The effect of intrauterine infusion of dextrose on clinical endometritis cure rate and reproductive performance of dairy cows

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ABSTRACT

The main objective of this study was to evaluate the intrauterine administration use of 200 mL of 50% dextrose solution as a treatment against clinical endometritis (CE); CE cure rate and reproductive performance were evaluated. Additionally, the association of several relevant risk factors, such as retained placenta (RP), metritis, CE, anovulation, hyperketonemia, and body condition score with reproductive performance, early embryonic mortality, and CE were evaluated. A total of 1,313 Holstein cows housed on 4 commercial dairy farms were enrolled in the study. At 7 ± 3 DIM cows were examined for metritis and had blood collected to determine serum β -hydroxybutyrate concentration. To determine if cows had ovulated at least once before 44 ± 3 DIM, the presence of a corpus luteum was evaluated by ovarian ultrasonography at 30 ± 3 DIM and at 44 ± 3 DIM. At 30 ± 3 DIM, CE was diagnosed using the Metrichick device (SimcroTech, Hamilton, New Zealand); cows with purulent or mucopurulent vaginal discharge were diagnosed as having CE. Cows diagnosed with CE ($n = 175$) were randomly allocated into 2 treatment groups: treatment (intrauterine infusion of 200 mL of 50% dextrose) or control (no infusion). Clinical endometritis cows were re-evaluated as described above at 44 ± 3 DIM, and cows that were free of purulent or mucopurulent vaginal discharge were considered cured. Intrauterine infusion of dextrose tended to have a detrimental effect on CE cure rate, and treatment did not have an effect on first-service conception rate and early embryonic mortality. A multivariable Cox's proportional hazard model was performed to evaluate the effect of several variables on reproductive performance; the variables RP, CE, parity, anovulation, and the interaction term between parity and anovulation were

associated with hazard of pregnancy. Cows that did not have RP or CE were more likely to conceive than cows that were diagnosed with RP or CE. Cows that had RP were at 3.36 times higher odds of losing their pregnancy than cows that did not have RP. In addition, cows diagnosed with CE were at 2.16 higher odds of losing their pregnancy than cows without CE. In conclusion, intrauterine infusion of 200 mL of 50% dextrose solution as a treatment for CE had a strong statistical tendency to decrease CE cure rate, did not improve first-service conception rate and early embryonic mortality, and did not decrease calving-to-conception interval.

Key words: dextrose, endometritis, uterine health, reproduction

INTRODUCTION

Reproductive efficiency is of great importance for the dairy industry and significantly affects the overall economic outcome of a dairy enterprise. A healthy reproductive tract is a prerequisite for satisfactory reproductive performance. Clinical endometritis (CE) refers to the presence of purulent or mucopurulent uterine exudates in the vagina occurring after the third week of lactation (Sheldon et al., 2006). *Escherichia coli*, *Trueperella pyogenes*, and *Fusobacterium necrophorum* are the primary bacterial causes of uterine diseases (Miller et al., 2007; Bicalho et al., 2010; Santos et al., 2011). In the first days postpartum, *E. coli* is the predominant bacterium in the infected uterus and is highly associated with uterine inflammation and impaired reproductive performance (Bondurant, 1999; Bicalho et al., 2012; Machado et al., 2012a). This early uterine contamination with *E. coli* leads to subsequent infection by *F. necrophorum* and *T. pyogenes* at 7 and >25 d postpartum, respectively (Dohmen et al., 2000; Bicalho et al., 2012), which are associated with both metritis (Santos et al., 2011; Bicalho et al., 2012; Machado et al., 2012a) and endometritis (Williams et al., 2005; Machado et al., 2012b).

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In North America, metritis affects 10 to 20% of cows (LeBlanc et al., 2011). Cows affected with metritis develop moderate to severe illness and are commonly treated with systemic antibiotics, such as penicillin or third-generation cephalosporins with reasonable efficiency (Smith et al., 1998; Drillich et al., 2001; McLaughlin et al., 2012). On the other hand, CE, a disease that affects approximately 28% of cows, with herd level prevalence ranging from 6.7 to 47.0% (Bicalho et al., 2010; Dubuc et al., 2011), is not accompanied by systemic signs of illness. However, CE impairs reproductive performance (Barlund et al., 2008; Plontzke et al., 2011), and consequently has a negative economic effect on the modern dairy industry (Lee and Kim, 2007). Many CE treatment strategies have been evaluated to date, such as intrauterine-administered antibiotics (Runciman et al., 2008; Galvão et al., 2009b; McDougall et al., 2013), chlorhexidine (Gilbert and Schwark, 1992), enzymes (Drillich et al., 2005), hypertonic dextrose (Brick et al., 2012), and the systemic administration of PGF_{2α} (LeBlanc et al., 2002; Kasimanickam et al., 2005; Lima et al., 2013).

Systemic use of PGF_{2α} is one of the most investigated treatments for endometritis, and its efficacy as a CE treatment remains controversial. LeBlanc et al. (2002) reported a detrimental effect of PGF_{2α} administration between 20 and 26 DIM to cows that were diagnosed with CE and did not have a corpus luteum (CL). Kasimanickam et al. (2005) reported that a single dose of PGF_{2α} between 22 to 33 DIM improved first-service conception rate (FSCR) and increased the overall hazard of pregnancy. Other studies reported that systemic administration of PGF_{2α} did not have any effect on uterine health or reproductive performance (Galvão et al., 2009a; Dubuc et al., 2011; Lima et al., 2013).

The use of intrauterine-administered antibiotics is currently not approved in the United States. However, a single intrauterine infusion of 0.5 g of cephapirin (first generation cephalosporin) has been reported as an efficacious treatment against CE (Runciman et al., 2008; McDougall et al., 2013). On the other hand, the intrauterine administration of ceftiofur (third generation cephalosporin) was reported to decrease the prevalence intrauterine infection by *T. pyogenes* but did not improve CE or reproductive performance (Galvão et al., 2009b).

Recently, Brick et al. (2012) suggested a novel treatment for CE; cows diagnosed with CE were treated with single intrauterine infusion of 200 mL of a 50% dextrose solution. It was proposed that the proportion of cows that were clinically cured was higher for treated cows, and the authors suggested that treated cows had increased FSCR. Their hypothesis was that the intrauterine administration of a hypertonic sugar

solution would inhibit bacterial growth (Chirife et al., 1983). Consequently, affected cows would recover from infection, mitigating the detrimental effects of endometritis on reproductive performance. Replication of these findings would provide more evidence that intrauterine administration of dextrose could be implemented as a routine practice in the reproductive management of dairy herds.

Therefore, the main objective of this study was to evaluate the effect of intrauterine administration of 50% dextrose solution on CE cure, FSCR, and early embryonic mortality (EEM). Our secondary objective was to evaluate the association of retained placenta (RP), metritis, CE, anovulation, hyperketonemia (HYK), and BCS with FSCR and EEM at first service, and reproductive performance.

MATERIALS AND METHODS

Farms and Management

In this study, 1,313 cows housed on 4 large commercial dairy farms located near Ithaca, New York, were enrolled from May 8, 2013, until July 8, 2013. Farm 1 milked 3,300 cows, farm 2 milked 1,000 cows, farm 3 milked 1,800 cows, and farm 4 milked 1,300 cows. Cows were housed in free-stall barns with concrete stalls covered with mattresses and bedded with manure solids in farm 1, waste paper-pulp in farm 4, and sand in farms 2 and 3. Cows were milked 3 times daily in milking parlors and their milk production was automatically recorded on a daily basis. Cow reproductive management was based on a combination of Presynch (Moreira et al., 2001), Ovsynch (Pursley et al., 1995), Resynch (Fricke et al., 2003), and detection of estrus.

Study Design, Case Definition, and Treatments

Prior to commencement of the study, sample size calculation was performed. We assumed that the CE cure rate in control cows would be 60%. Considering a β -value of 0.8, an α value of 0.05, and that intrauterine dextrose infusion would increase the cure rate to 75%, a sample size of 165 cows per treatment group was considered sufficient. Based on our assumptions that the CE incidence would be around 30% (Bicalho et al., 2010; Lima et al., 2013), we evaluated 1,313 cows, which would be sufficient to enroll over 165 cows per treatment group.

Cows were enrolled on a weekly basis; all cows at 7 ± 3 DIM available during the enrollment period were included in the study. At 7 ± 3 DIM, vaginal discharge was retrieved using the Metricheck device (SimcroTech, Hamilton, New Zealand). The presence of fetid, watery,

red-brown uterine discharge was defined as metritis. Blood was collected from the coccygeal vein/artery using a Vacutainer tube without anticoagulant and a 20 gauge $\times$ 2.54 cm Vacutainer needle (Becton, Dickinson and Company, Franklin Lakes, NJ). All blood samples were transported to the laboratory on ice and spun in a centrifuge at $2,000 \times g$ for 15 min at 4°C; serum was harvested and frozen at -80°C . Serum samples were tested for BHBA concentrations using an electronic BHBA measuring system (Precision Xtra, Abbott, Abingdon, UK) already validated for animal use. (Gordon et al., 2013). Cows with blood BHBA ≥ 1.2 mmol/L were diagnosed as having HYK, as previously reported (McArt et al., 2011). Additionally, BCS were determined for all study cows at 30 ± 3 DIM using a 5-point scale with a quarter-point system (Edmonson et al., 1989).

At 30 ± 3 DIM, vaginal discharge was visually evaluated using the Metrichick device. Vaginal discharge was scored using a modified 0 to 5 scale: 0 = no secretion material retrieved, 1 = clear mucus, 2 = flecks of pus in the vaginal discharge, 3 = $<50\%$ pus in the vaginal discharge, 4 = $>50\%$ pus in the vaginal discharge, 5 = watery, fetid vaginal discharge (McDougall et al., 2007; Dubuc et al., 2010). Cows that had score ≥ 3 were considered to have CE.

A randomized field trial study design was used; cows diagnosed with CE ($n = 175$) were randomly allocated into 2 treatment groups: control and treatment. Cows enrolled in the treatment group received intrauterine infusion of 200 mL of 50% dextrose solution; cows were restrained, the perineum area was cleansed and disinfected with 70% ethanol, and a plastic infusion pipette was introduced into the cranial vagina and manipulated transrectally through the cervix into the uterus. A total of 200 mL of sterile 50% dextrose solution was infused into the uterus. Control cows did not receive placebo. Vaginal discharge was re-evaluated at 44 ± 3 DIM using the same scoring system previously described. As the definition of clinical cure for CE is not well established yet; we approached clinical cure as the absence of signs characterizing CE. Therefore, cows that did not have a purulent or mucopurulent vaginal discharge at 44 ± 3 DIM (score <3) were considered cured from CE.

To determine if cows had ovulated at least once before 44 ± 3 DIM, ovarian ultrasonography was performed at 30 ± 3 DIM and at 44 ± 3 DIM using a portable ultrasound (Ibex, EI Medical Imaging, Loveland, CO) equipped with a 7.5-MHz linear-array transducer. Absence of a visible CL in both examinations was considered anovulation. Cows that were diagnosed with a visible CL at 30 ± 3 DIM were not re-evaluated at 44 ± 3 DIM. Pregnancy was diagnosed on d 30 ± 3 post-AI via rectal ultrasonography. Pregnancy per AI was

calculated as the number of pregnant cows on d 30 ± 3 divided by the number of cows that were inseminated. Cows diagnosed as pregnant were reexamined 35 d later for detection of viable pregnancy on d 65 ± 3 ; EEM was defined as the absence of a viable pregnancy on d 65 ± 3 .

Statistical Analyses

To facilitate data analysis and interpretation, the variable **BCSG** (BCSG = 1 if BCS at 30 ± 3 DIM was equal to or less than 2.75; BCSG = 2 if BCS at 30 ± 3 DIM was greater than 2.75 and equal to or less than 3.25; BCSG = 3 if BCS at 30 ± 3 DIM was greater than 3.25) was created. Descriptive statistical analyses were undertaken in SAS using the FREQ and MEANS procedures (SAS Institute Inc., Cary, NC).

To assess the effect of treatment on CE cure rate, FSCR, and EEM, 3 logistic models were fitted in SAS using the GLIMMIX procedure. To assess the effect of treatment on likelihood of conceiving, a multivariable Cox's proportional hazard was fitted using the PHREG procedure in SAS. Cows were right-censored if not diagnosed as being pregnant before culling, death, or the end of the data collection period, which was 200 DIM. Treatment (dextrose or control), parity, RP and metritis incidences, HYK, and BCSG were offered to the models. Independent variables and their respective interactions were removed from the models manually and in a stepwise manner when $P < 0.10$; the variable treatment was forced into the models even in the absence of statistical significance. Contrasts were performed to compare the effect of treatment versus control, and the effect of CE (treatment and control cows combined) versus cows not affected with CE in the logistic regression models. To illustrate the effect of treatment on reproductive performance, a Kaplan-Meier survival analysis was performed using Medcalc version 12.2.1.0; the Logrank test was used to compute the P -value.

To evaluate the effect of each risk factor on FSCR and EEM at first service, 2 groups of logistic models were fitted using the GLIMMIX procedure of SAS. For the first and second group of logistic regression models, the dependent variables were FSCR and EEM at first service, respectively. The independent variables of interest were RP, metritis, CE, anovulation, HYK, and BCSG. Parity and the interaction term between parity and the independent variable of interest were added to all models.

The effect of several variables on reproductive performance was analyzed by a multivariable Cox's proportional hazard using the PHREG procedure in SAS. The independent variables offered to the model included

parity, BCSG, and RP, metritis, CE, and anovulation incidences. Biologically plausible 2-way interactions were tested; independent variables and their respective interactions were kept when $P < 0.10$.

Farm was included under the STRATA statement in the Cox proportional hazard analysis and as a random variable in all other statistical models described above.

RESULTS

Descriptive Statistics

Descriptive statistics regarding the incidences of CE, metritis, RP, and anovulation for all cows screened in the study are presented in Table 1. The incidence of CE was 14.1%. Therefore, only 175 cows were eligible to be enrolled in the in the field trial. Additionally, descriptive statistics regarding average BCS at treatment, average blood BHBA concentration at 7 ± 3 DIM, number of animals enrolled in each farm, and parity of the 175 cows that were randomly allocated into 1 of the 2 treatment groups are presented in Table 2.

Effect of Intrauterine Infusion of Dextrose on CE Cure Rate and Reproduction

Effects of intrauterine infusion of dextrose on CE cure rate, FSCR, and EEM at first service are presented in Table 3. Intrauterine infusion of dextrose had a strong tendency to decrease CE cure rate compared with controls ($P = 0.06$). Control cows were at 1.85 higher odds of being cured than cows treated with intrauterine infusion of dextrose; cure rate was 63.2 and 48.4%, respectively ($P = 0.06$, power = 0.52). Addi-

tionally, treatment did not have an effect on FSCR ($P = 0.55$, power = 0.10) and EEM at first service ($P = 0.82$, power = 0.07). Treatment also did not affect the likelihood of conceiving during the lactation; treated cows were 1.07 times more likely to conceive than control cows ($P = 0.74$). The median calving-to-conception interval for control and dextrose-infused cows was 144 and 159 d, respectively ($P = 0.99$, Figure 1).

Effects of RP, Metritis, CE, Anovulation, HYK, and BCS at 30 ± 3 DIM on Reproduction

The effects of RP, metritis, CE, anovulation, HYK, and BCS at 30 ± 3 DIM on FSCR and EEM at first service are presented in Table 4. Cows that did not have RP were at 2.00 increased odds of conceiving at first service than cows that had RP ($P = 0.01$). Additionally, cows that did not have CE had 1.62 higher odds of conceiving at first service than cows that had CE. Moreover, cows with normal blood BHBA concentration at 7 ± 3 DIM tended to have better conception rate at first service than cows diagnosed with HYK; they had 1.30 times higher odds of conceiving than hyperketonemic cows ($P = 0.10$).

Cows that had RP were at 3.36 times higher odds of losing their pregnancy than cows that did not have RP ($P = 0.01$). In addition, cows diagnosed with CE were at 2.16 higher odds of losing their pregnancy than cows without CE ($P = 0.06$).

A multivariable Cox’s proportional hazard model was performed to evaluate the effect of several variables on reproduction; the variables RP, CE, parity, anovulation, and the interaction term between parity and anovulation were retained in the model (Table

Table 1. Descriptive statistics of 1,313 cows enrolled in the study: incidence of clinical endometritis, metritis, retained placenta, and anovulation by parity and farm

Item	n	Incidence of CE ¹	Incidence of metritis ²	Incidence of RP ³	Incidence of anovulation ⁴
Parity					
1	537	12.3	27.9	4.5	25.3
2	391	13.9	16.6	8.2	27.3
≥3	385	17.2	18.2	12.7	31.6
Total	1,313	14.1	21.7	8.0	27.7
Farm					
1	651	15.7	23.2	8.1	27.4
2	191	16.7	22.0	5.7	29.1
3	293	11.9	20.5	7.8	26.4
4	178	9.8	18.0	10.1	29.4
Total	1,313	14.1	21.7	8.0	27.7

¹CE = clinical endometritis, defined as the presence of mucopurulent vaginal discharge retracted using the Metrichick device (SimcroTech, Hamilton, New Zealand).

²Metritis, defined as presence of fetid, watery, red-brown uterine discharge at 7 ± 3 DIM.

³RP = retained placenta, where cows fail to release their fetal membranes within 24 h of calving.

⁴Anovulation, defined as the absence of corpus luteum on ultrasonography evaluated at 30 ± 3 DIM and 44 ± 3 DIM.

Table 2. Descriptive statistics of treatment groups

Item ¹	Control	Dextrose	<i>P</i> -value
Average BCS at 30 ± 3 DIM (±SE)	2.60 (0.04)	2.62 (0.05)	0.71
Average blood BHBA concentration (mmol/L) at 7 ± 3 DIM (±SE)	1.28 (0.10)	1.25 (0.09)	0.80
Enrolled animals on farm 1 (%)	47 (49.5)	48 (50.5)	0.44 ²
Enrolled animals on farm 2 (%)	19 (63.3)	11 (36.7)	
Enrolled animals on farm 3 (%)	18 (54.5)	15 (45.5)	0.79 ³
Enrolled animals on farm 4 (%)	7 (41.2)	10 (58.8)	
Enrolled animals in parity 1 (%)	34 (54.8)	28 (45.2)	
Enrolled animals in parity 2 (%)	25 (48.1)	27 (51.9)	
Enrolled animals in parity >2 (%)	32 (52.5)	29 (47.5)	0.42 ⁴
Enrolled animals in BCSG 1 (%)	54 (54.5)	45 (45.5)	
Enrolled animals in BCSG 2 (%)	27 (52.9)	24 (47.1)	0.05
Enrolled animals in BCSG 3 (%)	10 (40.0)	15 (60.0)	
Enrolled animals with RP (%)	29 (31.9)	16 (19.1)	0.73
Enrolled animals with metritis (%)	41 (45.0)	40 (47.6)	0.66
Enrolled animals with HYK (%)	35 (38.5)	35 (41.2)	
Total enrolled animals (%)	91 (52.0)	84 (48.0)	

¹BCSG = BCS group; BCS was measured at 30 ± 3 DIM, using a 5-point scale with a quarter point system as described by Edmonson et al. (1989). BCSG = 1 (BCS <2.75); BCSG = 2 (BCS ≥2.75 and <3.25); and BCSG = 3 (BCS ≥3.25). RP = retained placenta; HYK = hyperketonemia.

²*P*-value applies to enrolled animals on farms 1 to 4.

³*P*-value applies to enrolled animals in parity 1, 2, and >2.

⁴*P*-value applies to enrolled animals in BCSG 1, 2, and 3.

5). Cows that did not have RP were 1.38 times more likely to conceive than cows that had RP ($P = 0.04$). Additionally, cows not diagnosed with CE were 1.40 times more likely to conceive than cows that had CE ($P < 0.01$). Furthermore, anovulation was only associated with reproductive performance in second parity cows; cows in their second parity that ovulated at least once before 44 ± 3 DIM were 1.42 times more likely to conceive than cows in parity 2 that did not ovulate ($P = 0.02$).

DISCUSSION

The main objective was to evaluate the efficacy of a single intrauterine infusion of 200 mL of a 50% dextrose

solution in treating CE, and the subsequent effect of this treatment on reproductive performance. Such a treatment was proposed to be effective in a recently published study (Brick et al., 2012). However, in the present study, intrauterine infusion of a hypertonic solution of dextrose tended to be detrimental for uterine health; only 48.4% of treated cows were cured from endometritis 14 d after treatment, whereas 63.2% of control cows were cured from the disease ($P = 0.06$). Additionally, intrauterine dextrose infusion did not have an effect on calving-to-conception interval, FSCR, and EEM at first service. However, some key points should be discussed when comparing our findings with the ones reported by Brick et al. (2012). First, whereas we used the Metricheck device to classify the vaginal

Table 3. The effect of intrauterine infusion of dextrose on clinical endometritis (CE) cure rate, first-service conception rate (FSCR), and early embryonic mortality (EEM) at first service

Item		n	Cure rate (%)	Odds ratio (95% CI)	<i>P</i> -value	
Cows with CE	Dextrose	84	48.4	Baseline	0.06	
	Control	91	63.2	1.85 (0.98–3.46)		
			FSCR (%)		C1 ¹	C2 ²
Cows with CE	Dextrose	84	24.9	Baseline	0.55	0.02
	Control	91	29.3	1.25 (0.59–2.62)		
Cows without CE		1,065	39.5	1.96 (1.11–3.48)		
			EEM at first service (%)			
Cows with CE	Dextrose	91	24.8	Baseline	0.82	0.16
	Control	84	21.7	0.84 (0.18–3.88)		
Cows without CE		1,065	11.8	0.41 (0.12–1.35)		

¹C1 = contrast 1, dextrose versus control.

²C2 = contrast 2, cows without clinical endometritis versus dextrose and control.

Figure 1. Kaplan-Meier survival analysis of calving-to-conception interval by treatment groups: treatment (intrauterine infusion of 200 mL of 50% dextrose, $n = 84$) or control (no infusion, $n = 91$). The median calving-to-conception interval for control (solid line) and dextrose-infused (dashed line) groups were 144 and 159 d, respectively ($P = 0.99$).

discharge (Dubuc et al., 2010; Bicalho et al., 2010; Lima et al., 2013), Brick et al. (2012) scored the vaginal discharge with the vaginoscopy technique, using a different scoring system. In our study, cows were diagnosed with endometritis when they had any presence of pus beyond flecks in vaginal discharge. In Brick et al. (2012), they used a scoring system that did not take into account vaginal contents composed of less than 50% purulent content, but with more than only flecks of pus. Therefore, it is unclear if cows with less than 50% pus, but more than just flecks in vaginal discharge would have been diagnosed with CE, thus being eligible to be enrolled in their study. This variation in CE definition can partially explain the disagreement between the findings regarding the effect of dextrose infusion on clinical cure. Additionally, they defined clinical cure as a cow with CE (more than 50% pus in vaginal discharge) before treatment that subsequently had clear mucus 14 d after treatment; thus, cows with flecks of pus in their vaginal discharge were not considered as cured. Although they observed a beneficial effect of treatment on uterine health, reporting a significant higher proportion of treated cows having clear mucus, after 14 d of treatment, which could be considered the

healthiest mucus score, we believe that, based on their initial definition of CE, cows with flecks of pus should also be classified as clinically cured. It is important to highlight that their results regarding reproductive parameters might have been misinterpreted. Although the proportion of cows that conceived in the first service was numerically higher for cows that received the intrauterine infusion of dextrose when compared with control cows, the P -value presented by them was 0.10. This should be interpreted cautiously as it might lead to a type I statistical error. They admittedly mentioned in their discussion section that more cows diagnosed with CE would be needed to conclude that the intrauterine dextrose infusion has beneficial effects on FSCR and pregnancy maintenance.

Clinical endometritis incidence in the present study was 14.1%. We performed our original sample size calculations based on an expected a CE prevalence of 20 to 40% of the study population (Bicalho et al., 2010; Lima et al., 2013) and the lower than expected CE prevalence observed certainly decreased the statistical power of the study. However, despite the unexpectedly low CE incidence, we observed a potentially detrimental effect of dextrose infusion on CE cure rate ($P = 0.06$). Ad-

Table 4. The effect of retained placenta, metritis, clinical endometritis, anovulation, hyperketonemia (HYK), and BCS on first-service conception rate (FSCR) and early embryonic mortality (EEM) at first service

Variable	Level	n	Adjusted proportion of cows pregnant (%)	Adjusted proportion of EEM (%)	Odds ratio (95% CI)	P-value		
						Main ¹	Parity	Main × parity
FSCR								
RP ²	Yes	105	23.9	—	Baseline	0.01	0.01	0.33
	No	1,208	38.8	—	2.01 (1.15–3.53)			
Metritis ³	Yes	285	33.6	—	Baseline	0.21	0.01	0.33
	No	1,028	38.7	—	1.25 (0.88–1.77)			
CE ⁴	Yes	175	28.6	—	Baseline	0.01	0.05	0.84
	No	1,065	39.4	—	1.62 (1.11–2.37)			
Anovulation ⁵	Yes	334	33.5	—	Baseline	0.14	<0.01	0.24
	No	872	38.7	—	1.25 (0.93–1.70)			
HYK ⁶	Yes	409	32.8	—	Baseline	0.10	<0.01	0.14
	No	904	38.9	—	1.30 (0.95–1.78)			
BCSG ⁷	1	601	34.7	—	1.37 (0.55–3.42)	0.20	<0.01	0.45
	2	421	39.8	—	1.70 (0.68–4.26)			
	3	214	28.0	—	Baseline			
EEM at first service								
RP ²	Yes	105	—	31.5	3.36 (1.20–9.45)	0.02	0.26	—
	No	1,208	—	12.0	Baseline			
Metritis ³	Yes	285	—	16.3	1.38 (0.65–3.00)	0.40	0.24	0.70
	No	1,028	—	12.4	Baseline			
CE ⁴	Yes	175	—	22.3	2.16 (0.97–4.82)	0.06	0.46	0.92
	No	1,065	—	11.7	Baseline			
Anovulation ⁵	Yes	334	—	12.5	1.01 (0.42–2.40)	0.98	0.07	0.19
	No	872	—	12.4	Baseline			
HYK ⁶	Yes	409	—	12.8	1.02 (0.45–2.31)	0.95	0.90	0.04
	No	904	—	12.5	Baseline			
BCSG ⁷	1	601	—	13.9	2.43 (0.52–11.38)	0.52	0.24	—
	2	421	—	13.4	2.33 (0.51–10.52)			
	3	214	—	6.2	Baseline			

¹P-value of the main effect in the model.²RP = retained placenta, where cows fail to release their fetal membranes within 24 h of calving.³Metritis = presence of fetid, watery, red-brown uterine discharge at 7 ± 3 DIM.⁴CE = clinical endometritis, defined as the presence of mucopurulent vaginal discharge retracted using the Metricheck device (SimcroTech, Hamilton, New Zealand).⁵Anovulation, defined as the absence of corpus luteum on ultrasonography evaluated at 30 ± 3 DIM and 44 ± 3 DIM.⁶HYK = hyperketonemia, defined as BHBA blood concentration equal or greater than 1.2 mmol/L, at 7 ± 3 DIM.⁷BCSG = BCS group; BCS was measured at 30 ± 3 DIM, using a 5-point scale with a quarter point system as described by Edmonson et al. (1989). BCSG = 1 (BCS <2.75); BCSG = 2 (BCS ≥2.75 and <3.25); and BCSG = 3 (BCS ≥3.25).**Table 5.** Cox's proportional hazard analysis evaluating the effect of several variables on the hazard of pregnancy; the variables retained placenta, clinical endometritis, parity, anovulation, and the interaction term parity × anovulation ($P = 0.09$) were retained in the model

Variable	Level	n	Adjusted hazard ratio	P-value
RP ¹	Yes	105	Baseline	0.04
	No	1,208	1.38	
CE ²	Yes	175	Baseline	<0.01
	No	1,065	1.40	
Parity × anovulation ³	Parity 1 and anovulation	127	Baseline	0.32
	Parity 1 and cycling	374	1.13	
	Parity 2 and anovulation	100	Baseline	0.02
	Parity 2 and cycling	266	1.42	
	Parity >2 and anovulation	107	Baseline	0.48
	Parity >2 and cycling	232	0.90	

¹RP = retained placenta, where cows fail to release their fetal membranes within 24 h of calving.²CE = clinical endometritis, defined as the presence of mucopurulent vaginal discharge retracted using the Metricheck device (SimcroTech, Hamilton, New Zealand).³Anovulation, defined as the absence of corpus luteum on ultrasonography evaluated at 30 ± 3 DIM and 44 ± 3 DIM.

ditional studies have evaluated the effect of intrauterine infusion of monocarbohydrates on intrauterine health of animals (King et al., 1998; Machado et al., 2012a). It was reported that in mares, intrauterine infusion of mannose decreased the incidence of positive cultures 24 h after intrauterine challenge with *E. coli*, suggesting that mannose was effective in reducing bacterial infection in the equine endometrium (King et al., 1998). On the other hand, previous work from our group found no effect of intrauterine treatment of 50 g of mannose at 2 ± 1 DIM on the incidence of metritis or endometritis, and reproductive performance in dairy cows (Machado et al., 2012a). The rationale for the use of intrauterine treatment of mannose is the inhibition property of this carbohydrate on the adhesion of *E. coli* onto epithelial surfaces, which is mediated by the type 1 fimbriae adhesive protein FimH (Mooi and de Graaf, 1985; Krogfelt et al., 1990). Previous findings suggest that the fimbriae subunit FimA of *T. pyogenes* plays an important role in the virulence of *T. pyogenes* to the bovine endometrium (Santos et al., 2010; Bicalho et al., 2012); however, in contrast to FimH, FimA does not possess a carbohydrate binding site (Sharon, 2006). Therefore, it is unreasonable to speculate that dextrose would bind to FimA, inhibiting *T. pyogenes* to adhere on the uterus epithelial surface. Otherwise, it was suggested that the intrauterine infusion of dextrose might have controlled intrauterine bacterial infection by reducing the water activity (Brick et al., 2012), which is required for bacterial survival (Chirife et al., 1983). The water activity will only be reduced with highly concentrated carbohydrate solutions; perhaps, in our study cows, the intrauterine volume was too large and the dextrose infusion was not sufficient to alter the water activity in the intrauterine environment. In this case, it is also possible that the intrauterine dextrose infusion might have nurtured the intrauterine pathogens, and instead of inhibiting, it might have stimulated their growth. Consequently, the cows' immune systems might have faced a higher load of pathogens in the treated group, failing to eliminate the infection and leading to a decreased CE cure rate.

The secondary objective of this study was to evaluate the association between several parameters (such as RP, metritis, CE, blood BHBA concentration at 7 ± 3 DIM, BCS at 30 ± 3 DIM, and anovulation) and reproductive performance. Here, we report that RP was an important factor for decreased fertility; cows that had their placenta retained had decreased odds of conceiving at first service ($P = 0.01$), higher odds of embryonic death ($P = 0.02$), and had lower hazard of becoming pregnant throughout their lactation ($P = 0.04$). Other studies had reported association between RP and reproductive parameters; it has been reported

that RP increased time to first service (Stevens and Dinsmore, 1997), increased services per conception (Holt et al., 1989), increased days open (Melendez et al., 2003), increased first-service EEM (Pereira et al., 2013), and decreased pregnancy rates (McDougall, 2001). Many treatments for RP have been evaluated, such as administration of prostaglandins and oxytocin (Stevens and Dinsmore, 1997), infusion of collagenase-containing products into the umbilical arteries of RP (Eiler and Hopkins, 1993), and intrauterine ozone flush (Zobel and Tkalcic, 2013). However, they have been shown to be ineffective or impractical. Therefore, RP is still an important uterine disorder, and novel treatments should be evaluated to ameliorate its detrimental effects on fertility of dairy cows.

Anovulation in our study was not associated with first-service EEM ($P = 0.98$), which is not in accordance with many other studies, where anovulation tended to be associated with embryonic death (Galvão et al., 2004; Chebel et al., 2006; Bisinotto et al., 2010). Although FSCR was numerically higher for cyclic cows compared with anovulatory cows, this difference was not significant ($P = 0.14$). Dubuc et al. (2012) also reported that anovulation was not significantly associated with FSCR. Conversely, the majority of previous studies reported that FSCR is significantly lower for anovulatory cows compared with cyclic cows (Walsh et al., 2007; Bisinotto et al., 2010; Galvão et al., 2010). In the present study, survival analysis showed that cyclic cows were more likely to conceive throughout the lactation than anovulatory cows. However, this detrimental effect of anovulation was conditional to parity. Anovulation was associated with lower hazards of pregnancy only in cows in parity 2 ($P = 0.02$); for primiparous cows, and cows in parity greater than 2, anovulation was not statistically associated with hazards of pregnancy ($P = 0.32$ and 0.48 , respectively). A previous study had also reported a detrimental effect conditional to parity of anovulation on the likelihood of conceiving during the lactation (Dubuc et al., 2012). Unlike our findings, they reported that the detrimental effect of anovulation on likelihood of conceiving was only important for cows in parity >2 . A conditional effect of parity was not observed by Galvão et al. (2010).

Metritis was not associated with impaired fertility in our study; here, metritis was defined as the presence of fetid, watery, red-brown uterine discharge, regardless of fever. It has been recently published that only cows diagnosed with puerperal metritis (foul uterine discharge followed by fever) had impaired reproductive performance, whereas cows diagnosed with clinical metritis (foul uterine discharge not followed by systemic signs) and healthy cows had similar reproductive performance (Giuliodori et al., 2013). Perhaps, if fever was

part of our case definition for metritis, we could have observed detrimental effects of puerperal metritis on fertility. On the other hand, CE, another postpartum uterine disease, was associated with decreased FSCR at first service ($P = 0.01$), decreased hazard of becoming pregnant during the lactation ($P < 0.01$), and tended to be associated with increased embryonic mortality ($P = 0.06$). Many other studies have previously reported the detrimental effects of endometritis on reproductive performance of dairy cows; it has been associated with lower first-service pregnancy risk (Dubuc et al., 2011), increased calving-to-conception interval (Dubuc et al., 2010), and increased services per conception and embryonic mortality (Lima et al., 2013).

In conclusion, intrauterine infusion of a 50% dextrose solution failed to cure CE and had no effect on FSCR, pregnancy maintenance, and calving-to-conception interval. Further research is needed to develop and evaluate a proper treatment to cure CE and ameliorate the detrimental effect of this disease on the reproductive performance of dairy cows.

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