Sound knowledge of the bitch reproductive cycle is essential. Individual bitches may vary from normal, be presented at variable times during their estrous cycles for evaluation, and sometimes exhibit pathologic variations in cycles. Each of these scenarios requires veterinary interpretation. The normal canine reproductive cycle can be divided into 4 phases, each having characteristic behavioral, physical, and endocrinologic patterns, although considerable variation exists. Bitches with normal estrous cycles but unexpected patterns must be differentiated from those with true abnormalities. Detection of individual variation within the normal range of events in a fertile bitch can be crucial to breeding management. Evaluation of the estrous cycle for true abnormalities is an important part of the evaluation of an apparently infertile bitch.

PHYSIOLOGY OF THE ESTROUS CYCLE

Estrous Cycle

The onset of the first estrous cycle (puberty) of an individual bitch is expected between 6 and 10 month of age but may not begin until she has reached 2 years of age. The interestrous interval is normally 4-13 month, with 7 month the average.

Anestrus

The anestrus phase of the estrous cycle normally lasts 1-6 month. It is marked by ovarian inactivity, uterine involution, and endometrial repair. An anestrous bitch is not attractive or receptive to male dogs. No overt vulvar discharge is present, and the vulva is small. Vaginal cytology is predominated by small parabasal cells, with occasional neutrophils and small numbers of mixed bacteria.

The endoscopic appearance of vaginal mucosal folds is flat, thin, and red. The physiologic controls terminating anestrus are not well understood, but the deterioration of luteal function and the decline of prolactin secretion seem to be prerequisites. The termination of anestrus is marked by an increase in the pulsatile secretion of pituitary gonadotropins, follicle stimulating hormone (FSH), and luteinizing hormone (LH), induced by gonadotropin-releasing hormone (GnRH). Hypothalamic GnRH secretion is itself pulsatile, its intermittent secretion is a physiologic requirement of gonadotropin release. Mean levels of FSH are moderately elevated, and those of LH slightly elevated, during anestrus. At late anestrus, the pulsatile release of LH increases, causing the preovulatory surge. Estrogen levels are basal (2-10 pg/mL) and progesterone levels at nadir (<1 ng/mL) at late anestrus.

Proestrus

During proestrus, the bitch becomes attractive to male dogs but is still not receptive to breeding, although she may become more playful. A serosanguineous to hemorrhagic vulvar discharge of uterine origin is present, and the vulva is mildly enlarged.

Vaginal cytology shows a progressive shift from small parabasal cells to small and large intermediate cells, superficial-intermediate cells, and finally superficial (cornified) epithelial cells, reflecting the degree of estrogen influence. RBC are usually, but not invariably, present. The vaginal mucosal folds appear edematous, pink, and round. FSH and LH levels are low during most of proestrus, rising during the preovulatory surge. Estrogen rises from basal anestrus levels (2-10 pg/mL) to peak levels (50-100 pg/mL) at late proestrus, while progesterone remains at basal levels (<1 ng/mL) until rising at the LH surge (2-3 ng/mL). Proestrus lasts from 3 days to 3 weeks, with 9 days average. The follicular phase of the ovarian cycle coincides with proestrus and very early estrus.

Behavior correlates with decreasing estrogen levels and increasing progesterone levels. Serosanguineous to hemorrhagic vulvar discharge may diminish to variable degrees. Vulvar edema 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

Estrus lasts 3 days to 3 wk, with an average of 9 days. Estrous behavior may precede or follow the LH peak—its...
duration is variable and may not coincide precisely with the fertile period. Primary oocytes ovulate 2 days after the LH peak, and oocyte maturation is seen 2-3 days later; the lifespan of secondary oocytes is 2-3 days.

**Diestrus**

During diestrus, the normal bitch becomes refractory to breeding, with diminishing attraction of male dogs. Vulvar discharge diminishes and edema slowly resolves. Vaginal cytology is abruptly altered by the reappearance of parabasal epithelial cells and frequently neutrophils. The appearance of vaginal mucosal folds becomes flattened and flaccid.

Estrogen levels are variably low, and progesterone levels steadily rise to a peak of 15-80 ng/mL before progressively declining in late diestrus. Progesterone secretion depends on both pituitary LH and prolactin secretion. Proliferation of the endometrium and quiescence of the myometrium develop under the influence of elevated progesterone levels. Diestrus usually lasts 2-3 mo in the absence of pregnancy.

Parturition terminates pregnancy 64-66 days after the LH peak. Prolactin levels increase in a reciprocal fashion to falling progesterone levels at the termination of diestrus or gestation, reaching much higher levels in the pregnant state. Mammary ductal and glandular tissues increase in response to prolactin levels.

**Estrogens**

Increased estrogen causes an increased turnover rate of vaginal epithelial cells, resulting in the progressive cornification seen on vaginal cytology. Progressive edema of the vaginal mucosa also develops and can be visualized with endoscopic examination. Estrogen assays are performed by many commercial laboratories; however, the information is of little value for ovulation timing because peak estrogen levels vary from bitch to bitch, and even relative changes do not correlate to ovulation or the fertile period.

Estrogen is best assessed by serial vaginal cytologies and vaginoscopy. Estrogen levels do not indicate the fertile period because ovulation is triggered by the LH surge, not an estrogen peak. Examination of the cells on the surface of the vaginal epithelium can provide information about the stage of the estrous cycle. Proper technique is important so that the cells obtained are representative of the hormonal changes occurring. The sample should be collected from the cranial vagina; cells from the clitoral fossa, vestibule, or caudal vagina are not as indicative of the stage of the cycle.

Under the influence of rising estrogen levels, the number of layers composing the vaginal epithelium increases dramatically, presumably to provide protection to the mucosa during copulation. As estrogen rises during proestrus, the maturation rate of the epithelial cells increases, as does the number of keratinized, cornified epithelial cells seen on a vaginal smear. Full cornification continues throughout estrus until the “diestral shift” occurs 7-10 days after the LH surge, signifying the first day of diestrus.

The vaginal smear then changes abruptly, with appearance of neutrophils and epithelial cells changing from full cornification to 40-60% immature (parabasal and intermediate) cells over the next 24-36 hr. If vaginal cytology is performed until the diestral shift is observed, the LH surge, ovulation, and the fertile period can be analyzed retrospectively.

**Luteinizing Hormone**

At the end of the follicular phase of the estrous cycle, a marked increase in LH over usual baseline values develops over 24-48 hr, followed by a return to baseline values. This surge is thought to occur in response to the decline in estrogen levels and increase in progesterone levels. The LH surge triggers ovulation, making it the central endocrinologic event in the reproductive cycle of the bitch. Daily serial measurement of LH to identify the exact date of the LH surge is an accurate diagnostic tool for timing breedings.

Affordable semiquantitative in-house kits are available for measuring serum LH levels in the dog and for identifying the preovulatory LH surge and thus the time of ovulation and the true fertile period. Blood samples must be drawn daily (at about the same time) for LH testing, as the LH surge may last only 24 hr in many bitches. The kits can be subject to variable interpretation, so the same person should run the tests if possible.

**Progesterone**

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 edema of the vulva and vagina, which can be seen on vaginoscopic exam. Other observable clinical signs are minimal. Serial blood samples performed every 2 days may identify the initial rise in progesterone (usually >2 ng/mL), which indicates that the LH surge has occurred. Progesterone can be assayed by radioimmunoassay at most veterinary commercial laboratories. Several in-house semiquantitative kits are also available.

No single absolute value of progesterone correlates to any particular stage of the cycle. Progesterone varies from 0.8-3.0 ng/mL at the point of the LH surge, from 1.0-8.0 ng/mL at ovulation, and from 4.0-20.0 ng/mL during the fertile period. However, if accurate serial quantitative progesterone assays are obtained, the LH surge may be estimated as the day a distinct increase in progesterone level is seen. While this is not as accurate as actual identification of the LH surge by assay, estimation by progesterone levels is still very useful and is often more widely available and convenient.
SOME CONSIDERATIONS ON PROGESTERONE ASSAYS

Semiquantitative in-clinic progesterone assays

When timing breeding using semiquantitative in-clinic progesterone assays, only a range of progesterone is obtained, this makes it difficult to accurately identify the day of the initial rise in progesterone or the true fertile period. Technical problems with these kits have also been seen. Therefore, these assays should be used only for routine breedings in which a wider margin of error is acceptable.

Quantitative progesterone assays

1) It is critical that the result is available at least the day after collection.
2) The laboratory must be familiar with the detection of canine progesterone. Some of the human assays have the same technical problems with canine serum as the semiquantitative in-clinic progesterone assays. The results of most human assays have to be adapted to the canine species by a factor which is done by a accurate laboratory.
3) A safe rule of thumb is that when progesterone is >2 ng/mL, breeding should begin. Optimal ovulation timing should use quantitative progesterone assays from commercial laboratories—the cost difference is minimal.

Table - Some aspects of the estrous cycle in the bitch

<table>
<thead>
<tr>
<th>Duration</th>
<th>Progesterone</th>
<th>Estrogen (E₂) levels</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anestrus</td>
<td>Basal (&lt;1 ng/mL)</td>
<td>Basal (2-10 pg/mL)</td>
<td></td>
</tr>
<tr>
<td>Proestrus 3 days-3 weeks</td>
<td>Initial basal (&lt;1 ng/mL);</td>
<td>Rising to peak levels (50-100 pg/mL)</td>
<td></td>
</tr>
<tr>
<td>(9 days average)</td>
<td>At LH surge: 2-3 (0,8-3) ng/mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>At day of ovulation: 4-10 ng/mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(ovulation: range 1-8 ng/mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estrus 3 days-3 weeks</td>
<td>Abrupt decrease at the day of LH peak to 10-20 pg/mL</td>
<td></td>
<td>Primary oocytes ovulate 2 days after LH peak;</td>
</tr>
<tr>
<td>(9 days average)</td>
<td>Basal (2-10 pg/mL)</td>
<td></td>
<td>Oocyte maturation finished 2-3 days later;</td>
</tr>
<tr>
<td></td>
<td>during the following few days</td>
<td></td>
<td>lifespan of secondary oocytes: 2-3 days</td>
</tr>
<tr>
<td>Diestrus 2-3 month</td>
<td>Peak at 15-80 ng/mL</td>
<td>Basal (2-10 ng/mL)</td>
<td></td>
</tr>
<tr>
<td>(in the absence of pregnancy)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Vaginal cytology

Parabasals
Small Intermediate Cells
Large Intermediate Cells
Partly cornified
Superficial cells
Completely Cornified

Figure 1

Figure 2
Regardless of which assay is used, an additional test should always be performed 2-4 days after the first rise is detected to indicate that the cycle has progressed as expected, a functional corpus luteum has been formed, and ovulation has occurred.

**PATHOLOGY OF THE ESTROUS CYCLE**

**A) Prolonged interestrous interval**

*Primary anestrus* in the bitch is the lack of estrous cycling by 24 month of age.

*Previous ovariohysterectomy* may be the reason for a bitch not to cycle. Challenge testing of serum estrogen has been described: A paired blood sample is taken before and after 60 to 90 minutes of Buserelin administration intravenously at a dose of 0.02 to 0.03 µg/kg. Intact bitches, at any stage of the reproductive cycle, will show an elevation in serum estrogen concentration to greater than 15 to 20 pg/ml.

*Silent heat* is defined as ovarian activity with no concomitant vulvar swelling, exudation of serosanguineous vulvar discharge, or attraction of male dogs. It can be diagnosed by monthly assay of serum progesterone concentrations, with a value of greater than 2 ng/ml indicating presence of functional luteal tissue, or by weekly assessment of vaginal cytology, with increasing percentages of cornified vaginal epithelial cells indicative of rising serum estrogen concentrations. Pubertal bitches are more likely than normal adults to manifest a silent heat.

**Abnormalities of sexual differentiation.** Female appearing dogs may have a 78,XO; 79,XXX; 79,XXY; or 78,XX/78,XY karyotype. Male pseudohermaphrodites, that have male gonads and female external genitalia, also may present for primary / prolonged anestrus. Diagnosis of abnormality of sexual differentiation is made by visual inspection of abnormal external genitalia, histopathology of excised gonadal tissue, measurement of serum gonadotrophin concentration, and/or assessment of the karyotype.

**Drug induced anestrus** may be present in bitches that received anabolic drugs, androgens, progestogens or glucocorticoids. Such agents may be used to enhance performance of show or working dogs or for other reasons.

**Hypothyroidism** may lead to anestrus, prolonged or irregular interestrous intervals, prolonged proestrus or other reproductive signs.

*Bitches with systemic diseases*, such as renal failure or hypercorticism or cancer cachexia, may be less likely to cycle than normal bitches.

**Progestosterone-secreting ovarian cysts** with serum progesterone concentrations greater than 2 ng/ml may inhibit the ovarian cycle.

**Ovarian aplasia** is a rare congenital anomaly in dogs, in which there is defective prenatal germ cell migration. Serum gonadotropin concentrations are elevated in affected dogs.

**Immune-mediated oophoritis** has been described in the dog with autoimmune destruction of the ovary.

**Hyperprolactinaemia** during prolonged pseudopregnancy may lead to a prolonged interestrous interval.

**B) Shortened interestrous interval**

Premature drop of progesterone

**Split oestrus**

Pubertal bitches are more likely to manifest a split or false heat. The exact cause for split/false heat is unknown. One possible explanation is that the increased levels of estradiol-17β from follicular development during anestrus, and prior to the onset of a “true” proestrus, may initiate the clinical signs. During a split heat the bitch shows some of the signs of a true proestrus-estrous, such as a serosanguineous discharge passing from the vagina, vulvar swelling, and the attraction of male dogs. In some cases, bitches exhibiting split heats will even be receptive to mating. However, after a few days, true estrus begins in several more days or weeks. During a split heat, proestrus-estrous behaviour occurs in the absence of ovulation during the first part of the “split”. However, conception occurs during the second part of the split heat, or true estrus.

**Anovulatory cycle**

Anovulatory cycles, in which serum progesterone concentrations never rose above 3.5 ng/ml, were reported in 11 of 1152 (1%) bitches. Five of these 11 bitches (45%) had a normal, ovulatory estrus at their next season.

**Hypoluteoidism**

Luteal dysfunction is generally considered to be a primary ovarian problem, but secondary luteal insufficiency has been described in a Great Dane with a pituitary defect. Diagnosis requires documentation of low serum progesterone concentrations in diestrus. If serum progesterone falls to less than 2 ng/ml for greater than 48 hours, pregnancy may be terminated. From a more practical (but empirical) standpoint, serum progesterone values less than 10 – 15 ng/ml during the first 4 weeks after mating may be suspected as hypoluteoidism.

Supplementation of progesterone may be *progesterone in oil* administered parenterally (2 mg/kg every 3 days to not later than 58 days from ovulation or 52 days from the first diestrous vaginal smear). *Ally-trenbolone* (Regumate) is administered at a dose of 0.088 mg/kg once dayly orally, to not later than 61 days from ovulation or 55 days from the first diestrous vaginal smear. Bitches receiving the oral progestogen, ally-trenbolone, may have poor milk production in the early parturition period.

**Short anestrus syndrome**

In some breeds (e.g. German Shepherd) some individuals tend to have a shortened interestrous interval of less than 4 month. This condition is correlated with poor reproductive performance. Heritability is assumed/discussed.

**Embryonal death/resorption**

The exact incidence of embryonic and fetal loss is difficult to determine. In 22 pregnant beagle bitches from 22 to 54 days postcoitum, 13 of 117 embryos and fetuses (11%) were found to be in different stages of resorption. In another study, 13 resorptions were present among 98 fetal implantation sites (13%) in 12 beagle bitches examined by hysterotomy at 48 days of gestation.
In case, the whole litter is resorbed, the interestrus interval may be shortened.

C) Prolonged estrus

A prolonged estrogen influence may be caused by one of the following conditions:

**Ovarian cysts** (follicular cysts)

Cysts can be treated with GnRH (Fertagyl, Intervet), 10µg/kg once or repeatedly, it probably is advisable to administer GnRH repeatedly, IM for example thrice daily during two days. This treatment, however, does not always resolve the problem. If luteinisation of cystic follicles or further luteinisation of luteinised cysts take place the estrus will stop, progesterone concentration will increase and the vaginal smear will show intermediate and parabasal cells, and leukocytes. If the problem persists oestrus can be stopped by oral administration of low doses of megestrol acetate (Ovarid, Pitman Moore/ not available in Germany) (once daily first week: 0.1 mg/kg; second week: 0.05 mg/kg).

**Ovarian tumors** (granulosa cell tumors)

Tumors should be removed.

**Pathologic liver function** (metabolism of estrogens is reduced)

**Exogenous estrogens**

**Suggested readings**

Johnston et al. (2001): Canine and Feline Theriogenology, Saunders