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## Individual and combined effects of anovulation and cytological endometritis on the reproductive performance of dairy cows

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### ABSTRACT

The objective was to evaluate the individual and combined effect of anovulation and cytological endometritis (CTE) on the reproductive performance of dairy cows. A total of 1,569 cows from 3 data sets were used. In data set 1, 403 Holstein cows from 5 dairies in New York were used. In data set 2, 750 Holstein cows from 2 dairies, one in Florida and one in California were used. In data set 3, 416 dairy cows, 165 Holsteins, 36 Jerseys, and 215 Holstein-Jersey crossbreeds from a grazing dairy in Florida were used. Cyclicity and CTE was determined at  $35 \pm 3$  (data set 2) or  $49 \pm 3$  d in milk (data sets 1 and 3). A variable (VarCycCTE) containing all 4 possible permutations between cyclicity (cyclic = Cyc; anovular = Anov) and CTE (present = CTE; absent = Healthy) was created. In the combined data set (sets 1, 2, and 3), pregnancy per artificial insemination (P/AI) diagnosed at 30 to 38 d after first AI was affected by VarCycCTE, with AnovCTE cows having decreased P/AI compared with CycHealthy cows (21.3 vs. 46.7%), whereas AnovHealthy (37.9%) and CycCTE cows (36.0%) had intermediate P/AI. Pregnancy per artificial insemination for the individual data sets and for pregnancy diagnosed at 63 to 74 d after artificial insemination followed a similar pattern. Pregnancy loss was not affected by VarCycCTE. Hazard of pregnancy up to 300 d in milk was affected by VarCycCTE in the combined data sets 1 and 2, with AnovCTE [hazard ratio (HR) = 0.55], AnovHealthy cows (HR = 0.71), and CycCTE (HR = 0.8) having decreased hazard of pregnancy compared with CycHealthy cows. Median

days open were 200, 159, 145, and 121 for AnovCTE, AnovHealthy, CycCTE, and CycHealthy, respectively. Hazard of pregnancy for the individual data sets followed a similar pattern. In summary, both anovulation and CTE were negatively associated with reproductive performance and, when combined, they had an additive negative effect.

**Key words:** cyclicity, cytological endometritis, reproductive performance, dairy cow

### INTRODUCTION

Uterine diseases such as metritis and endometritis are highly prevalent in dairy cows (Sheldon et al., 2006). Cytological endometritis (CTE), which is characterized by increased PMNL migration into the uterine lumen (Kasimanickam et al., 2004; Gilbert et al., 2005), affects approximately 30% of lactating dairy cows, with the prevalence ranging from 5 to >70% around 50 DIM (Gilbert et al., 2005; Cheong et al., 2011). Delayed ovarian cyclicity or anovulation is another common disorder of dairy cows, with more than 20% of the lactating dairy cows being affected around 60 DIM (Cerri et al. 2004; Walsh et al., 2007; Santos et al. 2009) and with some herds having more than 40% anovulatory cows around that time (El-Zarkouny et al. 2004; Chebel et al. 2006; Santos et al. 2009).

Several studies have shown that both anovulation (Darwash et al. 1997; Santos et al. 2009; Galvão et al., 2010b) and CTE (Gilbert et al., 2005; Galvão et al., 2009a; Dubuc et al., 2010) are negatively associated with reproductive performance; namely, decreased pregnancy per AI (P/AI), increased pregnancy loss (PL), and increased time to pregnancy (Santos et al., 2004; Gilbert et al., 2005; Lima et al., 2013). Nonetheless, anovulation is positively associated with CTE (Galvão et al., 2010b), which could make the individual association between each condition and reproductive

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performance spurious, as either condition could be confounding the effect of the other. Therefore, the objective of the current study was to evaluate the individual and combined effect of anovulation and CTE on the reproductive performance of dairy cows.

## MATERIALS AND METHODS

### *Experimental Design and Sample Size*

This was a retrospective cohort study. The data were conveniently sampled from previous studies that had collected similar information (Bruno et al., 2007; Galvão et al., 2009a; Bittar et al., 2013; Ribeiro et al., 2013). A total of 1,569 cows from 3 separate data sets were used. Because the data were sampled by convenience, no a priori calculation of sample size existed.

For clarity, data are presented below as 3 separate data sets based on differences and similarities regarding the use of timed AI programs as part of the herd reproductive management (yes or no), housing type (confinement or pasture), and timing of CTE evaluation ( $35$  or  $49 \pm 3$  DIM).

### *Animals, Housing, and Feeding*

**Data Set 1.** A total of 403 Holstein cows (163 primiparous and 240 multiparous) from 5 commercial dairies located in upstate New York were used. Herd sizes ranged from 70 to 1,500 lactating cows, and the rolling herd average was approximately 11,500 kg of milk/cow per year. Cows were housed in freestall barns and milked 3 times per day. Cows were fed the same TMR within herd, formulated to meet or exceed the NRC (2001) nutrient requirements for lactating Holstein cows weighing 680 kg and producing 45 kg of 3.5% FCM. Cows from this data set were part of a previous study that evaluated the effect of  $\text{PGF}_{2\alpha}$  on uterine health and fertility (Galvão et al., 2009a).

**Data Set 2.** A total of 750 Holstein cows (308 primiparous and 442 multiparous) from 2 commercial dairies located in Northern Florida ( $n = 429$ ) and central California ( $n = 321$ ) were used. In Florida, the herd size was approximately 500 lactating cows, cows were housed in freestall barns and milked twice daily, and the rolling herd average was approximately 10,500 kg of milk/cow per year. A portion of the cows ( $n = 245$ ) from Florida were part of a previous study that evaluated the effect of GnRH administration early in lactation on uterine health and fertility (Bittar et al., 2013). In California, the herd size was approximately 1,500 lactating cows, cows were housed in dry lots and milked twice daily, and the rolling herd average was approximately 12,400 kg of milk/cow per year. Cows from

California were part of a previous study that evaluated the effect of uterine infusion with ceftiofur on uterine health and fertility (Bruno et al., 2007). Within herd, cows were fed the same TMR formulated to meet or exceed the NRC (2001) nutrient requirements for lactating Holstein cows weighing 680 kg and producing 45 kg of 3.5% FCM.

**Data Set 3.** A total of 416 multiparous cows (165 Holsteins, 36 Jerseys, and 215 Holstein-Jersey cross-breeds) from 1 commercial dairy located in north-central Florida were used. The herd size was approximately 1,200 lactating cows, the cows were housed on pasture and milked twice per day, and the rolling herd average was approximately 6,000 kg of milk/cow per year. Cows were maintained on irrigated pasture paddocks of 2.7 ha and managed in a daily rotational method, allowing a 15-d resting period. Cows were offered 7 to 13 kg of concentrates/d on an as-fed basis (86 to 88% DM) during and immediately after each milking, according to forage availability, which was estimated at the entrance to each paddock using rising plate meters. Cows from this data set were part of a previous study that evaluated the prevalence of periparturient diseases and effects on fertility (Ribeiro et al., 2013).

### *Evaluation of Cyclicity and CTE*

**Data Set 1.** Cyclicity was determined based on serum progesterone concentrations measured in samples collected at 21, 35, and  $49 \pm 3$  DIM as previously reported (Galvão et al., 2009a). Cows were classified as cyclic if the progesterone concentration was  $\geq 1$  ng/mL in at least 1 measurement, and were classified as anovular if the progesterone concentration was  $< 1$  ng/mL in all measurements. Uterine lavage was performed at  $49 \pm 3$  DIM to diagnose CTE, as previously described (Gilbert et al., 2005; Galvão et al., 2009a). Briefly, the perineum of the cow was cleansed and a 62.5-cm Flex Tip sterile plastic infusion pipette (Exodus Breeders Corp., York, PA) was manipulated through the cervix into the uterus; sterile saline solution (20 mL) was then injected into the uterus, agitated gently per rectum, and a sample of the fluid aspirated. The recovered fluid was centrifuged at  $50 \times g$  for 3 min at room temperature using a cytocentrifuge (Cyto-Tek; Sakura Finetechnical Japan Co. Ltd., Tokyo, Japan) directly onto a glass slide. After drying, the slides were fixed and stained using a Romanowsky type stain and examined under  $400\times$  magnification. Cells were identified as PMNL, large mononuclear leukocytes, small mononuclear leukocytes, and uterine epithelial cells. At least 200 cells were counted and results expressed as percentage of PMNL. The threshold used for CTE diagnosis was  $\geq 5\%$  PMNL, as previously reported (Gilbert et al., 2005).

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