Breeding, Timing, and Insemination

Historical methods for timing the estrous cycle of the bitch used observable criteria: color of discharge, swelling of the vulva, and / or flagging of the bitch’s tail. While observable, these signs cannot guarantee that the bitch is at the point in her cycle where ovulation can occur so that she can become pregnant.

Other methods of determining optimum time of breeding include a vaginal cytology, which can provide an estimate of the estrogen level in the bitch. This is called a “vaginal smear” and has been thought to help breeders pinpoint the point of ovulation. However, the vaginal smear merely gives a retrospective view of the cycle at Day 1 and at six days, post-ovulation. So, rather than being able to pinpoint ovulation, the vaginal smear is useful in providing information the timing for other tests that will better pinpoint ovulation.

The same can be true of vaginoscopy, a visual method for evaluating the swelling in the vaginal folds and the crenulation that tends to equivocate with estrogen rise and fall. Both methods of vaginal inspection, by smear, or by scopic methods give an indication that other methods of test can be run, the most commonly use, and most reliable are the LH test, and the Progesterone test.

Deciding When to Breed

Owners of breeding animals should be advised to notify the clinic when they first notice that a bitch for which timing is planned is in season, based on vaginal discharge or vulvar swelling/attraction to males. This is the proestrus phase of the estrous cycle and it should be monitored in order to know when to perform blood work testing, like Prog Tests, and to begin the search (if necessary) for stud dog semen. Additional knowing the phases of the cycle will also assist bitch owners in planning for artificial insemination. The three phases are Proestrus, Estrus, and Diestrus.
Phases of the Ovarian Cycle

**Proestrus:**
During proestrus, the bitch becomes attractive to male dogs but is still not receptive to breeding, although she may become more playful. Vulvar discharge of uterine origin is present, and the vulva is mildly enlarged (wells). Proestrus lasts from 3 days to 3 weeks, with 9 days average.

Early proestrus can be documented with vaginal cytology (vaginal smear). A baseline progesterone level might be informative if the true onset of the cycle is unknown. Vaginal cytology should be performed every 2 days until cornification progresses, and the veterinarian will know the progression is apparent; when it is apparent, progesterone testing may be done every other day, until a rise in progesterone of >2 ng/dL.

**Estrus:**
During estrus, the normal bitch displays receptive or passive behavior, enabling breeding. This behavior correlates with decreasing estrogen levels and increasing progesterone levels.

Vulvar discharge may diminish to variable degrees. Vulvar swelling tends to be maximal. Vaginal cytology remains predominated by superficial cells; RBC tend to decrease but may persist throughout.

Vaginal mucosal folds become progressively wrinkled (crenulated) in conjunction with ovulation and oocyte maturation. Estrogen levels decrease markedly after the LH peak to variable levels, while progesterone levels steadily increase (usually 4-10 ng/mL at ovulation), marking the luteal phase of the ovarian cycle.

Estrus lasts 3 days to 3 weeks, with an average of 9 days. Estrous behavior may precede or follow the LH surge and peak, and its duration is variable and may not coincide precisely with the fertile period. The LH peak refers to the Luteinizing Hormone peak, which is an indicator of the trigger for ovulation.

**Diestrus:**
During diestrus, the normal bitch will cause a diminishing attraction of male dogs. Vulvar discharge diminishes and edema slowly resolves. Estrogen levels are variably low, and progesterone levels steadily rise to a peak of 15 ng/dL before progressively declining in late diestrus.
Progesterone Levels (Prog Test)

Progesterone levels begin to rise at approximately the time of the LH surge (prior to ovulation). Rising progesterone acts synergistically with declining estrogen to reduce swelling of the vulva and vagina. Other observable signs are minimal.

Vaginal smears identify the cornification of cells that suggest the LH surge has begun, and that the bitch’s cycle is progressing. Progesterone testing is an excellent and reliable method for timing breeding because the testing can predict ovulation.

The Prog Test is a quick, simple blood draw from the bitch. The sample is spun down and then sent through a special instrument that is able to determine the level of the progesterone hormone present in the bitch.

The Progression of Results for the Prog Test:

Blood testing should begin on day 5 through 7 of the bitch’s cycle (the LH Peak); results from these days of testing are typically reported as 1.5 – 2.5 ng/dL.

Testing should continue every other day, until the prog levels are reported to be in the 3 – 5 ng/dL range.

Ovulation is considered to occur when the Prog Test reports results of 5 – 7 ng/dL; depending on the breeding being chosen, the date for breeding will vary.

Prog Tests that report results of $\geq 22$ ng/dL indicate that the bitch is in the diestrus phase, and breeding is not advisable.
Artificial Insemination and Semen Samples

The choices for breeding using artificial insemination vary. Knowing the type of breeding that is desired before running Prog Tests on the bitch is very helpful and recommended, especially when using frozen or fresh-chilled semen.

Receipt of the semen at ICSB-MA must be coordinated to ensure its use with the selected female. This is especially important for fresh-chilled collections and shipments, and for cryo-frozen semen stored at ICSB-MA. Typically, we require a minimum of three days pre-notification of an impending shipment of semen in the tanks, and a cut-off shipment time of 5:00 p.m. EST Monday through Friday; shipment of fresh-chilled semen can happen any week day at ICSB-MA, but requires a schedule appointment and has the same shipment time limits as shipping a frozen sample.

If you are planning to use fresh-chilled or cryo-frozen semen, keep in mind shipping limitations (holidays, weekends). Also keep in mind import/export issues, if the semen is being sent out of the USA, or received from a foreign country.
Choices and Timing for Artificial Insemination

This chart serves as a quick reference for breeding by artificial insemination. This is not an absolute reference, but a guide that you should find useful when planning procedures, and coordinating shipment or receipt of semen.

<table>
<thead>
<tr>
<th>Type of Insemination</th>
<th>*Prog Test Min – Max Values</th>
<th>Type of Semen Used</th>
<th>Performed at Ovulation Day #</th>
<th>**Repeat Performed at Ovulation Day #</th>
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<tbody>
<tr>
<td>Vaginal Insemination</td>
<td>5-7</td>
<td>Fresh</td>
<td>1 or 2</td>
<td>3 or 4</td>
</tr>
<tr>
<td></td>
<td>5-7</td>
<td>Fresh-Chilled</td>
<td>2 or 3</td>
<td>3 or 4</td>
</tr>
<tr>
<td></td>
<td>- - -</td>
<td>Frozen</td>
<td>NOT AVAILABLE</td>
<td>NOT AVAILABLE</td>
</tr>
<tr>
<td>TCI</td>
<td>5 -7</td>
<td>Fresh</td>
<td>1 or 2</td>
<td>3 or 4</td>
</tr>
<tr>
<td></td>
<td>5 -7</td>
<td>Fresh-Chilled</td>
<td>1 or 2</td>
<td>3 or 4</td>
</tr>
<tr>
<td></td>
<td>5 - 7</td>
<td>Frozen</td>
<td>Day 3</td>
<td>Day 4</td>
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<td>Surgical Implant</td>
<td>5 – 7</td>
<td>Fresh</td>
<td>2</td>
<td>Not performed</td>
</tr>
<tr>
<td></td>
<td>5 - 7</td>
<td>Fresh-Chilled</td>
<td>2</td>
<td>Vag. Insem 3 or 4 TCI 3 or 4</td>
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<tr>
<td></td>
<td>5 - 7</td>
<td>Frozen</td>
<td>3</td>
<td>TCI 4</td>
</tr>
</tbody>
</table>

*values are ng/dL on Idexx or Antec Scale.
Acknowledgements:

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References: