

## Optimizing the accuracy of detecting a functional corpus luteum in dairy cows

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### Abstract

The objectives were to evaluate the accuracy of detecting a functional CL by transrectal palpation and ultrasonography, and to optimize the accuracy of detecting a functional CL by ultrasonography in Holstein cows. In Experiment 1, four veterinarians performed transrectal palpation in 1250 cows at 37 d in milk (DIM), two veterinarians repeated transrectal palpation in 823 cows at 58 DIM, and one veterinarian performed 206 ultrasonographic examinations at 37 DIM. In Experiment 2, 987 and 983 ultrasonographic examinations were performed at 21 and 24 d after AI by one veterinarian for detection and measurement of CL. Cows with a blood progesterone concentration  $\geq 1$  ng/mL were assumed to have a functional CL. Sensitivity and specificity were optimized using receiver operating characteristic analysis. In Experiment 1, sensitivity of transrectal palpation for diagnosing a functional CL ranged from 33.3 to 59.9% at 37 DIM and from 48.3 to 68.4% at 58 DIM, whereas specificity ranged from 76.7 to 93.2% at 37 DIM and from 73.3 to 86.7% at 58 DIM. Sensitivity and specificity for ultrasonography were 89.4 and 45.7%, respectively. In Experiment 2, the sensitivity and specificity of ultrasonography were 97.3 and 38.1% at 21 d after AI, and were 97.9 and 51.0% at 24 d after AI. Sensitivity and specificity were optimized using a cutoff diameter of 23 mm at 21 d and 22 mm at 24 d, which resulted in sensitivity and specificity of 87.2 and 83.0% at 21 d, and 89.5 and 89.4% at 24 d after AI, respectively. Sensitivity was low and specificity was high for transrectal palpation, whereas ultrasonography resulted in high sensitivity and low specificity. Using a cutoff diameter during ultrasonography improved accuracy of detection of a functional CL compared with either ultrasonography without cutoff or transrectal palpation.

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### 1. Introduction

Transrectal palpation of the uterine contents and the ovaries is the most common method used by veterinarians for diagnosis of pregnancy and ovarian structures in beef and dairy cows; nonetheless, ultrasonography of the reproductive tract [1] is becoming more widespread [2]. The presence of a corpus luteum/corpora lutea (CL) before initiation of synchronization programs [3,4] or before the beginning of the breeding season [5] affects fertility and

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intervention may vary depending on the presence of a CL; cows that do not have a CL may be given intravaginal progesterone (P4) inserts (e.g. CIDR and PRID) and cows with a CL may receive prostaglandin (PG)  $F_{2\alpha}$  [6–8]. After diagnosis of nonpregnancy, it is also common to administer treatments based on the presence of a CL, as cows with a CL may receive  $PGF_{2\alpha}$  to induce a return to estrus, whereas cows without a CL may receive GnRH as part of protocols to synchronize estrus or ovulation [6,8].

In reviewing studies that evaluated methods for detection of a CL, the authors concluded that the positive predictive value (PPV) and the negative predictive value (NPV) of ultrasonography were greater than transrectal palpation; however, for growing and regressing CL, both methods resulted in low PPV and NPV [9]. Nevertheless, most studies evaluating accuracy of transrectal palpation used P4 concentration as the reference test, whereas studies evaluating the accuracy of ultrasonography used dissection of the ovary as the reference test [9]. Ultrasonography has been reported to have perfect agreement with dissection of the ovary [10]; however, if the intention is to detect a functional CL ( $P4 \geq 1$  ng/mL), dissection may not be a good reference test for evaluating ultrasonography, because CL structures may still be detected when blood P4 concentrations are  $<1$  ng/mL [7,11–13]. Detection of a CL when P4 is  $<1$  ng/mL may lead to poor specificity for ultrasonography if P4 concentration is used as the reference test; nevertheless, some studies have reported a high level of agreement between ultrasonography and P4 concentration [14–16].

Although several studies have evaluated the accuracy of transrectal palpation for the detection of a functional CL, either a small sample size was used, or only one technician evaluated all cattle. Furthermore, few studies have evaluated the accuracy of ultrasonography for the detection of a functional CL using P4 concentration as the reference test, and no attempts have been made to evaluate accuracy for different CL diameters. The CL diameter for cows with  $P4 < 1$  ng/mL was smaller than for cows with  $P4 \geq 1$  ng/mL [7,13], allowing for optimization of CL diameter cutoff for classifying cows as having a functional CL. Therefore, the objective of the current study was to evaluate the accuracy of functional CL detection by transrectal palpation and ultrasonography in lactating Holstein cows. An additional objective was to estimate a minimum diameter cutoff for considering a CL structure as a CL, to try to optimize the accuracy of detection of a functional CL by transrectal ultrasonography.

## 2. Materials and methods

### 2.1. Experiment 1

#### 2.1.1. Study farms

The experiment was conducted on five commercial Holstein dairy farms located in Cayuga County, New York, USA, between November 2004 and May 2005. Farms A–C each consisted of approximately 1000 milking cows, Farm D consisted of 2500 milking cows, and Farm E consisted of 800 milking cows. The rolling-herd averages were approximately 12,000 kg of milk. Lactating dairy cows were housed in freestall facilities and milked three times daily. Within herd, cows were fed the same total mixed ration, formulated to meet or exceed the NRC (2001) nutrient requirements for lactating Holstein cows weighing 680 kg and producing 45 kg of 3.5% fat-corrected milk.

#### 2.1.2. Transrectal examination and progesterone concentrations

Transrectal examination of the ovaries for detection of a CL was performed immediately before administration of the first  $PGF_{2\alpha}$  of the presynchronization program at  $37 \pm 3$  d in milk (DIM). The only objective of performing transrectal examination was to diagnose the presence or absence of a CL, as all cows received  $PGF_{2\alpha}$  (regardless of the diagnosis). Furthermore, no previous information on the cow's history was available to the veterinarians at the time of examination. Five veterinarians performed transrectal examinations in the farms enrolled in the study. Veterinarian-A (VET-A) performed transrectal palpation at Farm A, VET-BC performed transrectal palpation at Farms B and C, VET-D performed transrectal palpation on Farm D, and VET-E performed transrectal ultrasonography for diagnosing CL at Farm E, using a 7.5 MHz linear transducer (Sonovet 2000, Universal Medical System, Bedford Hills, NY, USA). For transrectal palpation, a CL was characterized as a distinct firm mass within the ovarian stroma and an ovulation papilla or crown could be protruding from the surface. For transrectal ultrasonography, a CL was characterized as a distinct grayish echogenic area within the ovarian stroma and could contain a cavity. No attempt was made to diagnose cystic ovarian follicles. Number and size of follicles and uterine characteristics such as echotexture, and intra-luminal fluid were not evaluated. Data regarding the number of CL, diameter of the CL, or presence of a cavity were not recorded. The diagnostic data from Veterinarians B and C were considered as one, because data were not recorded separately for the two

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