J. Dairy Sci. 99:1–13
http://dx.doi.org/10.3168/jds.2015-10483

Diagnosis of uterine and vaginal disorders by different methodologies is affected by concentration of estradiol in plasma from lactating Holstein cows

B. F. Silper,* A. M. L. Madureira,* T. A. Burnett,* A. C. C. Fernandes,* F. M. Abreu,† D. M. Veira,* J. L. M. Vasconcelos,‡ and R. L. A. Cerri*[1]
*Faculty of Land and Food Systems, University of British Columbia, Vancouver, Canada V6T 1Z4
†Department of Animal Sciences, The Ohio State University, Columbus 43210
‡Department of Animal Production, São Paulo State University, Botucatu, Brazil 18168-000

ABSTRACT

The relationship between plasma estradiol concentration at time of examination and prevalence of uterine disorders, agreement among methods, and associations of diagnosis with pregnancy hazard and milk yield was studied in 268 Holstein cows examined at 30 ± 3 (exam 1) and 44 ± 3 d in milk (DIM; exam 2). Purulent vaginal discharge was sampled using 2 methods: gloved hand and Metricheck (Simcro, Hamilton, New Zealand; PVD; score ≥3). Percentage of polymorphonuclear leukocytes was determined by endometrial cytology (CYTO; exam 1: ≥18%, exam 2: ≥10%); diameter of uterine horns (UTH; >20 mm), diameter of the inner layer of the cervix (CVX; >20.5 mm), presence of fluid in the uterine lumen (FL), and ovarian structures were evaluated by ultrasonography. A blood sample was collected at each exam for estradiol analysis. Prevalence at exams 1 and 2 was, respectively, 14.2 and 18.5% (PVD), 21.4 and 10.1% (FL), and 40.6 and 50.2% (CYTO). Prevalence of PVD at exam 1 was greater among cows with estradiol ≥2 pg/mL (19.4 vs. 8.2%). Agreement of all methods with CYTO was poor, the greatest being between CYTO and FL (exam 1; kappa = 0.19). Agreement between CYTO and PVD, and between CYTO and FL (exam 1; kappa = 0.15 and 0.35, respectively) was higher among cows with estradiol ≥2 pg/mL. Likelihood of PVD at exam 1 was greater if cows were positive for CVX [odds ratio (OR) = 3.0], FL (OR = 2.6) or had estradiol ≥2 pg/mL (OR = 2.7). Likelihood of CYTO increased with dystocia (OR = 2.3) and FL (OR = 2.5). Estradiol did not influence diagnosis at exam 2. Positive FL or CYTO at exam 1 was associated with reductions in milk yield of 59 to 180 kg by 45 DIM. Pregnancy hazard until 250 DIM was reduced by CYTO at exam 1 (hazard ratio = 0.74) and by PVD (hazard ratio = 0.68) at exam 2. However, FL and CYTO reduced pregnancy hazard only when estradiol was ≥2 pg/mL (exam 1), whereas PVD reduced pregnancy hazard when diagnosed at exam 2 with estradiol <2 pg/mL. Overall, agreement was poor and effects of positive diagnosis differed according to method and DIM at exam. Estradiol concentration influenced prevalence, agreement, likelihood of positive diagnosis, and its effects on days to pregnancy.
Key words: cytology, endometritis, estradiol, fertility

INTRODUCTION

Bacterial contamination of the uterus occurs in most cows during the early postpartum period. Inflammation, delayed uterine involution, and impairment of fertility result from the persistence of this contamination and its progression to infection (Sheldon et al., 2006). Subclinical or cytological endometritis (defined as elevated percentage of PMN in endometrial cytology, in the absence of purulent vaginal discharge, **PVD**; Kasimanickam et al., 2004) and clinical endometritis (diagnosed by detection of PVD; LeBlanc et al., 2002; Pleticha et al., 2009) have been associated with reduced pregnancy risk. Similarly, both conditions have been associated with reduced conception rates (Kasimanickam et al., 2004) and increased days open (Barlund et al., 2008).

Definitions for the various uterine pathologies that affect postpartum cows have been proposed by Sheldon et al. (2006). There is still controversy regarding PVD, because it could be of vaginal, cervical, or uterine origin. Detection and scoring of vaginal discharge is an indirect assessment of endometritis (Pleticha et al., 2009), as PVD has been observed in absence of endometrial inflammation (Dubuc et al., 2010) and uterine contamination (Westermann et al., 2010). There is weak (Metricheck, Simcro, Hamilton, New Zealand;

Received October 2, 2015.
Accepted February 12, 2016.
[1]Corresponding author: ronaldo.cerri@ubc.ca

Dubuc et al., 2010) to moderate (vaginoscopy; Barlund et al., 2008) agreement between PVD and cytological endometritis. The terminology "PVD" is considered more appropriate than "clinical endometritis" (Dubuc et al., 2010) because this is a nonspecific indication of an inflammatory process (de Boer et al., 2014).

Barlund et al. (2008) observed approximately 90% specificity but low sensitivity when criteria such as PVD, presence of fluid in uterus, or increased endometrial thickness were compared with endometrial cytology. It has been suggested that presence of fluid in the uterine lumen could reduce recovery of PMN and influence diagnosis (Barlund et al., 2008). Presence of fluid is suggestive of defense through clearance mechanisms, whereas presence of PMN relates to the cellular response to infection (Kasimanickam et al., 2004; Barlund et al., 2008). Ultrasonography allows objective measurements of the uterus and cervix and detection of intraluminal fluid, although it does not seem to provide more accurate diagnosis, having cytology as the reference method, compared with evaluation of vaginal discharge (Sheldon et al., 2006; Barlund et al., 2008). Vaginoscopy and ultrasonographic evaluation of the reproductive tract showed similar sensitivity and specificity for prediction of pregnancy by 150 DIM (Barlund et al., 2008).

The agreement between methods for diagnosis of diseases of the reproductive tract could be impaired by the stage of the estrous cycle at examination. An endocrine milieu of high progesterone impairs immune function via reduced production of mucus by the cervix, myometrial contractions, uterine gland secretions, and PMN phagocytic activity, whereas a profile of high estradiol likely improves immunity (LeBlanc, 2008). Estradiol increases blood flow to the reproductive tract, synthesis of mucus, and PMN function (Hussain, 1989). The effects of estradiol and progesterone in the reproductive tract could affect diagnosis by altering the percentage of endometrial PMN, recovery of PMN in cytology due to presence of mucus, and detection of discharge from uterine or cervical origin due to degree of cervix openness. In support of this, Brodzki et al. (2015) reported that the percentage of PMN on endometrial cytology slides obtained from healthy cows during the follicular phase was 2 times greater than that observed on luteal phase samples.

The objectives of this study were (1) to evaluate the association of plasma estradiol concentration and ovarian structures at time of examination with diagnosis and agreement among methods, and (2) to study the effect of positive diagnosis by various methods and under different estradiol concentrations on days to pregnancy and milk yield.

MATERIALS AND METHODS

Animals and Management

This observational study took place at the University of British Columbia's Dairy Education and Research Centre (Agassiz, British Columbia, Canada) following the guidelines of the Canadian Council on Animal Care (CCAC, 2009). The local institutional animal care committee approved all experimental procedures.

Lactating Holstein cows (n = 268) were enrolled at 27 to 33 DIM. Data were collected until dry off or culling. Cows were housed in a sand-bedded freestall barn and fed a TMR to meet or exceed the requirements of a 620-kg Holstein cow producing 40 kg of 3.5% FCM per day (NRC, 2001). Data related to AI, pregnancy diagnosis, and milk production were recorded for the entire experimental period. Calving difficulty was recorded on a 1 to 4 scale (1 = unassisted; 2 = assistance by one person with hands only; 3 = assistance from more than one person and using chains; and 4 = abnormal presentation); stillbirths were also recorded. Milk production is presented as kilograms of milk produced until 45 DIM (**M45d**) and kilograms of milk adjusted to 305 d (**M305d**).

The reproductive management protocols from the dairy were followed during the experiment. Cows were artificially inseminated upon detection of estrus (visual and automated activity monitor; Heatime, SCR Engineers, Netanya, Israel) after a voluntary waiting period of 60 d. Cows not detected in estrus by 90 DIM or diagnosed nonpregnant were enrolled in timed ovulation synchronization programs (Santos et al., 2004). Pregnancy diagnosis was performed at 35 ± 6 d after AI (positive in the presence of uterine fluid with amniotic vesicle containing a viable embryo). The number of days open was recorded for each cow.

Reproductive Tract Examination

Each cow was examined twice in the postpartum period, at 30 ± 3 DIM (exam 1) and at 44 ± 3 DIM (exam 2; n = 521 exams; Figure 1), to determine score of vaginal discharge, diameter of the inner layer of the cervix (**CVX**) and of each uterine horn (**UTH**; reported values correspond to the largest horn), presence of fluid in uterus (**FL**) and of corpus luteum (**CL**), and percentage of PMN among endometrial cells on cytology slides. One person performed all diagnostic procedures. Body condition score was measured at exam 1 (1 to 5 scale; Ferguson et al., 1994). The complete exam was performed following milking (morning or afternoon), before cows returned to their pens.