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What do the values really mean? - A comparison of an enzyme-linked fluorescent assay vs chemiluminescent immunoassay for measuring serum progesterone in bitches

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The determination of progesterone (P4) concentration in the peripheral blood of the bitch is the gold standard under practical conditions for the detection of ovulation, to control pregnancies with suspected luteal insufficiency and it is useful to verify luteolysis prior to parturition. There is a need to rapid measurement of quantitative P4 in clinical sites for fast detection of luteal activity in the estrous cycle and pregnancy. The enzyme-linked fluorescent assay (ELFA), performed on MiniVidas®, was developed as an automated analyzer which provides quantitative results within 45 min. A number of commercial laboratories offer quantitative determination of P4 (chemiluminescent immunoassay (CLIA) or radioimmunoassay (RIA)), whereby serum samples have to transported to the lab. This may lead to a significant delay of information. Brugger et al. (2013) evaluates the ELFA and compared the values to a RIA validated in the dog. Although RIA is still the gold standard for quantitative methods, these assays are often replaced by non-radio isotopic immunoassays, to avoid the problem of radioactive waste. CLIA seems to be a reliable, accurate and safe method to detect quantitative P4 concentrations. The purpose of this study was to compare concentrations of P4 measured with ELFA and CLIA for the first time. For this study, 250 serum samples were collected from 72 bitches of different breeds during estrous, the early luteal phase in pregnancy and the nonpregnant-cycle, respectively. First, ELFA was performed on the MiniVidas® analyzer in fresh serum samples with values between 0.25-80.0 ng/ml. Subsequently, all samples (stored frozen at -80°C until second measurement) were compared to a CLIA carried out at a commercial laboratory (Laboklin, Germany). The values were grouped and analyzed according to their range measured with ELFA (group a = 0-1 ng/ml, group b = 1.01-3 ng/ml, group c = 3.01-6 ng/ml, group d = 6.01-10 ng/ml, group e = 10.01-20 ng/ml, group f =20.01-30 ng/ml, group g = 30.01-40 ng/ml, Group h >40 ng/ml). The serum concentrations of progesterone as measured by ELFA correlated well with CLIA (Pearson’s r=0.943, n=250). Furthermore, ELFA measured concentrations were significantly higher than progesterone concentration measured with CLIA in groups d (n=16, 8.20 ± 1.1 vs. 4.36 ± 1.40), e (n=56, 14.93 ± 2.95 vs. 7.47 ± 4.36), f (n=41, 24.54 ± 2.61 vs. 10.16 ± 1.93), g (n=31, 34.43 ± 2.56 vs. 13.85 ± 2.49) and h (n=48, 50.58 ±10.98 vs. 20.06 ± 4.82). The presented data show that ELFA measured values are nearly twice as high as measured with CLIA for groups d-h on the one hand, but also higher by trend for groups a, b and c (p = 0.15). These findings should be considered in clinical exercise, e.g. the detection of ovulation or the interpretation of progesterone decline in luteal insufficient bitches. The use of factors, multiplied by ELFA values could be a possible solution to compare the methods in a better way. In this connection, the suitable factor depends on the range of progesterone concentration (factor 0.6 for 0-6 ng/ml; factor 0.5 for 6.01-10 ng/ml; factor 0.45 for 10.01-20 and factor 0.4 for >20 ng/ml). The determination of serum progesterone concentrations with MiniVidas® ELFA provides rapid and reliable results, but the significant higher values should be considered in the clinical interpretation, esp. in interpreting the need of progesterone supplementation in hypoluteoid bitches. [1] Brugger, N., C. Otzdorff, B. Walter, B. Hoffmann, and B. Braun. "Quantitative Determination of Progesterone (P4) in Canine Blood Serum Using an Enzyme-Linked Fluorescent Assay." Reproductive in Domestic Animals 46 (2011): 870-73.; [2] Kutzler, M. A., H. O. Mohammed, S. V. Lamb, and V. N. Meyers-Wallen. "Accuracy of Canine Parturition Date Prediction from the Initial Rise in Preovulatory Progesterone Concentration." Theriogenology 60, no. 6 (2003): 1187-96.