

**Impact of Intrauterine Dextrose Therapy on Reproductive Performance of
Lactating Dairy Cows Diagnosed with Clinical Endometritis Following a
Randomized Clinical Trial**

THESIS

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By

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ABSTRACT

The objective of this study was to determine if lactating dairy cows diagnosed with clinical endometritis (CE) treated with an intrauterine infusion of 50% dextrose in water (DEX) would have a similar percentage of pregnancy per AI (PAI) when compared to cows treated with parenteral ceftiofur crystalline free acid (CEF). Cows (n=760) from 2 herds were screened for CE using vaginoscopy at 26 ± 3 days in milk (DIM) to score the uterine discharge using a 0-3 scale. Cows with uterine discharge scores of 2 or 3 were stratified by parity and randomly allocated into 1 of 3 treatment groups: 1) CON (n=83), 2) single dose of subcutaneous 6.6 mg/kg CEF (n=75), or 3) intrauterine infusion (200 mL) of DEX (n=79). Fourteen days post-therapy (at 40 ± 3 DIM), treated cows (n=146) were re-examined to assess treatment responses. All cows were pre-synchronized with two injections of $\text{PGF}_{2\alpha}$ given 14 d apart (starting at 26 ± 3 DIM) followed by Ovsynch (OV; GnRH-7 d-PGF-56 h-GnRH 16 h-timed-AI; TAI) 12 days later. Cows displaying standing estrus any time during the protocol were artificially inseminated (AI), while the remaining cows were subjected to TAI-16 h after second GnRH of OV. Body condition scores (BCS) were recorded at calving, 26 ± 3 DIM and 40 ± 3 DIM. Pregnancy diagnosis was performed via transrectal ultrasonography at 39 ± 3 days post-AI. DIM to first service (DIMFS) and percentage of pregnancy per AI (PAI) were statistically analyzed. DIMFS, milk yield at first service, BCS at treatment, rectal temperature at treatment were not different among the treatment groups. Mortality within 10 days post-treatment and culling rate at 250 DIM were not different for cows with or without CE. Cows with CE had greater cervical diameters at the time of treatment compared to cows without CE.

Mean uterine discharge scores were reduced for DEX cows compared to CON and CEF cows ($P=0.02$). First service PAI in DEX ($29.8\pm4\%$) tended to differ from cows in CON ($21.1\pm4\%$) and CEF groups ($19.7\pm4\%$; $P=0.1$). To the contrary, PAI in DEX cows was not different from cows without CE ($39.1\pm2\%$). Based on these findings, the use of intrauterine DEX alone or as an adjunct of antibiotic therapy for the treatment of cows diagnosed with CE needs further investigation.

This work is dedicated to my wife, Liz, without whom I would have never entered the world of veterinary medicine. Thanks to the understanding of Liz and our children, Avery and Kenyon, I was able to endure the last three years.

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CHAPTER 1: INTRODUCTION

Post-partum uterine diseases such as metritis and endometritis are common disorders of lactating dairy cows that negatively impact reproductive performance, thus diminishing profitability and sustainability of dairy operations. Metritis (puerperal; usually within 14 days postpartum) is the inflammation of all layers of the uterus and characterized by fetid red-brown uterine discharge and systemically ill cows (e.g., fever, decreased milk yield and dry matter intake).¹ Clinical endometritis (CE; ≥ 26 days in milk) is the inflammation of the endometrial lining of the uterus characterized (clinically or cytologically) by mucopurulent uterine discharge without systemic signs of illness.¹ Reported risk factors such as calving pen environment^{2,3}, peripartum metabolic status⁴⁻⁷, parity and season^{9,2}, retained fetal membranes⁸⁻¹⁰, twinning⁵, and dystocia^{5,8} have all been associated with metritis and clinical endometritis in dairy cattle. CE contributes to ovarian dysfunction (e.g., smaller follicle size, lower plasma estradiol and prolonged luteal phase)⁹, poor reproductive performance^{9,2}, increased risk of culling due to reproductive failure^{10,11}, and reduced milk yield.⁴

Recent studies have shown that up to 50% of lactating dairy cows from conventional herds suffer from CE at 40-60 days in milk (DIM).¹² Administration of prostaglandin analogues¹³⁻¹⁵ and antimicrobial agents such as cephalixin¹¹ or oxytetracycline¹⁶ are frequently used to treat cows with postpartum uterine infections in conventional dairy herds. However, none of the above treatment strategies can be used in certified organic dairies. The use of alternative therapies such as garlic tincture, aloe vera, vitamins, homeopathy, and vegetable oils have been reported by organic dairy

producers.^{19,20} A study performed Eastern Europe compared the use of a lytic enzyme preparations, derived from *Bacillus subtilis*, to commonly used antibiotic treatments containing chloramphenicol, nitrofurazone, polymyxin, sulphanilamide (uterosan) and neomycin and furazolidone (neofur).²¹ The lack of convincing clinical response (with these products) makes it difficult to promote an effective therapy for certified organic dairies. An *in vitro* study showed that mannose (a sugar monomer) inhibited the adhesion of bacteria to the epithelial cells of the equine endometrium.²² This suggests that the use of non-pharmaceutical antimicrobial therapy such as a 50% dextrose (a hypertonic solution), may be a viable and effective strategy for conventional and certified organic dairy cows diagnosed with CE.

The objective of this study was to assess first service pregnancy per AI (PAI) in lactating dairy cows diagnosed with CE treated by an intrauterine infusion of a hypertonic solution (50% dextrose), parenteral ceftiofur crystalline free acid, or untreated animals. We hypothesize that CE will be detrimental to PAI, but treatment with ceftiofur crystalline free acid or intrauterine dextrose infusion will reduce the incidence of CE while improving PAI in lactating dairy cows.

CHAPTER 2: LITERATURE REVIEW

2.1 DEFINING UTERINE DISEASES AND DIAGNOSIS

Metritis and endometritis are terms that are often used interchangeably but they refer to two different uterine conditions. Timing and characteristic of the uterine discharge are key components when diagnosing uterine disease in postpartum dairy cattle.

Metritis

Metritis is defined as a uterine inflammation affecting all layers of the uterine lining (endometrium, mucosa, submucosa, and serosa) that occur within the first two weeks postpartum.⁹ A uterus that is affected by metritis is enlarged, containing a thick, tenacious malodorous fluid that is reddish-brown to off-white in color.^{9,23} A cow with metritis also exhibits the signs of systemic illness including: increased rectal temperature, anorexia, lethargy, increased heart rate, and decreased milk production.^{7,23,24} Various methods have been utilized to detect and diagnose uterine infections including vaginoscopy for identification and classification of uterine discharge^{10,25}, palpation to evaluate cervical diameter and detect fluid within the uterus^{10,26}, uterine biopsy, cytology of uterine fluid or modified cytobrush techniques to collect and classify endometrial cells.^{12,27}

Clinical Endometritis (CE)

Postpartum CE is defined as inflammation of the endometrial lining of the uterus without an association to systemic illness.^{23,28} An increased rectal temperature may or

may not be present depending upon the extent of the inflammatory process and stress level of the cow at the time of the examination. CE is characterized by mucopurulent and purulent vaginal discharge around 20 to 40 days postpartum.⁷ CE has been defined as the presence of mucopurulent uterine discharge (>50 % purulent material) after 26 days in milk or a cervical diameter greater than 7.5 cm (estimated by rectal palpation) at 20 DIM.^{7,9,23} Involution of the uterus begins immediately post-partum and the diameter of the uterine horns should be less than 3-4 cm by 25-30 DIM and the cervical diameter should measure <5 cm by 40 days in milk.^{23,29} The diagnosis of CE relies on appropriate visual characterization of the uterine discharges noted at 21 days or more after parturition.^{9,10,23} Vaginoscopy is a practical method for the diagnosis of CE (uterine discharge).^{10,25,30} The uterine discharges is scored using a 0-3 scale (0 = normal uterine discharge, 1 = flakes of purulent exudate in the uterine discharge, 2 = <50% of the uterine discharge is made up of purulent exudate, 3 = uterine discharge mixed with >50% purulent exudate.^{23,31} Palpation of the uterus per rectum to estimate the uterine diameter and the presence of intraluminal fluid is commonly the sole method used for diagnosing CE. Palpation per rectum combined with vaginoscopy results in a more accurate method of diagnosis than palpation alone.^{7,10,30} Additionally, the use of endometrial cytology is a valuable alternative for the diagnosis of CE.^{12,91} A study using endometrial cytology samples at 40-60 DIM reported the prevalence of CE in dairy cows at 53%.¹²

Subclinical Endometritis (SCE)

SCE is defined as uterine inflammation (without visible signs of vaginal discharge) characterized by high proportion (>5%) of polymorphonuclear cells (PMN's) diagnosed through endometrial cytology around 40 to 60 days postpartum.^{9,32} Greater than 5.5-10% PMN's recovered in an endometrial sample 5 weeks postpartum is a positive sign of subclinical endometritis.³² Others researchers have defined SCE as greater than 18% PMN's on a cytological preparation of uterine fluid or based on the ultrasonic diagnosis of fluid within the uterus at 20-30 days postpartum.^{12,33} A recent study has defined subclinical endometritis as greater than 18% PMN's present in a sample that is collected at 21-33 postpartum or greater than 10% PMN's at 34 to 47 days postpartum.²³ It is agreed upon by most researchers that the presence of neutrophils within the lumen of the uterus is indicative of an inflammatory process. Inflammation is considered significant if the ratio of neutrophils to epithelial cells is greater than 1:10 as determined by uterine cytology.^{3,10} The only time that a normal uterus contains neutrophils is between days 2–8 of the estrous cycle.³⁴ A recent study used a cutoff point of >8% PMN's to diagnose SCE. The 8% cutoff was determined by using survival analysis to find the lowest percentage of PMN's associated with time to pregnancy at 150 days postpartum.²⁸ Additionally, a cutoff point of >5% neutrophils to diagnose SCE in cows 40–60 days postpartum was proposed.^{12,27,35} It appears that the most common method for the diagnosis of SCE is the percentage of PMN's found on uterine cytology, although endometrial biopsy appears to be the better method to diagnose SCE,^{12,36} having a high sensitivity (92%) and specificity (77%).³⁶⁻³⁸ Even though uterine biopsy appears to

be the better method to diagnose uterine diseases, especially for SCE, the biopsy procedure itself may cause damage and inflammation to the uterine lining leading to a delay in conception.^{12,39} A low volume uterine flush with sterile saline has been utilized for the recovery and subsequent cytological analysis of the uterine fluid to determine the presence of subclinical endometritis.¹² The modified cytobrush technique has also been described for the cytological diagnosis of SCE.²⁷ Results from the cytobrush study provided evidence that uterine cytobrush samples highly correlated with SCE. Uterine cytobrush yielded results similar to samples obtained by uterine lavage.²⁷ Uterine lavage (flushing) may provide a more representative sample (sample is obtained from a larger uterine surface) for diagnosis SCE than uterine samples obtained through uterine biopsy or swabs.^{27,37,38}

2.2 UTERINE INVOLUTION POSTPARTUM

The postpartum uterus is a prime environment for the propagation of environmental bacteria. During normal parturition, the uterus becomes infected with bacteria. These bacterial contaminants are normally cleared within the first five weeks after parturition.^{25,31} Contamination of the postpartum uterus is expected in most dairy cows within the first weeks after parturition^{31,40-42} and during uterine involution.^{23, 25} One study found that all cows suffer from inflammation of the uterus during involution.¹² This inflammatory process, caused by bacterial contamination and trauma that occurs during parturition, is normally cleared within 14–28 days postpartum.⁷⁷ Mild mucopurulent discharge during the first month postpartum is normal and likely reflects a successful

immune response.^{7,10,11} The presence of bacteria and inflammation within the uterus leads to activation of the innate immune response, causing the release of proinflammatory cytokines.^{31,43,44} These proinflammatory cytokines stimulate the release and activation of acute phase proteins.⁴⁴ Acute phase proteins are present to limit tissue damage and promote tissue repair.^{31,43,44} A study evaluating endometritis and its effect on reproductive performance provides evidence that 90% of cows have microorganisms in the uterus at 1-2 weeks postpartum. Furthermore, the presence of uterine microorganisms at 2-4 weeks decreased progressively until 6-8 weeks postpartum.²⁵ These results suggest that postpartum bacterial contamination is common in cattle and also provided evidence that the normal postpartum uterine defense mechanisms gradually decreased the bacterial load as the cows' progress through lactation. This normal postpartum uterine infection can be exacerbated in cases of dystocia with the introduction of contaminants into the uterus during the obstetrical procedure.^{5,45} Postpartum uterine infections occur when the inflammation is too severe and the normal uterine defenses are overwhelmed.

2.3 DESCRIPTION OF UTERINE CONTAMINANTS

Between 80–100% of postpartum cows have a bacterial infection of the uterus within the first 2 weeks after calving.^{25,34} Uterine involution is considered a septic process with most uterine bacteria cleared within the first 2–3 week postpartum with no detrimental effects on future fertility.^{25,34,46} The bacteria normally involved in the postpartum infections include *Arcanobacterium pyogenes* (*A. pyogenes*), *Pseudomonas spp.*, *Streptococcus spp.*, *Staphylococcus spp.*, *Pasteurella multocida*, *Clostridium spp.*, *Fusobacterium*

necrophorum, *Bacteroides*, and *Escherichia coli* (*E. coli*).^{9,31,40,41,47} *E. coli* and *A. pyogenes* are the most prevalent bacterial isolates from cows with postpartum uterine disease.⁴⁰⁻⁴² It is accepted that *E. coli* is the most common uterine pathogen isolated from the infected uterus.^{31,48} *A. pyogenes* has the greatest negative impact on bovine fertility but is rarely isolated from the postpartum uterus as a lone pathogen.^{10,34,48} Intrauterine infection with *E. coli* during the early postpartum period has been linked to negative effects on the ovary. The lipopolysaccharide of the *E. coli* outer cell membrane has a disruptive effect on the hypothalamic-pituitary axis which controls the ovarian cycle.^{42,49} The most common uterine pathogens and their relationship to the uterine discharge have been described as follows: *A. pyogenes*, *Proteus spp.*, and *Fusobacterium necrophorum* are associated with purulent or mucopurulent uterine and vaginal discharges while *E. coli*, *A. pyogenes*, non-hemolytic *Streptococci*, and *Mannheimia haemolytica* are more likely to yield a uterine discharge that is associated with a fetid odor.³¹

2.4 RISK FACTORS ASSOCIATED WITH UTERINE DISEASES

There are numerous factors predisposing a postpartum cow to be afflicted with endometritis. A clean calving environment⁵, for example, is very important in limiting the presence of pathogenic bacteria that would not otherwise cause periparturient uterine disease. Anything that places undue stress on the cow can have detrimental consequences on the immune system. Stressors may be as simple as increased traffic, increased noise, or movement away from her normal environment. Metabolic disorders such as periparturient ketosis or hypocalcemia can predispose a cow to clinical endometritis,

especially if she becomes recumbent.⁵⁰⁻⁵² About 5–10% of heifers and cows that calve are also at risk for retention of fetal membranes, a condition shown to increase the risk of endometritis.⁹² A relationship between low body condition score at calving and increased incidence of uterine infection has been reported.^{49,53} Resumption of cyclicity during the postpartum period also plays a role in clinical endometritis. The risk of contracting CE is increased by a factor of 5.4 in postpartum cows that have inactive ovaries.^{15,54} One study suggests older cows, those past their third lactation, are at the greatest risk for developing clinical endometritis¹⁰ while other authors have reported that parity does not have an effect on the incidence of CE.^{9,12,55}

2.5 PREVALENCE OF ENDOMETRITIS

The incidence rate for endometritis in postpartum cows varies considerably among studies, perhaps owing to different methods used to classify the disease. A review of the literature shows that the percentage of cows affected with postpartum endometritis can be as high as 50% at 40–60 days in milk.¹² Additional studies have reported a range of 16.9% to 34% prevalence of CE for cows examined between 21 and 28 days postpartum.^{7,10,11,15,31} However, based on the method used (cytology, biopsy, vaginoscopy, or rectal palpation) for diagnosis, the incidence of endometritis likely lies between 7.5% to 34%.¹² A diagnosis of endometritis based on unspecified criteria has been reported to be 7.8–13.8%.^{56,57} An 18% incidence of endometritis was reported when the diagnosis was made using palpation per rectum.⁵⁶ A mini-review published in 2009 summarized the rates and diagnostic criteria for endometritis.⁹

2.6 IMPACT ON FERTILITY

Most dairy producers maintain a voluntary waiting period (VWP) of approximately 60 days postpartum,^{58,59} allowing the animals to recover from calving and undergo proper uterine involution. Many factors during the transition period, 45 days before (late gestation) until 45 days after (early lactation) calving, will impact the success of the reproductive program. Approximately 50% of dairy cows have irregular ovarian cycles during the immediate postpartum period.^{9,60} Twins and retained fetal membranes increase the risk of post-parturient uterine diseases and subsequent reduced fertility.^{30,61} Animals with abnormal uterine discharge (purulent or purulo-hemorrhagic fluid from the uterus) have a delayed resumption of the estrous cycle.^{12,60} The ultimate goal of a reproductive program is to get cows pregnant as soon as possible following the VWP. Cow suffering from endometritis will not rebreed as timely as healthy cows, resulting in more days open, and leading to decreased profits for the dairy. Under normal conditions, the reproductive tract of dairy cows should have recovered and able to establish and maintain a new pregnancy 45 days post-calving.⁹ Cows that exhibit CE take approximately 27% longer to get pregnant than normal cows¹⁰ and are 1.7 times more likely to be culled due to reproductive failure.^{10,12} Cows with CE had a 17% reduction in pregnancy rate relative to cows with normal uterine discharge.¹⁰ A 20 percentage points decreased conception has been reported in cows diagnosed with endometritis when compared to cows with a normal postpartum uterus.^{9,10} Another study showed that cows exhibiting endometritis had a 17.9% lower conception rate than non-endometritis cows.²⁸

Endometritis positive cows exhibited a 24 day increase in median days open when compared to normal postpartum cows.²⁸ A meta-analysis study showed a 19 days increase from first service to conception in cows diagnosed with postpartum metritis. This increase was due to a 20% decrease in the first service conception rate combined with 7 extra days to first service, when compared to cows without metritis.⁶² Cows with endometritis have a calving interval that is 30 days longer than cows without endometritis.^{9,63} Endometritis is responsible for increased culling of cows due to reproductive failure.⁹ Purulent or mucopurulent discharge was associated with significantly lower pregnancy and first service conception rates.²⁵ Even after successful treatment for periparturient uterine disease, affected cows are less fertile than normal cows of same age.⁶³ The early detection of uterine diseases (e.g., metritis and endometritis) can reduce costs incurred by the dairy allowing them to be more profitable. The costs associated with undetected and untreated uterine infection are due to reproductive inefficiencies that include: culling, treatment costs, milk losses, and increased labor.^{9,10,12,25,62} Using a conservative 20% incidence rate of postpartum endometritis, a study estimated the annual cost of metritis at \$650 million dollars to U.S. dairy producers.⁹ A meta-analysis of the effects of diseases on dairy cow reproduction concluded that uterine diseases will decrease the relative risk of pregnancy at 150 days by 31%.⁶² A field study conducted on 114 Holstein cows from 5 dairies revealed that cows diagnosed with endometritis were open on average 206 days compared to 118 days open for cows without endometritis.¹² This study also found that the pregnancy to first

service conception rate was lower for cows diagnosed with endometritis compared to cows without postpartum endometritis.¹²

The score assigned to the uterine discharge correlates with pathogenicity of the infecting bacteria and serves as a prognostic indicator.^{31,64} Uterine discharge characterized by clear mucus with only flakes of pus (score of 1) was not associated with a decrease in pregnancy rate.¹⁰ A malodorous and purulent discharge (score 2 or 3) was associated with 20% decrease in pregnancy rate.¹⁰ As the amount of purulent discharge increases so does the likelihood of impaired fertility.¹⁰ It was also found that a cervical diameter of greater than 7.5 cm, diagnosed by rectal palpation, was detrimental to fertility.¹⁰ The increased cervical size was associated with an overall decreased pregnancy rate. The greatest effect occurred when the diagnoses were made at 27–33 DIM.¹⁰ Cows having both a large cervix (≥ 7.5 cm) and purulent uterine discharge were the most severely affected.¹⁰ Palpable ovarian structures were lacking in cows that were diagnosed with CE.¹⁰ The absence of a corpus luteum (CL) or ovarian follicles at the time of the postpartum examination was associated with CE.¹⁰

2.7 TREATMENTS

Reducing bacterial load within the uterus while enhancing the uterine defense and repair mechanisms are key to control of uterine diseases.^{7,62} Numerous treatment regimens have been utilized, however, conflicting experimental evidence makes it difficult to promote a single treatment strategy.

Prostaglandin Administration

Prostaglandin ($\text{PGF}_{2\alpha}$), a lipid compound derived from the arachidonic acid, participates in numerous body functions including smooth muscle contraction. The uterus is composed of layers of smooth muscle, and systemic administration of $\text{PGF}_{2\alpha}$ at 5–7 days postpartum appears to aid in uterine expulsion of fluid and contaminants, helping to improve the postpartum uterine environment, thus leading to increases in subsequent pregnancy rates.^{7,55} $\text{PGF}_{2\alpha}$ also causes luteolysis of a responsive CL. Lysis of the CL leads to a decreased concentrations of blood progesterone, inducing estrus 2-5 days following its administration. The subsequent estrus cycle is characterized by increased estrogen and myometrial contraction^{8,15,65-67} The benefits of $\text{PGF}_{2\alpha}$ are due to induction of estrus caused by the lysis of a functional CL on the ovary.³³ The ensuing estrus promotes uterine contractions that physically expel uterine bacterial contamination and improves the estrogen-mediated uterine defense mechanisms.³³ One recent study reported that $\text{PGF}_{2\alpha}$ was equal to or more effective than intrauterine penicillin or oxytetracycline for the improvement of reproductive performance of cows with CE.⁶⁴ Another study showed that $\text{PGF}_{2\alpha}$ treatment had no effect on the prevalence of SCE diagnosed in cows 35 or 49 days post-partum.^{13,68} The presence of an active CL was the only variable shown to lower the prevalence of SCE at 35 and 49 DIM.^{13,68} These data showed how important it is for cows to return to a normal estrous cycle by 21 DIM. The first service PAI was increased in the cows that received $\text{PGF}_{2\alpha}$ but the treatment did not affect the time to first insemination, pregnancy rate, hazard of pregnancy, or median days open in cows that

exhibited SCE at 49 DIM.¹³ Furthermore, a recent study showed that PGF_{2α} had no effect on reproductive performance when treating cows diagnosed with endometritis.¹

Antimicrobial Therapy

Ceftiofur is a third generation cephalosporin with a spectrum of activity against most pathogens that cause uterine infection. The most common bacterial pathogens isolated from the infected uteri of cattle include: *E. coli* and *A. pyogenes* followed by a host of other bacteria.^{9,12,31,34} It was reported that ceftiofur reaches higher concentrations at the sites of bacterial inflammation than most antibiotics.⁶⁹ This study measured the tissue concentration of sodium ceftiofur using *Pasteurella haemolytica*-infected tissue chambers implanted into the paralumbar fossa of cattle. It showed a higher concentration of sodium ceftiofur in the infected tissues when compared to healthy tissues.⁶⁹ Relatively high concentrations have been found in the equine uterus after subcutaneous injections of 2 mg/kg of body weight.^{17,69} However, another study was unable to detect antimicrobial activity in the endometrium of the mare after repeated administration of sodium ceftiofur when the same dose of 2 mg/kg was used.⁷⁰ Subcutaneous injection of ceftiofur hydrochloride at 1 mg/kg of body weight yielded concentrations of active metabolites in uterine tissue and lochia that exceeded the minimum inhibitory concentrations of 90% of the bacterial isolates for common uterine pathogens including *E. coli*, *A. pyogenes*, *Fusobacterium necrophorum*, and *Bacteroides spp.*^{18,71} Desfluoroyl ceftiofur, an active metabolite of ceftiofur, has been isolated in the uterine tissue of sows up to 48 hours after a single intramuscular injection.⁷² Cows experiencing fever, vaginal discharge, or

dystocia, and treated parenterally with ceftiofur had improved cure rates, increased milk yield, and decreased rectal temperatures.²⁴ The success rate for the non-treated control cows that exhibited high rectal temperatures accompanied by a vaginal discharge was 28.9% as compared to 56% for the treated cows showing the same clinical signs.²⁴ Treated cows that exhibited high rectal temperatures and vaginal discharge also showed improvements in milk yield²⁴; however, reproductive information was not reported. Cephapirin is another cephalosporin antimicrobial that has been tested for treating CE. Single intrauterine treatment of cephapirin combined with cloprostenol, a synthetic PGF_{2α}, produced significantly greater pregnancy rates in treated animals when compared to untreated control cows.³³

Uterine Infusions

Intrauterine infusion allows for direct treatment of the endometrium. Treatment is applied at the site of infection but may not penetrate into the deeper tissues. Treatment may not be effective if the cow is suffering from metritis which affects not only the endometrium but the glandular and muscular tissues as well. Numerous elixirs have been infused into the bovine uterus for the treatment of infection including antibiotics and antiseptics: tetracycline, penicillin, cephapirin, chloramphenicol, gentamicin, spectinomycin, sulfonamides, nitrofurazone, iodine, and chlorhexidine.^{11,64,65,73} Treatment of uterine infection by the direct infusion of substances into the uterus has been debated for many years with discussions and research results showing great variability on the efficacy of these treatments. Intrauterine antibiotic therapy has failed to resolve CE and has not shown any benefit on reproductive performance in several

studies.^{64,65} Conversely, two separate studies reported that reproductive performance improved when cows were treated with an intrauterine infusion of cephalixin for periparturient diseases.^{11,16} A single intrauterine treatment of cephalixin or an intramuscular treatment of cloprostenol significantly improved the symptoms associated with sub-clinical endometritis in another study.³³ A study in the United Kingdom compared intrauterine infusions of oxytetracycline hydrochloride (only approved antibiotic treatment for uterine infection in the UK) with intrauterine infusions of a topical hemostatic, antiseptic agent (Policresulen).⁷⁴ Policresulen was significantly less effective for the treatment of endometritis than oxytetracycline.⁷⁴

Alternative Therapy

Sugar has been utilized in wound care due to its hypertonic effect on offending bacteria as well as its ability to expedite wound healing.⁷⁵⁻⁷⁷ Select carbohydrates (sugars) have been shown to inhibit the adherence of bacteria to various cells of the body. One sugar that has been studied is mannose. It has been shown that mannose suppresses or impedes the attachment of *E. coli* to the epithelial cells of most animals including equine endometrial cells.^{22,78-81} A clinical report stated that clinicians at the Western College of Veterinary Medicine, University of Saskatchewan, routinely infused the uterus of postpartum cows with 1 liter of 50% dextrose to treat uterine infections in post-partum cows.⁸² It was believed that the hypertonic solution increased uterine tone which assisted in evacuation of the uterine contents. Contraction and evacuation of the uterus aids in removal of bacteria as well as assists in uterine involution.⁸² The use of D-Mannose and N-acetyl-D-galactosamine was shown to stop the adhesion of *E. coli* and *Pseudomonas*

aeruginosa (*P. aeruginosa*) to epithelial cells of the equine endometrium through competitive inhibition.²² Mannose binds to the bacterial surface lectins, inhibiting adhesion of the bacteria to the epithelial cells.²² Furthermore, this *in vitro* study found that D-mannose inhibited epithelial adherence of *Streptococcus zooepidemicus* while other sugars did not affect the adherence of tested bacteria.²²

Studies have shown that mannose and N-acetyl-D-galactosamine can competitively inhibit the adherence of some bacteria (*E. coli*, *Streptococcus*, and *Pseudomonas*) to endothelial cells.^{22,80} An extensive literature review has not revealed whether D-glucose, also referred to as dextrose, has the same ability as mannose and N-acetyl-D-galactosamine. Mannose and dextrose are both 6 carbon molecules which differ only with respect to the position of the hydroxyl group on the second carbon. It seems logical to postulate the use of dextrose as alternative therapy (alone or as an adjunct of antibiotics) for post-partum uterine infections in dairy cows.⁷⁷ Although the mechanism by which dextrose might control the uterine infections is not definitively known, the addition of dextrose may change the bacterial environment (due to its hypertonicity) at the site of infection, interfere with bacterial growth-attachment to the endometrial lining, or increase the uterine contractions (thus, evacuation). Dextrose provides energy to the healing cells along with tissue contraction that aids in wound healing.^{77,82,95} This tissue contraction may aid by increasing uterine tone.¹⁰² A solution of 50% dextrose was shown to decrease bacterial colony numbers from cultures of canine duodenal contents.¹⁰⁴ Other homeopathic remedies have been used to treat uterine diseases (metritis, CE, and SCE). In a clinical trial, the administration of *Lachesis compositum* (*Lachesis*), *Carduus*

compositum (Carduus), and Traumeel LT (Traumeel) to cows with CE did not improve the overall reproductive performance when compared to untreated cows.⁸³ Intrauterine lysobutillin (broad spectrum preparation of lytic enzymes derived from *Bacillus subtilis*) mixed with distilled water resulted in 100% clinical recovery when compared to a 90% recovery following treatment with antimicrobials (neofur, uterosan).²¹

2.8 DESCRIPTION OF THE PROBLEM AND JUSTIFICATION

Although preventing the risk factors associated with uterine diseases is advised, there is not yet a clear consensus on how to treat these postpartum conditions (metritis, endometritis, subclinical endometritis) once diagnosed. Successful breeding of dairy cows in a timely manner is the goal of any reproductive management program. Postpartum endometritis negatively impacts fertility in dairy cows; thus, profitability and sustainability. Proactive on-farm identification and diagnosis of cows suffering from endometritis followed by administration of effective treatments should logically improve reproductive outcomes. Currently, the use of local or systemic therapies (e.g. ceftiofur, oxytetracycline, prostaglandins), are advised for cows with endometritis to resolve uterine infection and inflammation, and to enhance fertility. The effectiveness of treatments, as measured by the ability of cows to conceive following inseminations, varies greatly. Therefore, we propose to test the effectiveness of infusing a hypertonic dextrose solution into the uterus of cows diagnosed with endometritis as an alternative therapy to local and parenteral antibiotics. The epidemiological information obtained from this study will be valuable in understanding the dynamics of endometritis and its

association with repeat breeder cows while evaluating an antibiotic-free therapy for the treatment of endometritis in organic dairy herds. This project is significant for both conventional and organic dairy producers who need cost-effective strategies for the treatment of endometritis.

CHAPTER 3: MATERIALS AND METHODS

3.1 ANIMALS, FACILITIES AND FEEDING MANAGEMENT

A total of 833 lactating Holstein cows (primiparous (n=255) and multiparous (n=578)) from two commercial dairy farms located in central Ohio (approximately 2000- and 5000-cow operations) were enrolled in a randomized clinical trial. Briefly, cows were housed in free-stall barns and milked thrice daily at approximately 8-hour intervals. The herd rolling average milk production was 10,262 kg and the reported voluntary waiting period was 60 days. Cows were fed twice daily, in the morning and afternoon, with a TMR formulated to meet or exceed dietary nutritional requirements for lactating dairy cows.⁹⁷ This study was conducted from September, 2009 through September, 2010. The procedure described were reviewed and approved by the Institutional Animal Care Use Committee, The Ohio State University.

3.2 DIAGNOSIS OF CLINICAL ENDOMETRITIS AND TREATMENTS

Weekly, a list of cows was obtained according to calving dates from on-farm DairyComp 305 records (DairyComp 305 Valley Agricultural Software, Tulare, CA, Valley, California). Briefly, cows at 26 ± 3 DIM were sorted upon exiting the milking parlor and placed into a palpation rail. Once in the palpation rail, the uterus was massaged via rectal palpation, the vulva was wiped off with paper towel, and a single use vaginal speculum was introduced through the vulva. Using a light source (Mini-Maglite, Ontario, California), the vaginal vault and cervical os were visualized and the uterine

discharge scored. Cows were screened through vaginoscopy (single use speculum) for presence of CE at 26 ± 3 days in milk (DIM) and scored using the 0-3 scale (0 = normal uterine discharge, 1 = flakes of purulent exudates in the uterine discharge, 2 = >50% of the uterine discharge is made up of purulent exudates, 3 = hemorrhagic uterine discharge mixed with purulent exudates (adapted from^{23,31}).

Cows with uterine discharge scores of 2 or 3 were stratified by parity and randomly allocated into 1 of 3 treatment groups: 1) Control (CON; $n=83$), 2) 6.6 mg/kg single dose of subcutaneous ceftiofur crystalline free acid (CEF; $n=79$; Pfizer Animal Health, New York, NY), and 3) Intrauterine infusion (200 mL) of a 50% dextrose solution (DEX; $n=80$). Also, rectal temperature ($^{\circ}\text{C}$), the cervix diameter (cm), and presence of ovarian structures (e.g., CL, follicles, cysts) were recorded via ultrasonography. Additionally, cows had their body condition scores (BCS; scale 0-5;⁸⁴) recorded at calving, at 26 ± 3 , and at 40 ± 3 DIM. Fourteen days post-therapy (at 40 ± 3 DIM), all cows were re-examined through vaginoscopy to determine the response to treatment based on uterine discharge (Table 2).

3.3 PROGESTERONE RADIOIMMUNOASSAY

Blood samples (10 mL) for determination of blood progesterone (P4) level were collected at 26 ± 3 and at 40 ± 3 DIM by coccygeal venipuncture (BD Vacutainer[®], Franklin Lakes, NJ) immediately before the administration of treatments to determine cyclicity of cows.⁹⁹ Immediately after collection, blood samples were centrifuged at 2,785 g for 20 minutes and serum samples were stored at -20°C until assayed for P4.

Serum concentration of progesterone were determined in duplicated using a modified commercially available RIA kit (Coat-a-Count[®], Diagnostic Products Corporation, Los Angeles, CA) as previously described.¹⁰¹ The intra- and inter-assay CV were 8.8% and 10.3%, respectively.

3.4 BREEDING MANAGEMENT

For first postpartum services, cows were pre-synchronized with two intramuscular injections of PGF_{2α} (25 mg; Lutalyse, Pfizer Animal Health, New York, NY) administered 14 days apart at 26±3 and 40±3 days postpartum. Twelve days after the second injection of PGF_{2α}, all cows initiated Ovsynch (OV;^{85,86}). The initial GnRH dose (100 µg; Cystorelin, Merial, Duluth, GA) of OV was followed 7 days later by an injection of PGF_{2α} and 56 hours later, cows received the second dose of GnRH followed by timed AI (TAI; 72 hours after the PGF_{2α} injection). Following the first GnRH of OV, estrus was detected by visual observation plus tail chalking once daily, and all animals presenting visual signs of standing estrous behavior (cows staying still when mounted or presenting rubbed off tail chalking) received AI. Animals that did not display estrous behavior during the synchronization protocol were subjected to TAI 72 hours after the PGF_{2α} injection of OV. Reconfirmation of pregnancy was made approximately 30 days after the first pregnancy diagnosis (69±3 days post-AI).

3.5 BACTERIAL GROWTH AND IDENTIFICATION

Uterine swab samples were collected from cows diagnosed with CE for bacteriology. Briefly, individually wrapped and double guarded sterile equine swabs (Continental Plastics, Delavan, WI) were used to collect uterine samples for bacteriology. The swabs are packaged inside a sterile plastic wrapper and guarded with an outer sterile plastic sheath that is capped with a sterile, perforated rubber tip. The 3-piece swab was introduced through the vagina by parting the vaginal lips to avoid contact with the outside of the vulva. The swab device was advanced into the external cervical os and through the uterine body. The sterile swab was exposed to the uterine wall to collect the sample for bacteriology. Then the swab was pulled back into the inner capped tube keeping it protected from contamination while it was removed from the uterus and vagina. Immediately after collection, samples were placed into a transport culture media (BD, Franklin Lakes, NJ) and transported at ambient temperature within 4 hours to The Ohio State University Veterinary Medical Center Microbiology Laboratory.

Once at the laboratory the samples underwent culture, isolation, and sensitivity. All samples were cultured on Tryptic Soy Agar with 5% Sheep Blood and MacConkey's agar plates and incubated for 72 hours at ambient temperature under aerobic conditions. Plates were observed every 24 hours for growth, when colony growth was visually observed each unique colony was isolated and identified. Isolated colonies were placed on individual blood agar plates and storage at -80°C. All *enterobacteriaceae* colonies were identified for antimicrobial sensitivity using minimum inhibitory concentrations (MIC) on the 96 well BOPO6F microtiter plate (Trek Diagnostics, Cleveland, OH) and

analyzed using the sensititre system (Trek Diagnostics, Cleveland, OH). Sensitivities were then compared to available MIC's for specific antimicrobials and pathogens (Clinical and Laboratory Standards Institute, Wayne, PA).

3.6 STATISTICAL ANALYSES

Uterine swab samples from a subset of cows with CE (n=182) were collected immediately before treatment administration for bacteriology. The proportion of visible colonies after culture and the proportion of bacteria isolated (from the total number of identified isolates) in the 3 treatment groups (CON, DEX or CEF) were analyzed by Proc Freq (SAS Institute Inc., 2009). A P-value <0.05 was considered statistically significant.

Data from lactating dairy cows (e.g., DIM, milk yield, service number, pregnancy status) were exported from DairyComp 305 to an excel spreadsheet (Microsoft Corp., Redmond, WA). Prior to data analyses, enrolled lactating dairy cows that met the exclusion criteria (i.e., cows that were treated but died before the AI or pregnancy diagnosis, cows with a history of abortion before AI, dead and sold animals) were removed from the analysis. Body condition scores at the time of treatment administration (26 ± 3 DIM) were classified as <4.0, 4.0-5.0, >5.0).

Data were arranged in a randomized block design. Following treatment administration (at 26 ± 3 DIM), the proportion of cows with clear uterine discharge at 40 ± 3 DIM (response to treatment) was evaluated. Additionally, the proportion of cows that conceived to first service (PAI) in the 3 treatment groups (CON, n=83; CEF, n=75; or DEX, n=79) were evaluated. Data pertaining to response to treatments and PAI were

analyzed using generalized linear mixed models (Proc Glimmix; SAS Institute Inc., 2009). A model procedure that included treatment (CON, DEX, or CEF), parity (primiparous or multiparous), BCS, milk yield and DIM at the time of AI, sire, and SCC at the closest DHIA test relative to service was used to compare differences in FSPA between treatments. Non-significant variables were eliminated from the logistic model one at a time using the Wald statistic backward selection criterion ($P > 0.15$). Herd was included as a random effect. The estimates (proportions of PAI and response to treatment) from the final model were reported as least squares means.⁸⁸⁻⁹⁰ Standard errors of the means (SEM) for binomial outcomes (e.g., pregnancy) were computed as described by SAS⁹⁶ and reported elsewhere.⁸⁹ The differences between least squares means were calculated by including the PDIF option in the LSMEANS statement.^{88,90} Differences in individual least squares means were adjusted by using Tukey-Kramer method. A $P < 0.05$ was considered statistically significant.

Furthermore, the proportion of cows pregnant within 250 DIM for cows with and without CE was evaluated using LIFETEST procedure of SAS.⁹⁶ Data obtained from SAS output were plotted in Microsoft excel (Microsoft Corp., Redmond, WA) to graphically obtain the proportion of cows pregnant over time.^{91,92}

CHAPTER 4: RESULTS

Initially, 833 lactating dairy cows were screened for clinical endometritis at 26 ± 3 DIM, of which 255 cows (30.6%; Table 1) were diagnosed with CE (scores 2 or 3) and randomly assigned into 1 of 3 treatment groups. For PAI analysis, data from 73 cows (18 treated and 55 untreated cows) were not available because they died (49), were sold (17), or unknown (7) before the first AI. Therefore, 760 lactating Holstein dairy cows (240 primiparous and 520 multiparous) were available for the final PAI analysis (Table 5).

4.1. DIAGNOSIS OF CLINICAL ENDOMETRITIS

Postpartum lactating dairy cows were screened for CE at 26 ± 3 DIM using vaginoscopy technique (Table 1). The prevalence of CE at the time of treatment (26 ± 3 DIM) was 30.6% (scores 2 and 3 combined; Table 1). Cows that were diagnosed with CE (uterine discharge scores of 2 and 3) were stratified by parity and randomly assigned into 1 of 3 treatment groups. Fourteen days later (at 40 ± 3 DIM), a subset of enrolled cows were subjected to a second gynecological exam (Table 2).

The cervical diameter was estimated through transrectal ultrasonography at the time of first (26 ± 3 DIM; Table 1) and second gynecological exam (40 ± 3 DIM; Table 2). The majority of cows (45.5%) had a cervical diameter less than 4 cm, 37.7% of the cows had a cervical diameter of 4.0-5.0 cm, and 17.2% of the cows had a cervical diameter greater than 5.1 cm (Table 1). Although cervical diameter was not different for cows diagnosed with CE (score 2 and 3), cows without CE had reduced cervical diameter compared to cows with CE ($P = 0.04$).

The distribution of lactating dairy cows with and without CE were stratified with respect to DIM to first service (DIMFS), milk yield (kg), body temperature, body condition scores (BCS), and somatic cell count (SCC) at the closest DHIA test relative to treatment (Table 3). There was not significant differences between cows with or without CE for the parameters evaluated (Table 3).

4.2. BACTERIAL GROWTH AND IDENTIFICATION

From a subset of cows (n=182) with CE, swab samples were collected immediately before treatment (26 ± 3 DIM) for bacteriology. The proportion of positive bacterial growth (from 182 swab samples) was 68.13% (124 swabs; Table 4). A total of 54 swab samples (31.87%) were negative and yielded no bacterial growth. From the 124 positive cultures (68.13%), a total of 147 isolates were identified (Table 4). *Arcanobacterium pyogenes* and *Escherichia coli* were the most predominant isolates identified across the treatment groups (Table 4). Also, *Pasteurella spp.*, *Pseudomonas spp.*, *Corynebacterium spp.*, *Acinotebacter spp.*, and *Bacillus spp.*, were occasionally isolated (Table 4).

4.3. EFFECT OF TREATMENTS ON PREGNANCY PER AI (PAI)

SCC at treatment was significantly associated ($P < 0.05$) with PAI and stayed in the final model. For this study, location (herd), parity (primiparous and multiparous), and BCS at treatment were not significantly associated ($P > 0.05$) with PAI between treatment

groups. For first service only (Table 5), PAI in DEX ($29.8 \pm 4\%$) tended to differ from cows in CON ($21.1 \pm 4\%$) and CEF groups ($19.7 \pm 4\%$; $P = 0.1$), whereas the overall PAI in DEX cows was not different from cows without CE ($39.1 \pm 2\%$; Table 5). Furthermore, the survival curve of time to pregnancy for cows with (CON, DEX, or CEF) and without CE are presented in Figures 2.

CHAPTER 5: DISCUSSION

The objective of this study was to determine if an intrauterine infusion of 50% dextrose in water (DEX) would have similar first service PAI compared to cows treated with parenteral ceftiofur crystalline free acid (CEF) on lactating dairy cows diagnosed with CE. The primary findings of this study are (1) cows with CE (vaginal scores 2 or 3 combined) had greater mean cervix diameter compared to cows without CE, (2) the most predominant bacteria isolated at the time of treatment from CE cows were *Arcanobacterium pyogenes* and *Escherichia coli*, (3) the proportion of PAI for first service tended to increase in DEX cows compared to CEF or CON cows, and (4) increased PAI was observed in cows without CE compared to CEF or CON, but the overall PAI was not different from DEX cows.

The 30.4% prevalence of CE found at the first gynecological exam falls within the range of 13-50% cited in the literature.^{10-12,31,93} Postpartum CE has been defined as a presence of mucopurulent or purulent vaginal discharge by vaginoscopy after 26 DIM and a cervix diameter ≥ 7.5 cm (measured by rectal palpation) without systemic sign of illness.^{10,27,35} Cytological endometritis has been defined as an increased proportion of polymorphonuclear cells in endometrial cytology samples obtained by endometrial cytobrush^{27,35}, uterine lavage¹², or from uterine biopsy samples³⁶. The techniques for cytological diagnosis of CE have the potential to diagnose both clinical and subclinical cases of endometritis, vaginoscopic diagnosis of CE was used in this study.^{10,23,26,29} The use of ultrasonography to determine cervical diameter at the time of CE diagnosis has not been reported previously. In this study, the mean cervical diameter was greater for CE

cows (4.3 cm) compared to cows without CE (3.7 cm). Additionally, the mean cervix diameter was associated with reduced PAI at first service in cows diagnosed with CE compared to cows without CE. Other studies reported a cervical diameter of ≥ 7.5 cm at 20 DIM (based on rectal palpation) for CE cows.^{7,9,23} The assessment of cervical diameter as a mean to diagnose CE in cows may vary considerable according to the method used (e.g., ultrasonography vs rectal palpation). This may explain the values for cervical diameter reported in this study as opposed to those values reported through rectal palpation.^{1,3,96} Furthermore, as reported elsewhere¹¹, rectal temperature at the time of treatment (26 ± 3 DIM) was not different between cows with and without CE in this study. Immediately after CE diagnosis (vaginal discharge score 2 or 3 at 26 ± 3 DIM), cows were randomly assigned into 1 of 3 treatment groups. Fourteen days later, the proportion of cows with a vaginal discharge score of 0 (clear mucus) was significantly increased in DEX and CEF cows compared to CON cows (Table 2). The uterine discharge scores (and cervical diameter) are expected to decrease as the inflammation associated with CE resolves following the treatment administration.²⁵

In lactating dairy cows, many species of bacteria can be isolated in the first 10 days postpartum⁴². Recognized uterine pathogens (*A. pyogenes*, *Prevotella melaninogenica*, *E. coli*, *Fusobacterium necrophorum*, and *Proteus spp.*), potential uterine pathogens (*Bacillus spp.* and *Pasteurella spp.*), and opportunistic uterine contaminants (*Streptococcus spp.*, *Providencia spp.*, *Klebsiella spp.* and *Corynebacterium spp.*) have been associated with endometritis and decreased fertility in dairy cattle.^{31,42,49,68} Furthermore, it has been shown that the presence of *E. coli* and *A.*

pyogenes in the bovine uterus is associated with ovarian dysfunction such as smaller follicle diameter and corpora lutea with lower plasma estradiol and progesterone.⁴⁹ In this study, the most predominant bacteria isolated from CE cows were *E. coli* and *A. pyogenes*.

The reproductive success (pregnancy) following the treatment of CE cows was one of the primary outcomes of this clinical study. There was a tendency for cows in the DEX group (29.8±4%) to have a greater first service PAI than cows in CON (21.1±4%) or CEF (19.7±4%) groups. The use of intrauterine DEX in cows with CE may favor a quicker uterine recovery (by inhibiting bacterial growth locally, increase uterine tone, or by nurturing endometrial cells) as opposed to CON or CEF cows. Previous studies have shown the ability of sugar to aid in wound healing through inhibition of bacterial growth.^{76,77,79,82} Sugar has also been shown to treat bacterial infections with hypertonicity causing an osmotic draw of fluid (transudate) out of the affected area effectively reducing the water activity that is required for bacteria to survive.⁷⁷ Transudation also may bring more leukocytes into the uterine lumen allowing for an increase in bacterial phagocytosis.⁹⁵ Carbohydrates (sugar) have been shown to decrease bacterial production of proteases, leading to less tissue damage.⁹⁵ 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.⁹⁵ Furthermore, dextrose provides energy to the endometrial cells aiding in healing.^{77,100} The osmotic draw of fluid out of the tissues will aid in tissue contraction,¹⁰² thereby causing an increased uterine tone. Moreover, when all cows (with or without CE) were considered in the statistical analysis, cows without CE

had greater first service PAI as opposed to CON and CEF cows. However, the proportion of PAI at first service was not different between DEX cows and cows without CE. Also, the survival curve (proportion of cows not pregnant after the voluntary waiting period) showed this trend between treated cows and cows without CE. Administration of PGF_{2α} alone for cows diagnosed with CE has been reported to improve¹³ or to have no effect¹ on PAI in lactating dairy cows. Reproductive performance of lactating dairy cows diagnosed with CE was not improved following the administration of ceftiofur hydrochloride^{68,103} or ceftiofur crystalline free acid¹, which agrees with the current study. Previous studies on reproductive performance of CE cows following treatment were based on visual observation of the signs of standing estrous.^{1,68} Although in our study all cows were subjected to the same synchronization protocol (Presynch followed by Ovsynch), reproductive performance of CEF cows was similar to CON cows.^{1,68} A recent clinical study of 2,178 Holstein cows concluded that the treatment for CE through the use of PGF_{2α} alone or with ceftiofur crystalline free acid should to be reassessed due to lack of improved reproductive outcomes.¹

Blood samples for serum concentrations of progesterone (P4) were collected 14 days apart at 26±3 and at 40±3 DIM from cows diagnosed with CE. Cows were classified as cycling when the concentration of P4 from either blood sample was ≥1 ng/mL (High P4; High-High, Low-High, or High-Low). Non-cycling cows were identified when serum concentrations of P4 from both blood samples were <1 ng/mL (Low P4; Low-Low;⁹⁹

The presence of ovarian structures such as follicles, CL, and cysts were recorded through transrectal ultrasonography at 26±3 and at 40±3 DIM in cows diagnosed with

CE. The presence of cysts was defined as a follicle-like structures >25 mm in diameter.^{98,99} Ovarian structures did not differ between groups.

Studies involving sugar bandages and wound healing have shown an inhibitory effect on bacterial growth for many of the bacteria that cause CE in cows.^{77,79,81,95} The use of lytic enzyme preparations from *Bacillus subtilis* was assessed as alternative treatment for cows with post-partum endometritis compared to neofur® or uterosan®,²¹ but, the lack of clinical response (therapeutic outcomes) makes it difficult to promote this therapy in lactating dairy cows. Additionally, the use of homeopathic remedies such as *Lachesis compositum* (Lachesis), *Carduus compositum* (Carduus), and Traumeel LT (Traumeel) have not improved reproductive performance of lactating dairy cows diagnosed with CE.⁸³ The use of intra-abdominal hypertonic glucose (20%) was reported to control acute peritonitis in rabbits.⁹⁵ Furthermore, an *in vitro* study has shown that mannose (a sugar monomer) inhibits the adhesion of bacteria to the epithelial cells of the equine endometrium.²² These findings suggest that the use of a unique antimicrobial therapy (50% dextrose in water - a hypertonic solution) may be a viable and effective strategy for lactating dairy cows diagnosed with CE. In this study, lactating cows treated with an intrauterine infusion of 50% dextrose (200 mL) had similar PAI to first service compared to those cows without CE. The addition of a hypertonic solution (50% dextrose) may reduce the growth rate of bacteria in the uterus and increase uterine tone allowing the natural uterine defenses (e.g., macrophages, neutrophils) to control the infection (e.g., uterine environment) and improve the overall reproductive performance of cows diagnosed with CE.

In conclusion, this clinical study showed that the use of antimicrobial (ceftiofur crystalline acid) or $\text{PGF}_{2\alpha}$ alone were unsuccessful at improving the overall reproductive performance of dairy cows diagnosed with CE. Although the use of intrauterine dextrose was not significantly different from CON or CEF cows, it resulted in similar reproductive performance as those cows without CE. Therefore, the use of intrauterine dextrose and the underlining mechanisms by which the infection is controlled warrant further investigation under different herds and reproductive managements.

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APPENDIX A: TABLES

Table 1: Prevalence (%) of clinical endometritis (CE) at the time of treatment (26±3 DIM) of lactating Holstein cows (n=833) using vaginoscopy scoring technique and measurement of cervical diameter by ultrasonography.

Parameters	¹ First Gynecological Exam (n=833)
Clinical uterine discharge	
0 (clear mucus)	49.9
1 (mucus with flecks of pus)	19.7
2 (mucopurulent)	22.5
3 (brown-red foul)	7.9
Cervical diameter (cm)	
<4	45.5
4.0-5.0	37.3
≥5.1	17.2

¹Lactating dairy cows (n=833) were screened for CE at 26±3 DIM (First gynecological exam) using vaginoscopy technique and measurement of cervical diameter.

Table 2: Prevalence (%) of clinical endometritis (CE) at the time of second gynecological examination (40±3 DIM) in a subset of treated Holstein cows (n=146) using vaginoscopy scoring technique and measurement of cervical diameter by ultrasonography.

² Second Gynecological Exam (n=146)				
Parameters	CON	DEX	CEF	<i>P</i> -value
Clinical uterine discharge				
0 (clear mucus)	24.53 ^b	44.68 ^a	41.30 ^a	0.02
1 (mucus with flecks of pus)	33.96	27.66	21.74	
2 (mucopurulent)	32.08	21.28	28.26	
3 (brown-red foul)	9.43	6.38	8.7	
Cervical diameter (cm)				
<4	62.50	66.85	65.79	
4.0-5.0	31.25	29.7	23.68	
≥5.1	6.25	4.88	10.53	

²A subset of cows (n=146) diagnosed with CE (score 2 or 3) at the time of treatment (26±3 DIM) were subject to a second gynecological examination fourteen days later (40±3 DIM).

^{a,b} Values with different superscript letters within a row differ significantly at $P < 0.05$.

Table 3: Distribution of lactating dairy cows with and without clinical endometritis (CE) with respect to DIM to first service (DIMFS) and milk yield (kg), body temperature, body condition scores (BCS), mean cervix diameter (cm), and somatic cell count (SCC) at the closest DHIA test relative to treatment.

Variables	Cows with CE ¹			Cows without CE (n=523)	P-value
	CON (n=83)	DEX (n=79)	CEF (n=75)		
DIMFS (d)	64.1	61.5	62.5	63.1	0.20
Milk yield (Kg)	32.4	32.9	34.5	35.3	0.33
BCS (%)					
<3.0	65.4	68.2	76.3	68.3	0.47
≥3.0	34.6	31.8	23.7	31.7	0.47
Body temperature (°C)	38.6	38.7	38.5	38.6	0.33
Mean cervix diameter (cm)	4.3 ^a	4.3 ^a	4.1 ^a	3.7 ^b	0.001
SCC (×10 ³)	118	246	111	172	0.32

¹Lactating dairy cows (n=760) were screened for CE at 26±3 DIM using vaginoscopy technique and measurement of cervical diameter and randomly assigned to 1 of 3 treatment groups: Control (CON; n=83), intrauterine infusion (200 mL) of a 50% dextrose solution (DEX; n=79), or 6.6 mg/kg single dose of subcutaneous ceftiofur crystalline free acid (CEF; n=75). Variables from cows without CE (n=523) were reported for comparison purposes.

^{a,b} Values with different superscript letters within a row differ significantly at $P < 0.05$.

Table 4: Proportion of bacteria isolated from the endometrium immediately before treatment in lactating dairy cows with clinical endometritis (CE).

Species	Identified isolates (%) over total (n/n) ¹		
	CON	DEX	CEF
<i>Arcanobacterium pyogenes</i>	60.78 (31/51)	67.39 (31/46)	64.0 (32/50)
<i>Escherichia coli spp.</i>	29.41 (15/51)	23.91 (11/46)	30.0 (15/50)
<i>Pasteurella spp.</i>	3.92 (2/51)	6.52 (3/46)	--
<i>Pseudomonas spp.</i>	1.96 (1/51)	--	2.0 (1/50)
<i>Corynebacterium spp.</i>	1.96 (1/51)	--	2.0 (1/50)
<i>Acinotobacter spp.</i>	1.96 (1/51)	--	--
<i>Bacillus spp.</i>	--	2.17 (1/46)	2.0 (1/50)

¹From a total of 182 swab samples submitted for bacteriology, 124 (68.13%) yielded positive bacteria growth. A total of 147 isolates were identified and reported as a proportion (%) over the total (n) number of isolates of a particular genus from the total (n) number of identified isolates obtained for CON (51), DEX (46), and CEF (50) groups.

Table 5: Pregnancies per AI (PAI) from lactating dairy cows with clinical endometritis (CE) following an intrauterine dextrose infusion (DEX), ceftiofur crystalline free acid (CEF), or untreated animal (CON).

Variable	Cows with CE			Cows without CE (n=523)	P-value
	CON (n=83)	DEX (n=79)	CEF (n=75)		
First service for treated cows only (%)	21.1 ± 4 ^a	29.8 ± 4 ^a	19.7 ± 4 ^a	--	0.1
First service for all cows (%)	20.1 ± 4 ^b	30.5 ± 4 ^{ab}	19.4 ± 4 ^b	39.1 ± 2 ^a	0.0001

¹Lactating dairy cows (n=760) were screened for CE at 26±3 DIM using vaginoscopy technique and measurement of cervical diameter and randomly assigned to 1 of 3 treatment groups: Control (CON; n=83), intrauterine infusion (200 mL) of a 50% dextrose solution (DEX; n=79), or 6.6 mg/kg single dose of subcutaneous ceftiofur crystalline free acid (CEF; n=75). Variables from cows without CE (n=523) were reported for comparison purposes.

^{a,b} Values with different superscript letters within a row differ significantly at $P < 0.05$.

Table 6: Proportion (%) of ovarian structures (presence of follicles, CL, or cysts) and cycling status based on serum concentration of progesterone (P4) in lactating dairy cows diagnosed with clinical endometritis (CE).

Variable	Cows with CE			
	CON	DEX	CEF	<i>P</i> -value
Cycling status of cows based on serum concentrations of P4 ¹				
Non-cycling (%)	46.8 ± 7.2	44.2 ± 7.5	59.5 ± 8.0	0.35
Cycling (%)	53.2 ± 7.2	55.8 ± 7.5	40.5 ± 8.0	0.35
Ovarian structures at 26±3 DIM ²				
Follicles (%)	91.2 ± 4.1	91.8 ± 3.9	95.8 ± 2.9	0.76
CL (%)	50.9 ± 7.0	55.1 ± 7.1	56.2 ± 7.1	0.95
Cysts (%)	15.7 ± 5.1	20.4 ± 5.7	8.3 ± 3.9	0.41
Ovarian structures at 40±3 DIM ³				
Follicles (%)	93.6 ± 3.5	89.7 ± 4.8	94.4 ± 3.8	0.87
CL (%)	80.8 ± 5.7	64.1 ± 7.6	66.7 ± 7.8	0.34
Cysts (%)	14.9 ± 5.1	23.1 ± 6.7	13.9 ± 5.7	0.71

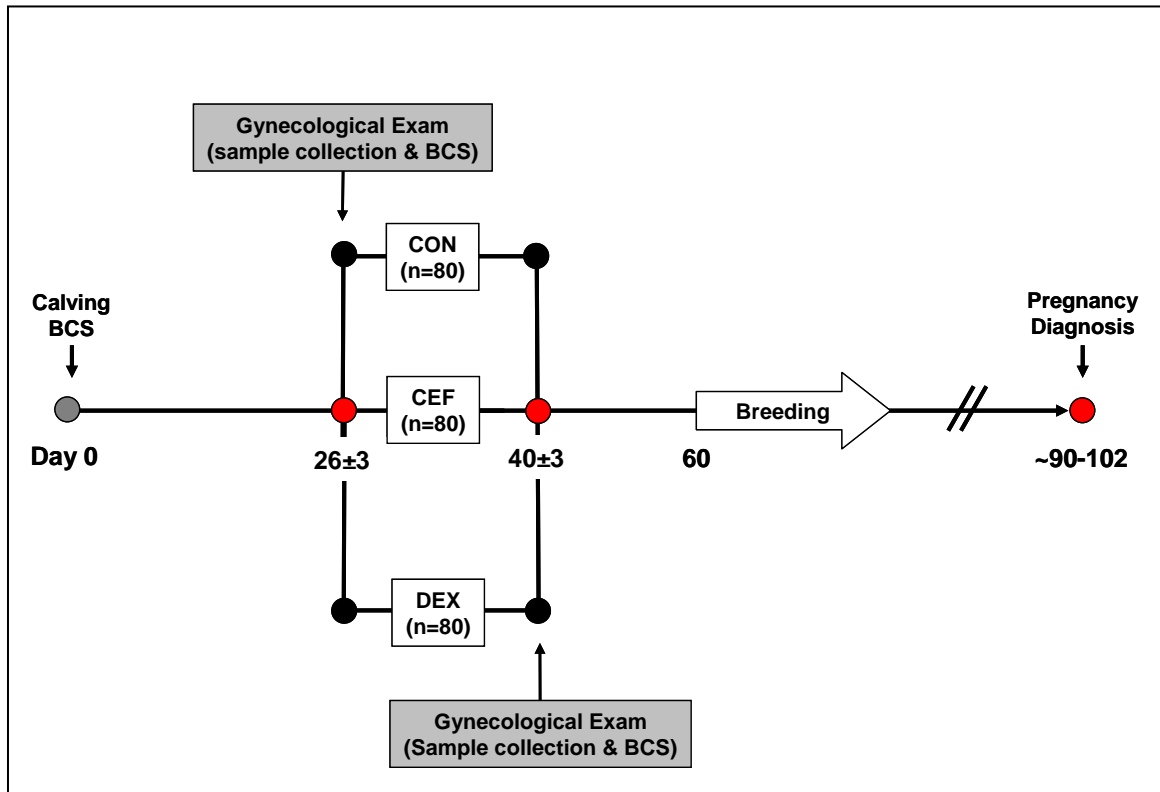
¹The proportion (%; LSM ± SEM) of lactating dairy cows with serum concentrations of progesterone ≥1 ng/mL (cycling) or <1 ng/mL (non-cycling) were reported. Blood samples were collected from cows diagnosed with CE at 26±3 and 40±3 DIM. Cows were classified as cycling when concentrations of P4 from 1 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).

²The proportion (%; LSM ± SEM) of ovarian structures (presence or absence of follicles, CL, or cysts) was recorded at 26±3 DIM in lactating dairy cows with and without CE.

³The proportion (%; LSM ± SEM) of ovarian structures (presence or absence of follicles, CL, or cysts) was recorded at 40±3 DIM in lactating dairy cows with and without CE.

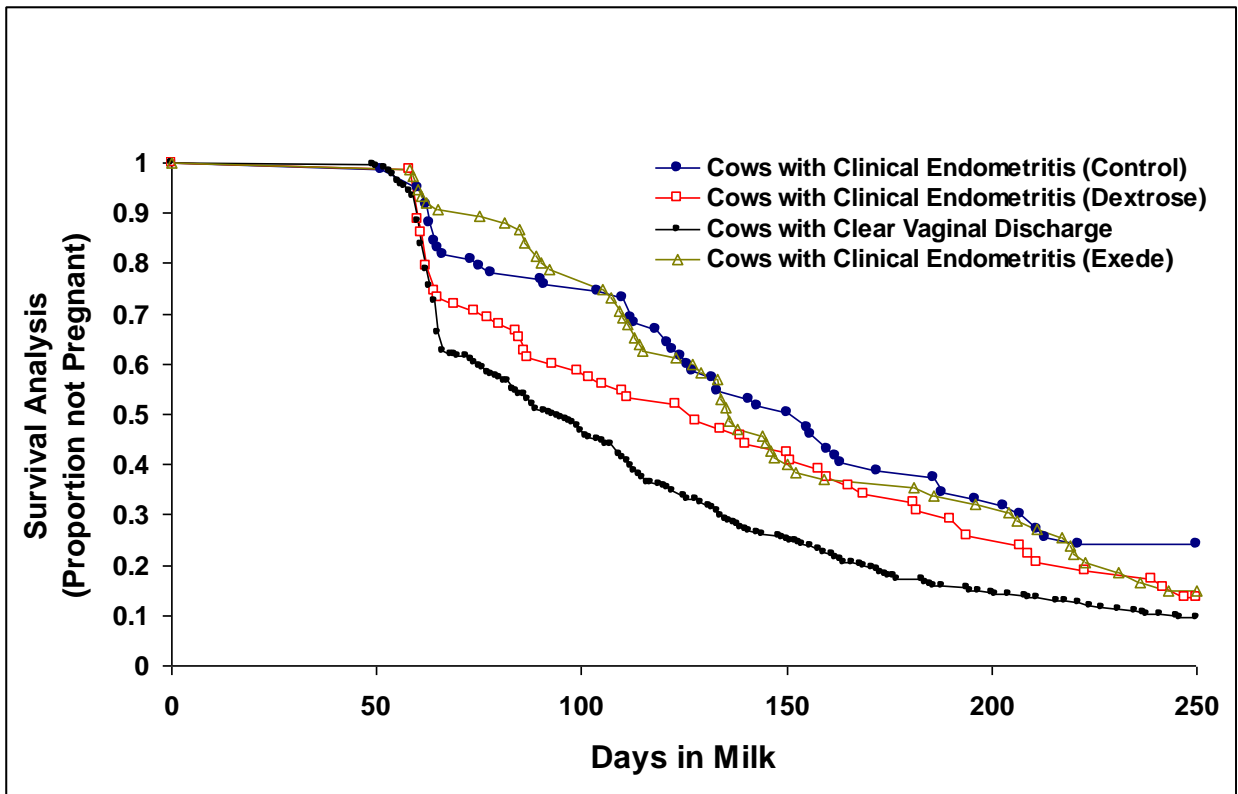
APPENDIX B: FIGURES

Figure 1: Scheme showing the outline of experimental design. At the time of calving, cows had their body condition scored (BCS). Lactating dairy cows were screened for clinical endometritis (CE) at 26 ± 3 DIM (first gynecological exam) and randomly assigned into 1 of 3 treatment groups.¹



¹Lactating dairy cows (n=833) were screened for CE at 26 ± 3 DIM using vaginoscopy technique and measurement of cervical diameter. Cows diagnosed with CE (score 2 or 3) were randomly assigned into 1 of 3 treatment groups: Control (CON; n=83), intrauterine infusion (200 mL) of a 50% dextrose solution (DEX; n=79), or 6.6 mg/kg single dose of subcutaneous ceftiofur crystalline free acid (CEF; n=75). Cows diagnosed with and without CE (n=523) were subjected to the same reproductive program.

Figure 2: Survival curves of open cows after voluntary waiting period to pregnancy over time (days in milk) for cows diagnosed with clinical endometritis (CE) or with clear vaginal discharge.¹



¹Lactating dairy cows (n=760) were screened for CE at 26±3 days in milk (DIM) using vaginoscopy technique and measurement of cervical diameter and randomly assigned into 1 of 3 treatment groups: control (CON; n=83), intrauterine infusion (200 mL) of a 50% dextrose solution (DEX; n=79), or 6.6 mg/kg single dose of subcutaneous ceftiofur crystalline free acid (CEF; n=75). Cows without CE (n=523) were included in the survival analysis for comparison purposes.