Simplifying the Canine Reproductive Cycle Using the Concentration of Progesterone

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Introduction

Many veterinary practitioners have limited access to bitches that are in estrus and they may lack the experience to interpret reproductive examinations such as vaginal speculum examinations and vaginal smears. Progesterone concentrations (PC) can aid both experienced and less experienced practitioners with reproductive consultations.

Progesterone assays have been available from commercial laboratories for some years, but the turnaround time impedes their usefulness in cases where immediate clinical decisions need to be made. Point-of-care (in-house) progesterone assays are now available, allowing for immediate results within the patient visit.

Experienced dog breeders are aware of the value of progesterone testing. It is recognized that in the dog up to 75% of failures to conceive can be attributed to incorrect timing of breeding. The reason it is so difficult to time ovulation in the absence of hormone testing is that the stages of the canine estrous cycle vary considerably in length. Proestrus is defined as the period from onset of vulvar bleeding to the first acceptance of copulation; its duration averages 9 days, but it can range from 1–27 days in extreme cases.[1]

Proestrus is followed by estrus. Estrus refers to the stage where the bitch shows outward signs of receptivity, and it is the stage during which the bitch allows mating. Estrus averages 9 days, but it can range from 3–21 days in extreme cases.[2] These extreme variations have led to the belief that the bitch's heat cycle is very elastic and variable. It is only in late estrus that the bitch ovulates and that, a couple of days later, eggs become fertilizable. Fertilization typically occurs between 3–4 days before the end of estrus. The problem is that in observing a bitch during her heat cycle, there is no way of knowing when the end of estrus will be. To complicate matters, some bitches may bleed throughout their entire heat period, whereas other may not. Furthermore, some bitches may stand for a stud willingly, even long before there are eggs available for fertilization, whilst others may only be receptive for a day or two. Certain bitches may not be willing to stand for a stud at all because they only stand for a particular stud. There are many other variations and idiosyncratic behaviors that can confuse the breeder, all contributing to a missed opportunity to breed the bitch successfully.

It is also a common misconception among breeders that the duration of the different stages within a heat cycle and the point at which a bitch will allow copulation replicates itself in each repeating heat cycle of each individual bitch. The breeder then uses the data from a previous cycle and applies it to the next cycle. For instance, because a bitch was previously successfully bred on day 10 (starting from first signs of heat), the breeder will keep on mating that specific bitch on day 10. While this might work in some cases, it most certainly won't work in all cases. Similarly, it is not always true that the stud can sniff out exactly when the bitch is at her peak (optimum time for fertilization). More precise timing of the breeding events is required when there is limited access to the stud, when artificial reproductive techniques are going to be used, or when the quality of the semen is questionable. The ability to accurately time breeding is of practical and economic importance to breeders. Optimal timing helps breeders in the following ways:

- Maximizes pregnancy rates and litter size (Semen and stud fees are expensive.)
- Allows the breeder to plan travel for matings
- Allows optimization of time for assisted breeding techniques (artificial insemination [AI] using fresh, chilled, or frozen sperm)
- Allows optimization of time for breeding when access to the stud is limited to one or two matings
- Prevents unnecessary use of male
- Helps with the planning of matings when the same male is used on two bitches simultaneously
- Allows optimization of time for breeding in bitches that have a history of unreceptiveness or show silent heats
- Ovulation timing for fair estimates of expected whelping dates

Hormonal events during the bitch's heat cycle

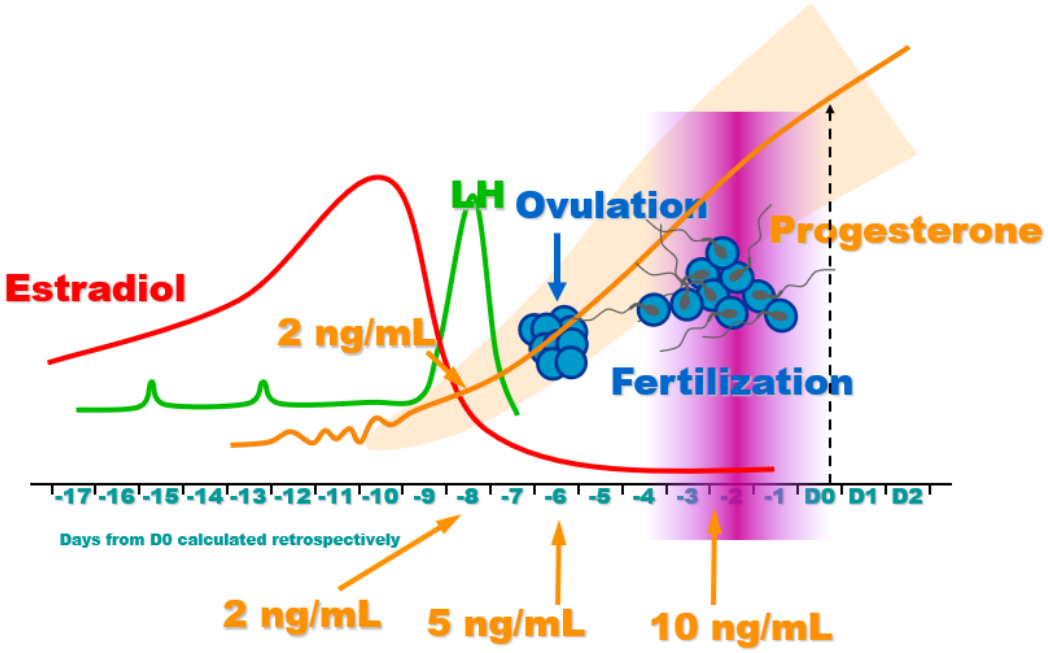

Figure 1. Chronological relationship between PC and key events in estrous cycle. Progesterone starts to rise just before ovulation, toward the last week of the heat cycle. It is well established that as soon as the progesterone levels are around 2 ng/mL, the ovulation hormone (luteinizing hormone or LH) peaks and that ovulation takes place 2 days later when the progesterone levels are around 5–8 ng/mL. Canine eggs cannot be fertilized immediately after ovulation because they need another 2 days to mature, and by that time the progesterone levels usually average 10 ng/mL or above.

Other methods used to monitor the estrous cycle to optimize the time of breeding in the bitch

As mentioned, the behavioral signs of heat can be very misleading and reveal little else other than the fact that the bitch is indeed in heat. Over the years, many methods have been made available to veterinarians and breeders to assist them in the timing of ovulation and breeding. These methods measured the vaginal pH, salinity, electrical conductivity or resistance, ferning patterns in the vaginal mucous, glucose content, and other parameters. The results of these methods did not correlate well, however, with the actual time of ovulation. Further, progesterone test kits that performed semiquantitative evaluation of the progesterone concentration proved much less useful and reliable than quantitative assays that give an accurate progesterone concentration numerical value.

Vaginal smears are only useful to confirm that the bitch is in heat and to see if there is pathology such as a severe vaginitis. Vaginal smears cannot accurately reflect the bitch's heat cycle; they cannot indicate at what point in the heat cycle the bitch is, when a mating should take place, or indeed predict in how many days her heat cycle will be over.[3,4] Vaginal smears are, however, very useful in determining when the bitch is out of heat, and this again is very helpful to accurately determining the date of whelp.[5]

Vaginal speculum examination in the hands of experienced operators is an acceptable estrous monitoring tool. It guides clinicians as to when they should start collecting blood samples for progesterone assay, especially in instances when breeding with fresh semen of good quality is planned or when there are no restrictions regarding the use of the stud. Vaginal speculum examinations may save the breeder the cost of progesterone assays; however, for critical breedings (compromised semen, chilled, or frozen semen) and aberrant heat cycles, this method lacks precision.[3]

Number of progesterone tests required to assess the heat cycle and subsequent decision making

Financial constraints and the inconvenience and time constraints to keep on presenting the bitch may limit the number of assays the veterinarian is permitted to perform. In some cases, the breeder might present a bitch too late in her heat cycle to allow for serial blood sampling in order to plot a progesterone curve. Experienced practitioners that are familiar with vaginoscopic examinations are also better equipped to correlate single progesterone results with clinical events. One of the most important reasons that a number of progesterone assays are required is to circumvent the following error: Clinical experience shows that some bitches have a spurious rise of progesterone (sometimes as high as 2–3 ng/mL) that occurs during estrus followed by several days where the progesterone remains low (well below 2 ng/mL). This spurious rise may then be followed by a second but true rise above 2 ng/mL and subsequent immediate and steep continued rapid rise in the days thereafter. If the practitioner, for instance, had noticed the progesterone rising to, say, 2.6 ng/mL and had only taken a single blood sample, the timing would be incorrect.

When the single progesterone value is around 6 ng/mL during estrus or thereabouts, the timing is likely to be more reliable and good results can be obtained when using fresh semen for breeding. However, even under these circumstances, a single progesterone reading is generally not advised. For critical breedings, an accurate estimate of the optimum time of fertilization is best achieved using three, four, or, if required, even more progesterone assays to cover the key events of the LH peak, ovulation, and onset of the fertile period.

Interval between blood collections to monitor the estrous cycle

The number of blood collections and deciding which days the collections should occur rely on the experience of the practitioner. Experienced practitioners are more likely to identify the stage of the cycle where the progesterone is likely to rise (and continue to rise) than less experienced practitioners. Less experienced practitioners are therefore advised to start collecting blood earlier in the estrous cycle rather than later. During proestrus and early estrus, the progesterone level may remain close to basal up to around 1 ng/mL for many days (10 days or longer). At this stage, the interval between collection may be extended to around 4 days. However, as soon as the bitch starts to exhibit authentic signs of estrus, blood collection and assay should be performed every second day. As soon as the progesterone has risen above 2 ng/mL, the practitioner may elect to collect blood daily or at least every second day to confirm a sharp and continued rise to 6 ng/mL or above.

Longevity of fresh sperm in the reproductive tract of the bitch

Although sperm can remain alive in the reproductive tract of the bitch for 4–6 days and longer, they do so in diminished numbers.[6] Furthermore, for many stud dogs, their sperm may survive no longer than 1 or 2 days in the female tract.[7] This is important because it gives us an indication that breeding should occur at the beginning of the fertile period and that intervals between breedings should ideally be 48 hours (2 days), even when good quality fresh semen is used.

Longevity of frozen sperm in the reproductive tract of the bitch

Frozen semen has a short survival in the female genital tract following artificial insemination, probably not exceeding 12 hours.[8–11] This is important because it gives us an indication that breeding should occur at the beginning of the optimum time of fertilization with an interval of 1 day when using frozen thawed semen.

Progesterone and pregnancy

The progesterone concentration increases to peaks of 15–90 ng/mL at midpregnancy and then gradually declines until it falls abruptly just prior to parturition or the end of diestrus in the nonpregnant bitch. The progesterone profiles of pregnant and nonpregnant bitches do not differ significantly; therefore, progesterone cannot be used for pregnancy diagnosis. However, progesterone levels above 2.6 ng/mL are required throughout the entire pregnancy to maintain pregnancy.[12] Hypoluteoidism is a condition in which the progesterone levels become too low and the bitch either aborts or resorbs. Serum progesterone should be monitored every 1 to 2 weeks in bitches with a history of pregnancy failure or luteal insufficiency.[13] Supplementation of progestogens should be done with caution and must be discontinued before the expected parturition date to avoid prolonged gestation and fetal demise.

Progesterone and whelping

Progesterone concentrations in the period around ovulation has value in that it assists with the prediction of whelping dates. In one study it was found that the date on which PC first exceeded 1.8 ng/mL (approximately 2 ng/mL) predicted the day of parturition within ±1 day, ±2 days, and ±3 days with a precision of 67%, 90%, and 100% respectively.[14] Pregnancy lengths based on time of the LH surge (synonymous with PC of ~2 ng/mL), ovulation (5–8 ng/mL), and fertilization (~10 ng/mL) are respectively 65 days, 63 days, and 61 days, with the latter two being less accurate than the former.

If progesterone is below 2.8 ng/mL, there is a 99% probability of whelp within 48 hours, and if the level is below 1.0 ng/mL this probability is 100%. Conversely, if the progesterone is 5 ng/mL or above, the probability of onset of spontaneous parturition within the following 12 hours is less than 2%.[15] Controlled studies that are sufficiently large to be convincing are required to confirm that it is safe to routinely perform a Cesarean section when the PC has decreased to below a defined threshold.

Table 1. Reference table for progesterone interpretation for bitches in heat or apparent reproductively quiescent bitches

Progesterone (ng/mL)	Likely events	Action
<1	Anestrus or proestrus	Retest in 3–4 days
1–2	Proestrus and early estrus Pre-LH surge	Retest in 2 days
2–3[14,16–21]	LH surge	Retest in 2 days to confirm continued rise in progesterone Aim for breeding 4–7 days after the rise above 2 ng/mL
3.1–4.9	Post-LH surge, preovulation	Retest in 2 days to confirm continued rise in progesterone Aim for breeding 3–5 days after this date
5–8[17,22–26]	At or near ovulation	Retest in 2 days to confirm continued rise in progesterone Aim for breeding 2–4 days after this date
8–10 and above[17,27–32]	In fertilizable period	Aim for breeding on this day and for another 2–3 days hereafter

Table 2. Reference table for breeding timing based on determination of LH surge with progesterone concentrations

Semen type	Breeding approach	Suggested breeding days (days after LH surge)
Fresh semen	Natural breeding	Days 4 and 6
Fresh semen/AI	Artificial insemination	Days 4 and 6
Frozen/chilled or compromised semen[33]	Artificial insemination	Days 6 and 7

Table 3. Reference table for progesterone interpretation around the preparturient period in pregnant bitches

Progesterone (ng/mL)	Likely events and action
>5[15]	Onset of parturition is likely 12 hours or longer away. Less than 2% of pregnant bitches with this PC are likely to enter spontaneous whelp within the following 12 hours Places bitch at low risk for need for overnight parturition observation
<2[34–36]	Most of the bitches will be within 48 hours of onset of parturition Bitch owners must be on high alert for the next 48 hours
<1[15]	All bitches are very likely within 24 hours of onset of spontaneous parturition Bitch owners must be on high alert for next 24 hours

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