

**DETECTION OF CANINE PROGESTERONE**

OVUCHEK® Premate is designed to be a rapid and simple method of determining the level of progesterone in canine serum and plasma. During anestrus and proestrus, the progesterone level is very low (0 to 1.0 ng/ml). Prior to the Luteinizing Hormone (LH) surge, the progesterone level begins to increase, reaching a level of 1.5 - 2.5 ng/ml during the LH surge, and will continue to rise. To identify the bitch's fertile period, compare the color in the sample well with the A and B control wells. The first day color in the sample well matches the color in A well indicates that progesterone has risen to 3 ng/ml and that the LH surge has likely occurred in the past 24-72 hours. Breeding should begin within 48-72 hours (depending on the type of breeding to be performed). It is recommended that an additional sample be tested 2 to 4 days later to ensure, confirming that ovulation has occurred.

**TEST PRINCIPLES**

Premate is a progesterone assay that provides a convenient semi-qualitative measurement of progesterone levels without special equipment. The quantity of progesterone present is indicated by a change in color, which is compared with low (A control = 3 ng/ml) and high (B control = 10 ng/ml) progesterone standards.

**WHEN TO START TESTING**

At least 1 serum or plasma sample should be run during the first 5 days of the cycle, during proestrus, to determine the bitch's baseline progesterone level. This varies in individual dogs, but will usually range between 0 and 1.0 ng/ml - giving a test result that is darker than the A and B controls.

If you are not comfortable performing and interpreting vaginal smears and the breeding is extremely important, or if the bitch has trouble conceiving, begin testing early - e.g., day 3 of 4 - and continue testing until you are certain of the progesterone rise indicating the LH peak has occurred. Sampling every other day is usually adequate.

If you are comfortable performing and interpreting vaginal smears, you may want to use vaginal cytology as a guideline for continued progesterone testing. Once vaginal cytology show 50% or 60% cornification, run progesterone levels every other day. (Cornification is defined as cells with roughened, angulated borders with or without a nucleus.

**TIMING OF THE FERTILE PERIOD**

Unsuccessful breeding results more often from improper timing than any other cause. Proper timing of breeding can be achieved conveniently with the use of hormonal assays. The outward signs of heat (flagging and changes in vaginal cytology) are primarily controlled by estrogen. Unfortunately, these changes are only an approximation of ovulation and can vary by more than a week. A much more precise measure is the LH surge, which actually triggers ovulation. Ovulation occurs 2 days after the LH surge. The oocytes then require an additional 2 to 3 days to mature and will live for about 48 to 72 hours. Thus, the fertile period of the bitch falls between days 4 to 7 after the LH peak, with the most fertile days beginning on days 5 and 6 post LH peak.

Premate progesterone assay allows you to estimate the day of the LH surge. During LH surge, progesterone levels will begin to rise to the 1.5 - 2.5 ng/ml level. Progesterone will continue to rise as the cycle progresses and remain elevated for 2 to 3 months in non-pregnant as well as pregnant bitches. Premate will initially detect the rise in progesterone when it reaches 3 ng/ml.

**WHEN TO CONDUCT INSEMINATIONS**

By properly planned multiple breeding or inseminations, you will optimize the probability of success.

*Natural Breeding or Fresh Artificial Insemination*

Fresh semen of a normal healthy stud often lives up to 5 or more days in the bitch. Therefore, semen inseminated a day or two before the fertile period will usually be viable at the time of peak fertility. Synbiotics recommends that breeding begin when the progesterone level has risen above 3 ng/ml. To most effectively cover the fertile period, breeding should be repeated every 2 to 3 days for a total of 2 to 3 breedings.

*Fresh Chilled Semen Breeding*

Fresh chilled semen will usually live for 2 to 4 days post collection - one day of this time is used in shipping. The reduced survival time for the sperm requires the breeding to be conducted closer to the short fertile period of the bitch. The LH surge can be most accurately identified using the Witness® LH assay kit. Synbiotics recommends that 2 inseminations, using separate ejaculates, be conducted on days 3 and 5, or 4 and 6 post-LH peak.

### *Frozen Semen Breeding*

Frozen semen survives less than 24 hours once it is thawed, so timing is even more crucial than with fresh chilled semen breeding. It is critical that the LH surge be identified using the Witness® LH assay kit. Frozen semen breedings should be conducted on days 4, 5, and 6 post-LH peak with multiple vaginal inseminations. If one surgical insemination is planned, it should be performed on day 5 or 6 post-LH peak.

### **PROGESTERONE-ONLY TESTING PROTOCOL SUMMARY**

- Progesterone levels from OvuCHECK® Premate and commercial labs will vary. The key is to get a baseline reading and see an increase from that level to know that the bitch is ovulating.
- Within the first 5 days of proestrus, take a progesterone reading to determine a baseline level.
- At 50% cornification, or around the 4<sup>th</sup> or 5<sup>th</sup> day or proestrus, start doing progesterone testing every other day.
- Around the time the LH surge is occurring, the progesterone level will be increasing, so the color in the sample well will be fading and approaching the color of the A well.
- The day the sample well matches the A well, progesterone has risen to 3 ng/ml, the LH surge has most likely occurred, and the breeding may commence within 48-72 hours (depending on the type of breeding to be performed). Perform another progesterone test 2-4 days later to ensure levels continue to rise, confirming that ovulation has occurred. Breedings should continue until the progesterone level is lighter than the B well.
- When the sample well is equal to, or lighter than the B well, the progesterone level is 10 ng/ml or greater. During peak fertility, the progesterone level is usually between 5-20 ng/ml, on the average.
- Progesterone will continue to rise and remain elevated for 2-3 months in both pregnant and non-pregnant dogs.

### **PROGESTERONE AND LUTENIZING HORMONE (LH) TESTING PROTOCOL SUMMARY (for optimal timing)**

- Within the first 5 days of proestrus, take a progesterone reading to determine a baseline level.
- At 50% cornification, or around the 4<sup>th</sup> or 5<sup>th</sup> day or proestrus, start doing LH testing daily and progesterone testing every other day.
- A positive Witness® LH test result indicates the LH level in the sample is >1 ng/ml. During this peak, progesterone levels are about 1.5-2.5 ng/ml. The progesterone level will rise and be as light, or lighter, than the A well by day 2-3 post LH surge.
- The day of the LH surge is counted as Day 0. The surge will last approximately 24 hours and will trigger ovulation 2 days after the LH surge. The fertile period is 4-7 days post LH surge.



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