It is often surprising how many well-seasoned breeders will have little knowledge of the canine estrous cycle. Basic biology of reproductive hormones and their interactions on the bitch reproductive tract is essential for appropriate timing of insemination. Fortunately, dogs as a species tend to be very fertile (which is evident by our pet overpopulation problem) but as humans have selected for other “more desirable” traits, fertility in some breeds can be very poor. Frequently, a veterinarian is consulted for insemination when a bitch has not become pregnant after several breeding attempts, but breeding management can be important in any situation to maximize conception success.

**Canine Estrous Cycle**

The canine estrous cycle is a complex event involving the interaction of many hormones. Typically bitches will cycle anywhere from 5-12 months, although interestrous intervals of 18 months have been seen. The cycle is divided into 4 stages:

<table>
<thead>
<tr>
<th>Stage</th>
<th>Duration</th>
<th>Signs</th>
<th>Hormone Dominant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proestrus</td>
<td>3-17 days</td>
<td>Vulvar swelling, bloody vaginal discharge</td>
<td>Estrogen dominant</td>
</tr>
<tr>
<td>Estrus</td>
<td>3-21 days</td>
<td>Flaccid vulva, blood to straw colored vaginal discharge</td>
<td>Progesterone dominant</td>
</tr>
<tr>
<td>Diestrus</td>
<td>56-58 days</td>
<td>Vulva no longer swollen, lactation may occur</td>
<td>Progesterone dominant</td>
</tr>
<tr>
<td>Anestrus</td>
<td>2-9 months</td>
<td>No external signs, period of involution</td>
<td>Baseline reproductive hormones</td>
</tr>
</tbody>
</table>

The bitch ovulates an immature (primary) oocyte. Unlike other domestic species, the canine oocyte will take approximately 48 hours to mature and become able to be fertilized. Canine oocytes are unique in that they remain viable for several (4-5) days after ovulation. Canine spermatozoa can live up to 11 days in the female reproductive tract so it is very difficult to predict an exact “due date” which owners utilize natural mating. If chilled or frozen semen is to be used, optimum timing of insemination is essential. Inappropriate timing is the number one cause of infertility in the bitch.
Breeding Management

Often breeders will use time alone to begin natural mating. A breeding scheme allowing the male to breed the female every other day during her receptive period will often result in excellent conception rates in normal healthy young animals. However, due to the large variation in proestrus duration, and the variability in behavioral response, this isn’t always a reliable method.

Vaginal Cytology
The cells lining the vagina will change reliably in response to circulating levels of estrogen and progesterone. As the vagina prepares for breeding, it thickens in response to estrogen. The superficial epithelial cells are pushed further away from the blood supply and therefore gradually die and are sloughed. A cytology can easily be performed using a cotton tipped applicator and a slide. The swab is placed between the vulvar lips, angled dorsally, and advanced cranially over the brim of the pelvis. The swab is then rotated to obtain a sample and then rolled gently on a slide. The cells are then evaluated on 400x microscopy after staining in Diff Quick solution.

Vaginal cytologies are invaluable for evaluating the estrous cycle and are a cheap and effective bioassay for the presence of estrogen. The presence of superficial epithelial cells confirms the rise in serum progesterone and that the bitch is entering her fertile period. There will be a gradual change from parabasal (plump cells with large healthy nuclei) to “cornified” cells (flat, dead, pyknotic to absent nuclei). Once the cells are at least 50% cornified, progesterone sampling can begin. This helps save clients money and can help you as the practitioner observe problems with the cycle without ever having to run a hormone level assay. Vaginal cytologies are usually begun on day 3-5 of the cycle and are obtained every 3-4 days until the change is noted and progesterone sampling begins.

Progesterone
Progesterone is a steroid hormone secreted by the corpus luteum (CL) in the ovary. The bitch is unique in that progesterone will rise prior to ovulation. This progesterone rise helps the practitioner to reliably predict when ovulation will occur and thus the appropriate time for insemination. Commercial, semi-quantitative, in-house testing kits are available to estimate progesterone levels, however these are often inaccurate for precise breeding timing (i.e. In the case of subfertile animals or breeding with frozen semen). It is recommended that a commercial laboratory be used to obtain an exact number.

During anestrus and proestrus, progesterone levels will be “baseline” or less than 0.1 ng/ml. During estrus progesterone will gradually rise coinciding with the surge of luteinizing hormone that must occur to stimulate ovulation. A level of 2-3 ng/ml coincides with the LH surge, and will cause ovulation to occur approximately 48 hours later. A level of 4-10 ng/ml typically indicates ovulation, with an abrupt jump of 3-4 ng/ml 24 hours later confirming ovulation has taken place. Progesterone levels are obtained every 24-48 hours once the vaginal cytology has become >50% cornified.
**Luteinizing Hormone**

Luteinizing hormone, or “LH” is a necessary hormone secreted from the anterior pituitary to induce ovulation. A “surge” of LH occurs approximately 48-60 hours prior to ovulation and lasts 12-24 hours in duration. A commercial in-house kit (Witness® LH Ovulation Timing Test Kit, Synbiotics, Kansas City, MO) is available to document the onset of the LH surge. Due to the short duration of the surge, it is recommended to test the bitch daily. Serum samples can be “banked” by freezing them and running the frozen-thawed semen once progesterone values coincide with 2-3 ng/ml to help reduce cost.

Often the best breeding timing involves a combination of all methodologies listed. This is especially important when dealing with subfertile animals or when breeding with frozen-thawed semen.

**Timing of Insemination**

The time of insemination will vary depending on type of semen and method of insemination used. For natural breeding, often breeding every other day for a maximum of three breedings once the LH surge is identified or estimated will be effective. For subfertile animals, artificial insemination will be required.

**Fresh or Cooled Semen**

For fresh semen that will be used via artificial insemination, it is recommended to breed 4 and 6 days following the LH surge, or 2 and 4 days following estimated day of ovulation. If the stud use is limited and only a single breeding can be performed, it is recommended to breed 4 days following the LH surge (2 days following ovulation). Fresh semen is often used when the male will not mount the female. Semen is collected, extender is added, and the semen either deposited into the cranial vagina (vaginal AI) or directly into the uterus using a rigid endoscope (trans-cervical insemination or TCI). Vaginal insemination can be performed with a standard AI pipette or modified foley catheter (MAVIC™ Vaginal Insemination Catheter, Minitube of America). For successful conception, a minimum number of 150-250 million live motile spermatozoa should be used for vaginal insemination. For intra-uterine insemination, much lower numbers of spermatozoa can be used if needed (20 million of fresh collected sperm). In general, intra-uterine deposition of semen will yield a 20-30% increase in conception rate when compared with vaginal insemination.

For studs that are housed elsewhere, semen can be collected, cooled, and shipped overnight for breeding at the optimum time. We will try to coordinate two shipments of semen collected 48 hours apart but this may not always be possible. Timing for insemination is essential when using this method and often times will need to be predicted as a 24-36 hour turnaround time will be needed to receive the semen. When received, the semen is opened and a drop evaluated on a warmed microscope slide for motility. Assuming that motility is good, the bitch is prepared and the semen either deposited in the cranial vagina, or in the uterus using either TCI or surgical insemination (breeders will often call this an “implant”). The semen does not need to be warmed prior to insemination.
Frozen Semen

Frozen thawed semen is beneficial for bitches that are being bred to internationally-housed males, deceased males, or males that may be unavailable at the time of insemination. As the freezing process causes the semen to live for far less than other methods, it is recommended to place the semen directly in the uterus as close to the period of maximum fertility as possible. For most bitches this will be a single insemination performed 5 or 6 days after the LH surge (surgical insemination). If multiple breeding doses are available, this can be paired with a trans-cervical insemination 12-24 hours after a surgical insemination. Two transcervical inseminations can also be performed 24 hrs apart. Some clinicians advocate waiting until the progesterone is 18-20 ng/ml before performing the insemination to ensure adequate ovulation and maturation of the oocytes. This level of progesterone is highly variable based on individual lab values. Not all bitches read the book in this regard so good timing of LH and ovulation is still essential! It is important to note that vaginal insemination is not advised for frozen-thawed semen as conception rates are extremely poor.

Semen can be received well before the date of insemination as most vapor nitrogen tanks (“dry shippers”) will remain at freezing temperatures up to two weeks. Due to this, there should be no emergency frozen semen breedings! The semen should be at the breeding location well in advance. Please note that if the shipment is coming internationally, there are special import requirements which should be determined prior to the planned to breeding to ensure the semen is imported in time.

Once the dry shipper arrives and breeding is to take place, it is important to note that the semen received is actually from the designated stud as accidents do happen! Semen can be used in pellets or in straws. Please follow the individual thaw requirements that should be sent with the frozen semen as these will vary depending on type of semen and method of freezing.

Surgical insemination

For surgical insemination, the bitch is placed in dorsal recumbency under general anesthesia. A small abdominal midline incision is made caudally and the uterine body and caudal horns exteriorized. Once the uterus is visualized, the thawed semen is drawn into a plastic air-tite syringe by the surgeon. A 22 gauge, intravenous catheter with stilette is used to pierce the uterine body. The stilette is removed and the catheter slid cranially to ensure it is within the uterine lumen. Pressure is placed on the caudal uterine body to ensure no backflow and the semen is slowly injected, filling the uterine horns. The catheter is removed and gentle pressure is placed over the hole until bleeding stops. The uterus is gently replaced and the incision closed routinely.
References


