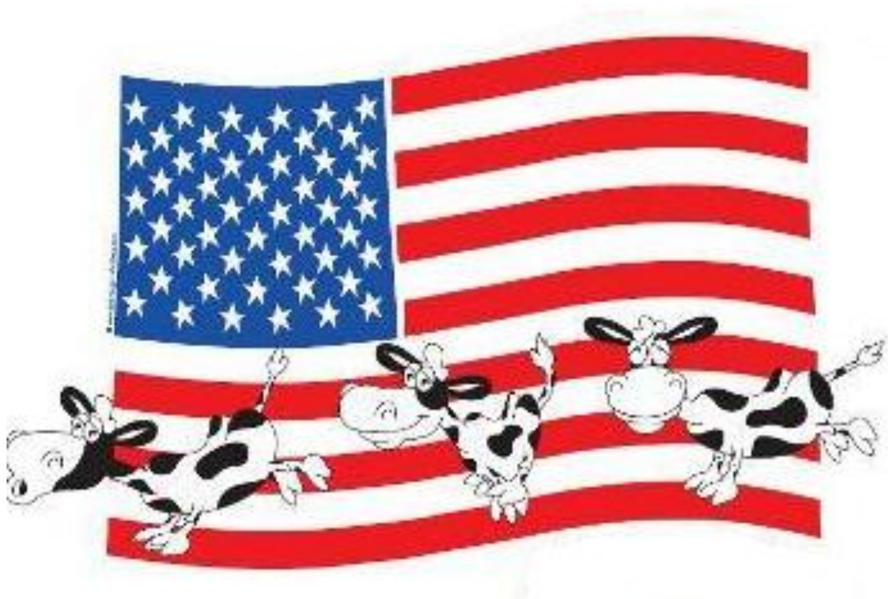
Effect of Intrauterine Dextrose Therapy on Clinical Recovery and Uterine Status in Lactating Dairy Cows with Clinical Endometritis



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Abstract

Post-partum uterine diseases such as clinical metritis and endometritis are common disorders of lactating dairy cows that diminish reproductive performance, thus diminishing profitability and sustainability of dairy operations. The objectives of the study were: 1) to assess the amount of fluid present in cows diagnosed with metritis at 7±3 DIM (experiment I) and 2) to assess the response (clinical cure 14 days post-therapy) of lactating dairy cows diagnosed with CE and treated with an intrauterine infusion of a hypertonic solution of 50% dextrose (DEX) as compared to untreated control cows (CON) (experiment II). A total of 228 Holstein cows from 1 commercial dairy operation were used in this observational study. The prevalence of clinical metritis was 20%. The presence of uterine fluid was confirmed in cows with and without clinical metritis via transrectal ultrasonography (experiment I). Only 2 cows (out of 14) diagnosed with clinical metritis presented extended uterus. Using a tubing line, the amount of fluid was attempted to measure by emptying the uterus, but due to the presence of necrotic tissue-debris, small amount of fluid was recovered.

Lactating dairy cows (n=158, experiment II) were screened for CE at 26±3 DIM using vaginoscopy technique (experiment II). The incidence of CE was 16.4% at Exam 1 (26±3 DIM). Cows with CE were stratified by parity (primiparous and multiparous) and randomly allocated in 1of 2 treatment groups: 1) Intrauterine infusion (~200 mL) of a 50% dextrose solution (DEX; n=10) or 2) Untreated control cows (CON; n=12). Fourteen days post-therapy (at 40±3 DIM), treated and control cows (DEX: n=10, CON: n=12) were re-examined to assess treatment responses. Cervix diameter was measured via transrectal ultrasonography at Exam 1 (26±3 DIM) and at Exam 2 (40±3 DIM). Cows that received DEX, had an increased proportion (66%) of reduced mean cervical diameter (<4 cm) and increased proportion of clinical cure (44.7%) compared to CON cows (P=0.12). The proportion (%) of cycling lactating dairy cows based on serum concentrations of progesterone (P4) and presence of ovarian structures were reported. The proportion (%) of ovarian structures (presence or absence of follicles, CL, or cysts) was recorded at exam 1 (26±3 DIM) and at exam 2 (40±3 DIM) in lactating dairy cows with CE. There was no significant difference between the DEX and CON cows at Exams 1 and 2. Based on these findings further research is needed on the use of dextrose alone or as an adjunct of antimicrobial therapy under different herd managements.

Introduction

Post-partum uterine diseases such as clinical metritis and endometritis are common disorders of lactating dairy cows that diminish reproductive performance, thus diminishing profitability and sustainability of dairy operations. Uterine function is often compromised by bacterial contamination of the reproductive tract during and after parturition, and recognized pathogenic bacteria present in the uterine environment cause the disease that leads to infertility in dairy cows (Sheldon *et al*., 2006; Dubuc *et al*., 2011). The primary goal of reproduction management in dairy cattle is to have cows become pregnant at a profitable time interval after calving. Losses due to uterine diseases are associated with reduced milk yields, subfertility and an increased culling risk or death of animals. Proactive management of the transition period (3 weeks prior to- and 3 weeks after calving) is the key to control the appearance of diseases such as clinical metritis or endometritis. Once the uterine conditions have been diagnosed (e.g., clinical metritis), the general therapeutic principles should control the bacterial infection and reduce the elevated body temperature to restore intake as soon as possible.

Therefore, the objectives of the study were: 1) to assess the amount of fluid present in cows diagnosed with metritis at 7±3 DIM (experiment I) and 2) to assess the clinical response (14 days post-therapy) in lactating dairy cows diagnosed with clinical endometritis (CE) following an intrauterine infusion of a hypertonic solution (50% dextrose) or untreated animals (experiment II). We hypothesize that treatment with intrauterine dextrose infusion would improve the clinical cure of CE dairy cows.

a. Uterine Involution Postpartum

Uterine involution is defined as reduction in the size of the reproduction tract (generally the uterus) after calving. The uterine involution process involves uterine contractions, physical shrinkage, necrosis of tissue, sloughing of the caruncles, and regeneration of the endometrium (Sheldon, 2004; Sheldon and Dobson, 2004). Uterine contractions continue for several days and decrease in regularity, frequency, strength and duration as the cow progresses through early days of lactation. Five days after parturition, the previously gravid horn is half of its size and the length of the horn is halved in 15 days. After this rapid involution phase, changes proceed more slowly, until the uterus reaches its pre-gravid state. Generally, the involution of the non-gravid

horn is more variable than the involution of the previously gravid horn, and this depends upon the degree of involvement in placentation.
Immediately after birth, the cervix constricts rapidly and it is almost impossible to insert a hand through it after 10-12 hours after parturition and it is only possible to insert two fingers through the cervix after 96 hours. The mean diameter of the cervix shrinks from 15 cm at 2 days postpartum to <5 cm at 60 days postpartum. Under normal conditions, expulsion of the uterine fluids and tissue-debris takes place (known as lochial discharge) within the first 7-10 days after parturition. The color of lochial discharge varies in color from yellowish brown to reddish brown as well as the volume varies from 2 L in pluriparae to ≤500 mL in primiparae. Under normal uterine involution process, the uterine discharge smells relatively fresh. Another event that occurs during uterine involution is the sloughing of the caruncles within 15 days post-partum and it is expulsed as part of the lochia. Complete re-epithelialization of the caruncles is completed around day 25. After all these uterine changes, the caruncles are getting smaller and smaller and they consist of small protrusions of 4-8 mm in diameter and 4-6 mm high at day 40-60 after parturition (Noakes *et al*., 2001). Factors that delay uterine involution such as dystocia, hypocalcaemia, retained placenta, metritis and endometritis (Sheldon, 2004) may cause subsequent infertility in dairy cows.

b. Uterine Contaminants

Bacterial contamination occurs in >90% of the dairy cows in the first two weeks after calving. During calving and immediately post-partum, the vulva is relaxed and the cervix is dilated, so bacteria can enter into the reproduction tract and contaminate the uterus. Bacterial contamination of the uterus postpartum is not consistently associated with uterine disease. To establish an infection, the bacteria need to adhere to the uterine mucosa and colonize or penetrate the endometrium. Infection of the postpartum uterus by recognized uterine pathogens depends on the immune status of the cow, bacteria, bacterial load and endocrine environment. It is known that progesterone suppresses uterine immune defenses. The pathogenic bacteria load may be high enough to overcome uterine defense mechanisms and can cause systemic illness, which can lead to uterine infection in dairy cattle associated with impaired reproductive performance (Sheldon *et al*., 2006). Bacterial contamination of the uterus can cause inflammation, histological lesions of the endometrium and myometrium and delays uterine involution. Most cows eliminate the bacteria within 5 weeks after parturition, but a proportion of cows (around 10%) will develop uterine disease such as metritis or CE (Williams *et al*., 2004).

In the early postpartum, a number of bacteria can be identified in the uterus. Recognized uterine pathogens such as *Escherichia coli, Arcanobacterium pyogenes, Prevotella melaninogenica, Fusobacterium necrophorum,* and *Proteus spp.*, potential uterine pathogens such as *Bacillus spp.* and *Pasteurella spp.*, and opportunist contaminants such as *Streptococcus spp., Providencia spp., Klebsiella spp.* and *Corynebacterium spp.* are transiently isolated from the uterus (Sheldon *et al*., 2002; Williams *et al*., 2007). The most common recognized uterine pathogens present in the uterus of dairy cattle are *E. coli and A. pyogenes* (Williams *et al*., 2005). *E. coli* is most frequently isolated from an early postpartum uterus and dominates the uterine flora in the first couple of days after parturition (Williams *et al*., 2007). *A. pyogenes* (associated with anaerobes such as *F. necrophorum* and *P. melaninogenicus*) is associated with purulent vaginal discharge and reduced reproductive performance (Sheldon *et al*., 2002; Williams *et al*., 2007; Galvão *et al*., 2009a).

c. *Defining Uterine Diseases, Diagnosis, and Treatments*

Metritis:

Puerperal metritis is defined as a bacterial infection of the uterus within 21 days postpartum that causes systemic illness. Puerperal metritis can be recognized by the following clinical signs: foul smelling red-brown watery uterine discharge and pyrexia ($\geq$39.5°C or $\geq$103°F). In severe cases other symptoms such as decreased milk production, dullness, reduced dry matter intake (anorexia), increased heart rate and dehydration could be observed (Sheldon *et al*., 2006). Most puerperal metritis (i.e., acute form) often requires antibiotics, pain and fluid (electrolytes) therapy to avoid severe dehydration or death (Noakes *et al.,* 2001).

Clinical metritis is defined as a delayed involution of the uterus with a foul smelling red-brown watery uterine discharge in the absence of pyrexia in the first 21 days after parturition. Clinical metritis has a negative impact on fertility and can be treated with antibiotic therapy, fluid therapy and pain management (e.g., flunixamine meglumine; Sheldon *et al*., 2006).

A recent randomized clinical study showed that the incidence of metritis was decreased when treating lactating dairy cows at high risk of developing metritis (cows with retained fetal membranes, twins, or dystocia) with a subcutaneous administration of ceftiofur crystalline free acid (CCFA) within 24 hours after parturition (Dubuc *et al*., 2011). It is important to note that CCFA is approved for respiratory disease in the U.S. In the Netherlands, CCFA is approved for acute post partum metritis ($\leq$10 days post partum). Dairy producers and veterinarians in the U.S. should be aware that the administration of CCFA is not approved for metritis or clinical endometritis in the U.S. Dairy producers and veterinarians in the Netherlands should be aware that CCFA can only be used on individual animals when bacterial research in combination with an antibiogram showed that the bacteria are not sensitive for other antibiotics than CCFA. (WVAB guideline, KNMvD).

Clinical Endometritis (CE):

CE is defined as a mucopurulent (>50% pus present in the exudate) or worse vaginal discharge and a cervical diameter >7.5 cm (transrectal palpation; measurement of the cervical diameter incl. cervical wall) or >4 cm (transrectal ultrasonography; measurement of the inner diameter of the cervix) within 21-40 days after parturition, without systemic signs of illness (e.g., fever) (Sheldon *et al*., 2006; Dubuc *et al*., 2010a). Histologically, cases of CE show a disruption of the epithelium with marked infiltration of inflammatory cells such as neutrophil cells (Dubuc *et al*., 2010a). The diagnosis of CE is based on clinical findings such as presence of mucopurulent (>50% pus) or worse vaginal discharge associated with increased cervical diameter within 21-40 days postpartum (Dubuc *et al*., 2010a). Transrectal palpation, vaginoscopy (single use speculum), gloved hand (single use palpation sleeve), metricheck® (metal rod devised with rubbed cup), ultrasonography (presence of fluid inside the uterus) and cytology (cytobrush technique) are used to diagnose CE in lactating dairy cows. CE is associated with postpartum infection of the uterus, primarily with *A. pyogenes* (LeBlanc *et al*., 2002); leading to lower conception to first service, increased intervals from calving to first service, and increased culling due to failure to conceive (Williams *et al*., 2008). The use of intrauterine cephapirin (Metricure®, Intervet International, Boxmeer, The Netherlands) is prescribed to treat cows with CE (McDougall, 2001; LeBlanc *et al*., 2002). Cows treated with intrauterine cephapirin had a shorter time to pregnancy compared to untreated cows (McDougall, 2001; Leblanc *et al*., 2002). The use of intrauterine ceftiofur hydrochloride (Galvão *et al*., 2009a) or prostaglandin $F_{2\alpha}$ (Dubuc *et al*., 2011a) has been unsuccessful for treatment of CE in lactating dairy cows.

d. Risk Factors for Uterine Diseases

Several risk factors such as reduced dry matter intake (due to poor nutrition management), difficult births (dystocia), twins at birth, stillbirths, abortion, retained fetal membranes, body condition score (BCS) at calving (≤2.75), increased hyperketonemic cows (i.e., Non Esterified Fatty Acids (NEFA), Beta-Hydroxybutyrate (BHBA) and haptoglobin in the first week postpartum), and hygiene of the perineum of cows at calving (Schuenemann *et al*., 2011) have been associated with metritis (Dubuc *et al*., 2010b). Risk factors such as twins at birth, dystocia, metritis and BCS at calving (≤2.75), hyperketonemia (≥1,100 µmol/L) and increased haptoglobin (≥0.8 g/L) in the first week after parturition have been associated with CE or cytological endometritis (>5% neutrophils, no purulent discharge, using the cytobrush technique for cell collection) (Dubuc *et al*., 2010b). The negative impact of metritis or CE is associated with reduced conception risk; increased days open and calving interval; reduced milk yield (Dubuc *et al*., 2011b); increased risk for displaced abomasums; increased risk for culling and mortality; and increased treatments and replacement costs.

e. Description of the Problem and Justification

Uterine diseases such as metritis and CE (Sheldon *et al*., 2006), are closely related with decreased reproductive (Galvão *et al*., 2009) and productive performance (Dubuc *et al*., 2011b). The primary goal of any reproduction management program for dairy herds should focus on getting cows pregnant in an efficient way and at a profitable time span after calving. Proactive management strategies should focus on preventing the risk factors for metritis or CE, especially pre- and post-partum and at calving as well as improve cow comfort and accuracy of diagnosis to minimize the negative impact of herd productivity. Even though preventive management of uterine diseases should be a top priority in any dairy herd, the treatment principles for clinical cases should focus on control bacterial infection (i.e., restore electrolytes), reduce fever, and restore dry matter intake.

Several studies have investigated the efficacy of prostaglandin $F_{2\alpha}$ administration (Heuwieser *et al*., 2000; Kasimanickam *et al*., 2005; Galvão *et al*., 2009b; Dubuc *et al*., 2011a) and antimicrobials such as intrauterine ceftiofur hydrochloride (Galvão *et al*., 2009a), ceftiofur crystalline free acid (CCFA; Dubuc *et al*., 2011a) and cephapirin (McDougall, 2001) for treatment of metritis in conventional dairy herds. The administration of intrauterine oxytetracycline is used as an off label therapy for the treatment of metritis, but its efficacy can depend on the amount of fluid present in the uterus (Sheldon, 2004). The minimal inhibitory concentration (MIC) of *E. coli* for oxytetracycline is 1µg/ml, so even if a cow has a large amount of fluid in the uterus (>10 liters), the concentration of oxytetracycline will still be higher than the MIC of *Escherichia coli* for oxytetracycline (Sheldon *et al* 2004). Judicious administration of antimicrobials in food animals is recommended due to resistance of bacteria to antimicrobials as well as the residues in meat or milk from cows that have been treated with antibiotics.

The development of effective alternative treatment options for conventional and certified organic dairy herds is warranted. The use of alternative therapies such as garlic tincture, aloe vera, vitamins, pH modifiers (e.g., vinegar), and vegetable oils has been reported by organic dairy producers (Pol and Ruegg, 2007; Arlt *et al*., 2009). Limited information is available in the literature to support the use of these strategies. Intrauterine administration of lysosubtilin, a broad-spectrum preparation of lytic enzymes naturally produced by *Bacillus subtilis,* has been showed to improve clinical recovery and reduce the calving-to-conception interval in cows diagnosed with CE (Biziulevichius and Lukauskas, 1998). An *in vitro* study showed that mannose, which is a sugar monomer, inhibited the adhesion of bacteria to equine endometrial cells (King *et al*., 2000). Similarly, the use of hypertonic sucrose solutions has been shown to inhibit growth of *E. coli* in human wounds (Cheriffe *et al*., 1983; Ambrose *et al*., 1991). Therefore, these findings suggested that a hypertonic solution of dextrose (50% dextrose in water) may provide a therapeutic option for the treatment of metritis and CE in conventional and certified organic dairy farms.

Materials and Methods

a. Animals and Facilities

A total of 228 Holstein cows from 1 commercial dairy operation were used for experiment I and II in this observational study. Individual cows were only used in one study (experiment I or experiment II). Holstein dairy cows were housed in free-stall barns bedded with sand. Cows were fed twice daily, in the morning and afternoon, with a total mixed ration (TMR) formulated to meet or exceed dietary nutritional requirements for dry dairy cows (NRC, 2001). Pregnant cows were located in the prepartum pen, which was located right next to the maternity pen. Cows at labor were placed in an individual maternity pen (approximately 20 m^2) padded with wheat straw bedding and constantly monitored until birth. This study was conducted from August to November, 2011.

b. Diagnosis of Clinical Metritis and Endometritis, Ovarian Structures, and Treatments

Experiment I: This field study investigated the amount of fluid in cows diagnosed with clinical metritis. Every week, a list of cows was obtained from the on-farm computer records (DairyComp 305, Valley Agricultural Software, Tulare, CA, USA) based on their days in milk (DIM). Cows at 7±3 DIM were sorted upon exiting the milking parlor and were placed in a palpation rail. Rectal body temperature (^{o}C; DeltaTRAK®, Pleasanton, CA, USA) was recorded and the uterus of cows was palpated via transrectal palpation. Clinical metritis was defined as vaginal discharge with foul smelling red-brown watery uterine discharges (by massaging the uterus) and rectal body temperature ≥39.5 ^{o}C. Cervix diameter (cm) and the presence or absence of fluid in the uterus were assessed by transrectal ultrasonography. Involution of the uterus was estimated by transrectal palpation according to its size as: 1) the entire reproductive tract (cervix and uterine horns) was palpated or 2) unable to reach the entire reproductive tract (deep uterus). We tried to measure the volume of fluid (e.g., in mL or liters) present in the uterus.

Experiment II: A list of cows was obtained every week from the on-farm computer records (DairyComp 305, Valley Agricultural Software, Tulare, CA, USA) based on their DIM. Cows at 26±3 DIM were sorted when exiting the milking parlor and were placed in a palpation rail for diagnosing CE. In the palpation rail, the uterus was massaged by transrectal palpation, the vulva was cleaned with paper towels and a single use vaginoscope was introduced through the vulva into the vagina. A light source (Mini-Maglite, Ontario, Canada) was used to visualize the vaginal and cervical mucosa and the discharge was scored. The vaginal discharge was scored from 0-3 (0 = normal clear uterine discharge, 1 = flakes of purulent exudate in the uterine discharge, 2 = >50% purulent material in the discharge, 3 = hemorrhagic uterine discharge mixed with purulent exudates (adapted from Williams *et al*., 2005; Sheldon *et al*., 2006). Figure 1 shows the different types of vaginal discharge. CE was defined as a score 2 or 3 at the first gynecological exam. The cervix diameter was measured via transrectal ultrasonography (Ibex pro®, EI medical, Colorado, USA). Ultrasonography was also used to assess the presence or absence of ovarian structures such as corpus luteum (CL), follicles (>3mm), or cysts. The presence of a cyst was defined as a follicle like structure >2.5 cm in diameter (Stevenson *et al*., 2004, Vanholder *et al*., 2006). BCS was also measured at the time of the first gynecological exam (DIM 26±3), using a 0-5 scale (Ferguson *et al*., 1994). Cows with CE were stratified by parity (primiparous and multiparous) and randomly allocated in 1of 2 treatment groups: 1) control group (CON; no placebo) and 2) administration of 200 mL of 50% dextrose (DEX; Vedco, Saint Joseph, MO, USA). Individually wrapped, single use infusion pipettes (Continental Plastic, Delavan, WI, USA) were used for intrauterine administration of DEX. Cows with CE were rechecked (Exam 2) 14 days after the first gynecological exam to assess the response to the treatment (clinical cure). A positive response to treatment was defined as a cow with CE at Exam 1 (26±3 DIM) which scored a 0 at Exam 2 (40±3DIM). Figure 2 shows the experimental design of both experiments in this study.

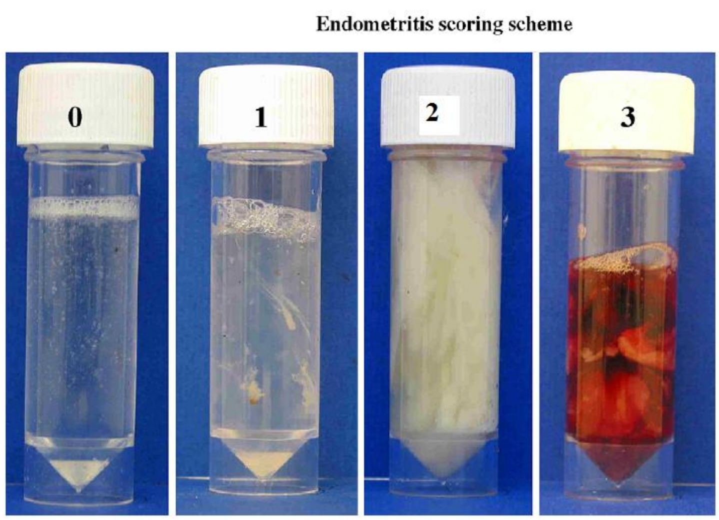

Figure 1: Typical samples of vaginal discharge.

Score 0 = normal clear uterine discharge, 1 = flakes of purulent exudate in the uterine discharge, 2 = >50% purulent material in the discharge, 3 = hemorrhagic uterine discharge mixed with purulent exudate.

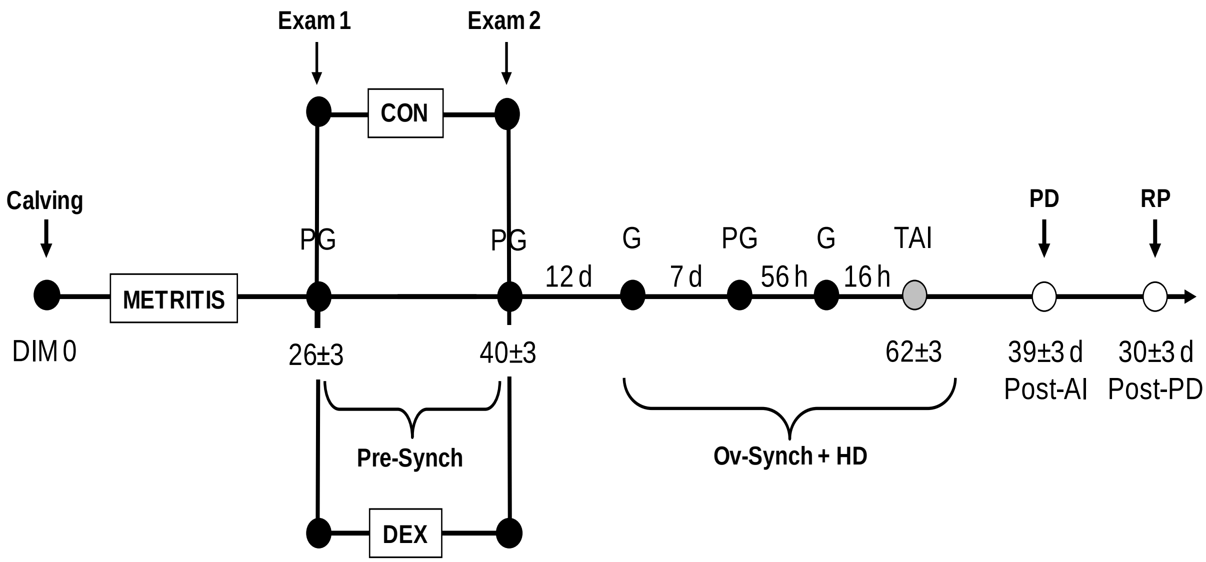

Figure 2. Scheme of the experimental study.

Experiment I: Periparturient Holstein cows were screened at 7±3 DIM for clinical metritis (n=70).

Experiment II: Periparturient Holstein cows were screened at 26±3 DIM for clinical endometritis (CE; n=158). Cows with CE (n=26) were randomly allocated to 1 of 2 treatment groups: 1) Intrauterine infusion (~200 mL) of a 50% dextrose solution (DEX; n=10) or 2) Untreated control cows (CON; n=12). All cows with CE were screened at exam 2 to assess the clinical response to treatments. All cows (experiment I and II) were subjected to the same reproductive program.

Legend: DIM: days in milk. PG: Prostaglandin F2α injection (Pre-Synch). G: GNRH injection (Ov-Synch). DEX: dextrose group; intrauterine infusion with 200 ml hypertonic (50%) dextrose. CON: control group; no placebo used. HD: Heat detection. TAI: Timed artificial insemination. PD: pregnancy diagnosis. RD: Recheck pregnancy diagnosis. .

c. Progesterone Radioimmunoassay

Blood samples (10 mL) were collected for determination of blood progesterone (P4) level at 26±3 and at 40±3 DIM by coccygeal venipuncture (BD Vacutainer®, Franklin Lakes, NJ, USA) to determine cyclicity status of cows (Stevenson *et al.*, 2006). Blood samples were centrifuged at 2,785 x g for 20 minutes immediately after collection and serum samples were stored at -20 ºC until assayed for P4 using a modified commercially available RIA (radioimmunoassay) kit (Coat-a-Count®, Diagnostic Products Corporation, Los Angeles, CA, USA) as described by Burke *et al*., 2003.

d. Statistical Analysis

Information from individual lactating dairy cows (e.g., lactation number, DIM, milk yield, service number, pregnancy status) were exported from DairyComp 305 to an Excel spreadsheet (Microsoft Corp., Redmond, WA, USA). The distribution of cows with and without CE with respect to milk yield (lbs) at the closest DHIA test(Dairy Herd Information Association; e.g. milk sampling for i.a. amount of milk, fat%, protein% per individual cow), mean cervix diameter (cm), and BCS (≤2.75 or ≥3) at Exam 1 were analyzed using MIXED procedure of SAS (Table 4; SAS, 2009). The response to treatment (clinical cure), and cycling status (presence of ovarian structures and P4) were assessed for cows with CE. Data pertaining to response to treatments (clinical cure at Exam 2; Table 2), and cycling status of cows measured by the serum concentration of P4 (<1 ng/mL or ≥1 ng/mL) and presence of ovarian structures (Yes or No) were dichotomized and analyzed using GLIMMIX procedure of SAS. Least square means and standard errors of the means (±SEM) were reported. A P value < 0.05 was considered statistically significant.

Results

a. *Diagnosis of Clinical Metritis and Endometritis*

Experiment I: A total of 70 lactating Holstein dairy cows were screened for clinical metritis (foul smelling red-brown watery discharge and rectal body temperature) at 7±3 DIM.
The prevalence of clinical metritis was 20%. The distribution of cows (with and without metritis) with respect to mean cervical diameter at the time of the gynecological exam (7±3 DIM) is reported in table 1. This group of cows was assessed to determine the amount of fluid in the uterus of cows with clinical metritis. The presence of uterine fluid was confirmed in cows with and without clinical metritis via transrectal ultrasonography. Only 2 cows (out of 14) diagnosed with clinical metritis presented extended uterus and we were unable to palpate the entire uterus to assess the amount of fluid. Using a tubing line, the amount of fluid was attempted to measure by emptying the uterus, but due to the presence of necrotic tissue-debris, small amount of fluid

was recovered. In the remaining cows with metritis, the uterus was successfully palpated and not much fluid was observed in the uterus via ultrasonography.

Experiment II: A total of 158 lactating Holstein dairy cows were screened for CE at 26±3 DIM, of which 26 cows were diagnosed with CE (scores 2 or 3). The prevalence of CE at the time of treatment was 16,4% (scores 2 and 3 combined; Table 2). The distribution of cows (with and without CE) with respect to milk yield at the closest DHIA test, body condition score (BCS) and mean cervical diameter is reported in table 4.

b. ***Effect of Dextrose on Uterine Diseases and Cycling Status (experiment II)***

Cows with CE (DEX and CON) were subjected to a second gynecological exam 14 days post-therapy (40±3 DIM) to assess the response to intrauterine dextrose (Table 3). Cows that received DEX, had increased proportion (66.02%) of reduced mean cervical diameter (<4 cm) and increased proportion of clinical cure (44.7%) compared to CON cows (P=0.12; Table 3). The proportion (%) of ovarian structures (presence or absence of follicles, CL, or cysts) was recorded at exam 1 (26±3 DIM) and at exam 2 (40± 3 DIM) in lactating dairy cows with CE. There was no significant difference between the DEX and CON cows at Exams 1-2 respectively (Table 5).

Discussion

The primary goal of reproduction management in dairy cattle is to have cows become pregnant at a profitable interval after calving. Losses due to uterine diseases are associated with reduced milk yields, subfertility and an increased culling risk or death of animals. The development of effective alternative treatment options for conventional and certified organic dairy herds is warranted. It is important to know the volume of fluid present in the uterus in cows with metritis, because the active compound or drug, like dextrose is diluted when there is a large amount of fluid in the uterus. The efficacy of intrauterine infusions with antibiotics is questionable, especially in cows with large amount of fluids into the uterus. Therefore, the uterine fluid should be removed to improve the clinical response of intrauterine infusions. On the other hand, when you calculate with the MIC of oxytetracycline for *E. coli*, even if the cow has more than 10 liters of fluid in the uterus, the concentration of oxytetracycline is still higher than the MIC (Sheldon *et al* 2004). However, it is questionable if the drug has diluted evenly throughout the fluid in the uterus, and it will be better for the cow when you remove the fluid from the uterus, because you remove i.a. toxins, bacteria and necrotic debris. In experiment I, we were not able to empty the uterus and measure the amount of fluid, because necrotic debris got stuck in the infusion line. Another possibility is to use an infusion line with a larger diameter (> 2 cm in diameter), but this can be a problem when the cervix is closed.

In the present study, during experiment II we investigated the efficacy of an intrauterine infusion of 50% dextrose solution to treat cows diagnosed with CE. The prevalence of CE at the time of treatment was 16.4%, which falls within the range of 13% – 50% cited in the literature (LeBlanc *et al*., 2002; Gilbert *et al*., 2005; Williams *et al*., 2005). Statistical analysis showed that cows that received DEX, had an increased proportion (66.0%) of cows with reduced mean cervical diameter (<4 cm) and an increased proportion of clinical cure (44.7%) compared to CON cows. Although the proportion of cured cows was large, we were unable to detect statistical significance, probably due to small sample size. Previous studies have shown the ability of sugar to aid in wound healing through inhibition of bacterial growth (Chirife *et al*., 1983; Callahan and Hortzmon, 1987; Archer *et al*., 1990; Sharon, 2006). Sugar has also shown to control bacterial infections with hypertonicity causing an osmotic draw of fluid (transudate) out of the affected area and effectively reducing the water activity that is required for bacteria to survive (Chirife *et al*., 1983). Transudation also may attract more leukocytes into the uterine lumen resulting in an increase of bacterial phagocytosis. Carbohydrates have shown to decreases bacterial production of proteases, leading to less tissue damage. Limiting absorption of bacterial toxins and promoting exudation is one of the principles behind the use of hypertonic glucose injections for the treatment of peritonitis (Narat, 1923). Furthermore, intrauterine dextrose may provide energy to the endometrial cells aiding in healing, but due to the high osmolarity it is not a good energy source for bacteria (Chirife *et al*., 1983; White, 1995). The osmotic draw of fluid out of the tissues may aid in tissue contraction (Kilic, 2001); and hence causing an increased uterine tone.

In experiment II, we also registered the ovarian structures during exam 1 and exam 2. In exam 1, the cows have less cysts than in exam 2, this can be the result of prostaglandin F2α (luteal cyst), GNRH (follicular cyst) or a combination of those two. Prostaglandin F2α can dissolve a luteal cyst and progesterone levels decrease as a result of the prostaglandin F2α.

Conclusions

Based on these preliminary findings, CE cows treated with an intrauterine infusion of 50% dextrose had improved clinical cure 14 days post-therapy compared to untreated CON cows. The efficacy of intrauterine dextrose (alone of as an adjunct of antimicrobial) to treat lactating dairy cows diagnosed with CE needs further investigation under different herds and reproductive managements.

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References

Archer, H.G., S. Barnett, S. Irving, K.R. Middleton, and D.V. Seal. 1990. A controlled model of moist wound healing: comparison between semi-permeable film, antiseptics and sugar paste. J. Exp. Pathol. (Oxford) 71:155-170.

Ambrose, U., K. Middleton, and D. Seal. 1991. In vitro studies of water activity and bacterial growth inhibition of sucrose-polyethylene glycol 400-hydrogen peroxide and xylose-polyethylene glycol 400-hydrogen peroxide pastes used to treat infected wounds. Antimicrob. Agents Chemother. 35:1799-1803.

Arlt, S., W. Padberg, M. Drillich, and W. Heuwieser. 2009. Efficacy of homeopathic remedies as prophylaxis of bovine endometritis. J. Dairy Sci. 92:4945-4953.

Biziulevichius, G.A., and K. Lukauskas. 1998. In vivo studies on lysosubtilin. 2. Efficacy for treatment of post-partum endometritis in cows. Vet. Res. 29:47-58.

Burke, C.R., M.L. Mussard, C.L. Gasser, D.E. Grum, and M.L. Day. 2003. Estradiol benzoate delays new follicular wave emergence in a dose-dependent manner after ablation of the dominant ovarian follicle in cattle. Theriogenology 60:647-658.

Callahan, C.J., and L.A. Horztmon. 1987. Treatment of early postpartum metritis in a dairy herd: Response and subsequent fertility. Bovine Prac. 22:124-128.

Chirife, J., L. Herszage, A. Joseph, and E.S. Kohn. 1983. In vitro study of bacterial growth inhibition in concentrated sugar solutions: microbiological basis for the use of sugar in treating infected wounds. Antimicrob. Agents Chemother. 23:766-773.

Dubuc, J., T.F. Duffield, K.E. Leslie, J.S. Walton, and S.J. LeBlanc. 2010a. Definitions and diagnosis of postpartum endometritis in dairy cows. J. Dairy Sci. 93:5225-5233.

Dubuc, J., T.F. Duffield, K.E. Leslie, J.S. Walton, and S.J. LeBlanc. 2010b. Risk factors for postpartum uterine diseases in dairy cows. J. Dairy Sci. 93:5764-5771.

Dubuc, J., T.F. Duffield, K.E. Leslie, J.S. Walton, and S.J. LeBlanc. 2011a. Randomized clinical trial of antibiotic and prostaglandin treatments for uterine health and reproductive performance in dairy cows. J. Dairy Sci. 94:1325-1338.

Dubuc, J., T.F. Duffield, K.E. Leslie, J.S. Walton, and S.J. LeBlanc. 2011b. Effects of postpartum uterine diseases on milk production and culling in dairy cows. J. Dairy Sci. 94:1339-1346.

Ferguson, J.D., D.T. Galligan, and N. Thomsen. 1994. Principal descriptors of body condition in Holstein dairy cattle. J. Dairy Sci. 77:2695-2703.

Galvão, K.N., L.F. Greco, J.M. Vilela, M.F. Sá Filho, and J.E. Santos. 2009a. Effect of intrauterine infusion of ceftiofur on uterine health and fertility in dairy cows. J. Dairy Sci. 92:1532-1542.

Galvão, K.N., M. Frajblat, S.B. Brittin, W.R. Butler, C.L. Guard, and R.O. Gilbert. 2009b. Effect of prostaglandin F2alpha on subclinical endometritis and fertility in dairy cows. J. Dairy Sci. 92:4906-4913.

Gilbert, R.O., S.T. Shin, C.L. Guard, H.N. Erb, and M. Frajblat. 2005. Prevalence of endometritis and its effects on reproductive performance of dairy cows. Theriogenology 64:1879-1888.

Heuwieser, W., B.A. Tenhagen, M. Tischer, J. Lühr, and H. Blum. 2000. Effect of three programs for the treatment of endometritis on the reproductive performance of a dairy herd. Vet. Rec. 146:338-341.

Kasimanickam, R., T.F. Duffield, R.A. Foster, C.J. Gartley, K.E. Leslie, J.S. Walton, and W.H. Johnson. 2005. A comparison of the cytobrush and uterine lavage techniques to evaluate endometrial cytology in clinically normal postpartum dairy cows. Can. Vet. J. 46:255-259.

Kilic, A. 2001. Healing of diabetic ulcers with granulated sugar. Plast. Reconstr. Surg. 108:585.

King, S.S., D.A. Young, L.G. Nequin, and E.M. Carnevale. 2000. Use of specific sugars to inhibit bacterial adherence to equine endometrium in vitro. Am. J. Vet. Res. 61:446-449.

LeBlanc, S.J., T.F. Duffield, K.E. Leslie, K.G. Bateman, G.P. Keefe, J.S. Walton, and W.H. Johnson. 2002. Defining and diagnosing postpartum clinical endometritis and its impact on reproductive performance in dairy cows. J. Dairy Sci. 85:2223-2236.

Narat, J.K. 1923. Experimental study upon the use of intra-abdominal injections of hypertonic glucose solution in the treatment of peritonitis. Ann. Surg. 78:357-363.

Noakes, D.E., T.J. Parkinson, and G.C.W. England. 2009. Veterinary reproduction and obstetrics. Chapter 7: The puerperium. 9th ed., Saunders.

NRC. 2001, Nutrient Requirements of Dairy Cattle. 7th rev. ed. National Academy Press, Washington, DC.

Pol, M., and P.L. Ruegg. 2007. Treatment practices and quantification of antimicrobial drug usage in conventional and organic dairy farms in Wisconsin. J. Dairy Sci. 90:249-261.

SAS Institute Inc. 2009. SAS/STAT 9.2 User’s Guide. 2nd ed. SAS Institute Inc., Cary, NC, USA.

Schuenemann, G.M., I. Nieto, S. Bas, K.N. Galvão, and J. Workman. II. Dairy calving management: Effect of perineal hygiene scores on metritis. J. Dairy Sci. 94 (E-Suppl. 1):744 (Abstr.).

Sharon, N. 2006. Carbohydrates as future anti-adhesion drugs for infectious diseases. Biochim. Biophys. Acta 1760:527-537.

Sheldon, I.M. The postpartum uterus. 2004. Vet. Clin. Food Anim. 20:569-591.

Sheldon, I.M., and H. Dobson. 2004. Postpartum uterine health in cattle. Anim. Reprod. Sci. 82-83:295-306.

Sheldon, I.M., D.E. Noakes, A.N. Rycroft, D.U. Pfeiffer, and H. Dobson. 2002. Influence of uterine bacterial contamination after parturition on ovarian dominant follicle selection and follicle growth and function in cattle. Reproduction 123:837-845.

Sheldon, I.M., M. Bushnell, J. Montgomery, A.N. Rycroft. 2004. Minimum inhibitory concentrations of some antimicrobial drugs against bacteria causing uterine infections in cattle. Veterinary Record 155: 383-387.

Sheldon, I.M., G.S. Lewis, S. LeBlanc, and R.O. Gilbert. 2006. Defining postpartum uterine disease in cattle. Theriogenology 65:1516-1530

Smit, W., P. van Dijk, M.J. Langedijk, N. Schouten, N. van den Berg, D.G. Struijk, and R.T. Krediet. 2003. Peritoneal function and assessment of reference values using a 3.86% glucose solution. Perit. Dial. Int. 23:440-449.

Stevenson, J.S., and S.M. Tiffany. 2004. Resychronizing estrus and ovulation after not-pregnant diagnosis and various ovarian states including cysts. J. Dairy Sci. 87:3658-3664.

Vanholder, T., G. Opsomer, and A. De Kruif. 2006. Aetiology and pathogenesis of cystic ovarian follicles in dairy cattle: a review. Reprod. Nutr. Dev. 46:105-119.

Wenz, J.R., D.A. Moore, and R. Kasimanickam. 2011. Factors associated with the rectal temperature of Holstein dairy cows during the first 10 days in milk. J. Dairy Sci. 94:1864-1872.

White, G.W. 1995. Maltodextrin NF powder: A new concept in equine wound healing. J. Equine Vet. Sci. 15:296-298.

Williams, E.J., S. Herath, G.C.W. England, H. Dobson, C.E. Bryant and I.M. Sheldon. 2008. Effect of *Escherichia coli* infection of the bovine uterus from the whole animal to the cell. The Animal Consortium. Doi:10.1017/S1751731108002413.

Williams, E.J., D.P. Fischer, D.E. Noakes, G.C.W. England, A. Rycroft, H. Dobson, and I.M. Sheldon. 2007. The relationship between uterine pathogen growth density and ovarian function in the postpartum dairy cow. Theriogenology 68:549-559.

Williams, E.J., D.P. Fischer, D.U. Pfeiffer, G.C.W England, D.E. Noakes, H. Dobson, and I.M. Sheldon. 2005. Clinical evaluation of postpartum vaginal mucus reflects uterine bacterial infection and the immune response in cattle. Theriogenology 63:102-117.

WVAB guideline: Werkgroep Veterinair Antibioticabeleid, Royal Dutch Society of Veterinary Medicine (KNMvD), the Netherlands. (http://wvab.knmvd.nl/wvab).

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Table 1. Prevalence (%) of clinical metritis at 7±3 DIM of lactating Holstein cows (n=70) using vaginal discharge technique and rectal body temperature.

Parameters	Exam 1 (%)	
Clinical vaginal discharge		
0 (mucus without smell)	80	
1 (brown-red, foul smell, pyrexia)	20	
Cervical diameter (cm)	Metritis (%)	Non Metritis (%)
<4	0	0
4.0-5.0	21.1	16.6
≥5.1	78.9	83.3

Table 2. Prevalence (%) of clinical endometritis (CE) at Exam 1 (26±3 DIM) of lactating Holstein cows (n=158) using vaginoscopy scoring technique and measurement of cervical diameter by ultrasonography

Parameters	**Exam 1 (%)**
Clinical vaginal discharge	
0 (clear mucus)	71.3
1 (mucus with flecks of pus)	13.5
2 (mucopurulent)	10.1
3 (brown-red foul)	5.2
Cervical diameter (cm)	
<4	32.5
4.0-5.0	37.3
≥5.1	17.2

Table 3. Prevalence (%) of clinical endometritis (CE) at Exam 2 (40±3 DIM) in lactating Holstein cows using vaginoscopy scoring technique and measurement of cervical diameter by ultrasonography

	Exam 2(*)		
Parameters	**CON (%)**	**DEX(%)**	***P*-value**
Clinical uterine discharge			
0 (clear mucus)	24.5	44.7	0.12
1 (mucus with flecks of pus)	34.0	27.7	
2 (mucopurulent)	41.6	27.6	
3 (brown-red foul)	0	0	
Cervical diameter (cm)			
<4	62.5	66.0	
4.0-5.0	31.3	29.1	
≥5.1	6.3	4.9	

(*)Cows diagnosed with CE (at Exam 1) were screened 14 d post-therapy (at Exam 2) to determine the response to treatments based on vaginoscopy technique (Figure 1). A positive response to treatments (clinical cure) was defined a cow with CE at Exam 1 (26±3 DIM) that scored 0 (clear mucus) at Exam 2 (40±3 DIM).

The proportion of cows that cured after intrauterine DEX (44.7%) was ~20 percentage points greater than untreated CON (24.5%) cows..

Table 4. Distribution of lactating Holstein cows with and without clinical endometritis (CE) with respect to milk yield (lbs), body condition scores (BCS) and mean cervix diameter (cm).

Exam 1

	Cows with CE[1]		**Cows without CE**	***P*-value**
Parameters	**CON**	**DEX**		
Milk yield (lbs)	75	82	90	0.33
BCS (%)				
≤2.75	71.4	72.7	56.8	0.49
≥3.0	28.6	27.3	43.2	0.49
Mean cervix diameter (cm)	4.9	4.5	4.0	0.3

[1]*Lactating dairy cows (n=158) were screened for CE at 26±3 DIM using vaginoscopy technique. Cows with CE were randomly assigned to 1 of 2 treatment groups: 1) Intrauterine infusion (~200 mL) of a 50% dextrose solution (DEX; n=10) or 2) Untreated control cows (CON; n=12). Information from cows without CE (n=132) were included in the analysis.*

Table 5. Proportion (%) of ovarian structures (presence of follicles, CL, or cysts) and cycling status based on serum concentration of progesterone (P4) in lactating Holstein cows diagnosed with clinical endometritis (CE)

Parameters	Cows with CE		
	CON	DEX	*P*-value
Cycling cows (%)[1]	53.2 ± 7.2	55.8 ± 7.5	0.81
Ovarian structures at 26±3 DIM[2]			
Follicles (%)	81.0	72.7	0.76
CL (%)	53.3	52.3	0.95
Cysts (%)	22.0	33.3	0.41
Ovarian structures at 40±3 DIM[3]			
Follicles (%)	91.0	88.0	0.87
CL (%)	80.8	64.1	0.34
Cysts (%)	14.9	23.1	0.71

[1]*The proportion (%) of cycling lactating dairy cows based on serum concentrations of progesterone (P4) were reported. Blood samples were collected from cows diagnosed with CE at Exam 1 (26±3 DIM) and Exam 2 (at 40±3 DIM). Cows were classified as cycling when concentrations of P4 from 1 of 2 blood samples were ≥1 ng/mL (High P4; High-High, Low-High, or High-Low). Cows were classified as non-cycling when serum concentrations of P4 from both blood samples were <1 ng/mL (Low P4; Low-Low).*

[2]*The proportion (%) of ovarian structures (presence or absence of follicles, CL, or cysts) was recorded at 26±3 DIM in lactating dairy cows with CE.*

[3]*The proportion (%) of ovarian structures (presence or absence of follicles, CL, or cysts) was recorded at 40±3 DIM in lactating dairy cows with CE.*