Sample handling has minimal effect on blood progesterone concentrations in jennies

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Abstract

Consistency of assay results is important for practitioners and investigators. In this context, for progesterone assays, appropriate handling and processing of blood samples is critical. Objective was to compare progesterone assay results from jennies on the island of St. Kitts in Eastern Caribbean from samples subjected to a variety of time and temperature treatments. Five blood samples drawn via jugular venipuncture from 7 jennies at various stages of estrous cycle were randomly assigned to various treatments prior to storage at -80°C and subsequently thawed and assayed. Handling methods were: immediate placement of blood in plain vacutainer tubes on ice for 1 hour (Tr1); 1 hour of clot time before placing on ice for 24 hours (Tr2); 1 hour of clot time at room temperature followed by serum separation and freezing (Tr3); allowing 24 hours at room temperature before serum separation (Tr4); and 1 hour at room temperature for blood collected in an EDTA tube before plasma separation (Tr5). Time and temperature treatment of samples used in this study did not influence progesterone assay results.

Keywords: Progesterone, assay results, time, temperature, jennies

Introduction

Progesterone concentrations in serum or plasma are often utilized in reproductive medicine as basis for clinical decision-making and are equally important in research. Reproducible results are important in both situations. Evidence exists that sample handling before analysis may influence quantifiable concentrations of progesterone. For example, in bovids, there was appreciable loss of progesterone concentrations if whole blood was left to incubate at ambient temperature prior to analysis.[1,2] Although the rate of decline in detectable progesterone concentrations was different between bovine serum and plasma, immediate refrigeration and incubation at 4°C was recommended to slow the decline.[3]

In contrast, progesterone concentrations in canine serum declined when blood was refrigerated at 4°C following collection and processed after 3 hours, whereas concentrations remained relatively stable when kept at room temperature (22°C).[4] However, plasma progesterone concentrations remained stable throughout 5 hours of storage at 4 or 22°C.[4] Hence, at least 2 hours of clotting time was suggested prior to centrifugation for blood samples used for progesterone assays to determine luteinizing hormone surge in bitches.[4] For equine serum samples, a decline in detectable progesterone concentrations was noticed when samples were held at room temperature for 2 or 5 days.[5] No significant differences in progesterone concentrations were reported in equine serum/plasma samples kept at 4 and 22°C.[3] It was noteworthy that type of collection tube selected for storage of whole blood influenced assay results, with significantly higher progesterone concentrations in serum compared to plasma.[6]

Objective of the study was to compare progesterone assay results from jennies on the island of St. Kitts in the Eastern Caribbean from samples subjected to a variety of time and temperature treatments. Goal was to ascertain whether immediate handling of samples was necessary.

Materials and methods

Animals

Seven nonpregnant (3 - 12 years, ~ 250 Kg), jennies in estrus and diestrus (as determined by daily transrectal ultrasonographic examination of ovarian structures) were used for each treatment. Research protocol was approved by Ross University School of Veterinary Medicine IACUC. All jennies were housed together in paddocks from February 2016 to July 2017. They were fed fresh cut guinea grass ad libitum daily, had free access to fresh water and mineral blocks, and were given grain when handled for procedures.

Experimental design

Jennies were subjected to jugular venipuncture with 18 gauge 1.5'' needle and 7 ml of blood was collected in 4 plain glass tubes and in 1 glass tube with EDTA anticoagulant. In treatment 1 (Tr1), blood was immediately placed on ice for 1 hour. In treatment 2 (Tr2), blood was allowed to clot at room temperature (~ 21°C) for 24 hours. In treatment 3 (Tr3), blood was allowed to clot outside (~ 32°C) for 1 hour. In treatment 4 (Tr4), blood was allowed to clot for 1 hour at ambient temperature and then placed on ice for 24 hours. In treatment 5 (Tr5), blood drawn in EDTA anticoagulant tube was kept outside (~ 32°C) for 1 hour. Tubes were centrifuged at 3,000 x g for 3 minutes at 4°C and aspirated serum/plasma was stored into labeled microcentrifuge tubes in an -80°C freezer until thawed for progesterone analysis.

Progesterone concentrations were determined using a commercial enzyme immunoassay kit (Arbor Assays K025-H5, Ann Arbor, MI). Partial validation was conducted to assess possible matrix effects. A serum pool was prepared and diluted in a serial manner to determine parallelism with reference standard curve. Four of the 6 dilutions were within the linear range of standard curve, with a coefficient of variation of 26%, indicative of parallelism (European Medical Agency, 2011).[7] Assay was conducted as per manufacture's protocol using 50 µl of sample diluted 1:16 with assay buffer. Progesterone concentrations that were outside the range of reference standard were further diluted and reanalyzed as necessary. Within assay coefficient of variation was 17% and sensitivity was 0.05 ng/ml.

Data analyses

Effects of time and temperature handling of samples on progesterone concentrations were examined using mixed effects linear regression, with treatments (sample handling) regarded as fixed effects. Jenny was treated as random effect to account for repeated measures. When this approach indicated no significant differences, all possible pairwise comparisons were made by paired Student's t-tests to ensure that no between-group differences were overlooked, although not strictly appropriate for this experimental design. Stata®/IC version 11.1 (StataCorp, College Station, TX) was used for data analyses.

Results

Progesterone measured ranged from 1.81 to 33.60 ng/ml (Figure). There was no difference ($p = 0.26$) in assay results between sample treatment protocols and there was no difference ($p > 0.20$) in all possible pairwise comparisons.

Discussion

Although some variation was present across progesterone concentrations, in each group, differences were not significant. Samples from diestrus jennies had expected progesterone concentrations, whereas jenny's E and G progesterone concentrations correlated to their estrous cycle stage (estrus or entering into estrus). Findings on effects of time and temperature handling (up to 24 hours) agreed with an earlier study involving equine serum and plasma.[3] In field conditions, if whole blood cannot be immediately refrigerated nor put on ice, samples can be kept at room temperature up to 24 hours.

Conclusion

With no clinically relevant depreciation of serum or plasma progesterone obtained from jennies, whole blood can be drawn and allowed to clot at room temperature safely when indicated for progesterone assay. It is suggested that clinicians, owners, and researchers who prepare samples for progesterone assay in jennies need not handle whole blood differently within first 24 hours after collection.

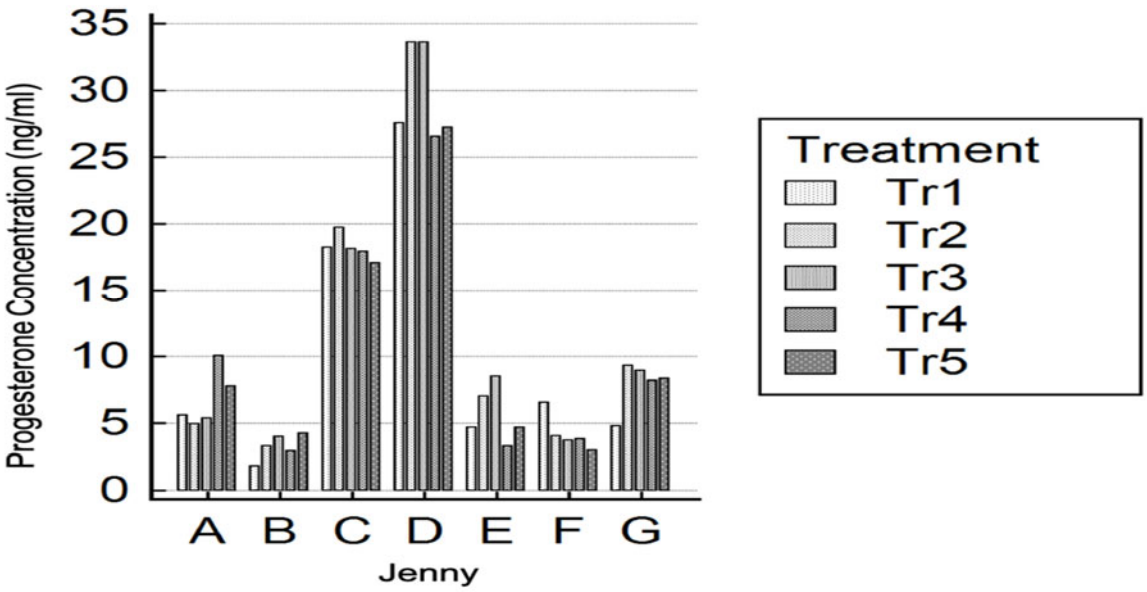

Figure. Progesterone concentrations within 5 treatment groups for each of the 7 jennies sampled. Immediate placement of blood in plain vacutainer tubes on ice for 1 hour (Tr1); 1 hour of clot time before placement on ice for 24 hours (Tr2); 1 hour of clot time at room temperature followed by serum separation and freezing (Tr3); 24 hours at room temperature before serum separation (Tr4); and 1 hour at room temperature for blood collected in an EDTA tube before plasma separation (Tr5).

Conflict of interest

Authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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