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# Categorization of endometritis and its association with ovarian follicular growth and ovulation, reproductive performance, dry matter intake, and milk yield in dairy cattle

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## ABSTRACT

The objectives were to evaluate the effect of different categories of endometritis on follicular growth and ovulation, reproductive performance, dry matter intake (DMI), and milk yield (MY) in dairy cows. Lactating Holstein cows ( $n = 126$ ) were examined for endometritis on  $25 \pm 1$  day postpartum (DPP) using vaginoscopy, transrectal ultrasonography, and endometrial cytology to determine the presence and type of vaginal discharge, uterine fluid, and proportion of polymorphonuclear (PMN) cells, respectively. Cows that had mucopurulent vaginal discharge and/or presence of uterine fluid, no mucopurulent vaginal discharge or uterine fluid but 8% or more PMN, and mucopurulent vaginal discharge and/or uterine fluid and 8% or more of PMN were defined as having clinical (CLIN;  $n = 45$ ), cytological (CYTO;  $n = 15$ ), and clinical and cytological (CLINCYTO;  $n = 30$ ) endometritis, respectively. Cows that had none of the above pathological conditions were classified as unaffected (UNAF;  $n = 36$ ). The diameter of the largest follicle at first examination, intervals from calving to first dominant (diameter = 10 mm) follicle, preovulatory size (diameter = 16 mm) follicle, ovulation, presence of follicular cyst, and proportion of ovular cows at 35 and 65 DPP were recorded as the measures of follicular growth and ovulation. None of the ovarian follicular parameters analyzed was affected by categories of endometritis. The first service conception rate tended ( $P = 0.06$ ) to differ among categories of endometritis; cows that had CLIN and CLINCYTO endometritis were four times less likely to conceive to the first insemination compared to UNAF cows. Cows that had CLIN (hazard ratio: 0.52) and CLINCYTO (hazard ratio: 0.40) endometritis had decreased likelihood of pregnancy at 150 DPP compared to UNAF cows. Similarly, cows diagnosed as having CLINCYTO endometritis had decreased likelihood (hazard ratio: 0.48) of pregnancy at 250 DPP compared to UNAF cows. The DMI and MY up to 5 weeks postpartum were not affected by categories of endometritis. In summary, categories of endometritis as determined at 25 DPP did not affect follicular growth and ovulation, DMI, or MY. However, the combined (CLINCYTO endometritis) category had a negative impact on first service conception rate and subsequent services.

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## 1. Introduction

Postpartum uterine infection (metritis or endometritis) is considered as one of the most important factors affecting both longevity and profitability of a dairy herd [1]. Metritis is the inflammation of the entire thickness of the uterine

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wall, which typically occurs within 21 days postpartum (DPP) and generally accompanied by a fetid watery red-brown uterine discharge [2]. Endometritis is the inflammation of the endometrium and usually categorized as clinical (CLIN) and subclinical (cytological [CYTO]), which typically occurs at or after 21 DPP [2].

Clinical endometritis is defined as the detection of purulent (>50% pus) discharge between 20 and 33 DPP or mucopurulent (approximately 50% pus and 50% mucus) discharge between 26 and 33 DPP either by external observation of perineum or using vaginoscope [3]. Even though the observation and characterization of vaginal discharge remains a convenient method for determining CLIN endometritis, several other studies have contemplated cervical diameter [3–5], presence of uterine fluid [5–8], and endometrial thickness [5,7] as alternative measures to define CLIN endometritis.

Cytological endometritis is diagnosed by determining the proportion of polymorphonuclear (PMN) cells on a uterine cytology sample collected via cytobrush or uterine lavage techniques [6,7]. To determine whether a cow has CYTO endometritis, a diagnostic threshold for PMN percentage must be pre-established. Several thresholds have been reported, but usually, the PMN percentage used decreases as the DPP of sample collection increases. In this regard, Kasimanickam et al. [6] used a threshold of greater than 18% PMN from cytobrush samples collected between 21 and 33 DPP or greater than 10% PMN between 34 and 47 DPP. However, lower threshold (4%–8%) values have also been used when cytobrush samples were collected between 25 and 45 DPP [7–9].

Dubuc et al. [4] were the first to classify endometritis into CLIN, CYTO, and a combination of clinical and cytological (CLINCYTO) on the basis of type of vaginal discharge determined using the Metricheck device and percentage of PMN using the cytobrush technique. Although the combined category (CLINCYTO) had an additive effect on subsequent reproductive performance, the study did not evaluate the presence of uterine fluid [4]. A recent study [5] reported that cows with echogenic or anechogenic iu fluid detected between 15 and 21 DPP had lower odds of becoming pregnant to the first insemination at 70 DPP compared to cows without iu fluid. Therefore, in the present study, we took a different approach in defining CLIN endometritis as detection of mucopurulent vaginal discharge at vaginoscopy and/or presence of uterine fluid at transrectal ultrasonography, and CLINCYTO endometritis was defined as detection of mucopurulent vaginal discharge at vaginoscopy and/or presence of uterine fluid at transrectal ultrasonography combined with 8% or more of PMN.

It has been previously described that cows with uterine infections in the first week postpartum are susceptible to have perturbed ovarian follicular growth, specifically the growth of dominant follicle into a preovulatory size follicle, which leads to delayed ovulation [10,11]. However, the effect of categories of endometritis on ovarian follicular growth and ovulation has not been studied. Furthermore, it is unknown how different categories of endometritis may affect postpartum dry matter intake (DMI) and milk yield (MY) in lactating dairy cows.

Therefore, we hypothesized that categories of endometritis will compromise ovarian follicular growth and ovulation and alter reproductive performance, DMI, and MY. The main objective of this observational study was to evaluate the effect of categories of endometritis (CLIN, CYTO, and CLINCYTO) on early postpartum ovarian follicular growth (i.e., size of first largest follicle, intervals from calving to first appearance of dominant follicle [10 mm], preovulatory size follicle [16 mm] and ovulation, presence of follicular cyst and proportion of ovular cows at 35 and 65 DPP) and reproductive performance (i.e., first service conception rate and probability of pregnancy by 150 and 250 DPP). The secondary objective was to determine the association between the categories of endometritis and postpartum DMI and MY.

## 2. Materials and methods

### 2.1. Animals and housing

A total of 126 lactating Holstein cows (44 primiparous and 82 multiparous) was enrolled in this retrospective observational study. The study was conducted at the Dairy Research and Technology Centre of the University of Alberta, Edmonton, Canada. All cows were housed in tie-stalls and placed on a common ration containing alfalfa hay, barley silage and concentrate balanced for a lactating dairy cow of 690 kg body weight, producing 45-kg milk per day, according to National Research Council (2001) guidelines. All animal experimental procedures were approved by the University of Alberta Animal Care and Use Committee for Livestock, and animals were cared for according to the Canadian Council of Animal Care (1993) guidelines.

### 2.2. Examination of ovarian follicular growth, ovulation, and uterine fluid

Transrectal ultrasonography (Color Doppler scanner equipped with a multifrequency 5–10 MHz linear transducer, MicroMAXX, SonoSite Inc., Bothell, WA, USA) was used to examine the ovaries, twice weekly, from 7 ± 1 DPP until confirmation of first ovulation or until 35 DPP whichever occurred earlier. The diameter of the largest follicle at first examination, intervals from calving to first dominant (10 mm) follicle, preovulatory size (16 mm) follicle, and ovulation, presence of follicular cyst, and proportion of ovular cows at 35 and 65 DPP were recorded as the measures of ovarian follicular growth and ovulation.

Ovulation was confirmed by the absence of a dominant (≥10 mm in diameter) follicle that had been detected at the previous examination and subsequent CL formation [12]. Anovulatory follicles 25 mm or more in diameter that persisted for at least 10 days in the absence of a CL were defined as cysts [13]. The uteri of all cows were scanned on 25 ± 1 DPP to determine the presence or absence of fluid in uterine lumen, and if present, its type (anechogenic or echogenic). A single ultrasonographic examination was also performed at 65 ± 3 DPP to determine the presence of CL.

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