Frequently-Asked Questions: Assessment of Reproduction

Are progesterone test results affected by the type of collection tube and handling? Yes

Recent reports and preliminary data from our laboratory have demonstrated that concentrations of progesterone in canine serum will be decreased if the sample is collected in a separator gel tube. The extent of the decrease is time-dependent. The comparisons have been examples where blood samples were divided into a plain tube (serum removed after centrifugation), a separator gel tube (serum removed after centrifugation), and another separator tube (serum held in separator tube overnight after centrifugation). In comparison to progesterone results from the plain tube the values from freshly harvested separator tubes were slightly lower (10-15% decrease). Serum concentrations of progesterone in the serum held overnight in separator tubes were approximately 50% of values from the plain tubes with freshly harvested serum.

Until determined otherwise, it is presumed that the effect will be the same in serum from other species. In sampling for progesterone assay, the take home message is to be consistent in sample collection and processing with the ideal protocol of using plain tubes with serum harvested soon after clotting and centrifugation. If separator tubes must be used, remove the serum from the gel tube immediately after centrifugation.

What are approaches in sampling for testosterone in diagnosis of cryptorchidism?

Dogs and Cats – In these species and others including cattle and swine, starting with a random baseline serum sample for testosterone assay is usually sufficient. In neutered males, serum testosterone is usually well below 0.5 nmol/L, while testosterone is typically above 1.0 nmol/L if there is a retained testicle, with values sometimes in excess of 10 nmol/L. In the infrequent instance of an equivocal baseline testosterone result, an HCG stimulation test can be performed. In cats, identification of barbs on the penis is a useful indicator for the presence of a retained testicle. There may be exceptions. In dogs with estrogen-secreting Sertoli cell tumors, serum testosterone may be low due to suppression of gonadotropin release by estrogens. Another rare exception is with testosterone-secreting adrenocortical tumors, which we have seen in two cats. These cats showed late-age onset of male behavior years after castration.

Horses – In the horse, the gap between serum concentrations of testosterone between geldings and males with a retained testicle is narrower than that seen with other species. Thus there is an added benefit of diagnostic accuracy in performing an HCG-simulation test. Clinical studies have utilized HCG doses of 5,000-12,000 IU per horse (probably related to half or one vial), given by IM or IV route. The best test protocol employs collection of a baseline sample with post samples collected at 30 minutes, 1 and 2 hours. If there are constraints where there is limited time to collect all samples, the 1-hour post sample is the most likely to identify a significant increase of testosterone.

What are approaches for determining the presence of an ovarian remnant in dogs or cats?

In both species, a diagnosis of ovarian remnant is made with integration of behavior and physical changes consistent with estrus and subsequent serum concentrations of progesterone indicative of ovulation and development of luteal tissue. Typically, these events are seen within a few months following ovariohysterectomy.
**Dogs** – If there is behavior and/or physical changes suggesting of estrus, the initial diagnostic evaluation is histological examination of vaginal epithelium. If there is suggestion of cornification, a series of smears are taken to see if the progression of changes is typical for estrus. If the smears indicate progression past estrus, a baseline progesterone assay is done to determine the presence of functional luteal tissue.

**Cats** – Diagnostic evaluation is initiated when the cat exhibits estrous behavior. Vaginal smears can identify changes indicative of estrogen influence. There must be induction of ovulation to document the development of luteal tissue. If there is not exposure to a male for breeding attempts, the most consistent means to induce ovulation is by administration of human chorionic gonadotropin (HCG, 200 IU) or gonadotropin hormone-releasing hormone (GnRH, 25-50 µg) when there is active estrous behavior. Typically there is cessation of estrus within a day after administration of gonadotropin. A blood sample for progesterone assay is collected 7-14 days after induction of ovulation to document the presence of functional luteal tissue. There may be false negative results with this test if the gonadotropin is given too late in estrus where the follicles are undergoing atresia and have lost the capacity to luteinize.

### How is assay for progesterone used in estimation of pregnancy in llamas and alpacas?

In these species, ovulation is induced by mating. If the female is pregnant, the CL will persist and progesterone will be elevated until parturition. If the female is mated and ovulates but does not become pregnant, the CL will produce progesterone for 2-3 weeks and then regress. Thus interpretation of a progesterone result is based on when the sample was collected in reference to known breeding dates or the time or previous exposure to a male. In these species, progesterone concentrations at or above 6 nmol/L are consistent with the presence of functional luteal tissue. Thus if a progesterone result is above 6 nmol/L in a sample collected a month or more after exposure to a male, the most likely reason for the result is pregnancy.