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REVIEW ARTICLE

Complementary and Alternative Treatments for Postpartum Uterine Diseases in Dairy Cows

Rejean Cleophas Lefebvre

Faculty of Veterinary Medicine, University of Montreal, Saint-Hyacinthe, Québec, Canada

rejean.lefebvre@umontreal.ca

ABSTRACT

The goal of treating postpartum uterine diseases in dairy cows is to reach clinical cure in a reasonable amount of time and to improve reproductive performance while minimizing milk and meat residue issues. The ultimate goal is to reduce the economic losses caused by uterine diseases. This article focuses on postpartum uterine diseasess in dairy cows, in particular acute puerperal metritis, pyometra and endometritis. The usual treatment approach for these conditions is systemic or local antibiotic therapy to control bacterial growth. Based on clinical signs and bacteria identification using dependent- and independent-based methods, antibiotic use appears to be the most logical and effective therapeutic approach. However, despite considerable research, the treatment of uterine diseasess remains a subject of considerable controversy in the literature. Although some local and systemic antibiotic therapies are effective at mitigating the consequences of postpartum uterine diseases, antibiotic use in the current global context is a serious concern because it is associated with selective pressures on bacteria and the emergence of resistant bacteria in humans and animals. Research on mucosal immunology and the genital microbiota of cows has improved our understanding of uterine involution and how pathological agents behave during uterine diseases in dairy cows. And while antibiotic therapy remains the most common therapeutic option chosen by veterinarian practitioners to treat PUDs, complementary and alternative veterinary medicine is increasingly being considered in response to a demand from animal owners. However, because of the small number of randomized clinical trials and a lack of evidence-based medicine, there is widespread concern regarding the adequacy of evidence for the efficacy and safety of complementary and alternative veterinary medicine. The objectives of this article are to describe uterine involution and the main postpartum uterine diseases that occur during the postpartum period in dairy cows, briefly review the current approaches to treating postpartum uterine diseases, and critically review the evidence pertaining to complementary and alternative veterinary medicine in postpartum reproductive veterinary medicine.

Introduction

In the past two decades, the decreasing fertility of dairy cows has been given considerable attention in veterinary science (Fourichon et al., 2000).[1] Since reproductive performance is strongly related to uterine health status at the end of the voluntary waiting period (VWP, minimum time necessary to establish a new pregnancy)[2] (Sheldon et al., 2006), the assessment and treatment of uterine pathologies in postpartum cows has been the focus of much research. Based on an objective and replicable overview of efficacy and safety, science-based medicine (so-called conventional or orthodox medicine) has traditionally proposed antibiotics for treating uterine infections. But as antibiotic use continues to increase, so does the selective pressure for resistant bacteria in humans and animals. To promote a more judicious use of antibiotics, a number of strategies are now being used, including the systematic use of antibiograms to target specific infectious agents and/or the use of anti-inflammatory drugs to improve treatment efficacy and reduce the quantity of antibiotics used. The current global context has produced fertile ground for complementary and alternative veterinary medicine (CAVM). For conventional dairy farmers, the absence of a withdrawal period for milk and meat after CAVM treatment has become an important economic consideration. Furthermore, organic farms have an urgent need for CAVM so they can meet their objectives:

- produce food of high nutritional quality
- ensure optimal living conditions for livestock that ensure they can perform all aspects of their innate behaviour
- protect consumers from pharmaceutical residues
- reduce antibiotic resistance

In addition, herd management and preventive medicine methods are not progressing quickly enough to meet the needs of the livestock industry. As in conventional medicine, decisions concerning CAVM therapies also need to be based on the best available evidence and supported by scientifically valid data. This article provides an overview of postpartum uterine involution and the major postpartum uterine diseases (PUDs) in dairy cows, and reviews conventional and CAVM approaches to treating PUDs.

Postpartum uterine involution

Based on its structure and the number and form of the layers intervening between fetal and maternal circulation, the cow has a cotyledonary and synepitheliochorial placenta. Normal uterine involution after calving allows the reproductive tract to return to normal function before a new pregnancy is established. This process is very complex and involves morphological, endocrine, metabolic, histologic, bacteriological, biochemical and immunological changes. It lasts about 45 days (VWP), and during that time the weight of the uterus decreases from 9 kg to approximately 1 kg and the uterine horns return to their normal dimensions of about 3 cm in diameter and 30 cm in length.[3] At the end of the first week postpartum, the caruncles have necrotized and the stratum compactum is infiltrated by neutrophils.[4] In the second week, the necrotic tissues detach and mix with blood, endometrial exudates and cellular debris in the uterine cavity to make the lochia, which is then gradually expelled during the first rise in estrogen during the first follicular wave.[5,6] Even after the first ovulation, the process of sloughing and regeneration of the endometrium will not allow for implantation of a potential embryo.[7] About 20 days after calving, tissue sloughing and hemorrhaging have stopped and uterine size has decreased by more than 80%, but full restoration of all layers of the uterine wall requires more time (about 45 days).[6] The epithelialization of the endometrium then progresses to completion by the end of the VWP, at which time the uterine environment is normal and ready to establish a new pregnancy.[8] If uterine involution does not proceed in an orderly fashion, complications can occur and lead to PUD and subfertility.

Bacteria in the postpartum uterus

After calving, approximately 95% of cows present some degree of uterine contamination with bacteria, regardless of whether or not there are signs of disease.[9-11] The loss of anatomical barriers and the negative pressure created by the phenomenon of uterine contraction and relaxation enhances the vacuum effect and facilitates the ascent of bacteria into the uterus, allowing for rapid colonization within minutes.[12] There is also evidence that bacteria can reach the uterus by blood circulation[13] and that they can even be present in the uterus during pregnancy.[14] In humans, amniotic fluid and meconium contain bacterial DNA and a microbiota that is similar to that of the fetus.[15-16] Gram-negative bacteria (e.g., *Escherichia coli*) are dominant during the first week, before gradually being replaced by Gram-positive bacteria by day 15 postpartum, and 78% of cows still have bacteria in the uterus at day 15.[17] During normal uterine involution, the uterus clears bacteria by week 6 after calving, but 40% of dairy cows are not able to eliminate the infectio.[6] The persistence of pathogenic bacteria in the uterus is associated with delayed uterine involution, PUDs and reduced reproductive efficiency.[19-21]

Based on culture-dependent studies, the most common bacteria involved in PUDs are *Trueperella pyogenes*, *Escherichia coli*, *Fusobacterium necrophorum*, *Prevotella melaninogenica* and *Bacteroides* spp. .[19,22-24] Culture-independent approaches based on 16S rRNA gene sequencing using PCR and metagenomic DNA sequencing techniques have shown that diseased and healthy postpartum dairy cows present similar phylum abundance (Firmicutes, Bacteroides, Proteobacteria and Fusobacteria), although there is considerable variation at the genus level. The uterine microbiota of diseased cows has reduced richness and diversity compared to healthy cows.[25-27] Currently, it is unclear whether changes in the normal composition of the uterine microbiota (dysbiosis) is associated with the occurrence of PUDs.[28] The etiological importance of new and unclassified bacteria is also unclear.

Definitions of the most serious postpartum uterine diseases: acute puerperal metritis, pyometra and endometritis (clinical and subclinical)

Acute puerperal metritis (APM) is the most serious uterine inflammatory disease during the postpartum period in dairy cows. It typically occurs within the first 10 days after parturition and has an incidence of about 20%.[2] This PUD is characterized by the following systemic signs of illness: fever ≥ 39.5°C, anorexia, dehydration, poor general condition, reduced milk, and a foul-smelling and brown-red watery uterine discharge. The risk factors are retained fetal membranes, dystocia, twins and stillbirth. The condition is assigned a grade from 1 to 3 based on the severity of clinical signs.[2] Several bacteria have been found in the uterus of cows with APM: *E. coli*, *F. necrophorum*, *P. melaninogenica*, *Proteus* species and *T. pyogenes*.

Pyometra is characterized by an accumulation of purulent or mucopurulent exudate in the uterine lumen, causing distention of the uterus, persistence of the corpus luteum and closed cervix, and loss of cyclicity.[6] The persistence of progesterone released from the corpus luteum downregulates uterine immunity and creates conditions favourable to cervical closing and the growth of opportunistic bacteria like *T. pyogenes* and *F. necrophorum*. Pyometra occurs most often at the end of VWP, after normal cyclicity has resumed.

Clinical endometritis is defined as inflammation of the endometrium with a normally sized uterus and vaginal purulent discharge 3 weeks after parturition in the absence of systemic clinical signs.[2] Even though the presence of vaginal discharge is only an indirect sign used to diagnose inflammation and infection of the uterus, the presence of purulent exudate in the anterior vagina or cervix has been consistently associated with reduced fertility in dairy cows [20,2330-33] Clinical endometritis affects about 20% of all dairy cows and is diagnosed by vaginoscopy, gloved hand or Metricheck curette.[20,34]

Lastly, **subclinical endometritis** is a superficial endometrial inflammation that is identified by a high neutrophil count on endometrial cytology in the absence of purulent vaginal discharge or the accumulation of pus in the uterine lumen, and the absence of systemic clinical signs. The threshold neutrophil count for the diagnosis of subclinical endometritis varies with time postpartum, ranging from 5 to 18%. This condition, which has a prevalence of 20 to 30%, is associated with detrimental effects on the reproductive performance of cows.[35]

Conventional treatments for postpartum uterine diseases

The goal of treating PUDs in dairy cows is to reach clinical cure in a reasonable amount of time and to improve reproductive performance while minimizing milk and meat residue issues. The ultimate goal is to reduce the economic losses associated with PUDs. APM in cows is usually treated with systemic antimicrobial drugs like third-generation cephalosporins (ceftiofur), tetracycline, penicillin, ampicillin and nitrofurazone, and with fluid therapy in severe cases.[36] Administration of ceftiofur for 3 to 5 days represents an effective treatment of cows diagnosed with.[37] As the World Health Organization (WHO) considers ceftiofur to be a critically important antimicrobial for human medicine and because of concerns about antibiotic resistance and residues, some veterinarians advocate waiting 24 to 48h before beginning antimicrobial therapy, thus allowing for a potential spontaneous cure.[38] In severe cases (grade 3 APM), this is frequently not advised due to animal welfare concerns. Indeed, manipulation of the uterus and drainage of the fetid watery red-brown discharge are not recommended because of the risk of bacteremia and of perforating the friable and sensitive uterine wall.[39] Before initiating antibiotic therapy, or in combination with it, anti-inflammatory drugs can be used to reduce the symptoms associated with APM. A combination of antibiotic and anti-inflammatory drugs seems to improve condition, although the reproductive efficiency of APM cows is less than that of healthy cows.[40] There is no clear evidence of improvement in clinical condition or reproductive efficiency with other antimicrobials.

Since systemic inflammation is present around the time of parturition in dairy cows, systematic anti-inflammatory drugs (NSAIDs) like acetylsalicylic acid and flunixin have been proposed for use in early postpartum, although the evidence does not clearly support this approach.[41] Complementary drugs like estrogen[42], which is now illegal in many countries, oral calcium[43] and prostaglandins do not improve the prognosis. However, in cases of pyometra, PGF2a or its analogue[44] is the treatment of choice, promoting luteolysis of the persistent corpus luteum, opening of the cervix, expulsion of uterine exudates and a reduction in bacterial load in 90% of cases.[45] This last study showed a recurrence rate of about 10%, in which case the PGF2a treatment would have to be repeated.

Local antibiotic therapy informed by efficacy testing in the lab may be used once the uterine contents have been completely evacuated. Veterinarians need to remember that the use of intrauterine nitrofurazone infusion has been known to reduce conception rates. Endometritis, both clinical and subclinical, is most often treated with an intrauterine infusion of a specific formulation of cephapirin (first-generation cephalosporin; Metricure, Merck), which has beneficial effects on clinical cure and subsequent reproductive performance.[20,30,32,33] Although prostaglandins are still used for the treatment and prevention of endometritis, there is no evidence of their efficacy, neither for improving reproductive efficiency nor for reducing its incidence.[38,47] However, this hormone is essential in the systematic synchronization protocols used in controlled breeding programs.

Complementary and alternative veterinary medicine

Veterinary professionals are now looking at new options for improving the health of animals under their care. This trend is driven by a number of advances, including the

WHO's guidelines on the use of medically important antibiotics by health professionals, the One Health approach, which recognizes the interconnectedness of people, animals, plants and their shared environment, and the demand of their clienteles. Practitioners without a veterinary medical education have been at the forefront of complementary and alternative veterinary medicine (CAVM).[48] In veterinary medicine, CAVM is gradually attracting interest as an option in the treatment of chronic conditions where conventional medicine has proven not very effective or is used more as palliative therapy. Since veterinary students are not trained or formally instructed in CAVM, it has never become a possible approach for specific conditions. The absence of objective discussions concerning the efficacy of CAVM at universities and by professional associations has hampered critical research on this subject. CAMV has been more widely used for prevalent chronic diseases like allergies, chronic asthma (heaves) and degenerative joint diseases in small animals and horses, conditions that conventional medicine has had poor success in treating.[49] In the dairy industry, organic farmers have been the driving force behind CAVM as an alternative to synthetic medicines. However, it has been difficult to assess CAVM therapies despite the willingness of farmers because treatments and prevention practices are not always recorded and in a consistent way. For example, Espadamala et al. showed significant disparity in how farmers treat PUDs. [50]

To mitigate the impact of PUDs, several non-antimicrobial therapies to treat or prevent uterine diseases have been proposed. However, the efficacy of most of these has still not been critically assessed. Regarding the few clinical trials that have been conducted, the scientific merit of most in vivo studies is compromised by poor experimental design, including an absence of precise clinical definition, inconsistent outcomes, lack of negative controls, insufficient number of animals per treatment group, use of inadequate or rebutted diagnostic methods, disparate study populations, lack of randomization, and faulty experimental design.

In general, CAVM therapies cover a wide range of therapies, including acupuncture, herbal medicine, homeopathy, aromatherapy, gold therapy and vibration therapy. All therapies need to be assessed by studies informed by evidence-based medicine and using solid experimental designs and statistical inference. Such efforts would advance our understanding and provide potentially new solutions for treating PUDs in dairy cows. In this article, I review the following therapies: chitosan microparticles, essential oils, mannose, bacteriophages, dextrose, paraffin, ozone, probiotics, antiseptics, homeopathy, apitherapy, vaccines, pegbovigrastim, recombinant IL-8 and genomic selection. The goal of these alternative therapies is to improve the ability of the cow's defence mechanisms to avoid (chitosan, mannose, dextrose), tolerate (probiotic, paraffin, ozone) or resist (pegbovigrastim, recombinant IL-8, genomic selection) bacterial infection in the genital tract.

Complementary and alternative therapies for treating postpartum uterine diseases

CHITOSAN: This substance, which is FDA approved for some uses, is synthesized from chitin in the exoskeleton of arthropods and in the cell walls of certain fungi and yeast. It exhibits a broad antimicrobial spectrum in neutral and acidic environments.[51] The pH of the cow's genital tract is normally between 6.84 and 7.51, or neutral.[52] It is claimed that chitosan improves resistance to PUDs by reducing the uterine bacterial load. More specifically, chitosan microparticles disrupt the bacterial cell membrane by interacting with outer membrane protein A (OmpA), eventually causing cell death.[51] Cows with metritis were administered 24 g (in 40 ml of sterile distilled water) of chitosan particles in the uterus for 3 days (D0 = day of diagnosis, D2 and D4) and compared to cows administered a ceftiofur crystalline-free treatment (6.6 mg/kg SC in the base of the ear at D0 and D3) or no treatment at all (the control).[53] Cows treated with chitosan showed no clinical improvement and also showed decreased milk production, reproductive performance (conception rate at first AI and pregnancy rate at 300 DIM) and survival rate compared to the control group. Issues with case definition and the number of local treatments render the results even more uncertain. In the case of acute conditions like APM, manipulating a fragile uterus increases the risk of further traumatizing the genital tract and negatively affects outcome. Therefore, the intrauterine administration of chitosan is not recommended in dairy cows.

ESSENTIAL OILS: These volatile substances, of which there are some 3,000 in total, are naturally produced by plants as secondary metabolites. They are known for their antibacterial, antifungal, insecticidal and antiviral properties, many of which are due to their constituent terpenes and phenylpropanoids.[54] It is important to remember that an essential oil is composed of different compounds and that its bioactivity results from a complex interaction of different classes of compounds. Essential oils have antioxidative, anti-inflammatory and antimicrobial properties, making them good candidates for treating infectious processes like PUDs.[55] One study has examined the effects of using Optimum UterFlush (Van Beek Natural Science, Orange City, IA), a certified organic blend of essential oils, on the performance of dairy cows on organic farms. This product was infused every other day for a total of 3 times between 1 and 12 DIM in the uterus of APM dairy cows (n=107). Another group was administered an intrauterine iodine infusion (n=113) and another received no treatment (the control, healthy cows, n=400).[56] The first service conception rates were 23%, 37% and 38% for the iodine, essential oils and control groups, respectively (p=0.05). At 150 DIM (days in milk after calving), 75%, 31.1% and 44.8% were pregnant in the control, iodine and essential oils groups, respectively. The survival analyses suggested a shortening of the interval from calving to first AI (P=0.08). The odds of pregnancy at 150 DIM for cows in the essential oils group was 1.81 times that for cows infused with iodine. All cows in the study received intravenous calcium, hypertonic saline and oral aspirin. The safety of Optimum UterFlush was not tested on healthy cows. These first results are very encouraging and further research on a larger scale is needed.

CARBOHYDRATES: Three rationales have motivated researchers to use sugars as an intrauterine remedy for PUDs: 1) sugars disrupt the attachment of bacteria to tissues, 2) sugars inhibit microbial growth, and 3) sugars are hypertonic and attract fluid to the uterus and facilitate the evacuation of debris and bacteria when the cervix opens. Lectins on the surface of the infectious microbe bind to complementary carbohydrates on the surface of the host tissue, thereby preventing the pathogen from being washed away by the host's natural cleansing system. This adhesion also provides nutrients to the bacteria, facilitates the delivery of toxins, and eventually enables the bacteria to penetrate the tissues. The lectins have elongated multi-protein appendages with sugars (typically saccharides) that increase the microbe's infectiousness (e.g., the fimH lectin in *E. coli*).[57] Anti-adhesion therapy for preventing or treating microbial diseases consists of inhibiting these lectins using suitable carbohydrates. **Mannose**[26] and **50% dextrose**[58,59,60,61] have been tested in cows with metritis and endometritis. Since the discovery that mannose can block the growth of *E. coli* in human wounds and also inhibit the adhesion of *E. coli* to endometrial cells in mares with endometritis,[62] further studies have been done on cows with PUDs. The adhesion of pathogenic organisms to host tissues is often a prerequisite for the initiation of an infectious disease. By inhibiting bacterial adhesion to the cell membrane of endometrial cells, the risk of initiating infection should be lower. The hyperosmotic effect of high sugar concentrations may also inhibit bacterial growth by breaking down the bacterial membrane. One study examined the use of mannose to reduce the risk of metritis in dairy cows.[63] The treatment, 50 g of sugar intrauterine 2 days after calving, was not successful in improving uterine health in the treatment group compared to the placebo group. Similarly, 200 mL of 50% dextrose solution infused intrauterine in cows with endometritis at about 30 DIM did not improve either cure rate, first conception rate or calving-to-conception interval.[26,59,58] By contrast, another study showed that the cure rate for endometritis improved with dextrose treatment.[61] Nevertheless, the dextrose improved median days open compared to systemic treatment with ceftiofur or no treatment at all (control). However, the accumulation of transudate in the uterus that occurs after infusing dextrose has not been investigated. In sum, treating metritis and endometritis using a 50% dextrose solution remains controversial and will continue to be until we have consistent, repeatable and valid results showing its efficacy and safety.

PARAFFIN: The intrauterine infusion of liquid paraffin for endometritis has been proposed as an effective way of increasing uterine resilience during the postpartum period. Cows with endometritis infused with 100 ml of liquid paraffin at around 30 DIM showed no improvement in condition and also showed a reduction in reproductive performance.[58] Therefore, liquid paraffin should not be considered a viable treatment option for endometritis in dairy cows until more studies have been conducted.

OZONE: This gas, which is composed of three atoms of oxygen in a dynamic unstable structure, is essential for life on earth due to its ability to absorb harmful UV radiation from the sun.[64] Ozone is used in a variety of medical therapies because of its strong bactericidal, fungicidal, viricidal, yeasticidal and protozocidal properties. Ozonated vegetable oil has emollient, cicatrizing, antibacterial and disinfection properties, is available in a variety of forms.[65] Ozone inhibits bacterial growth by damaging its capsule and cell membrane through the activity of peroxidases, and possibly also by blocking the replication of bacterial DNA. It can therefore act as a supportive therapy when treating infectious diseases. Gram-positive bacteria seem more sensitive to ozone than Gram-negative bacteria. The most important activity of ozone is probably its synthesis of cell membrane enzymes like superoxide dismutase, catalase and glutathione peroxidase, all of which counter the damaging effects of O2 free radicals.[66] Ozone reduces the pro-inflammatory cascade by promoting the immunosuppressive cytokines produced by neutrophils, monocytes and lymphocytes, such as like IL-10 and TNFß1.[67] The therapeutic efficacy of ozone is most likely dependent on the strength of the oxidative stress produced. Moderate oxidative stress activates nuclear transcriptional factors (NrF2), which suppresses NFkB and stimulates the inflammatory response. Ozone has been used to prevent endometritis[68] and to treat metritis[69,70] with some positive effect. Although ozone therapy is used in human medicine in therapies like autohemotherapy and antineoplastic therapy, and to treat conditions like obstructive arterial disease, macular degeneration, autoimmune disease and diabetes, it's medical use is still not accepted in all countries.[67] In terms of the efficacy of ozone in treating endometritis in dairy cows, the sample size in studies conducted so far is too small to draw any conclusions.

BACTERIOPHAGES: The use of bacteriophages and phage-derived endolysins may represent an alternative treatment for PUDs in dairy cows.[71] Bacteriophages are host-specific viruses that affect particular species or strains of bacteria. They produce endolysin, which digests the bacterial wall, enters the bacterial cell, integrates the genome (prophage) of the host cell, and replicates. This results in lysis of the cell wall[72] in the biofilms produced by certain bacteria, such as *E. coli*, *T. pyogenes*, coagulase-negative *Staphylococcus* and *S. aureus*.[73] Treatment with an intrauterine infusion of bacteriophages specific to *E. coli* and *T. pyogenes* 2 days postpartum had no effect on uterine health and reproductive performance, and in fact increased the incidence of retained fetal membranes and metritis.[26] As is the case for paraffin, the use of bacteriophages to treat PUDs in dairy cows is not recommended and more research is needed.

PROBIOTICS: These are mixtures of live bacteria and/or yeasts that, when administered in adequate amounts, confer a health benefit on the host. Probiotic products include probiotic-containing drugs, medical and conventional foods, dietary supplements and animal fodder. However, the term is not applied to microbial transplants, fermented foods, dead microbes and microbial products such as proteins, polysaccharides and nucleotide components. Probiotics work by producing substances that have a desirable effect on the body, for example, by improving immune response or by ensuring the maintenance of a healthy microbiome.[74] In humans, probiotics have shown promise in preventing antibiotic-associated diarrhea (*Clostridium difficile*), necrotizing enterocolitis and sepsis, and in treating infant colic, periodontal disease and ulcerative colitis. Prebiotics are not the same as probiotics since prebiotics are food components that selectively stimulate the growth or activity of desirable microorganisms. Probiotics may contain a variety of microorganisms, but one of the most common is *Lactobacillus*, a major member of the vaginal microbiome in women. In dairy cows, several doses of a cocktail of lactic acid were infused in the vagina before calving and in the uterus after calving to measure the effect on the incidence of metritis and endometritis.[75-77] Treatments with the lactic acid cocktail reduced the incidence of metritis and endometritis, improved vaginal mucus secretion of immunoglobulin A, and decreased the inflammatory response by reducing neutrophil and dendritic cell gene expression and reducing the blood concentration of cytokines (IL-1β, IL-6, IL-8 and TNF)).[78] *Lactobacillus* can inhibit pathogen growth by promoting the production of organic acids and bacteriocin, which keep the vagina acidic.[79] Probiotics improve the epithelial barrier of the vagina, facilitate the formation of biofilms on the vaginal mucosa, produce other antimicrobial molecules like bacteriocin, reduce pathogen adhesion, compete with other bacteria for nutrients, and modulate mucosal immunity (Bermudez-Brito et al., 2012).80 Therefore, the intravaginal or intrauterine infusion of probiotics appears to be effective in reducing the incidence of PUDs and improving animal health. Probiotic functionality and efficacy is species specific and therefore different cocktails may have different effects.

APITHERAPY: Another alternative way to treat PUDs is by using products that come from honeybees, or apitherapy. Honey products have been used extensively since ancient times as anti-putrefactive agents (e.g., on mommies), antiseptics, cicatrizants, anti-inflammatories, antimicrobials, antioxidants,[81] and antibiofilm agents.[82,83] Honey is a powerful antioxidant and free radical scavenger. Unfortunately, in modern medicine, propelled by new developments and the wide applications of antibiotics, the use of honey as a medical treatment has been sunk into oblivion. Given the ongoing dangers of antibiotic resistance, there has recently been renewed interest in using honey products like propolis, venom, royal jelly and pollen to treat conditions in livestock. The active components of honey, such as glucose, fructose, flavonoids, polyphenols and organic acids, are largely responsible for its therapeutic effect. For example, the flavonoids found in honey have been shown to induce apoptosis and prevent the release of IL-1ß, Il-6, TNFa, iNOSm and Cox-2.[84] Propolis has antifungal properties and can be used as an antibiofilm agent.[85]

In general, honey possesses significant therapeutic potential and its broad-spectrum antimicrobial activity has been widely documented in a large number of in vitro studies with MIC and MBC testing.[86] The immunomodulatory response to honey products involves the activation of lymphocytic function, the activation of macrophages and the stimulation of cytokine release by monocytes.[87] Abdul-Hafeez et al. [88] infused 100 ml of 70% Egyptian cotton honey for 3 consecutive days in the uterus of repeat breeder dairy cows (n=16) with purulent vaginal discharge unresponsive to standard antibiotic therapies. The conception rate was 75% at first insemination after treatment. However, the small number of animals in this study does not allow us to draw any definitive conclusions. In terms of innocuity, honey products do not seem to cause irritation of the reproductive tract mucosa. Since there have been no randomized clinical trials on the efficacy of honey products, a proper assessment of their benefits in treating PUDs in dairy cows is currently impossible.

HOMEOPATHY: This therapeutic approach was invented by Samuel Hahnemann in 1797 as a new way of treating disease based on the use of diluted remedies. The underlying concept is that diseases can be cured by a highly diluted substance that produces the same clinical signs in healthy people.[89] Homeopathy is based on three fundamental principles: 1) the law of similia, 2) the law of simplex, and 3) the law of minimum. The law of similia means "like cures like", that is, the medicine must be capable of producing similar symptoms of the disease. The law of simplex states that only one single and simple medicinal substance is to be administered. The reasoning is that if more than one remedy is used, the doctor will never know which element was curative and the different ingredients may even result in interactions that have adverse effects in the body. The remedies are often so diluted that not a single molecule of the original product is likely present.[90] The third law is probably the most debated. It states that the quantity of medicine given to a patient is minimal and appropriate for producing a gentle remedial effect and avoiding any aggravation.

In both the scientific community and veterinary practice, the use of homeopathy in food-producing animals is controversial, despite it being a common practice on organic farms. As is the case for other alternative therapies, the use of the homeopathy is fueled by the overuse of antibiotics in humans and animals, and the resulting increase in antibiotic resistance.[91] However, the exact mechanism of action of homeopathic drugs is not known. For example, Lachesis is used to treat several inflammatory conditions, including uterine infection, because it is believed to stimulate the local immune defense and help mucosa regenerate (Boitor et al., 1994; Dorenkamp 1991).[92,93] In ruminants, clinical trials have reported no effect of Lachesis on uterine infections in dairy cows.[94] Homeopathic studies on fertility during the peripartum period (retained placenta, endometritis) have only examined its preventive effects, not its therapeutic benefits. In a randomized, controlled, double-blinded clinical trial, Arlt et al., [95] compared the effects of three different homeopathic remedies (Traumeel, Lachies and Carduus administered IM) at 7 to 13 DIM, 14 to 20 DIM, and 21 to 27 DIM in dairy cows with clinical endometritis. In violation of the second law, the researchers did not individualize treatments. None of the homeopathic treatments were effective in preventing endometritis or in improving metabolic condition and reproductive performance. Another randomized, controlled, blinded trial with 105 cows testing the efficacy of compound remedies showed a significant reduction cyclicity in endometritis cows compared to the control group.[96] Homeopathic treatments were found to significantly reduce the number of days to pregnancy[97] and result in fewer services per conception.[92]. Due to small sample sizes and study quality (high risk of bias), we cannot rule out the possibility that the effects of the homeopathic remedies were due to a placebo effect.

ANTISEPTICS: This class of medicines includes chlorhexidine, propylene glycol, Lugol's iodine, iodine, polyvinyl-pyrrolidon-iodine, formaldehyde and Betadine solution. Antiseptics have been used routinely in veterinary practice for many years to treat PUDs in dairy cows.[98,99] It has been hypothesized that the intrauterine infusion of irritating antiseptics triggers an acute inflammation, thereby transforming chronic endometritis into an acute condition (local irritation) by stimulating the uterine immune defense system.[100] Antiseptics also have an microbiocidal effect. Furthermore, a local condition like endometritis can be treated locally using an intrauterine infusion of antiseptic. Intrauterine infusions of 2% and 4% polycondensated m-cresol-sulphuric acid formaldehyde in dairy cows with endometritis between 22 and 28 DIM did not improve conception rate compared to prostaglandin treatment.[101,102] Similar results were obtained with an intrauterine infusion of 2% polyvinylpyrrolidone-iodine at 35 DIM (n = 531).[99] However, no detrimental effects were observed in cows without endometritis. Knutti et al.[103] infused 100 ml of Betadine into the uterus but there was no improvement in reproductive performance compared to intrauterine infusion of antibiotics or intramuscular prostaglandins.

The reduction in reproductive performance with intrauterine infusion of antiseptics may be associated with endometrial irritation and the resulting fibrosis. Another concern with the infusion of iodine derivatives into the uterus is the significant increase in milk iodine concentrations.104,[105] In conclusion, irritation of the endometrium by intrauterine infusion of antiseptics has a negative effect on uterine defense and reduces self-healing ability. Unfortunately, studies typically lack negative controls, have an insufficient number of animals per treatment group, and use a faulty experimental design that does not include randomization. Since subepithelial tissue (stroma) is more sensitive to irritation than is the epithelium and remains exposed before the end of the VWP, intrauterine infusion could cause a major inflammation with serious consequences.

Complementary and alternative therapies for preventing postpartum uterine diseases

METRITIS VACCINE: While research in the past decade on innate immunity has provided a better understanding of PUD etiopathology in dairy cows, the potential role of adaptive immunity in combatting PUDs is not yet perfectly understood. Furthermore, the development of a vaccine against metritis is still in the early stages of development.[106,107] The endometrium of cows houses a lymphocyte population composed of B-cells, T-cells and NK cells. Researchers have noted that compared to the population of CD4, CD8 and NK cells, the B-lymphocyte population in water buffaloes is widely distributed throughout the endometrium, the stroma, the luminal and glandular epithelium, and the myometrium. Four studies have used different vaccine formulations targeting *E. coli*, and *T. pyogenes* as major pathogens, both with or without virulence factors (FimH, leukotoxin, pyolosin), and Bacteroides and *Streptococcus uberis* as secondary bacteria.[108-110] Of the four studies, only one measured serological response based on blood levels of IgG specific to the antigens used in the vaccine formulation.[109] Inoculation of the vaccine was done with whole cells orally, intravaginally or subcutaneously in heifers or cows. Two of the four studies succeeded in reducing the incidence of metritis, but only one led to an improvement in reproductive efficiency.[109] In addition, milk production was increased in the first 30 DIM in multiparous cowsThe difference in efficacy among these studies may be related to the particular vaccine formulation used, and so more research is warranted to replicate the findings and determine the efficacy of metritis vaccines.

PEGBOVIGRASTIM: Another approach to improving the immune defense of cows in peripartum has been the use of pegbovigrastim. This compound is a recombinant bovine granulocyte colony-stimulating factor (G-CSF), a hematopoietic growth factor that stimulates the production and differentiation of neutrophils by the bone marrow.[111] Pegbovigrastim administered subcutaneously 7 days before expected calving date and 1 day after calving increased circulating neutrophil counts but did not decrease the incidence of PUDs[112], a result that led to pegbovigrastim being removed from the market.

RECOMBINANT IL-8: IL-8 is a pro-inflammatory chemokine that attracts and activates neutrophils in inflammatory tissues, stimulates their chemotaxis, and increases phagocytosis and killing ability (Mitchell et al. 2003). Intrauterine infusions of high and low doses of recombinant IL-8 within 12 h of calving in dairy cows decreased metritis incidence and increased milk production compared to control cows (Zinicola et al., 2019 a, b). If large studies confirm these preliminary results, recombinant IL-8 could be a promising treatment.

GENOMIC SELECTION: The best way to increase the efficiency of farms is to prevent disease. Genomic and genetic studies have attempted to validate genomic prediction analysis by taking advantage of the variation associated with the incidence of reported diseases between best and worst herds[115] and incorporating these data into selection programs. For a long time, practitioners have observed large differences in PUD incidence rate among herds. Tison et al.[33], showed 3 out of 17 herds had an incidence close to 0. Even though the genetic heritability for metritis (0.05) and endometritis (0.04) is actually very low, geneticists have used Bayesian statistical models and high-density single nucleotide polymorphisms (SNP) panels to estimate genomic predictive values of uterine health traits (PUDs) in Holstein cows in a more efficient way.[116] Given the increase in genomic testing, these tools may soon become a routine part of PUD prevention programs.

Conclusion

The efficacy of the intrauterine infusion of antibiotics for treating PUDs in dairy cows and mitigating the impact of PUDs on reproductive performance and productivity has been well documented. However, the current global context of antimicrobial resistance is now compelling veterinarians to substitute antibiotics with complementary and alternative treatments. A wide range of non-antimicrobial therapies to treat and prevent PUDs have been proposed, although most of these therapies are still in the early stage of testing.

Corresponding author:
Rejean Cleophas Lefebvre
Faculty of Veterinary Medicine
University of Montreal
3200 rue Sicotte,
Saint-Hyacinthe, Québec, Canada
J2S 2M2
Email: rejean.lefebvre@umontreal.ca

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