**Canine & Feline Reproductive Function Tests**

**Progesterone Testing for Determination of Ovulation in Dogs**

Progesterone analysis of serum samples is useful for distinguishing the different reproductive states. For bitches, the increase in progesterone following the LH can predict ovulation. This will enable a planned breeding to be at the optimal time of fertility and better coordination of the logistics involved for either natural or artificial breeding.

1. Collect a baseline blood sample in a plain red-top collection tube with no additives.  
   *Do not use serum separator tubes due to assay interference in some instances.*
2. Allow whole blood to clot at room temperature for up to 1 hour. Refrigerate after this if longer time is needed before centrifugation. Centrifuge at a speed and time that will allow for adequate separation and specimen yield.
3. Draw off serum and transfer to a tube or vial suitable for shipping and/or frozen storage. The aliquoted serum sample can then be stored either refrigerated or frozen (better). Frozen storage is recommended if time before shipping will be 12 hours or more.
4. Optimally, ship samples frozen with cold packs. A frozen specimen is not necessary for Progesterone testing, but does better ensure arrival of a chilled sample for more accurate test results.

**Anti-Müllerian Hormone (AMH) Test for Determination of Spayed vs. Intact in Cats and Dogs. Also to be considered for the determination of Cryptorchidism.**

The qualitative AMH test for dogs and cats is meant to distinguish between spayed and ovarian intact bitches and queens after they have reached the age of sexual maturity (varies by breed). A negative AMH test is consistent with a spayed female, whereas a positive AMH test indicates the presence of ovaries. An inconclusive AMH result cannot distinguish between the intact and spayed conditions. Intact females tested before reproductive maturity or after reproductive senescence may have a negative AMH test. The AMH test has also been evaluated for ovarian remnant syndrome (ORS), and a positive AMH test is consistent with the presence of an ovarian remnant in animals that had been previously spayed. A negative AMH test does not rule out ORS, and progesterone baseline testing is recommended in conjunction with AMH whenever ORS is suspected.

The AMH test has NOT been fully evaluated for cryptorchidism, but a positive AMH test is consistent with the presence of testicular tissue in these situations.

1. Draw a baseline blood sample in a plain red-top tube with no additives.
2. Allow whole blood to clot at room temperature for up to 1 hour. Refrigerate after this if longer time is needed before centrifugation. Centrifuge at a speed and time that will allow for adequate separation and specimen yield.
3. Separate serum from cells within 4 hours after collection and transfer to a vial *(plastic preferred)* suitable for shipping and frozen storage. Freeze serum promptly, especially if time before shipping to the laboratory will be 12 hours or more.
4. Optimally, ship samples frozen with cold packs. Sample should be received at least “cold or partially thawed” for accurate AMH testing. Sending frozen serum with frozen cold packs using a two-day or overnight courier service will usually ensure the sample is received chilled.

**Note:** *Avoid using serum collection tubes with materials/additives, including gel or physical barriers and clot activators and/or anticoagulants due to potential assay interference in some instances.*

5. *Do not use serum separator tubes due to suspected assay interference in some instances.*
**Progesterone Test for Detection of Ovarian Remnants in Cats using GnRH or hCG**

If ovarian tissue is present as a result of incomplete ovariohysterectomy, follicles will develop naturally or can be artificially developed with exogenous FSH (follicle-stimulating hormone). In either case, ovulation must be induced with exogenous GnRH (gonadotropin-releasing hormone) or hCG (human chorionic gonadotropin).

**If the animal is in heat now...**
1. Inject 25 μg of GnRH intramuscularly or 250 IU of hCG subcutaneously.
2. Collect a blood sample approximately 10 days after GnRH or hCG injection. Follow the sample processing procedure above, steps 1 to 4 and request progesterone analysis.

**For cats not in heat or if the owner or vet is unwilling to wait, heat can be induced...**
1. Inject 2 mg/day of FSH. Stop treatment when cat shows heat signs or after 5 days, whichever is soonest.
2. Follow the sample processing procedure above, steps 1 to 4 and request progesterone analysis.

**Note:** GnRH is preferred over hCG because of a decreased risk of an anaphylactic reaction.

1 IU = 1 USP (1500 USP = 1 mg of hCG)

**GnRH- or hCG-Response Test for Male Dogs and Cats**

The GnRH- (gonadotropin-releasing hormone) or hCG- (human chorionic gonadotropin) response tests are useful for distinguishing fully castrated males from those with either retained or missed testicles or testicular remnants.

1. Draw a baseline blood sample in a plain red-top clot tube. Follow the sample processing procedure above steps 1 to 4.
2. Inject GnRH at 2.2 μg/kg intramuscularly for dogs or 25 μg intramuscularly for cats or 250 IU of hCG subcutaneously for dogs and cats.
3. Collect an additional blood sample at 2 hours after GnRH or hCG injection.
4. Follow the sample processing procedure above. If the patient is a male, Testosterone testing should be done on the paired samples.

**Note:** GnRH is preferred over hCG because of a decreased risk of an anaphylactic reaction.

1 IU = 1 USP (1500 USP = 1 mg of hCG)