Proceedings of the Society for Theriogenology Annual Conference 2015

Aug. 5-9, 2015 – San Antonio, TX, USA

Next SFT Meeting:

Jul. 27 – 30, 2016 – Asheville, NC, USA

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Comparative progesterone assay

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Abstract
Analysis of systemic progesterone concentrations is routinely used for ovulation timing in bitches. Ability to accurately determine when ovulation has occurred can improve pregnancy rates, litter size, and reduce the need for multiple inseminations during an estrous cycle; it is of utmost importance when utilizing cooled shipped or frozen semen to correctly identify the appropriate time for insemination. It has anecdotally been recommended that progesterone analysis be performed at the same laboratory for a given estrous cycle to reduce confounding factors related to differences between sample handling and different analysis techniques. However, it is sometimes necessary to send samples to different laboratories during a given estrous cycle which can lead to difficulties in interpretation and determination of the fertile window.

There are many options available for quantitative progesterone assay available to the practitioner today. Radioimmunoassay (RIA) is considered the gold standard for endocrinology testing, but is only available at reference laboratories and may have delayed turnaround times as a result. Point-of-care assay is becoming increasingly common in private practice settings, which utilizes methods such as chemiluminescence (CLIA), enzyme-linked fluorescent assay (ELFA), or fluorescent enzyme immunoassay (FEIA). It is important to determine the level of agreement between different point-of-care analyzers as well as to determine whether point-of-care analyzers are consistent with gold-standard testing techniques such as RIA. These differences could impact the accuracy of ovulation timing in bitches with a subsequent negative impact on pregnancy rate and litter size.

Keywords: Progesterone, radioimmunoassay, chemiluminescence, enzyme-linked fluorescent assay, fluorescent enzyme immunoassay, ovulation timing

Introduction
A variety of diagnostic assessments are utilized for ovulation timing in bitches, including vaginal cytology, vaginoscopy, behavior, serum luteinizing hormone (LH) measurements, and serum progesterone measurements. Great variability has been noted regarding estrus behavior and ovulation, so progesterone assay has been utilized to further define and identify the fertile window.1 The most common cause of failure to conceive is poor ovulation timing and breeding management, which makes accurate progesterone assay of vital importance to bitch owners and veterinarians alike.2-4 The bitch is unique among the domestic species in that preluteinization of follicles results in a preovulatory rise in progesterone above baseline, allowing both estimation of the LH surge and ovulation indirectly. Conveniently, progesterone is not a species-specific molecule which allows human progesterone assays to be utilized effectively in a variety of species.5 Several immunoassay methods have been evaluated in a variety of species, including dissociation enhanced lanthanide fluorescence immunoassay (DELFIA), multianylate immunoassay, luminescence immunoassay, and enzyme-linked immunosorbent assay (ELISA).5-9 However, there is very little research examining the repeatability of these methods or directly comparing the agreement of these methods against each other.

With the increased use of artificial insemination, determination of ovulation and the optimal time of breeding have become even more important. Likewise, some veterinarians are utilizing point-of-care machines, with quantitative progesterone results available the same day. Semi-quantitative ELISA test kits are available for patient-side use such as Target Canine Ovulation Test (BioMetallics; Princeton, NJ) and Ovucheck® Premate 10 (Zoetis; Florham Park, NJ). These tests are not considered highly accurate, even when correlating results with other parameters.11 Some veterinarians consider these semi-quantitative test kits to be most useful in confirming progesterone values that are very high (>10 ng/ml) or
very low (<2 ng/ml), but they are not accurate enough for determination of quantitative values for the purposes of breeding management, and thus these types of tests are excluded from this discussion. Rather, the purpose of this review is to compare different types of quantitative progesterone assays that are commonly used for ovulation timing in the bitch (RIA, CLIA, and ELFA/FEIA).

Radioimmunoassay has long been considered the gold standard for assessment of hormones and endocrine compounds as it is considered to be both highly accurate and repeatable. However, RIA is only available at select reference laboratories due to equipment cost, maintenance and handling of radioactive materials; this often results in 24 hours or longer prior to reporting of results. Recent changes in availability of reagents utilized for RIA has led to a decrease in the number of reference laboratories offering progesterone assay via this method. At the time of publication, the only reference laboratory in the United States utilizing RIA that was identified by the authors was Colorado State University Reproductive Endocrinology Laboratory. The reference laboratories that the authors previously utilized for RIA have since transitioned to CLIA as a result of reagent unavailability.

Chemiluminescence (CLIA) has become an increasingly popular method of progesterone analysis due to rapid turnaround time and decreased costs compared to RIA. Some practitioners report anecdotally that this assay type is less reliable than other methods, but the literature reports that chemiluminescence is equivalent to, or even more accurate than RIA. For perspective, one commonly used CLIA machine is the Immulite® system (Siemens Medical Solutions USA, Inc, Malvern, PA) now used by at least two universities that have recently switched from RIA to CLIA. Even more recently, ELFA/FEIA machines have become available and affordable for individual practitioners to maintain within the hospital setting to allow rapid, cost-effective, quantitative progesterone assays that have been specifically validated for use in dogs. Some common models for ELFA/FEIA testing include mini VIDAS® (Biomerieux, France) and TOSOH™ (Tosoh USA, Inc, Grove City, OH). To date, no research has directly compared multiple methods to each other, nor compared the effects of ovulation timing when multiple laboratories must be used for analysis. When new diagnostic methods are validated, they are typically assessed by testing known concentrations of progesterone created from stock solutions rather than comparing to another assay type. Determining agreement between the most commonly utilized methods (RIA, CLIA, and ELFA/FEIA) could confirm commonly accepted anecdotal paradigms regarding the necessity of ideally utilizing a single laboratory for progesterone analysis, and may provide insight into means to adapt data when multiple laboratories or analysis techniques are used.

There are several machines available for in-clinic progesterone assay, including the mini VIDAS® and TOSOH™ models. These utilize enzyme-linked fluorescent assay and fluorescent enzyme immunoassay, respectively. Dependent on the particular machine, a conversion chart may be utilized to convert raw data. Initial set up costs and individual test costs will vary with the particular brand and model purchased, but are estimated to range between $8-15 per test (clinic cost). Cost of testing at reference laboratories (RIA or CLIA) can vary considerably, ranging from $20 per test to considerably more. Practice owners will have to balance the cost of purchase/rental and maintenance of equipment relative to the number of blood samples assayed on a regular basis.

Sample handling

Good laboratory technique suggests that consistent handling of blood samples should result in greater consistency in reported results. Several studies have reported alterations in reported results when serum separator tubes are used. therefore plain glass (red top) tubes are recommended. Progesterone appears to remain relatively stable in canine blood, with no significant differences noted from freeze-thaw cycles or prolonged storage up to 14 days at room temperature. Significant differences were also not noted between silicone, lithium heparin, and EDTA blood tubes.

Determining agreement between samples

Comparison of different laboratory techniques can be achieved by measuring accuracy, precision, repeatability, and/or the level of agreement between sample values. Accuracy is defined as the degree of conformity of a measure to a standard or “true” value. Precision describes the closeness of two or more
measurements to each other, which is its repeatability. Therefore, it is possible for any type of measurement to be very precise (repeatable), yet inaccurate. Another question that must be answered is how to best determine the level of agreement between samples. Even if samples between different laboratories had poor agreement, if the bias between analytical technique is consistent this can be accounted for, and values adjusted accordingly. Bland-Altman methods\textsuperscript{18} of statistical analysis can be utilized to account for both of these issues. Other statistical tests, such as analyzing the coefficient of variance, may give a false sense of security as the values between different laboratories are likely to be closely associated. The agreement between samples can be summarized by calculating the bias as a function of progesterone concentration; if the bias is consistent, this would allow adjusted values to be calculated between those two methods. However, differences in values (and potentially standard deviation in those differences) will likely vary depending on the range that the actual progesterone measurement falls within. As the progesterone level rises, the limits of agreement for clinical acceptability increase as well, which is another factor to consider when determining if two methods are interchangeable.

While a proper comparison of these testing methods can seem confusing and academic in nature, such comparisons are necessary in order for veterinarians to make sound clinical decisions regarding ovulation timing and breeding management. At the time of publication, a collaborative study involving all authors is underway to determine these comparisons using different methodologies and different laboratories. Formal results are not yet available but will be presented at the August meeting in San Antonio, and will also be available for reference in a separate publication at that time.

Acknowledgement
The authors thank the Theriogenology Foundation for generous financial support for this project.

References