

Faera

Rhonda Hovan

Perspectives

Timing Is Everything

Okay, maybe not everything. But in a sport/hobby/passion where the future always begins in someone's whelping box, timing does play a pivotal role. After all the blood, sweat, and tears that breeders often go through to reach the point where they are ready to breed a litter, there is one last hurdle to overcome before a breeder has wonderful puppies to bring into the world: the bitch has to get pregnant. And that's where the timing comes in, because the most common reason for missed breedings and small litter size is sub-optimal breeding dates.

Historically, breeders have developed traditions that work relatively well to achieve pregnancies. First and foremost is simply to permit the dogs themselves to determine breeding dates. Experienced stud dogs paired with cooperative bitches actually do pretty well when their owners permit them to breed on days of the dogs' choosing, particularly when the dogs are bred for as many days as they are both willing. With little scientific knowledge, but with experienced stockmen's common sense, the tradition developed to breed dogs every other day throughout the bitch's standing heat. This is a generally workable method that remains the most common of all breeding practices.

But there are many circumstances in which the above scenario is not feasible or advisable, or may not result in a successful pregnancy. These may include uncooperative dog and/or bitch; dog and bitch separated by distance; limited availability due to show, travel, or breeding conflicts; unsatisfactory reproductive history; and myriad other circumstances. Whatever one's motivation for doing so, breedings managed through more precise ways of determining optimal timing increase the chances of successful outcomes.

The Hormone Story

The three hormones that are most commonly tracked and most influential in determining correct breeding time are estrogen, lutenizing hormone (LH), and progesterone.

Estrogen: The first hormone that starts to change when a bitch comes into season is estrogen. Estrogen was actually already slightly elevated before she even came into heat, and begins a more noticeable rise at the beginning of her cycle. Estrogen levels peak several days prior to ovulation, and then fall fairly quickly throughout the fertile period.

In many respects, estrogen is the hormone that is most familiar, because it is responsible for the bitch's outward signs of breeding readiness, such as flirting and flagging. It is also the hormone that is tracked indirectly with vaginal cytology (smears). Smears can be used to detect changes (cornification) in vaginal cells that are produced by rising estrogen levels. However, there is a time lag between the actual rise in estrogen and the cellular changes, so that cytology does not measure the estrogen level directly, but rather follows behind the actual rise. In addition, a rising estrogen level only loosely corresponds with

ovulation, and thus is not an accurate predictor of the fertile period. Further complicating factors with smears are that the cellular changes follow a bell-curve in which their appearance on either side of the peak is similar, and interpretation is somewhat subjective based on the skill and experience of the reader. Overall, one cannot make accurate timing predictions based on following the effects of rising estrogen levels, whether by observing the bitch's signs of breeding readiness, or by using vaginal cytology.

More accurate, though less frequently done, is direct visualization of vaginal folds using an endoscope or proctoscope. Shortly before or during the LH surge, the vaginal surface becomes noticeably more wrinkled ("crenulation"), as estrogen levels fall. Extensive folds appear approximately two days after LH surge, near the time of ovulation. An experienced veterinarian can use visualization of vaginal folds more accurately than cytology to track the effects of falling estrogen as the fertile period approaches.

Lutenizing hormone (LH): Near the end of the first week of the average bitch's cycle (plus or minus several days), there is a rapid peaking of LH, called the LH surge. This is the event that actually triggers ovulation, and on breeding timelines, the LH surge is referred to as "Day 0." It is considered to be the most reliable predictor currently available of ovulation and the fertile period. Unfortunately, because the LH level falls as rapidly as it rises, it is measurable with blood tests for approximately only 24 hours. As a result, daily testing is required to monitor LH closely enough to be useful, because missing even one day can result in missing the entire rise and fall. Daily LH testing can be done in cases where timing is the most critical, as when using semen of compromised quality (such as frozen); but with the vast majority of breedings, LH testing is not practical or necessary to achieve a good result.

Progesterone: Also near the end of the first week of the average bitch's cycle, progesterone begins to rise. And although progesterone does not stimulate ovulation directly, it corresponds closely enough with ovulation to be considered the most practical hormone level available to determine breeding timing.

Progesterone levels can be measured either by semi-quantitative (enzyme-linked immunosorbent assay, or ELISA) tests such as "in office" test kits, or by quantitative tests (radioimmunoassay, or RIA) such as are generally done at off site laboratories. One difference is that the test kits approximate progesterone levels within several defined ranges (usually: under 2.0 ng/ml; between 2.0 and 5.0; and over 5.0), whereas laboratory testing provides actual precise values (e.g., 1.7 ng/ml; 3.8, 14.6, etc). Another difference is that kit tests can provide results in about 20 minutes, whereas lab results may take several hours or overnight. Because laboratory testing offers more detail than kit tests, the information is often more useful in guiding breeding decisions. Both of these tests are run using blood samples drawn by the veterinarian. At home urine progesterone tests are also available, but their reliability is currently questionable.

Canine progesterone values are usually reported in "nanograms per milliliter" or ng/ml. However, there are some labs that report the value as "nanomoles per milliliter" or nmol/ml. Nanomoles can be converted to the more familiar nanograms by dividing by 3.17 (e.g., 6.34 nmol/ml = 2.0 ng/ml).

Progesterone values offer a great deal of information to the breeder and the veterinarian. Values below 2.0 ng/ml indicate that the LH peak has not yet occurred, and that the bitch is fairly early in her cycle. (Values below 0.2 ng/ml indicate that she is not in heat.) LH peak occurs very close to 2.0 ng/ml, so 2.0 ng/ml of progesterone is also considered to be Day 0. This is the most important day relative to calculations of timing of breeding and whelping.

The next two critical changes occur at approximately two day intervals. On Day 2 (two days post LH peak), progesterone levels are very close to 5.0 ng/ml, and this is considered to coincide with ovulation. However, canine oocytes (eggs) are not “mature” and are unable to be fertilized at the time of ovulation, needing approximately two more days to develop (“capacitation”) before accepting the sperm. Thus, Day 4 is considered to be the first day of the fertile period, and progesterone values have usually risen above 12.0 ng/ml by this time. The eggs remain viable and able to be fertilized for 2-3 days, thru approximately Day 7.¹ By Day 7, progesterone values have typically risen into the mid 20’s.

After the fertile period, progesterone continues to rise – usually well over 30 ng/ml – and remains elevated for 65 days post-LH peak whether the bitch is pregnant or not. This hormone is necessary to maintain pregnancy, and in certain situations it may be useful to test progesterone again at other points in the pregnancy. For example, if there are any signs that the pregnancy may be in trouble, evaluating the progesterone level may help determine some treatment options.

Finally, at the end of a normal pregnancy progesterone returns to baseline levels, and this is one of the initiating events of labor. The typical drop in body temperature that signals imminent labor is actually caused by the drop in progesterone below 2.0 ng/ml. Progesterone testing at the end of pregnancy can verify that the litter is full term, and can help determine safe timing for an elective C-section.

What Counts, And How to Count

In the natural course of a bitch's cycle, there are certain time periods that are constant and valuable to note, and others that are less so. The time intervals discussed above beginning with Day 0 are based on measurable progesterone (or LH) levels, and are relatively stable. Another time interval that is relatively stable is the length of time between Day 0 and whelping – that is, the length of the pregnancy. The majority of bitches whelp 65 days +/- 1 day post LH peak², which is equal to 63 days after ovulation, which is equal to 61 days after the beginning of the fertile period. A handy rule of thumb is that a bitch is due to whelp on the same day of the week as she ovulated (i.e., ovulation on a Wed = whelping on a Wed).

Some other periods of time related to the heat cycle, while often closely tabulated, offer less valuable information. For example, even though it is somewhat typical that many bitches are fertile around days 11-13 from the beginning of the cycle, those days are so widely and frequently variable as to be little more than a general guess. Thus, the time period from the beginning of the heat until the fertile days is not predictable and need not be counted after the first progesterone test. This is true not only from one bitch to another, but also for the same bitch from one heat cycle to another. There is a general trend for bitches to ovulate later in the cycle as they age, but this also is not dependable.

¹ There is a recent research report (Verstegen JP, et al) suggesting that cervical closure may be the event that prevents fertilization after Day 7, rather than reduced viability of the eggs. This report suggests that canine eggs may remain viable up to eight days after ovulation, providing that the sperm are not blocked from reaching the eggs by a closed cervix. In the study, Beagles inseminated directly into the uterus conceived normal litters as late as Day 10 (post LH peak). The variability noted in that report also suggests that cervical closure may occur as early as Day 5 in 2-3% of bitches, thereby preventing fertilization after Day 5 using sperm deposited vaginally in this small fraction of bitches.

² A recent study (Kutzler MA, et al) showed that using a progesterone value of 2.02 +/- 0.18 ng/ml as Day 0, 67% of bitches whelped within one day of Day 65, and 90% of bitches whelped within two days of Day 65. This was not affected by litter size.

Another time interval that has little stability but is commonly counted is the number of days from breeding to whelping. Productive breedings can easily range from up to several days prior to ovulation (Day -1) to at least five days after ovulation (Day 7), which is a nine day span. (More on this below) But since pregnancy is measured from either LH peak (65 days) or ovulation (63 days), the breeding date is meaningless in determining an anticipated whelping date.

Finally, there is another useful way of counting that takes advantage of the relatively stable number of days from the beginning of the fertile period to whelping (61 days). In the case of a bitch bred without using progesterone testing, one may wish to verify by hindsight which days the bitch had been most fertile. For example, in the case of a small litter, determining whether the timing of the breeding was a likely cause for reduced litter size might impact future breeding decisions. Counting backward 59-61 days from the whelping date will help indicate when the bitch's most fertile period occurred.

Timing the Timing Test

While quantitative (laboratory) progesterone tests provide information at any stage of the cycle, for the purposes of planning a breeding, it is typically recommended to begin testing near the end of the first week of the cycle. Early "baseline" tests are not necessary unless the owner is uncertain of when the bitch came in season. Follow-up tests can then be scheduled at 2-4 day intervals, depending on each previous result and the breeding method. For example, with a very low value (under 1.0 ng/ml), the fertile period is not imminent, and testing again in four days may be reasonable. But as the fertile period nears, and especially if breeding arrangements need to be planned ahead (as with fresh-cooled semen), progesterone tests may be advisable on alternate days, or sometimes on consecutive days. Although most bitches will ovulate two days after LH peak (approximately 2.0 ng/ml), it is always wise to continue progesterone tests until ovulation has been verified by values above 5.0 ng/ml. There are a few percent of bitches that fall outside the norms, and may ovulate more than two days post LH peak (in which case, calculate breedings dates by adjusting Day 2 to coincide with 5.0 ng/ml of progesterone); and though very infrequent, there are occasional bitches that fail to ovulate at all.

Progesterone tests done by the kit method offer less detailed information and slightly less accurate information³, so there is reduced leeway for multiple day intervals between tests. These are also recommended to begin near the end of the first week of the cycle, and are generally repeated every other day until ovulation has been confirmed. Semi-quantitative kits offer no progesterone information after ovulation, because the highest reading available is "5.0 ng/ml or above." Thus, once the bitch has ovulated, there is no way to determine using a kit test if she may still be within her fertile period, or past it. By comparison, a laboratory progesterone test continues to give precise values through the entire cycle, and can indicate the fertile period with greater accuracy.

Finally, even if progesterone testing has not been a scheduled part of the breeding plan as early as the fifth to seventh days, it can still be initiated at any time that problems arise. That is, no matter what day of the cycle it is, if the breeding is not proceeding smoothly, a progesterone test may well offer valuable information.

³ A comparison of the "Target" brand kit to radioimmunoassay (RIA) laboratory results showed approximately 75% agreement when progesterone values were below 5.0 ng/ml, rising to 96% agreement at progesterone values above 5.0 ng/ml. (Manothaiudom K, et al)

Timing Considerations of Sperm

While understanding how to time the bitch's fertile period is requisite knowledge, a successful breeding also requires coordinating the delivery of healthy sperm. Fortunately, it is a somewhat easier task to predict sperm viability. Canine breedings are usually done with either fresh sperm/semen, chilled (fresh-cooled) sperm, or frozen sperm.

Fresh sperm/semen: Fresh sperm is sperm that results from a natural breeding, or (as defined by AKC) sperm that is collected with the bitch on the premises, for immediate use, to be artificially inseminated (AI) vaginally, transcervically, or surgically. It may or may not be mixed with a nutritious preservation medium called semen extender, and may or may not include prostatic fluid. As might be expected, fresh sperm is longer lived than chilled or frozen sperm, and healthy fresh sperm remains viable inside the bitch for at least 5-7 days. This long period of viability provides great flexibility with regard to timing the breeding. Combined with a 2-3 day fertility period of the eggs, the long life of fresh sperm extends the breeding window to about eight days. Ideally however, when opportunity permits, fresh breedings are recommended to begin by Days 3-4, and include at least one breeding after ovulation has been verified by a progesterone reading above 5.0 ng/ml.

Chilled (fresh-cooled) sperm: Chilled sperm is sperm that is collected for use in a bitch at some other location and/or near future time. (And AKC requires veterinarian signatures on both the sire's and dam's end of the procedure.) Optimal viability of chilled sperm typically requires that the semen collection is spun in a centrifuge to separate the sperm from the prostatic fluid. The prostatic fluid is then removed, and semen extender is added prior to chilling. Alternatively, experienced veterinarians may collect only the sperm rich fraction (removing the collection vessel prior to ejaculation of the prostatic fluid), to which the semen extender is added. Properly prepared in this manner, chilled sperm remains viable for 3-5 days. This permits plenty of time for an overnight shipment, with inseminations usually planned immediately upon arrival. Sperm always lives longest inside the bitch, and there is no value to storing the chilled sperm (or a portion of the sperm) and performing the AI at a later date. (This is sometimes incorrectly advised, for example, if the sperm arrives prior to the bitch's fertile period; or in the case of splitting a single collection into two inseminations in an attempt to "cover" a longer portion of the fertile period). Chilled sperm breedings are recommended to include at least one breeding after ovulation has been confirmed with a progesterone reading over 5.0 ng/ml.

Frozen sperm: Frozen sperm has a very limited span of viability, usually only 12-24 hours. This short life span, combined with substantially reduced or absent motility, necessitates precise timing and optimal handling to produce a successful result using frozen sperm. Because the sperm can neither wait for the eggs to be ready, nor go in search of the eggs, frozen breedings should be timed for after the eggs are already mature, and the sperm should be placed directly into the uterine horns via a surgical insemination. To allow ample time for the eggs to mature, frozen breedings are recommended to be scheduled three to five days after ovulation, which are Days 5 thru 7 post LH peak⁴. It is very important that ovulation has been confirmed with a progesterone reading above 5.0 ng/ml.

Breeding Timelines

⁴ A study of 16 litters conceived from frozen semen showed that of 87 total puppies, 54% were conceived on Day 7 and 46% on Day 5. (Tsumagari S, et al)

Clearly, there are numerous signs, tests, and examinations available to assist in determining the optimal time for breeding. The Breeding Timelines included with this review are intended to align so that the breeder can locate a point on any one of them (for example, a progesterone kit reading of "C3"), and follow that point vertically up and down the page to visualize how that point corresponds to time periods of interest (for example, the breeding zone for chilled sperm). Traditional breeding practices such as breeding every other day on days of the dogs' mutual choosing may certainly have their place, but breeders also have other tools at their disposal which can increase options and maximize success. Understanding the choices available allows the breeder and veterinarian to make decisions which best serve the individual circumstances.

References

1. *J Reprod Fertil Suppl.* 2001;57:31-4. Determination of the role of cervical closure in fertility regulation after mating or artificial insemination in beagle bitches.
Verstegen JP, Silva LD, Onclin K.
2. *Theriogenology.* 2003 Oct 1;60(6):1187-96. Accuracy of canine parturition date prediction from the initial rise in preovulatory progesterone concentration.
Kutzler MA, Mohammed HO, Lamb SV, Meyers-Wallen VN.
3. *J Am Anim Hosp Assoc.* 1995 Jan-Feb;31(1):57-64. Evaluation of the ICAGEN-Target canine ovulation timing diagnostic test in detecting canine plasma progesterone concentrations.
Manothaiudom K, Johnston SD, Hegstad RL, Hardy SK.
4. *J Vet Med Sci.* 2003 Sep;65(9):1003-5. Optimal timing for canine artificial insemination with frozen semen and parentage testing by microsatellite markers in superfecundity.
Tsumagari S, Ichikawa Y, Toriumi H, Ishihama K, Morita M, Kanamaki M, Takeishi M.
5. Canine and Feline Endocrinology and Reproduction, Feldman, EC, Nelson, RW. WB Saunders, 1996
6. *Canine Theriogenology Continuing Education Conference*, Walter R. Threlfall, DVM, PhD, Diplomate, American College of Theriogenologists, Ohio State University College of Veterinary Medicine, Dec 10, 2000.