ABSTRACTS

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Evaluation of serum hormone measurements in the bitch

Bergeron, LH¹ and Gartley, CJ²

¹Department of Biomedical Sciences and ²Department of Population Medicine; University of Guelph, Guelph, ON, Canada, N1G 2W1, Canada.

lbergero@uoguelph.ca

OBJECTIVES AND METHODS: The first day serum progesterone concentrations exceed 2ng/mL is used as an indirect indication of the LH surge, an accepted reference for monitoring periovulatory events in the bitch (1). Chemiluminescence progesterone assays (CLIA) are more readily commercially available than radioimmunoassays (RIA), although RIA has a higher intra-assay precision (2). The objectives of this research were to 1) determine the RIA and CLIA progesterone concentrations on the day of the LH peak and on the day LH exceeded 1ng/mL; and 2) compare RIA and CLIA progesterone concentrations.

Mature bitches (n=12) were examined from proestrus until four days after the LH peak. Bitches were Beagle, hound-cross or Beagle-cross and weighed 7.5kg to 27.5kg. The onset of proestrus was based on the appearance of serosanguineous discharge and/or vulvar swelling. Jugular blood samples were collected once daily and transferred into plastic red top vials (BDTM Vacutainer, Becton Dickinson). Serum was transferred into polystyrene vials and assayed the same day for progesterone by CLIA, or stored at -20°C until RIA. Experimental procedures were carried out in accordance with Canadian Council on Animal Care guidelines and were approved by the Animal Care Committee at the University of Guelph (Guelph, Ontario, Canada).

CLIA (IMMULITE®, Diagnostic Products Corp., Los Angeles, CA, United States) was performed for the quantitative measurement of progesterone in serum at the Animal Health Laboratory (University of Guelph, Ontario, Canada). These assays were performed on the first day of examination and then every other day. Canine validated RIA was performed for the quantitative measurement of LH and progesterone at the Animal Health Diagnostic Center (Cornell University, Ithaca, NY, United States). The mean and standard deviation RIA (n=12) and CLIA (n=6) progesterone concentrations on the day of the LH peak were calculated. The mean and standard deviation RIA (n=10) and CLIA (n=5) progesterone concentrations on the day LH exceeded 1ng/mL were calculated. A simple linear regression (n=30) was performed in SAS (SAS Institute, Inc., Cary, NC), where dog was the repeated/random subject using the variance component error structure.

RESULTS: At the LH peak, RIA progesterone concentrations were 3.35ng/mL ± 1.058ng/mL (mean ± SD) and CLIA progesterone concentrations were 3.90ng/mL ± 1.559ng/mL (mean ± SD). On the first day LH exceeded 1ng/mL, RIA progesterone concentrations were 2.70ng/mL ± 0.973ng/mL and CLIA progesterone concentrations were 2.73ng/mL ± 1.942ng/mL. Only 6 of 11 bitches had their LH peak occur on the day RIA progesterone concentrations first exceeded 2ng/mL. This analysis could not be performed for CLIA progesterone concentrations.

On average, CLIA progesterone concentrations were higher than RIA progesterone concentrations. CLIA and RIA concentrations were significantly correlated (r²=0.96093). They were not, however, in agreement with one another, as demonstrated by their linear regression line having an intercept unlikely to be 0 (P=0.0007) and a slope unlikely to be 1 (P<0.0001). The regression equation was y = 0.8002x + 0.9780 [where y = CLIA (ng/mL) and x = RIA (ng/mL)].

CONCLUSIONS: Although CLIA and RIA progesterone concentrations both exceeded 2ng/mL on the day of the LH peak, only 54.5% had their LH peak occur on the day RIA progesterone concentrations first exceeded 2ng/mL. This demonstrates that RIA progesterone can serve as an estimate of the LH peak but should not be used to reliably monitor periovulatory events. CLIA and RIA progesterone concentrations may not both first exceed 2ng/mL on the same day. Thus, neither RIA nor CLIA progesterone may accurately identify the LH surge. Our results demonstrate that, on average, CLIA progesterone concentrations were higher than RIA progesterone concentrations. CLIA would thus identify the LH surge to occur earlier than RIA would. Overall, our data suggest that CLIA and RIA for progesterone analysis should only be used as estimates of the LH peak and that CLIA is not an ideal alternative for RIA.

(2) Kutzler MA, Mohammed HO, Lamb SV, Meyers-Wallen VN. Accuracy of canine parturition date prediction from the initial rise in preovulatory progesterone concentration. Theriogenology. 2003;60:1187-1196.