ABSTRACTS

ISCFR 2012

July 26-29, Whistler, Canada

7th International Symposium on Canine and Feline Reproduction

In a joint meeting with

EVSSAR 2012

15th Congress of the European Veterinary Society for Small Animal Reproduction

Editors: Gary England, Michelle Kutzler, Pierre Comizzoli, Wojciech Nizanski, Tom Rijsselaere and Patrick Concannon

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Accurate determination of serum progesterone using a Fluorescence Enzyme Immunoassay in the bitch

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OBJECTIVES AND METHODS: Veterinarians dealing with small animals reproduction often need the efficient quantification of serum progesterone, that is ordinarily used to identify the appropriate time of mating or artificial insemination, to predict the parturition date and to monitor pathological conditions, such as hypoluteoidism (1, 2, 3). The Radio Immunoassays (RIA) technique is considered the “gold standard” technique for progesterone quantification, but nowadays its replacement by other analytical techniques is prompted by the necessity to avoid the negative impact of handling hazardous radioactive material, the restriction of use to licensed laboratories, the waste handling problem, as well as the high cost of the counters (4, 5). Thus also in human laboratories, RIA has been replaced by alternative methods, primarily represented by Chemiluminescent (CLIA) and Fluorescent Immunoassay (FEIA) (4). In veterinary medicine there are few publications on the detection of progesterone concentration in canine serum with the use of CLIA (3, 5) and FEIA (6), but a deepen on this subject would be desirable. Thus the aims of the present study were: 1) to compare in the canine species the accuracy of serum progesterone detected by fluorescence enzyme immunoassay technique (FEIA; AIA®-360, TOSOH Corp. Japan) and by chemiluminescence (Access®, Beckman Coulter Inc. USA); 2) to detect the repetitiveness of FEIA by means of intra-assay test, inter-assay test and linearity-of-dilution test; 3) to compare the results of progesterone concentration in samples obtained in serum, in plasma-EDTA and in plasma-Litium Heparin; 4) to evaluate the interference of haemolysis on progesterone concentration detection by comparing the results of a blood sample purposely haemolysed with a non-haemolysed serum sample.

In order to compare the serum progesterone concentration obtained with the two different techniques (FEIA vs CLIA), a total of 40 clinically healthy, normocyclic, bitches of different breeds and ages were included in the study. Blood samples were collected by cephalic vein puncture and left to clot at room temperature prior centrifugation at 3.000 rpm for 10 minutes. Two aliquots of serum were subsequently prepared and frozen at -20°C until the time of the assay, always within 60 days after collection. An additional 24 samples obtained from as many bitches were used for the other purposes: a) 4 for the intra-assay test, five replicates each, during the same session; b) 6 for the inter-assay (10 replicates in 30 days); c) 3 neat serum samples, after four serial dilutions (1/2, 1/4, 1/8 and 1/16), were tested for the linearity of dilution test in a single series; d) 11 were used for the serum vs plasma test. Seven out of the eleven serum samples from the latter group (group d) were also tested in haemolysed conditions.

Data from the comparative study between FEIA and CLIA were tested by Simple Linear Regression. In the Intra and the Inter-assay study for the FEIA method the Coefficient of Variation (CV) was calculated, whereas results for the linearity-of-dilution test is described by the percentage of Observed value/Expected value. Results from the serum vs plasma vs haemolysed test were described in terms of Concordance Correlation Coefficient (CCC).

RESULTS: In the comparative test the results confirms the very high level of agreement between FEIA and CLIA (r²=0.978). The overlapping results on serum progesterone concentration obtained with the two techniques indicate that they are interchangeable in this hormonal determination and lead to the same clinical decision. This results confirmed what it has been recently described by Brugger N et al (2011), that compared the enzyme-linked fluorescence assay (ELFA, Biorieux, France) to RIA. Moreover the intra-assay reproducibility tested on four samples (mean values 0.62, 2.66, 12.16 and 23.6 ng/ml) demonstrated low coefficients of variation in the 5 replicates (CV= 6.67%, 2.58%, 4.62% and 2.01%, respectively), confirming the high accuracy of the results regardless the concentration of analyzed samples, and thus sustaining the reliability of the technique in different moment of canine estrus cycle. The inter-assay variability on five serum samples (mean value 0.25, 2.82, 10.13, 24.87, and 30.74 ng/ml) from different bitches were assayed singly in 10 separate series and demonstrated a coefficient of variability of 15.88%, 7.21%, 6.04%, 5.8% and 5.09 % respectively. These results indicate the high accuracy of the technique in determining progesterone concentration regardless the value of the sample, the time between collection and test, and the batch used. The accuracy of the assay, tested by means of the linearity-of-dilution method on 3 serum samples (neat value 16.66, 20.18 and 34.7 ng/ml), was confirmed by the mean recovery in terms of percentage for observed/expected concentrations, that was 100.75%, 107.51% and 103.41%, respectively. These data have important clinical applications demonstrating that hormonal determination is accurate even when a dilution of the sample is required, as for example when testing very high concentration (pregnancy or diestrus).

The results in progesterone concentration obtained with serum, plasma-EDTA and plasma-Litium Heparin were highly correlated (CCC=0.99), thus excluding interference of anticoagulant used. This renders very practical the use of FEIA that guarantees accurate result regardless the substrate of samples analysed. Moreover the comparison of the result of serum and
haemolysed serum showed a negligible lower degree of agreement (CCC=0.94), but the differences obtained never brought
to different clinical decisions.

CONCLUSIONS: The high clinical and statistical agreement between FEIA and CLIA, the reproducibility (intra-assay), the
low variability in results over time (inter-assay), the flexibility of use with different substrates (serum, plasma) and a
moderate impact on test results due to the interference of haemoglobin represents the confirmation of the eligibility of the
Fluorometric Enzyme Immunoassay method as a valid alternative to more expensive equipments, offering advantages to
veterinarians dealing on a daily basis with bitches and their reproductive needs and problems in terms of speed, costs and
reliability of results.

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