

Progesterone Testing: What is it and Why??

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Client Educational Handout

Progesterone and Breeding: A Quick Reference

What is progesterone?

A simple explanation is progesterone is a hormone produced by the bitch that creates an ovulation and maintains a pregnancy until the puppies are ready to be born.

Why do we need to understand progesterone?

Understanding progesterone allows us to better create, monitor, maintain, and control a pregnancy. By knowing the bitch's progesterone levels during a heat cycle and pregnancy, we can detect important changes in her levels that give us a tremendous amount of helpful information. It helps us identify her ovulation, which we use for helping us time her breeding's during her peak fertility to better assure conception and an optimal litter size. Also, by knowing the time of her peak fertility, we can carefully chose the best day (and time of the day) to breed depending on which method of breeding we are planning. Whether it is fresh semen with a live coverage, fresh semen with artificial insemination, chilled or frozen semen with artificial insemination by a transvaginal, surgical, or transcervical insemination method, there are different times following ovulation to breed accordingly that optimize success. Identifying her ovulation also allows us to understand when she is due. If ovulation is correctly detected with an accurate progesterone analyzer, a bitch is very commonly going to go into labor 63 days after her date of ovulation. She should maintain a certain level of progesterone during her pregnancy, which if she dips below acceptable levels we can supplement her with synthetic progesterone in order to avoid a pre mature abortion. Understanding progesterone helps us in a number of ways which creates a need for us to be able to test the values.

How do we test for progesterone?

Progesterone is circulating in the blood of the bitch. Careful venipuncture allows us to obtain a sample that we are able to use for testing. The blood sample is analyzed by a test or tests in a variety of different ways. Most serious breeders use veterinarians who send the blood samples overnight to laboratories or that have their own in-clinic laboratory machines that are very accurate in measuring hormones such as progesterone. The IMMULITE 2000 is a highly accurate machine used in many well known reliable laboratories such as Idexx laboratory and Michigan State University. This machine is not to be confused with machines that most veterinary clinics possess in their clinics, which is the IDEXX Catalyst machine that doesn't fully compare to the IMMULITE 2000 in accuracy or reliability. Clinics that do a lot of breeding and reproductive work often own and use a Biomerieux Mini Vidas machine at their

clinic which has a very comparable result to the IMMULITE 2000 as far as its trustworthiness for its accuracy and reliability.

How accurate is the progesterone testing?

Because progesterone is a hormone, it can be more troublesome to accurately measure and understand. With all diagnostic laboratory tests, errors can be made and can create wrong and misleading information. We think about errors as *Pre-Analytical, Analytical, and Post Analytical*.

Pre-Analytical errors can occur depending on the way the blood is collected, including how and when. Care and skill should be used by a professional to minimize the number of needle pokes during the venipuncture to minimize hemolysis which can directly interfere with accurately measuring the sample. Lipemia, fat in the blood that peaks hours after a meal and can interfere and affect the accuracy of the results. Making sure a proper amount of blood is collected to ensure you have enough serum is very important. Not collecting enough of the sample will directly result in falsely lower than accurate levels that can be misleading due to less of the sample than what is required for the test. It is important that the clinician follows the GLP (Good Laboratory Procedures) to ensure that enough sample is collected per the recommendation of the blood vial, and the GLP to ensure the proper sample and enough of the sample is collected for the analyzer planning to be used. Pre analytical errors can occur from how the blood is handled, including transferring to a blood vial, keeping out of sunlight, keeping protected from extreme temperatures, keeping it chilled until measuring, running the blood sample in a timely manner. Typically, it is recommended that the blood is collected by a syringe with a needle, the needle is removed, the cap to the "white top tube" is removed, the blood is transferred to the vial, the cap is replaced on the vial and the sharps and bio-hazardous waste is disposed of properly, the blood is allowed to sit at room temperature for around 30 minutes out of direct sunlight, the blood is centrifuged down for around 5-10 minutes, the serum is removed from the sample, and finally the serum is either run immediately or chilled until testing. The blood vial must not contain any serum separating gel as this gel will sequester progesterone out of the sample and cause the results to be inaccurately low. Sunlight will degrade the progesterone, as it degrades and ruins other hormones rendering the sample unreliable as the progesterone will not be detectable by the analyzer. Drawing the blood through a needle into a syringe, then pushing the blood back through the needle, and through the top of the blood vial will create hemolysis. Poking into a vessel several times to collect a sample will also cause hemolysis to the sample. Hemolysis causes a red tinge to the serum sample that will affect the chemiluminescence immunoassay color change chemical reaction occurring which will throw off the progesterone results.

Analytical Errors can occur in numerous different ways. Often, the analyzers/ machines have reagents that are used to perform the testing.

The reagents typically must be kept chilled until use, sometimes refrigerated, sometimes frozen. Keeping the reagents stored properly affects the values obtained when the sample is tested. There is usually a time period for warming the reagents up from storage before using. If the reagents aren't handled properly and warmed before use the validity of the results can be affected. The reagents are typically perishable and expire. Using expired reagents will affect the accuracy of the test results. The

machines typically require maintenance and calibration. The maintenance should be as guided, directed, and trained from the manufacture including proper cleaning. The calibration is often time-consuming and expensive, but must be done as directed by the machine you are using to ensure accurate results. Assuming the machine is clean and calibrated, the reagents are not expired, they are stored and handled as the manufacturer suggests, results still can be compromised by the analyzer machine itself. Machines differ in accuracy and reliability. The better-quality machines such as the IMMULITE 2000 made by Siemens and the Mini Vidas made by the manufacturer Biomerieux are known to be very accurate and reliable. Although on occasion breeders will gain possession of one of these two machines, they are unable to acquire reagents to use in the machines unless they are a veterinarian and have purchased the machine directly from the manufacturer. There are a handful of cheap inaccurate machines breeders possess that claim the accuracy and reliability of these machines, however they are a scam, and most veterinarians will not perform any breeding's or breeding related services such as ultrasound or cesarean sections based off the values obtained from anywhere other than a veterinarian. This will be discussed in another section. Other machines owned by veterinary clinics other than these two machines are usually either an IDEXX Catalyst machine or a Heska analyzer. These machines are less accurate, but better than any of the other machines on the market and are the lowest quality analyzers considered worthy of use for a veterinary clinic. Results from the in house Idexx veterinary machines need to be considered to be mildly accurate, and in my opinion shouldn't be used alone to determine ovulation unless no other option exists. Results from the IMMULITE and the Mini Vidas will rarely have analytical errors, the Idexx catalyst has analytical errors on occasion and make it more important to look beyond just the progesterone results obtained, and all other machines are considered highly inaccurate and have analytical errors very regularly meaning the results cannot be trusted alone without further information.

Post Analytical Errors can exist, as another way of making the results misleading and inaccurate. Reading the results, interpreting the results, converting/translating the results when necessary, recording the results to her medical record, and using the results to make decisions and predictions about her heat cycle and or pregnancy are common places that post analytical errors can occur. For example, many of the results need to be translated and converted into a number that is used by the industry, which is typically considered the IDEXX number. This is easy to read the results from the machine and forget to translate them before the owner gets the results. Interpreting the numbers meaning requires an educated and experienced professional, and the counseling dictates how the results are used. Not all veterinarians know how to use this information, however, they are all capable if they desire to learn how to. Veterinarians with reproductive medicine interests are best equipped to minimize these post analytical errors.

Assuming the blood is collected and handled properly, the machine is a high-quality machine trusted by veterinarians that has been maintained and calibrated properly with valid well-handled reagents, and the user of the machine is educated and wise to interpreting the results acquired, progesterone testing is very accurate and very reliable.

Where do you take your pet to have progesterone testing done?

It is highly recommended and advised to use a veterinarian that is experienced and comfortable with progesterone testing and consulting. There are many client owned cheap unreliable progesterone analyzers that breeders are starting to utilize and convince other uneducated new breeders to use. These machines are not only highly inaccurate, but the people using them lack the education or understanding of how to handle how to calibrate the machine and how to interpret the results obtained from these unreliable machines. Since progesterone is a hormone, there are certain ways to handle the blood sample in order to get reliable and accurate results, including avoiding all of the pre analytical, analytical, and post analytical errors that were discussed in the last section under the reliability and accuracy section of the progesterone hormone.

What are the progesterone levels and what do they mean?

Progesterone levels of a dog are usually 0.2 - 0.5 while a female is not in heat. Usually as the female begins cycle, and around 5 to 7 days into her heat cycle she begins to have increased levels of progesterone circulating her blood. Pre-ovulatory progesterone levels are usually around or below a 3.0. Usually sometime between days 5 and day 17 a female will ovulate. Her progesterone levels during ovulation are usually between a 4.5 up to a 10. A very confusing part about progesterone levels near an ovulation is that there is no one number that represents ovulation. However, it is a jump in her number in less than a 24 hour by at least 2 when her levels are between a 4.5 to 10. For example, if a female is a 4.5 today and retested tomorrow to be a 6.5 she has ovulated sometime between today and tomorrow's blood draw. An alternative example would be if she were a 4.5 today and a 5.5 tomorrow no ovulation has occurred and we would recheck the blood sample the following day. If that blood sample was checked the day after tomorrow and she jumps from a 5.5 to a 7.5, than her ovulation would have occurred in between those two blood draws. There is also such a thing as a split heat, which means that a females progesterone numbers can escalate up to or just under the value of a 10 with no ovulation occurring (an increase of 2 in a 24 hours period or less while her numbers are between a 4.5 and a 10), and she can spontaneously regress without ovulating. Usually she will stay at a lower number for an undetermined amount of time from one day to months before her progesterone levels will escalate and finally ovulate. Your reproductive veterinarian can walk you through a protocol of monitoring and measuring progesterone levels through a split heat in hopes not to miss the breeding. When the bitch's progesterone values have indicated a spike in progesterone of 2 points or higher in a 24 hour period when her number is a greater than a 4.5 but not greater than a 10, it is typical that her progesterone values will continue to rise by numbers greater than 2 daily following her ovulation. For example, a female that was today a 4.5 and tomorrow is 6.5 I would expect two days from now that her progesterone value would be a 9 or 10 or above in the following day. That number may rise to a 20 or 30 within four days post-ovulation. Her progesterone levels would likely be 20+ all the way up to 80 or even higher, and sustained for 60 days through her pregnancy until she is close to delivering her puppies when there will then be a sharp drop in her progesterone to the number zero at which time she will begin to deliver her puppies.

Another common misconception about interpreting a bitch's progesterone results is that a particular number such as a 14 or a 19 or a 25 is an indication of how many days ago she has ovulated and that this number would indicate her breeding readiness and optimal fertility. This, however, is incorrect. Her

numbers post ovulation do not determine her breeding readiness or optimal fertility. In fact, recent research has led us to believe that her numbers after ovulation could be representative of her size of ovulation. For example, if a female that ovulated today at a 5.5 and tomorrow she is a 7.5 or higher, the second day after ovulation if her number was 10 this could mean that she is ovulating only a few eggs and will have a smaller litter size compared to if her progesterone value was a 14 - 20 2 days post ovulation which could mean she has ovulated more eggs and will have a larger litter. Although there is no concrete facts that this is true at this current time, there are many factors that support this theory.

What is a reverse progesterone?

As mentioned, progesterone levels during a pregnancy can assist us in identifying her likelihood of going into labor to a certain degree. These values can be tricky to interpret, however, definitively knowing her ovulation date from accurate and timely progesterone testing during breeding helps us use a reverse progesterone more reliably. Understanding that most females will maintain a fairly high progesterone level between a 10 and an 80 during pregnancy, we will normally see a sudden drop in the numbers down to or below a 2.0 just before she is ready to start delivering puppies. Along with monitoring and watching for other signs of labor, we usually begin testing reverse progesterone's 2-3 days before her anticipated due date, or sooner if there are other signs or symptoms of labor. Drops in her body temperature often correlate with drops in her progesterone values. If we are planning a cesarean section to deliver the puppies, when her reverse progesterone is below a 2.0, she is 61 days or more along since her ovulation, she has milk production and let down when stimulated, and she has other obvious signs of labor, we are usually interested in completing the c-section.