# Accuracy of color flow doppler ultrasonography to diagnose nonpregnancy in dairy cows 21 days after last insemination

**J. Houle**, DMV, IPSAV; **S. Buczinski**, Dr Vet, MSc, DACVIM; **J. Dubuc**, DMV, MSc, DVSc Faculté de médecine vétérinaire, Université de Montréal, 3200, rue Sicotte, St-Hyacinthe, QC, J2S 2M2, Canada

### Introduction

A previous study (Siqueira et al, 2013, J Dairy Sci 96:6461-6472) demonstrated that the doppler ultrasonography could be used to identify cows not pregnant 20 days after last insemination (DALI). Unfortunately, such study was not repeated and it remains unclear if this diagnostic tool could also be used 21 DALI. Therefore, the objective of this study was to quantify the accuracy of using Doppler ultrasonography to diagnose nonpregnancy (NPG) in lactating dairy cows 21 DALI.

#### **Materials and Methods**

A total of 618 dairy cows from 5 commercial herds were enrolled in a prospective observational study. Participating cows were examined by a veterinarian and enrolled in the study 21 DALI. At that time, the ovaries were examined by transrectal palpation and ultrasonography (Exago, ECM, Angoulème, France). Presence and size of corpus luteum (CL) and follicle were noted. When a CL was present, the color flow doppler function (Freq: 6.0 MHz; PRF: 4000 Hz) was activated to estimate the blood flow in the structure. A score was attributed based on the color activity observed. A score of D0 was given when there was no luteal activity; D1 when 10-20% of the CL surface was colored; D2 when 30-50% of the surface was colored; and D3 when >50% was colored. A blood sample was collected from coccygeal vessels to quantify progesteronemia. No matter the ovarian structure found at the time of examination, no intervention was made. Cows were then examined 32 DALI for pregnancy diagnosis by the regular herd veterinarian. The herd manager and veterinarian were blinded to ovarian findings found previously. Statistical analyses were calculated considering the cow as the unit of interest. Prevalence of NPG at 32 DALI was computed. Independent variables (CL size, progesteronemia, and doppler score) were dichotomized in various categories. The dependent variable was the pregnancy status at 32 DALI as the reference standard test for defining NPG. Contingency tables (2x2) were created to calculate sensitivity (Se), specificity (Sp) as well as positive and negative predictive values (PPV

and NPV). Intra- and inter-observer agreements (subsample of 100 cows; kappa value) were calculated.

#### Results

The prevalence of NPG at 32 dali was 63 %. At 21 DALI, the prevalence of NPG was 21, 7, and 50 % when using, respectively, doppler score D0, CL size < 15 mm, and progesteronemia < 1ng/mL as diagnostic criteria for NPG. The Se, Sp, PPV, and NPV for doppler score D0 were 52, 100, 100, and 57 %, respectively. The Se, Sp, PPV, and NPV for CL size < 15 mm were 11, 100, 100, and 42 %, respectively. The Se, Sp, PPV, and NPV for progesteronemia < 1 ng/mL were 67, 75, 81, and 59 %, respectively. Intra- and inter-observer agreement (kappa) values were 0.91 and 0.90, respectively.

## **Significance**

Overall, these results show that doppler ultrasonography has an excellent specificity to identify NPG cows but only a fair sensitivity. These results are not surprising as the doppler ultrasonography is targeted for ruling out pregnancy (cows that are positive with doppler ultrasonography have no active CL and are not pregnant) and not for confirming pregnancy (cows that are negative with the doppler ultrasonography have an active CL and may or may not be pregnant). This approach also provides excellent intra- and inter-observer agreement. In herds where a lot of ovulation synchronization protocols are used and a lot of cows are bred on the same day, this procedure could be useful to diagnose NPG earlier than the current transrectal examination procedure. Interestingly, the use of CL size < 15 mm or progesteronemia < 1 ng/mL as diagnostic criteria for NPG had poorer accuracy performance than the doppler ultrasonography. Further investigation should be done to refine the use of this diagnostic tool. For example, further analysis should be done to find the optimal diagnostic criteria (D0 only or D0-D1) for predicting NPG as well as to quantify the impact of using this diagnostic test on reproductive performance of cows and economics.