Optimizing Fertility: The Importance of Uterine Health

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Take Home Messages

- Up to 40% of dairy cows have evidence of uterine disease, and an additional 20% have subclinical endometritis.
- First-calf heifers, cows with an abnormal calving, and those with a profound negative energy balance, are at increased risk for uterine infections.
- Uterine infections delay uterine involution, suppress ovarian functions, reduce fertility, and delay pregnancy.
- Even after clinical endometritis is successfully treated, conception rates are ~20% lower than in unaffected cattle.
- To improve immune function and reduce uterine infections, manage cows to optimize dry matter intake and calve with a BCS of 3.5 (1 to 5 scale).
- Prompt detection and appropriate treatment of uterine infections reduce their negative effects on fertility.
- Minimize contamination of the uterus during any interventions and ensure a clean environment before, during and after calving.

Introduction

The number and variety of microbes in the genital tract of cows depend on her reproductive state. Although the cervix provides a physical barrier (particularly during pregnancy), there are numerous bacteria in the uterus, during and particularly after calving. Bacteria can be cultured from the uterine lumen of most dairy cows within the first 2 weeks after calving (Sheldon et al., 2006). Although many cows will eliminate these bacteria within 5 weeks after calving, in large dairy herds in North America and Europe, up to 40% of cows have evidence of uterine disease, and an additional 20% of cows have

subclinical endometritis (Sheldon et al., 2009). In another report (LeBlanc, 2008) it was noted that clinical endometritis affects 15–20% of cows at 4–6 weeks after calving, whereas another 30–35% of cows have subclinical endometritis between 4 and 9 weeks after calving. Furthermore, 5 to 10% of dairy cows have retained fetal membranes, predisposing them to metritis and endometritis. First-calf heifers, cows with an abnormal calving, and those with a profound negative energy balance (i.e., high non-esterified fatty acids and low IGF-1) before calving are at increased risk for metritis (Giuliodori et al., 2013). A meta-analysis of 23 studies showed that endometritis increased the average interval to re-establishment of pregnancy by 15 days, decreased the relative risk of pregnancy at 150 days after calving by 31%, and reduced the rate at which cows became pregnant by 16% (Fourichon et al., 2000). It is well known that uterine infections reduce fertility and milk production; overall, the economic impact of uterine infections, including treatment, infertility and the value of milk (reduced production and milk discarded due to treatment) was estimated as ~\$2 x 10^9 annually in North America and Europe (Sheldon et al., 2009). Furthermore, even after clinical endometritis was successfully treated, conception rates were ~20% lower than in unaffected animals.

Uterine infections are relatively common in dairy cows; however, there are inconsistencies in definitions, particularly in older literature. The following descriptions from Sheldon et al. (2006) are intended to provide standard terms.

- Puerperal metritis: enlarged uterus with a foul, watery red-brown uterine discharge, clinically systemically ill (reduced milk production, depressed), temperature >39.5^{0}C, <21 days after calving.
- Clinical metritis: enlarged uterus, purulent uterine discharge detectable in vagina, not systemically ill, <21 days after calving.
- Clinical endometritis: purulent (>50% pus) uterine discharge detectable in the vagina >21 days after calving, or mucuopurulent (equal mixture of pus and mucus) discharge detectable in the vagina >26 days after calving.
- Subclinical endometritis: >18% neutrophils in uterine cytology at 21–33 days after calving or >10% neutrophils at 34–47 days (in absence of clinical endometritis).
- Pyometra: uterine lumen with considerable purulent material, persistent corpus luteum and closed cervix.

Diagnosis of Uterine Conditions

Regarding diagnosis of various uterine conditions, puerperal metritis and pyometra are usually readily diagnosed based on clinical signs. Although endometritis is best confirmed by histological examination of endometrial biopsies, the biopsies are somewhat invasive and costly. Cytology is more

practical and is necessary to diagnose subclinical endometritis (Sheldon et al., 2006). However, neither of these methods provides an immediate answer. To diagnose clinical endometritis, examination of vaginal contents for purulent material is recommended. This can be done with a clean, gloved hand, with a vaginoscope or with a specialized device (Metricheck) to retrieve vaginal contents. Minimizing contamination of the uterus during any manipulations or treatments, in addition to ensuring a clean environment in the peripartum period, is recommended to minimize contamination of the reproductive tract.

■ Bacteria Affecting the Bovine Uterus

Approximately 80–100% of cows have bacteria in their uterus for 2 weeks after calving (Sheldon, 2004). During this interval, cows often have one or more of the following bacteria: *Trueperella pyogenes*, *Escherichia coli*, *Pseudomonas* spp., *Streptococcus* spp., *Staphylococcus* spp., *Pasteurella multocida*, *Clostridium* spp., *Fusobacterium* spp., and *Bacteroides* spp. (Bondurant, 1999). By 3 to 4 weeks after calving, the number of bacteria and the variety of species have usually diminished in cows with an uncomplicated postpartum interval and healthy uterus. *Trueperella pyogenes* is one of the most important pathogens of the bovine uterus; it is consistently associated with chronic uterine inflammation (Bondurant, 1999). The presence of *T. pyogenes* in the bovine uterus more than 3 weeks after calving is associated with purulent vaginal discharge, persistent infection endometrial inflammation and reduced reproductive performance (reviewed in LeBlanc, 2008). Uterine infections cause uterine inflammation, which delays uterine involution, is toxic to embryos, suppresses ovarian follicular development and reduces corpus luteum size, reduces fertility, and delays re-establishment of pregnancy (reviewed in LeBlanc, 2008).

Organisms most commonly isolated from cows with postpartum uterine infections include *E. coli* and *T. pyogenes*, plus gram-positive anaerobes (Sheldon, 2014) which are often resistant to several antimicrobials (Sheldon *et al.*, 2004). Specific strains of these bacteria have adapted to live in the reproductive tract (termed endometrial pathogenic *E. coli*; Sheldon et al., 2010). *T. pyogenes* has pyolysin, a cholesterol-dependent cytolysin that causes hemolysis of erythrocytes and cytolysis of endometrial cells (Amos *et al.*, 2014). The pylolysin inserts into cholesterol-rich portions of the plasma membrane, forming a pore, allowing movement of water, which results in cellular damage because of osmosis (Amos et al., 2014). That endometrial stromal cells have a high cholesterol content (Amos *et al.*, 2014) makes them particularly susceptible to this toxin.

■ Immunity to Uterine Diseases

A normal, healthy bovine uterus is relatively resistant to bacterial infection. The initial line of defence is phagocytosis by white blood cells, in particular neutrophils (reviewed in LeBlanc, 2008). The endocrine environment affects uterine defence mechanisms. Increased progesterone concentrations suppress production of cervical mucus, contractility of the myometrium, uterine gland secretions, and phagocytosis by uterine neutrophils, all of which generally promote uterine infections (at least on a short-term basis; LeBlanc, 2008). Prostaglandin F2α can cause luteolysis (if the corpus luteum is susceptible), but may also have pro-inflammatory actions that increase neutrophil function (Lewis, 2004). In contrast to progesterone, high estrogen concentrations generally enhance uterine immune function (LeBlanc, 2008).

Immune function in dairy cows, including neutrophil functions, lymphocyte responsiveness, antibody responses and cytokine production, is suppressed from 1 to 2 weeks before calving to 2 to 3 weeks after calving (LeBlanc, 2008; Esposito et al., 2014). This suppression is related to several factors, including decreased nutrient intake, calving and initiation of lactation, a pre-calving increase in blood cortisol concentrations, and profound changes in blood estrogen and progesterone concentrations (LeBlanc, 2008). Furthermore, there is a direct association between the degree of negative energy balance and impairment of immune function. In cows with retained fetal membranes or metritis, suppression of innate immunity occurs earlier and is more severe than in unaffected cows. (reviewed in Leblanc, 2008).

■ Innate Immunity of the Reproductive Tract

The innate immune system of the cow's reproductive tract includes several forms of defence against microbes. Mucus forms a protective layer (its production is increased by estradiol) and a cervical seal during pregnancy. Epithelial surfaces, many with tight junctions between cells are another physical barrier. Epithelial cells secrete peptides with antimicrobial properties (e.g. beta-defensins; Davies et al., 2008) and various forms of complement, all with important roles in innate defence (Sheldon et al., 2014).

A critical aspect of the innate immune response is that host cells have specialized pattern recognition receptors that bind pathogen-associated molecular patterns (PAMPs), highly conserved molecules in bacteria (including lipopeptides, lipopolysaccharides and bacterial nucleic acids). Although there are several kinds of mammalian pattern recognition receptors that recognize and bind PAMPs, the best characterized in cattle are the Toll-like receptor (TLR) family, with 10 specific TLR in cattle (Sheldon et al., 2014). When the TLR recognizes and binds a PAMP, this causes dimer formation and activates Toll/IL-1 receptors, which in turn activates various other cell

signalling mechanisms. In addition to membrane-bound TLRs, there are intracellular TLRs that detect invading microbes. Activation of TLRs with bacteria usually triggers production of various cytokines (e.g. IL-1, IL-6 and TNF-alpha). Release of cytokines causes increased vascular permeability and stimulates release of antimicrobial peptides, prostaglandins and reactive oxygen species, which result in fever, systemic vasodilation, increased acute phase proteins (produced by the liver) and chemo-attraction of inflammatory cells.

It is well known that uterine infections in cattle reduce fertility. In addition to direct effects on the uterus, there is also evidence of an effect on the ovary (reviewed in Sheldon et al., 2014). In dairy cows, postpartum uterine infections suppressed growth of dominant ovarian follicles, decreased plasma estradiol concentrations and reduced the probability of ovulation (Sheldon et al., 2002). In addition, cows given bacterial endotoxin during the luteal phase had transient decreases in serum progesterone concentration, implicating effects of uterine infections on the corpus luteum. Perhaps inflammatory mediators from the uterus reach the ovary via the counter-current mechanism that delivers PGF2α from the uterus to the ovary (Sheldon et al., 2014).

■ Retained Fetal Membranes

Retained fetal membranes (in place 24 hours after calving) have a median incidence of 8.6%, and is more likely to occur in cattle with abortion, dystocia, birth of a stillborn calf, twins, induced calving, milk fever, higher non-esterified fatty acid (NEFA) concentrations, lower circulating vitamin E, and increasing age (reviewed in LeBlanc, 2008). The primary cause is the lack of a prompt breakdown of the cotyledon–caruncle attachment after calving, attributed to failure of the immune system to cause placentome degradation (reviewed in LeBlanc, 2008). In that regard, cows with retained fetal membranes have suppressed neutrophil chemotaxis and oxidative burst activity, and reduced concentrations of interleukin (IL)-8 concentrations at least 2 weeks before calving (LeBlanc, 2008). Retained fetal membranes increase bacterial contamination of the uterus and often impair uterine involution; the overall impact on reproductive performance ranges from none to a severe metritis with toxemia and suppressed feed intake and milk production (reviewed in LeBlanc, 2008).

■ Survey of Postpartum Dairy Cows

In a large study, 5719 postpartum dairy cows were monitored for 65 days after calving. In those cows, first-service pregnancy rates were 39.4, 38.7 and 51.4% for cows with metritis, clinical endometritis, or no uterine disease, respectively (Santos et al., 2010). Peripheral blood leukocytes of cows that developed endometritis had less efficient phagocytosis prepartum than cows

without endometritis (Kim et al., 2005). Furthermore, cows that subsequently developed metritis had impaired immune function (various aspects), prepartum and at calving (reviewed in Esposito et al., 2014).

■ Effects of Nutrition on Reproduction

Nutrition has a profound effect on various aspects of reproductive function. Retained fetal membranes, metritis and endometritis are generally attributed to reduced immune function and are usually associated with peripartum energy metabolism and nutrition and in particular, feed intake during transition (reviewed in LeBlanc, 2008). In one study (Kasimanickam et al., 2013), dairy cows with persistent uterine inflammation after calving had increased serum concentrations of TNF-α, IL-6, and leptin, and decreased concentrations of insulin and IGF- (compared to normal and spontaneously recovered cows). Furthermore, concentrations of adiponectin, IL-1β, IL-6, and TNF-α were increased in cows with a lower body condition score. Interestingly, body condition was lower for cows with metritis or clinical endometritis (Kasimanickam et al., 2013). Therefore, it was concluded that body condition loss mediates increases in anti- and pro-inflammatory cytokines, whereas increased anti- and pro-inflammatory cytokines may cause loss of body condition, resulting in persistence of uterine inflammation. Overall, the goal is to optimize immune function in the peripartum period, in particular by promoting feed intake during transition (Nordlund and Cook, 2004).

Ketosis

Ketosis (characterized by hypoglycemia and hyperketonemia) is the imbalanced use of body fat early in lactation; approximately 50% of dairy cows have at least temporary ketosis during early lactation (reviewed by Esposito et al., 2014). Ketosis and fatty liver both impair immune responses (Esposito et al., 2014). Therefore, cows should be managed to optimize dry matter intake, avoid excessive body weight gain during the non-lactating period and calve with a body condition score of 3.5 (on the 1–5 scale)

Transition Period

The transition period is commonly defined as the 6-week interval which is centered on calving. Although fetal nutrient requirements typically peak at the start of the transition period, dry matter intake decreases by 10 to 30%, and the cow undergoes the stress of calving, switches from a high-forage to a high-concentrate diet, and begins to produce milk, which collectively results in a negative energy balance (NEB; reviewed in Esposito et al., 2014). Although NEB is unavoidable, excessive NEB indicates poor adaptation and predisposes cows to health disorders and decreased production. The best method to assess NEB is to determine serum NEFA concentrations before

and particularly after calving. The best way to manage NEB is controlling energy intake during the dry period and feeding monensin (McArt et al., 2013).

Effects of Nutritional Deficiencies on Reproduction

There are numerous associations between nutritional deficiencies and immune function (reviewed in Bicalho et al., 2014). For example, subclinical deficiencies of the following minerals had the following associations: Se and immunosuppression and reproductive failure; Zn and impaired growth; Ca and decreased milk production, reduced blood neutrophil counts, decreased neutrophil function and increased metritis. Regarding trace minerals, the pre-calving diet should include 0.3 ppm selenium (ideally 5 mg/d and 1000–2000 IU/cow/d of vitamin E (reviewed in LeBlanc, 2008). Furthermore, it is well known that the greater the degree of NEB (manifested as elevated prepartum NEFA concentration and postpartum BHBA concentration), the greater the suppression of the immune response and increased susceptibility to uterine disease (reviewed in Bicalho et al., 2014).

■ Conclusions

Uterine disease is common in dairy cows and can have a profound impact on health and productivity. There is good evidence that nutrition affects the incidence and severity of uterine disease. It is important to optimize nutrition and to detect and treat uterine disease, so as to promote animal health and productivity and decrease losses.

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