F. necrophorum* is in the rumen; and *P. melaninogenica* is part of the normal bovine oral and vaginal flora. Bacteria that cause abortion are known to gain access to the pregnancy by a hematogenous route of infection or by ascending infection from the vagina (Yaeger and Holler, 2007). Some of the bacteria that cause metritis may be resident inside the uterus as part of the placental microbiome. We collaborate with Aaron Ericsson from MU [Director of the MU Metagenomic Center (MUMC) and co-PD]. A Caesarian section was used to collect samples from the pregnant uterus and newly born calf (meconium). We collected bovine feces and rumen papillae as positive control samples. The MU Metagenomic Center generated and sequenced 16S rRNA amplicons to characterize taxonomy. We found that the bacteria that we identified in the pregnant uterus [*Bacteroidetes* (includes *Prevotella* sp.) and *Firmicutes* (includes *Oscillospira* sp.)] were similar to those that were identified in postpartum metritic cows by Santos and Bicalho (2012) in their metagenomics study (**Figure 2**). These new data demonstrate that there is a microbiome associated with the bovine pregnancy. This microbiome may contribute to the pathogens that cause disease postpartum.

Figure 2. Metagenomic data for feces, placenta, meconium and rumen generated by the MU Metagenomic Center (MUMC). As expected, feces and rumen contained an abundant and diverse microbiome. There were also bacteria in the placenta and the meconium. The primary operational taxonomic unit (OTU) identified in placenta was *Mycoplasma haemobos* which accounted for 66% of the sequences. *Mycoplasma haemobos* was found only in placenta. The primary bacteria from meconium were *Bacteroides* sp., *Prevotella* sp., and *Oscillospira* sp.



The immunological imprint of early postpartum uterine disease on uterine function later postpartum.

We recently published a paper on lymphocytic foci within the bovine endometrium of postpartum dairy cows (Lucy et al., 2016). Their presence in the pregnant uterus at approximately 100 d postpartum was associated with chronic inflammation. The number of small and intermediate foci found in the histological sections was greater in cows with uterine infection postpartum. Foci were distributed within the caruncle and the intercaruncular tissue and comprised CD3 positive cells (T cells) surrounding CD79 positive cells (B cells). They were similar, therefore, to tertiary lymphoid structures currently being investigated in other tissues (Leslie, 2016). Some have likened these structures to Mobile Army Surgical Hospitals ("MASH") units that are positioned near immune battle fields. Cows with a high foci count had lesser placental weight on d 42 of pregnancy. Two cows with embryonic loss were in the highest quartile for foci count. The association of foci with embryonic loss is consistent with the well-recognized clinical biology of the mare where chronic inflammation that includes lymphocytic foci leads to infertility and early embryonic loss (Evans et al., 2007). Whether the foci themselves are associated with a unique microbiome or altered uterine transcriptome is one of the subjects that will be addressed in our future work. We believe that chronic inflammation of the uterus is a major cause of early embryonic loss in dairy cows.

Conclusions

The endocrine and metabolic environment of the lactating cow affects the capacity of the cow to become pregnant postpartum. There is ample evidence that the hormones responsible for the homeorhetic mechanisms that support lactation can also act on the ovary and uterus to affect their function prior to and during the breeding period. In addition to the hormonal environment, the metabolic environment created by lactation that includes low blood glucose and elevated NEFA and BHBA impinges upon the ovary as well as the immune system that plays a critical role in restoring uterine health in the postpartum cow. The specific mechanism through which the metabolic environment of early lactation deposits a lasting imprint on ovarian and uterine function is less clear. Also less clear are the mechanisms that link lactation to a predisposition for pregnancy loss in the lactating cow. Uterine inflammation in lactating cows may be an important mechanism explaining pregnancy loss.

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