

ACCURACY OF A RAPID EQUINE ENZYME IMMUNOASSAY TO MEASURE PROGESTERONE IN MARES

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Use of a qualitative enzyme immunoassay (ELISA kit) for rapid assessment of progesterone (P4) would be advantageous in equine reproductive management. The assay involves enzyme-induced color changes, which do not require the use of radioactive materials and can be done without elaborate laboratory equipment. Determination of presence or absence of a functional corpus luteum in different clinical situations like irregular estrous cycle in transitional mares, silent estrus, mares with incoherent findings after genital examination, mares that ovulation needs to be confirmed, monitoring P4 levels in the first trimester of high risk pregnancy, or mare synchronization in embryo transfer would help to maximize reproductive efficiency in broodmares. The aim of this study was to validate an enzyme-linked immunoassay for the measurement of P4 in mares. Specifically, the objectives were to: (1) determine the specificity and sensitivity of the ELISA test for determination of P4, (2) measure the potential agreement between the readers, (3) evaluate the effect of time on the outcome. Ten mares were sampled on the day before ovulation (D - 1), on day one (D1), three (D3) and five (D5) following ovulation during reproductive season (May 1–August 31, 2004). Mares were maintained on 16 h of artificial light between December 2003 and April 2004. While mares were cycling regularly they were induced in estrus by injection of 5 mg of prostaglandin SC (Lutalyse, UpJohn, Orangeville, Canada) and they were monitored starting on the fourth day by daily palpation per rectum and ultrasonographic examination to determined time of ovulation. Blood was collected by jugular venipuncture in heparinized tube, centrifuged and the plasma was stored at -20°C between collection and analysis. All samples were assayed for P4 by both the ELISA and by radioimmunoassay (RIA). The qualitative kits (Equine Premate, Biovet, Saint-Hyacinthe, Canada) was performed according to the manufacturers' instructions. A total of 96 blood samples were analyzed in a double blind setup experiment and a statistical analysis was done with a repeated measures of analysis of variance (ANOVA). Based on RIA, values of P4 on D - 1, D1, D3 and D5 were significantly different ($p < 0.0001$) with mean $\pm$ S.D. of 0.04 ± 0.52 , 2.05 ± 2.58 , 8.37 ± 4.17 and 12.76 ± 4.00 , respectively. The sensitivity and specificity were 94% and 95%, respectively, for the lowest values of P4. An evaluation of agreement between quantitative and qualitative tests resulted in a 0.87. The value of kappa was 0.90 between the readers. The accuracy of the test ELISA seemed better with 30 min than 15 min incubation period. In conclusion, these preliminary results suggest that Equine Premate ELISA (Biovet) test may be reliably and economically used to evaluate P4 levels in equine plasma in clinical situations.

Keywords: Mare; Progesterone; ELISA