

Canine breeding management – optimising fertility in bitches

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STEFANO ROMAGNOLI explains the different stages of a female dog's reproductive cycle, as well as oestrus-monitoring tests and techniques, to maximise the ability to conceive

THE canine reproductive cycle is divided into the oestrogen-dominated phases of pro-oestrus (average duration of nine days, initial male attractiveness, male-female interplay and female refusal to being bred) and oestrus (average duration of nine days, stronger male attractiveness and the female accepts breeding); the progesterone-dominated phases of dioestrus (range of 50 to 70 days from cessation of sexual receptivity) or (if a fertile mating occurred) pregnancy (63 or 55 to 71 days when timed from ovulation or breeding, respectively); and the hormonally quiescent phase of anoestrus (range of three to five months).

“Oestrous cycle” generally refers to the phases characterised by relevant clinical manifestation of the bitch, which includes vulvar oedema, sanguineous or clear vulvar discharge, male attractiveness, receptivity to mating and a cornified vaginal smear.

In normal bitches these behavioural and clinical signs of heat generally last 18 days (nine days of pro-oestrus and nine days of oestrus), but may vary in length and intensity, which may confuse owners and sometimes veterinarians. The range of normal pro-oestrus is three to 17 days, while normal oestrus range is three to 21 days. This stretches the normal length of an oestrous cycle from as short as six days to as long as 38 days.

Most owners tend to consider as abnormal any heat lasting more than a month. However, when considering maximum length of pro-oestrus (17 days) and maximum length of oestrus (21 days),

the longest possible heat period is 38 days. Such long heats are sometimes observed, and when duration approaches 38 days, it often becomes difficult to differentiate between a normal and an abnormal oestrus.

Differential diagnosis of prolonged oestrus behaviour includes normal heat, split heat, functional ovarian follicular cyst or ovarian neoplasia. Prolonged oestrogen stimulation, due to an ovarian cyst or ovarian neoplasia, can be dangerous for the bitch as it may cause pyometra and/or bone marrow depression. Therefore, one should be cautious when evaluating a bitch for prolonged oestrus, and assessing bone marrow function through haematology should always be taken into consideration.

Presence of heat needs to be confirmed through a careful history and exfoliative vaginal cytology as owners may:

- misinterpret signs such as vulvar discharge or male attractiveness (which may also be due to a pyometra or vaginitis); or
- observe their bitches occasionally rather than regularly (and therefore may be led astray by a split heat).

Furthermore, receptivity to mating can be present in early dioestrus, which may also confuse owners on what is the actual length of oestrus. Clinical and behavioural signs of oestrus in the bitch and their relative importance as indicators of presence of oestrogens in the general circulation are presented in [Table 1](#).

Split heat

At puberty, bitches may experience an anovulatory cycle, which is typically referred to as false or split heat. A false heat may last from two to seven days up to 10 to 15 days, and is generally followed by a normal heat after an interval of a few days to a few weeks, or even after a normal interval.

Owners, especially if unfamiliar with canine reproductive physiology, may not notice there is an interval between the false and the subsequent (normal) heat – especially if such interval is short. Therefore, a normal bitch at its pubertal season may be presented with a complaint of prolonged oestrus.

Split heats may occur in the adult bitch as well, thus leading owners astray – especially when a bitch has not had a false heat at puberty. These could be due to follicular development during late anoestrus producing transient elevations of oestrogen concentrations to pro-oestrus/oestrus levels.

Ovulation timing

Most bitches ovulate on day 12 of their season, therefore there is a widespread tendency of owners to assume day 12 is the ideal breeding day for all bitches. As a matter of fact, some bitches ovulate early (such as on day eight, six or even four from the onset of pro-oestrus), while others may ovulate as late as day 17, 19 or 22 to 24. One should never assume a given bitch will ovulate on day 12 unless proven.

Managing a canine breeding requires the client taking the bitch to the vet clinic as soon as the first signs of pro-oestrus display (vulvar discharge, male attractiveness) for a first check (to be done no later than day five), then coming back every two to three days to monitor how quickly the female is progressing towards ovulation through vaginal smears and serum progesterone assays.

Vaginal cytology

Vaginal cytology is still considered the simplest and most practical way to confirm oestrus, stage of the oestrus cycle and define length of the follicular phase, and it should always be performed on a bitch in oestrus. In normal bitches, maximal vaginal epithelial cornification is generally reached towards the end of pro-oestrus and persists for an average of nine to 15 days until the first day of dioestrus (D1).

The vaginal exfoliative cytology pattern of D1 is characterised by a sudden per cent drop of cornified vaginal epithelial cells, a concomitant sudden per cent increase in intermediate vaginal epithelial cells and a short-lasting (few days) increase of polymorphonuclear cells. D1 occurs six (ranging from five to eight) days after ovulation, therefore fertility at this time is very low or absent. Identifying D1 can be helpful to confirm ovulation and assess potential for conception based on timing of mating.

Vaginal endoscopy

Vaginal endoscopy is a good way of identifying and monitoring the oestradiol curve. During pro-oestrus the vaginal epithelium is pink and edematous, with vaginal folds appearing round and swollen, with some blood-tinged fluid in between them. This feature tends to progressively decrease as pro-oestrus comes to an end and the bitch enters oestrus – a phase that in the canine is characterised by declining oestrogen production.

As soon as follicular oestrogen production stops, fluid is reabsorbed from the vaginal mucosal lining and therefore the vaginal folds become whitish and wrinkled (a process called “crenulation”). As the wrinkling process is associated with a decrease in oestradiol rather than an increase in progesterone, the crenulation is only an indirect indicator of ovulation.

At the onset of dioestrus the vaginal epithelium changes abruptly becoming blotchy and red, and the wrinkles flatten out. If one touches the dioestrus mucosa with an instrument (an endoscope or catheter), the mucosa will blanch and then redden – a phenomenon called rosette formation.

During the rest of dioestrus the mucosa keeps flattening and remains blotchy pink-red – a feature that becomes even more evident during anoestrus.

Vaginal endoscopy is a good way of monitoring oestradiol (and not progesterone) concentration through its effects on the vaginal mucosa. Similarly, instruments that assess vaginal mucus electrical conductivity (such as the Draminski ovulation detector) allow monitoring the oestradiol curve and are only indirectly associated with ovulation. Vaginal endoscopy or vaginal mucus conductivity cannot be used to identify ovulation directly; serum progesterone remains a vital indicator in this respect.

Ovarian ultrasound

Ovarian structures can be visualised with ultrasound using 5.0 to 7.5 sectorial MHz probes; follicular growth can be followed and ovulation can be estimated based on disappearance of the hypoechogenic areas representing follicles (which become luteinised) and on appearance of a hypoechogenic area at the periphery of the ovary representing follicular fluid accumulation within the ovarian bursa.

Luteinising hormone assay

The luteinising hormone (LH) peak is the most important landmark of the canine reproductive cycle as it triggers ovulation. The LH peak is characterised by a rising phase of approximately 12 to 24 hours and a subsequent declining phase of approximately 12 to 36 hours for a total length of 1.5 to 2.0 days. The onset of oestrus behaviour is generally coincident with the LH peak, but it is often displayed one to four days later and, in some cycles, it just never occurs, regardless of a normal sequence of endocrine events.

Although the magnitude of hormonal secretion has never been accurately documented in cases of silent heat, failure to display oestrus behaviour may be related to an insufficient oestradiol secretion or to a varying sensitivity to changes in oestradiol: progesterone ratio.

Because of its importance in timing ovulation, LH assay would be very useful in clinical practice. Unfortunately, due to costs and short duration of its surge (several days of consecutive daily samplings would be necessary to catch the LH peak), the use of LH assay in a clinical setting tends to be restricted to very specific cases, such as when using frozen semen.

Progesterone assay

Unlike LH, serum progesterone (P4) assay is extremely helpful in managing canine reproductive cases. Determining P4 concentration helps to:

- confirm presence of corpora lutea;
- characterise length of the luteal phase; and
- stage the oestrous cycle (especially if used with vaginal cytology; [Table 2](#)).

In the normal bitch, a cornified vaginal smear with low (less than 2ng/ml) serum P4 concentrations indicates early oestrus (ovulation has not occurred yet), whereas a fully cornified vaginal smear with high (greater than 2ng/ml) serum P4 concentrations indicates late oestrus (ovulation has occurred).

Serum P4 has a concentration of 2ng/ml to 3ng/ml on the day of the peak of LH, 4ng/ml to 10ng/ml on the day of ovulation, and 10ng/ml to 34ng/ml during the two days following ovulation, which is when oocytes are reaching maturity in the ampulae of the oviducts and fertilisations are taking place.

Reproductive behaviour

The importance of vaginal cytology and P4 assay should not lead us to disregard the observation of reproductive behaviour. Although male attractiveness and acceptance of mating may not necessarily correlate with blood oestrogen concentration, observing the bitch's reaction to the male can help identify bitches that ovulate early in their cycle.

Clinical signs of oestrus are typically displayed coincidentally or shortly after the onset of the LH peak. Although strictly related to the LH peak, oestrus behaviour is under the control of both oestradiol and P4, with the former directly stimulating and the latter potentiating the clinical manifestations of heat.

In ovariectomised bitches, the subcutaneous administration of oestrogen implants causes a slow and progressive increase of pro-oestrus-like behaviour; if a P4 implant is administered at the time of oestrogen implant removal, full oestrus behaviour is displayed within eight to 12 hours. Probably, it is the decline in oestrogen:P4 ratio, which triggers acceptance behaviour and the secretion of pheromones responsible for male attraction.

Oestrus behaviour ceases around the D1, although in some bitches, male acceptance can be observed also on D2 or D3. Oestrus behaviour is not extremely well-correlated with endocrine events, but it is, nevertheless, reliable in approximately 80 to 90 per cent of cases and, as such, its importance in breeding management should never be underestimated.

Canine breeding management

A bitch to be bred should be examined for the first time no later than day five of its cycle as some

owners may miss the early signs of the oestrous cycle, so the bitch may be much further along in its cycle than believed. If breeding is to be accomplished with frozen semen, breeding management should be started earlier in the cycle, since determination of the LH surge is significantly more important when using frozen semen. Vaginal cytology is typically performed every other day.

It is not necessary to start assaying serum P4 until after full cornification of the smear is attained; however, bitches that have slides indicative of early to mid pro-oestrus may have blood samples drawn, and the serum frozen and stored to be able to backtrack to evaluate P4 or LH concentrations, thus increasing accuracy of ovulation staging. The percentage of anucleated squames is a useful parameter, as these cells tend to increase as ovulation approaches. Therefore, P4 concentrations should be performed every other day (EOD) once vaginal cytology indicates 50 per cent superficial cells and 20 per cent anucleated superficial cells ([Figure 1](#)).

The EOD frequency of blood sampling is acceptable if one is only measuring P4, while frequency should be daily if LH is to be assayed as the LH peak is very short-lived (average 18 to 24 hours) and it is easy to be missed when sampling daily. As the bitch approaches ovulation, EOD to daily sampling may be required depending on the type of breeding being performed and access to laboratory services.

At least one vaginal cytology slide should be obtained on the day of presentation in case the initial P4 concentration is very elevated, in which case a vaginal smear is necessary to determine if the bitch is still within its fertile window or if it has already entered dioestrus.

In some practices only LH testing is performed, and inseminations are planned based on the LH surge. However, the routine use of LH measurement may only be inadequate for bitches that may experience anovulatory cycles or have an abbreviated LH surge, so use of LH and progesterone or progesterone alone is better than LH alone.

Ovulation occurs when P4 reaches values between 4ng/ml and 10ng/ml, and is confirmed by a rise in P4 of at least 3ng/ml in 24 hours ([Table 3](#)). Some bitches rise 0.03ng/ml in 24 hours, while others jump by 10ng/ml or more in 24 hours. The amount of the rise is due to the number of follicles ovulated, the size of the follicles and the amount of luteal tissue present. Increases of less than 3ng/ml/day should be a reason for concern about ovulation failure, and additional samples should be obtained over the next one to two days.

Failure to confirm ovulation is a common source of problems, as often, low fertility bitches may reach the 4ng/ml to 5ng/ml stage and then stop there or go back to lower levels. If bred, these bitches normally do not conceive or may have a small litter size, and determination of due date is often complicated.

Types of insemination

Canine semen survival in the female genital system is variably long, and depends on age and reproductive health of both male and female dogs.

Fresh semen survives in the bitch from a minimum of 48 hours up to 11 to 14 days. Fresh, chilled semen can be expected to survive in the bitch at least 24 hours and may live significantly longer, depending on the dog. Frozen semen has a relatively short lifespan after thawing – on average, eight to 12 hours.

The type and routine of hormone analysis recommended depends on the type of breeding selected. With natural breeding and vaginal fresh semen insemination, assaying serum P4 every two to three days is considered adequate. Testing should be done at least once or twice after P4 has reached 4ng/ml to 5ng/ml.

EOD serum P4 testing is also sufficient for transcervical (or surgical) artificial insemination (AI) with fresh or chilled semen, while LH assay should be added when using frozen semen to help pinpoint ovulation. Ovulation should always be confirmed prior to inseminating with frozen semen, and always prior to anaesthetising the bitch for a surgical AI.

Natural breeding is recommended for fertile bitches and dogs with normal semen quality; when using good quality semen, AI performed intravaginally has the same conception rate of natural breeding. If semen quality or vaginal/uterine conditions are suboptimal, intrauterine AI (performed transcervically using endoscopy or surgically) may have a better success rate.

When to do it

When using natural breeding, bitches should be bred after ovulation, starting one or two days later and continuing EOD for up to three matings or until receptivity is not observed any longer. When using vaginal AI, breeding should start two days after ovulation and should be repeated in two days.

When using transcervical AI with fresh or fresh chilled semen, inseminations should be done two and four days after ovulation.

If only one insemination is planned, then day two, three or four post-ovulation should be picked as there is no known difference in fertility when picking one of the above three days.

When using frozen semen, two inseminations are preferable to one, with breedings planned on days five and/or six after the LH surge, or days three and/or four after ovulation. Surgical AI with fresh or fresh chilled semen can be performed on day two, three or four after ovulation. If semen longevity is poor, waiting to day three or four after ovulation is preferable. When using frozen semen, surgery should be done on day three or four after ovulation (days five or six after LH surge).

Further reading

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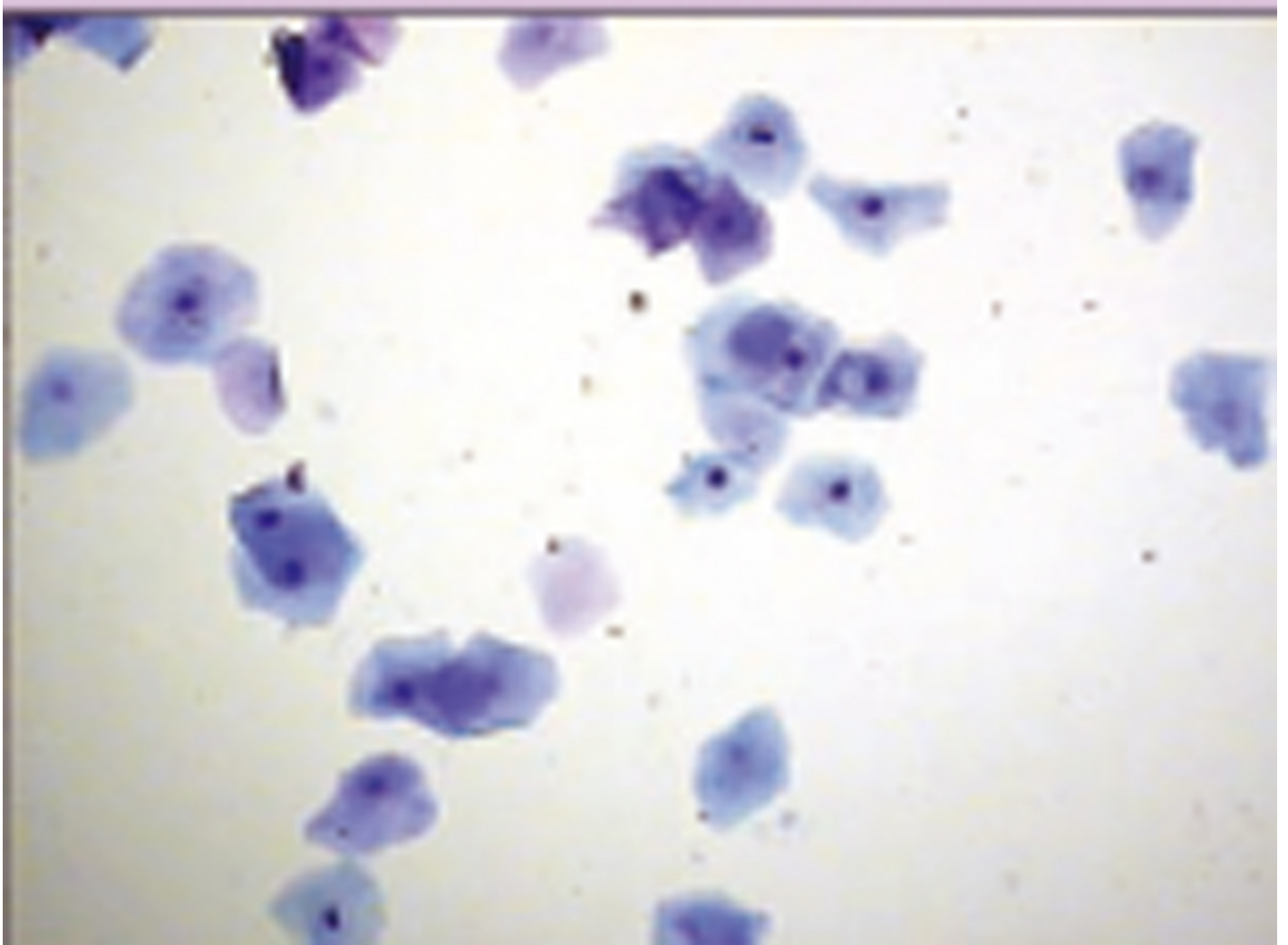


Figure 1. A fully cornified vaginal smear indicates the bitch might be anywhere between early and late oestrus. However, early oestrus smears show quite a variability of cellular types (including blood line cells), may also feature relevant amounts of debris and many cornified cells still show their nuclei. Full (late) oestrus smears typically feature only cornified cells, no red blood cells, no leukocytes, little – if any – debris, and at least 20 per cent of the cornified cells have no nucleus (also known as anuclear squames) as in the vaginal smear portrayed here. Such a late oestrus vaginal cytological pattern may last several days – up to greater than 10 days in some cases. Serum progesterone assay helps discriminating between bitches in early oestrus (which might not achieve the best conception rate, or might not even conceive if bred by a male of low fertility) and those who are ovulating and are more fertile.

Vaginal smear >50% cornified	(100%)
Male attractiveness	(95%)
Vulvar oedema	(90%)
Vulvar discharge	(80%)
Standing behaviour	(70%)
Mounting behaviour	(30%)

Table 1. Clinical and behavioural signs of oestrus in the bitch and their incidence. A fully cornified vaginal smear is always present. Male attractiveness is also extremely common, although silent heats may occur. Vulvar oedema and discharge also reflect the action of oestrogens. Standing behaviour can be affected by male preference, and as such, may not always be observed. Occasionally, bitches in heat will tend to mount other bitches.

Vaginal smear	Progesterone	Stage of the cycle
Cornified	<2ng/ml	Early oestrus
Cornified	>2ng/ml	Late oestrus
Non-cornified	>2ng/ml	Dioestrus
Non-cornified	<2ng/ml	Anoestrus/pro-oestrus

Table 2. How to stage the canine oestrous cycle using vaginal smear and serum/plasma progesterone concentration.

Stage of the cycle	Serum P4 concentrations, ng/ml	Serum P4 concentrations, nmol/L
Anoestrus or early pro-oestrus	0-1ng/ml	0-3.14nmol/L
LH surge (two days prior to ovulation)	1.1-1.9ng/ml	4.08-5.97nmol/L
Progesterone surge (one day prior to ovulation)	2-3.9ng/ml	6.28-12.25nmol/L
Ovulation	4-10ng/ml	12.56-31.40nmol/L
Oocytes mature and available for fertilisation	10-34+ng/ml	31.40-106.76nmol/L

Table 3. Serum progesterone (P4) values in different stages of the canine reproductive cycle. Values are in ng/ml and the so-called standard international (SI) units of nmol/L. The conversion factor from 1.0ng/ml to SI units is 3.14nmol/L.