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The effects of storage time and temperature and anticoagulant on laboratory measurements of canine blood progesterone concentrations

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Abstract

The effects of anticoagulant, storage time, storage temperature, and assay method, on laboratory measurements of blood progesterone concentrations of dogs is unclear; these factors have had a dramatic effect on blood progesterone concentrations in other species (particularly cows). In six experiments, we determined the effects of assay technique (chemiluminescence versus radioimmunoassay (RIA)), storage time, and temperature, as well as the use of heparinized plasma versus serum (coagulated blood) on measured progesterone concentrations of bitches. The studies showed that: (a) RIA measured significantly higher serum progesterone concentration (SPC) than chemiluminescence; (b) refrigeration of whole blood during the first 2 h after sample collection significantly decreased measured SPC; (c) progesterone concentration in heparinized plasma was not affected by storage temperature of whole blood for at least 5 h; (d) refrigeration of whole, clotted blood did not affect SPC, provided that samples were held at room temperature for the first 2 h after collection. These findings are of particular importance when blood samples are collected for determination of the initial rise in SPC that is associated with the LH surge in estrous bitches.
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1. Introduction

Progesterone concentrations of bitches are routinely measured in either serum or plasma. Textbooks on canine reproduction generally refer to serum and plasma concentrations of progesterone as though they were interchangeable [1,2]. Using an in-house ELISA method, Thuroczy et al. recently showed that heparinized canine plasma contains approximately 10% less progesterone than serum [3]. Progesterone concentrations in whole bovine blood decrease rapidly, regardless of whether the sample is allowed to clot or not or storage temperature [4–6]. Similar, though less dramatic, degradation of plasma progesterone has been recorded for ovine blood [7]. Prompt separation of the serum or plasma and the use of sodium fluoride as anticoagulant (coupled with prompt refrigeration of the sample) best preserved progesterone concentrations in bovine serum or plasma samples [8]. Storage of whole, heparinized porcine or canine blood at room temperature or at 4 °C did not affect progesterone concentration [6]. The variable impact of hemolysis on a large variety of blood hormone assay results have been documented in a comprehensive study by Reimers et al. [9].

Whole blood samples are usually kept refrigerated during storage or shipping. After analysis of the results for a few bitches from which the samples were not centrifuged promptly (but stored at 4 °C), it was apparent that the interval between the first rise of serum progesterone concentration (SPC) to >2 ng/mL and the

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first day of cytologic diestrus was often shorter than the expected mean of 8 days [12]. This prompted the formulation of the hypothesis that delayed separation of canine serum from the blood clot results in lower than expected SPC.

2. Materials and methods

Five beagle bitches at variable stages of diestrus, were used in each experiment. In Experiment 1 (Exp. 1), a jugular blood sample was collected from each bitch and divided among four glass tubes (free of anticoagulant or any serum separator agent); the tubes were centrifuged 0, 18, 24, and 30 h later, respectively. After centrifugation, serum was aspirated and stored at −18 °C. In Exp. 2, the same procedure was followed, but samples were centrifuged after 0, 8, 12, and 24 h, respectively. In Exp. 3, the original sample was divided into five aliquots that were centrifuged after 0, 1, 2, 4, and 6 h, respectively. Except for the first sample in each series, all blood samples in Exps. 1–3 were stored at 4 °C between sampling and centrifugation. In Exp. 4, the original sample was divided into nine aliquots, of which the first one was centrifuged immediately, whereas the others were paired. One tube of each pair was held at room temperature (22 °C), whereas the other was stored at 4 °C until centrifugation. The intervals between collection and centrifugation were 0, 1, 2, 3, and 5 h. Exp. 5 was identical to Exp. 4, except that the nine aliquots of blood were put into 1 mL plastic, heparinized blood collection tubes. For Exps. 1–5, serum and plasma samples that were harvested after centrifugation of the blood samples were frozen at −18 °C for 12–36 h prior to the determination of SPC and PPC. In Exp. 6, blood samples were divided into six aliquots in serum tubes. One aliquot was transferred from room temperature to 4 °C after 0, 0.5, 1, 2, 4, and 19 h, respectively. After 22 h, all samples were centrifuged and the SPC determined.

All serum and plasma progesterone concentrations (SPC and PPC) were determined by chemiluminescent immunoassay system (CLIA, Immulite®, Diagnostic Products Corporation, Los Angeles, CA, USA) [10]. A radioimmunoassay (RIA, Coat-A-Count, Diagnostic Products Corporation) was also used to measure SPC in all samples of Exps. 1 and 2. The SPC in each sample was expressed as a percentage of the SPC in the sample that was centrifuged at 0 h (T_0). Mean concentrations determined by RIA and CLIA (Exps. 1 and 2) were compared by two-tailed *t*-test for paired variables, while their relationship was determined by their correlation coefficient and a simple linear regression model [11].

For all experiments, one-tailed *t*-tests for paired variables were used to test the hypothesis that the mean SPC at selected time points was smaller than the mean (100%) at T_0.

3. Results

The results of Exps. 1 and 2 are shown in Fig. 1. The SPC declined to 58.9, 63.5, 59.0, 54.1, and 48.9% of initial concentrations at T_0 in samples that were centrifuged 8, 12, 18, 24, and 30 h after collection, respectively ($P < 0.005$ for the difference between T_0 and all other time points). The results of the two assay methods (CLIA and RIA) were highly correlated ($r = 0.97$; $P < 0.0001$), but concentrations determined by RIA were nearly 1.5 times as high as those detected by CLIA (Fig. 2). As canine SPC is routinely determined by CLIA in our laboratory, all further assays were performed by CLIA only. In Exp. 3, 88.7, 53.1, 47.7, and 50.3% of the original SPC was still detected after 1, 2, 4, and 6 h, respectively ($P = 0.0001$ for the difference between T_0 and 2 h; $P > 0.1$ for 1 h; no significant changes after 2 h). In Exp. 4 (Fig. 3), there were no differences ($P > 0.1$) between the original SPC and that measured after 1, 2, 3, and 5 h of storage at 22 °C, whereas the samples stored at 4 °C prior to centrifugation contained 91.0, 72.3, 60.8, and 57.5% of the original SPC after 1, 2, 3, and 5 h, respectively ($P < 0.01$ for the difference between T_0 and 2, 3, and 5 h). Mean SPC tended to be different at 2 h ($P = 0.08$) and differed at 3 and 5 h ($P < 0.05$) between

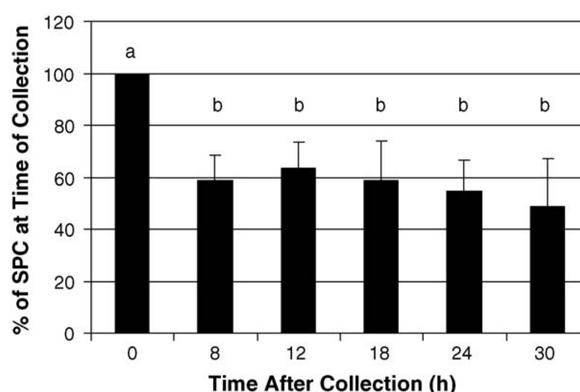

Fig. 1. Mean serum progesterone concentrations (expressed as percentage of the concentration at the time of blood collection) after storage of five samples of whole, clotted canine blood at 4 °C for 8, 12, 18, 24, and 30 h (Exps. 1 and 2). Subsequent to storage at 4 °C, harvested serum samples were stored at −18 °C for 12–36 h before progesterone concentrations were determined by chemiluminescent immunoassay. Means with different letters (a and b) are different ($P < 0.0005$).

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