

Breeding Management and Ovulation Timing in the Bitch

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Introduction

The bitch is sometimes seasonal, mono-estrus, polytocous and a spontaneous ovulator. Ovulation is followed by a prolonged luteal diestrus or pseudopregnancy, in turn followed by anestrus. This has been reviewed in detail (Concannon 2011). Gonadotrophin-releasing hormone (GnRH) secreted from hypothalamic nuclei into the portal hypophyseal circulation causes pulsatile release of stored pituitary gonadotrophins inducing steroid synthesis and gametogenesis by the gonad. Steroids mediate reproductive behavior and gametogenesis. Steroids and gonadal inhibin have a negative feedback on the hypothalamus and pituitary glands. Follicle-stimulating hormone (FSH) acts on granulosa cells in the wall of the ovarian follicle inducing steroidogenesis, including estrogen synthesis for transport to luteal cells. A surge of luteinizing hormone (LH) is precipitated by a fall in estrogen:progesterone ratio and is responsible for ovulation and luteinization of follicular theca and/or granulosa cells to luteal cells which produce progesterone. All progesterone is luteal. LH and prolactin are luteotrophs. The preovulatory LH surge can be temporally associated with all other events of estrus. Multi-oocytic follicles occur (Reynaud 2012) and may ovulate, though probably only one oocyte is functional. LH causes ovulation within 2 days. Primary oocytes are ovulated (requiring still the extrusion of the first polar body and completion of the first meiotic division), mature 60 hours after ovulation, and reach the distal oviduct in hours and remain there and degenerate from 6 days after ovulation. Although sperm can penetrate the immature oocyte, the fertilization process is not understood; more typically sperm penetrate the secondary oocyte causing completion of meiosis and extrusion of the second polar body (England, Concannon 2002).

Inter-Estrous Interval (IEI)

The IEI has a mean of 7 months, range of 4.5 to 12 months, and varies within and between bitches. Pregnancy does not affect IEI.

Proestrus (7–9 days; range 3–27 days). Proestrus and estrus together comprise heat. Estradiol rises and falls, progesterone rises, vaginal cornification begins, and the bitch is attractive but not receptive to males. Proestrus to estrus behavior changes about the time of the LH surge (mean 1 day after, range 3 days before to 5 days after).

Estrus (7–9 days; range 1–24 days). Progesterone continues to rise in response to the pre-ovulatory LH surge, the bitch is receptive to males, and ovulation occurs in response to and about 2 days after the LH surge. There is no difference in the time of ovulation of left and right ovary. Primary oocytes

are ovulated and cannot be fertilized until the completion of the first meiotic division to form the secondary oocyte, which takes a further 2–3 days. Ovulated oocytes may be fertilizable for 2 to 5 days; however, there may be breed differences.

Diestrus/Pregnancy (2 months). The bitch is a spontaneous ovulatory - every estrus will necessarily be followed by diestrus or pregnancy. The luteal support for pregnancy is 64 ± 1 day. Non-pregnant diestrus is about 70 days. Progesterone levels peak at 2–4 weeks of diestrus. Diestrus finishes when progesterone is < 2 nmol/L.

Anestrus (2 to 10 months). Anestrus is an obligatory period characterized by a quiescent endometrium and the absence of circulating sex steroids. Prolactin levels decline. Gonadotrophin levels come in pulsatile bursts toward the end of anestrus.

The Fertile Period

The following factors can result in mistimed matings and failure of conception; they can also provide an explanation why matings at very different times relative to behavioral and clinical signs can result in conception.

- Ovulated secondary oocytes are fertilizable for 2 to 5 days.
- Ejaculated spermatozoa can be stored in a reservoir in the tubular tract, which may be the uterus, uterine glands or the oviduct, for 2 to 5 (and reported up to 11 days) - this seems to vary with individual dogs and bitches.
- The clinical signs and receptive behavior of the bitch may not be closely related to underlying endocrine and gamete events.

The period of possible fertility after mating is 3 days before to 7 days after ovulation (Tsutsui 1989; England, Concannon 2002); however, the optimum opportunity for fertilizable oocytes to be fertilized exists from 2 days to 5 days after ovulation. After this period, the sperm reservoir is lost, oocytes degenerate, and the cervix closes.

Aberrations are:

- Very early mating in proestrus might result in conception if sperm storage is prolonged.
- Very late mating after the cervix has closed and after the fertile period can result in conception if the sperm deposition is intrauterine.
- Sperm that have been stored (frozen or chilled) seem to have reduced longevity in the bitch.

Broadly, pregnancy rates following natural mating are similar when bitches are mated on any day from 2 days before ovulation to 4 days later (LH + 0–6 days). Natural mating should be clustered in this period, 24–48 hours apart and preferably on LH + 3–5 days to allow for sperm capacitation. The period of potential fertilization of mature oocytes is 4 to 6 days after the LH peak - breeding with damaged or cryopreserved sperm should be limited to this period. Breeding outside these times can result in conception but at lower rates of fertility and litter size.

Recommendations

- Natural mate: LH + 3–6 d (1 to 4 days after ovulation)
- Chilled semen: LH + 4–6 d (2–4 days after ovulation)
- Cryopreserved semen: LH + 5–6 d (3–4 days after ovulation)

Counting Days

In a beagle colony, ovulation occurred 11.1 ± 0.2 (sem) days after the onset of vulval bleeding (Hori *et al.* 2012), but the range was 3 to 31 days and was unrelated to cycle length and varied between bitches, and cycles within bitch, so day counting cannot be relied on for planning mating.

Receptivity

The onset of behavior estrus (bitch receptivity) roughly approximates the LH surge; however, variations between and within bitches in receptivity and behavioral responses to dogs, and vice-versa, and mating situations mean this is not uniformly predictive.

Vaginal Discharge and Vulvar Morphology

In proestrus, the vulva is swollen and turgid under the influence of estrogen, with varying amounts of sanguinous vulvar discharge of red cells from uterine diapedesis. As estrus progresses, the vulva is swollen but with increasing flaccidity as estrogen falls, and progesterone rises and the vulva softens and turgidity is lost, and there may or may not be sanguinous to serous vaginal discharge. Typically vulvar softening approximates the LH peak but is subjective and not solely predictive. Vaginal discharge is too variable to be useful other than as a rough indicator of the estrus + proestrus "heat" period.

Vaginal Cytology

Vaginal cytology is possible because the vaginal epithelium, which is simple cuboidal at rest (in anestrus), becomes a stratified squamous epithelium under the influence of rising then falling estradiol and rising progesterone during proestrus and estrus presumably in preparation for mating. Exfoliated vaginal cells can be harvested by rolling a cotton-tip swab wet with saline over the anterior vagina, with care taken to avoid the squamous epithelium of the vestibule. Cells can be rolled onto a glass slide, air dried, and stained with a Wright-Giemsa or trichrome stain. Harvested epithelial cells change shape.

- Parabasal cells are the simple cuboidal epithelium of the non-estrous vagina, which are small and round and with a modest cytoplasm.
- Intermediate cells are partially cornified, nucleated, and are flatter with irregular cell borders.
- Superficial cells have small, dark-staining pyknotic nuclei and are flat and with generous cytoplasm compared to nucleus volume.
- Nuclear cells are fully cornified cells.

Cytologic estrus occurs when 80% or more of epithelial cells harvested are superficial and anuclear cells. The fertile period falls within generally cytologic estrus; however, there is sufficient variation in the onset and progression of cornification between and within bitches that cytology is useful more to

determine the progression through proestrus (and so the time to begin endocrine assays) than as a sole test for the fertile period. Cytologic estrus finishes and diestrus begins when the keratinized epithelium is lost and there is a sudden reappearance of 20% or smaller intermediate and parabasal cells.

The vagina has a bacterial flora and contains neutrophils at rest. As cornification occurs neutrophils are unable to migrate into the vaginal lumen and bacterial numbers increase. At the end of cytologic estrus, when the stratified epithelium is sloughed, there is a sudden "shower" of neutrophils marking the beginning of cytologic diestrus and a simple cytologic test for the end of the fertile period. This may be confused with proestrus.

Serial Serum Progesterone Assay

Luteinization of the follicular wall, induced by the LH surge, results in rising serum progesterone coincident with the LH surge. Serial progesterone measurements can be collected by sampling every 1 to 4 days - increasing in frequency as the sharp rise in progesterone approaches.

Continued sampling will confirm ovulation. Broadly:

- Progesterone is over 6.5 nmol/L (2 ng/ml) at the LH peak: breed 4–6 days later.
- Progesterone is over 16 nmol/L (5 ng/ml) at ovulation.
- Progesterone is over 25 to 30 nmol/L (8 ng/ml) at the start of the fertile period (LH + 3 days): breed 1–3 days later.

Hollingshead (2012) demonstrated no difference in whelping rate for insemination on LH + 2 to LH = 6. Progesterone concentrations vary between bitches from LH + 5, making its use unreliable (Hollingshead 2012). The end of the fertile period cannot reliably be determined by progesterone assay. It is thought that the cervix closes at about progesterone = 90 nmol/L (Verstegen, personal communication); however, this does not preclude vaginal matings resulting in fertility when progesterone is > 90 nmol/L. Assays available are radioimmunoassay (RIA), chemiluminescence (CLIA), fluorescent immunoassay and ELISA. There are qualitative and semiquantitative assays for in-clinic use, but these are most useful for natural matings where precision is of reduced importance. Progesterone is affected by the vessel into which it is collected, prolonged exposure to red blood cells, clot activators, and inter-laboratory difference, and eating prior to sample taking can increase hepatic metabolism of progesterone. RIA is the preferred assay with CLIA reading slightly higher (Bergeron, Gartley 2012). Good assays have a 20% coefficient of variation (so 10 nmol/L might be 8 or 12). There is a diurnal variation in pregnant bitches (progesterone is higher in the morning). **Note to convert 3.18 nmol/L = 1 ng/mL.**

Serial Serum LH Assay

The LH peak can be measured, and it is predictive and reliable; however, it carries the shortcoming that the LH surge is short-lived, so sampling must be frequent (daily at the same time every day) and concentrated at the appropriate time in order to detect the peak. Laboratory radioimmunoassays

are sporadically available. An in-clinic semiquantitative ELISA is available, which gives a result of low < 1 ng/ml or high > 1 ng/ml (Root Kustritz 2001). The test kits have a short shelf-life.

Vaginoscopy

Endoscopy allows monitoring of vaginal epithelial changes which grossly appear as changes in folding and colour. In **proestrus**, the mucosa is pink, edematous, and thickened under the influence of estrogen and thrown into primary longitudinal folds in late proestrus. Pink changes to white with epithelial cornification obscuring submucosal capillaries. As estrogen falls and the LH peak occurs in early **estrus**, the folds shrink. During the fertile period, secondary transverse folds develop, and folds become angular with white/cream colour - called crenellation; this cobblestone appearance is coincident with the fertile period and is present for about 3 days. In **diestrus**, the epithelium is sloughed so then in **anestrus** the mucosa is flat and dry and red.

Other Techniques

Vaginal glucose concentration, observations of the **crystallization of cervical glandular mucus** from the anterior vagina, and measuring **electrical resistance of vaginal fluid** are not predictive of ovulation. **Sonography** can be used to detect follicular growth and ovulation; however, variability in image quality and operator, the necessity for daily examinations, and the subtle changes associated with ovulation make this impractical.

Breeding Management

Beginning within the first week of estrus, include physical and reproductive system examination, serology, bacteriology, vaginal cytology, serial progesterone, and/or LH assays and vaginoscopy with planned natural or artificial breeding with follow-up progesterone assay to confirm complete ovulation and full luteal phase.

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