

Managing the Reproductive Cycle in the Bitch

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KEYWORDS

• Bitch • Reproduction • Estrus • Breeding management

This article reviews the normal physiology and endocrinology of the estrous cycle of the bitch and how to use that information to guide decisions about breeding management. This article also explains the mechanism of action of pharmaceuticals and strategies for estrus induction and suppression. There are many excellent review articles published on these topics. The references cited here include some of those review articles, from which readers may locate articles from the primary literature if they wish, and recent publications. Topics that will not be addressed are general methods of contraception and pregnancy termination.

THE CANINE ESTROUS CYCLE

The canine estrous cycle consists of 4 recurring stages (**Table 1**).¹ *Proestrus* is defined as the first outward evidence of fertility in the bitch. *Estrus* is defined by the bitch's behavior; she will allow the male to mount and breed her during this stage. For this reason, many owners call this stage "standing heat." *Diestrus* is defined as the bitch no longer being receptive to mounting and breeding ("going out of heat"). The end of diestrus is defined as a decline in progesterone below that needed to maintain pregnancy (1 to 2 ng/mL [3.1 to 6.2 nmol/L]).^{2,3} *Anestrus* is defined as absence of outward signs of fertility and low serum progesterone concentrations.¹

The *interestrus interval* is defined as the duration from onset of a given proestrus to onset of the subsequent proestrus. Interestrous interval varies between bitches and may vary within bitches, with reported average of 6 to 7 months and reported range of 5 to 12 months.³ Underlying causes of variation in interestrous interval include breed and environment. German shepherd dogs and rottweilers frequently are described as having interestrous intervals shorter than the average, although one study refutes this in one population of German shepherd dogs.^{4,5} Basenjis and Tibetan mastiffs cycle only once yearly, and some populations of wild dogs also may cycle seasonally, with time of estrous activity such that pups are born during clement weather early enough in the season so they are full grown before seasonally inclement weather redevelops.^{3,6,7} Domestic

The author has nothing to disclose.

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Vet Clin Small Anim 42 (2012) 423–437

doi:10.1016/j.cvsm.2012.01.012

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Stage (Duration)	Physical Changes	Behavioral Changes	Vaginal Cytology	Primary Endocrine Event(s)
Proestrus (9 d, range 3–21 d)	Vulva swollen and turgid, serosanguinous vulvar discharge	Males interested, bitches will not allow mount or breeding	Gradual increase in percentage cornified cells, decrease in PMNs	Follicular development; rise in serum estrogen concentration
Estrus (9 d, range 3–21 d)	Vulva softens, straw-colored vulvar discharge	Bitch allows mount and breeding	All cells cornified with greater than 50% anuclear, no PMNs	Fall in estrogen with subsequent rise in LH and ovulation, preovulatory rise in progesterone
Diestrus (pregnant bitches 62–64 d, nonpregnant bitches 49–79 d)	Slight mucoid discharge early in diestrus, mammary development possible with true or false pregnancy	None	Abrupt return to noncornified epithelial cells, large number of PMNs early in stage	Progesterone rises and then falls over this stage, falling abruptly at end (pregnant dogs) or more gradually diminishing (non-pregnant dogs). Progesterone production is supported by LH and prolactin secretion.
Anestrus (1–8 mo)	None	None	Noncornified epithelial cells, occasional PMNs	FSH relatively elevated throughout, LH concentrations increase late in stage after estrogen priming

bitches generally are described as nonseasonal in their estrus activity, although one study demonstrated some seasonality dependent on exposure to natural light and temperature.^{3,4} Studies disagree as to whether interestrus interval is dependent on whether the bitch became pregnant and whelped.^{4,5}

Puberty in bitches is defined as the first overt sign of fertility and therefore usually is defined as the first obvious proestrus. Time of puberty varies with breed, with small

breed bitches entering estrus as early as 4 months of age and giant breed bitches as late as 2 years of age. Average range for onset of puberty in bitches is 6 to 14 months of age.³

Proestrus

The bitch's owner or handler is the person who usually identifies onset of proestrus, using the physical clues of turgid vulvar swelling and serosanguinous vulvar discharge. The author recommends that owners watch for vulvar discharge as that is a more definable sign than is swelling and so is easier to note consistently from one cycle to the next. This serosanguinous discharge arises from the uterus, by extravasation of red blood cells (RBCs) through endometrial venules.⁸ Amount of vulvar discharge varies between bitches and neither an unusually small nor an unusually large amount is associated with subfertility or infertility. Owners call the onset of proestrus "coming into heat," and when an owner says a bitch has "been in heat" for so many days, they are saying it has been that many days since onset of proestrus. Male dogs are interested in urine and vulvar secretions from bitches in heat and may approach and try to investigate her hindquarters or mount her. The bitch will not permit mounting or breeding during proestrus, which lasts an average of 9 days but may last anywhere from 3 to 21 days.^{1,3}

Ovarian follicles are undergoing maturation during this stage of the cycle as they transition from secondary, or preovulatory follicles, to tertiary follicles, also called ovulatory or Graafian follicles. Ovulatory follicles are large (>4 mm in diameter) and lined with granulosa cells that produce estrogen.⁹ The primary form of estrogen secreted in bitches is estradiol-17 β . It is this estrogen that is responsible for the physical changes of proestrus described earlier, and that will stimulate the vaginal epithelium to divide, causing characteristic changes in vaginal cytology that will be described with breeding management. Serum estradiol concentrations peak at values of 50 to 100 pg/mL during this stage of the cycle.¹

Estrus

Estrus most often is defined by owners by evaluating the behavior of experienced males, many of whom will not show significant interest in a bitch until she is receptive to breeding and near her fertile period, and the behavior of the bitch, who will permit the male to mount and breed her during this stage of the cycle. Classically, the vulva softens as the bitch enters estrus, and the vulvar discharge changes in color from serosanguinous to straw-colored. Owners often call this "the color change" and worry if it does not occur. Some bitches will have vulvar turgidity and serosanguinous discharge throughout estrus, with no apparent effect on fertility. Estrus lasts an average of 9 days, with a possible range of 3 to 21 days.^{1,3}

Ovarian follicles in bitches undergo preovulatory luteinization, a process whereby granulosa cells and surrounding theca cells in the follicles change morphologically and start to produce small amounts of progesterone.² Coincident with this, there is a decline in serum estrogen concentrations. This triggers release of a large pulse of luteinizing hormone (LH) from the pituitary, which stimulates ovulation 36 to 50 hours later.^{1,10} After ovulation, serum progesterone concentrations continue to rise.

Diestrus

All bitches go through a prolonged luteal phase with significant progesterone production whether they were bred or not and whether they conceived and established pregnancy or not. Vulvar discharge wanes as the bitch no longer permits mounting and breeding

behavior; in some bitches, a small to moderate amount of mucoid discharge may be passed early in diestrus. Because progesterone concentrations are high during this stage, bitches may show physical changes owners may attribute to pregnancy whether or not the bitch is pregnant. These include mammary development and, in some bitches, rib spring with apparent abdominal enlargement. Elevated progesterone concentrations also are associated with uterine changes appropriate for pregnancy, including endometrial hyperplasia and increased secretory activity of endometrial glands. Diestrus lasts an average of 50 to 80 days.³ If the end of diestrus is defined by decline in progesterone to <1 to 2 ng/mL, the length of diestrus in pregnant dogs averages 62 to 64 days from ovulation as progesterone falls abruptly at the time of whelping. In nonpregnant dogs, diestrus may be prolonged as there may be a more variable decline in serum progesterone concentrations, such that diestrus may last anywhere from 49 to 79 days from ovulation.² It has been shown that corpora lutea (CLs) no longer produce progesterone after 60 to 120 days from ovulation but may be visible on the ovary until the next proestrus.¹¹

All progesterone during this stage of the cycle is produced by CLs formed at the ovulation sites on the ovaries. These CLs are maintained spontaneously early in diestrus but are dependent on secretion of LH and prolactin from the pituitary in the latter half of diestrus.² Secretion of progesterone from luteal CLs is stimulated by LH and prolactin may play a role by suppressing ovarian responsiveness to follicular development.^{10,12} It is not clear what is the driving force in luteal regression in dogs. Prostaglandin release may play a role as may decline in LH and prolactin. Opioids also may be involved, perhaps through association with secretion of luteotrophic LH.²

Anestrus

Anestrus has historically been considered a time of reproductive quiescence. It is now better understood that while there are no outward signs of reproductive activity during this stage, there are histologic and endocrine changes occurring that are required for onset of the next proestrus. Involution of the uterus takes 135 days (4.5 months), and this is the minimum length of anestrus considered normal in dogs.⁷ Reported ranges for anestrus range from 1 to 8 months.³ Serum concentrations of follicle stimulating hormone (FSH) remain stable throughout anestrus at anywhere from 50 to 100% of preovulatory concentrations, and while there are FSH receptors present on the ovary, follicular development is suppressed until very late in this stage.^{10,13} Serum estrogen concentrations rise slightly in late anestrus. This rise in estrogen primes the hypothalamo-pituitary-ovarian axis, causing increased responsiveness to gonadotropin releasing hormone (GnRH) and subsequent increases in frequency and magnitude of pulses of LH secreted.^{10,14} This stimulates follicular growth and the onset of the next proestrus.

BREEDING MANAGEMENT

Several factors unique to bitches make breeding management more challenging than in other species. Because we have limited access to the ovaries physically or visually, we must infer what is happening on the ovaries by diagnostic testing. Bitches ovulate an immature oocyte, which must undergo one more meiotic division before it can be fertilized. For this reason, optimal breeding is offset from ovulation day. Proestrus and estrus are prolonged, with behavioral estrus not well correlated with ovulation. Many of the diagnostic tests used approximate the day of ovulation but only endocrine assays provide the general practitioner with any accuracy in prospectively determining ovulation day. Increasing accuracy is required with increasing sophistication of insemination. With natural breeding or use of fresh semen, spermatozoa may live in

the bitch's reproductive tract for up to 1 week, making them available whenever ova have matured and can be fertilized.³ With chilled semen, life span of spermatozoa decreases to days, and with frozen/thawed semen, to hours, necessitating accurate timing of ovulation and subsequent insemination.

Duration of Estrous Stages/Breeding Management History

The average bitch is in proestrus for 9 days and ovulates about the second day of estrus. For this reason, many people breed bitches on days 9, 11, and 13, counting day 1 as the onset of proestrus and assuming they have an average bitch. There is great variability between bitches and one cannot assume that all bitches bred over this window are being bred near their most fertile time; it is reported that bitches may first show behavioral estrus anywhere from 2 days before to 5 days after the LH peak that causes ovulation and that number of days from proestrus onset to ovulation may vary from 5 to 30.^{1,15} Once a bitch has had ovulation date determined, usually by measurement of progesterone as described later, breeders may assume that she will ovulate on about that same day of her cycle repeatedly. This has not been demonstrated to be true.¹⁵ The author prefers to use history to help determine when to see the bitch for breeding management rather than as a predictor of events in this cycle; for example, if a given bitch ovulated very late in her last season, the owner probably does not need to rush in with the bitch on the second day after proestrus onset in this cycle.

Physical Changes

As described previously, vulvar tone and characteristics of the vulvar discharge vary as the bitch progresses from proestrus into estrus. The average bitch shows these changes at the onset of estrus and ovulates about 2 days later.¹⁵ However, there is great variability and, again, one cannot use this parameter alone to accurately determine ovulation day.

Vaginal Cytology

Under the influence of estrogen, vaginal epithelial cells are stimulated to divide. As samples are collected over the estrous cycle, characteristic changes in cell populations are noted (**Table 1**).

The author collects vaginal cytology specimens using a nonsterile, cotton-tipped applicator that is moistened with tap water. The swab is inserted dorsally in the vulvar cleft and passed craniodorsally at a 45° angle, to bypass the ventral clitoral fossa. The swab is passed until it passes the pelvis and can be directed more cranially, is rolled gently against the vaginal wall, and then removed. The swab is rolled several times over a clean glass slide. The slide is allowed to air-dry and then routinely stained. Cells are examined under the ×10 objective.

Four epithelial cell types are identified. Parabasal cells are those lining the basement membranes (**Fig. 1**). They are round with a 1:1 or smaller cytoplasm:nucleus ratio. Intermediate cells lie above parabasal cells (see **Fig. 1**). They also are round with a large, well-defined nucleus and are slightly larger than parabasal cells. These are the noncornified epithelial cell types and are always present in the vagina. As epithelial cell division is stimulated by elevated serum concentrations of estrogen in proestrus, a layer of nonviable cells develops as the vaginal epithelium thickens. Histologically, there will be 5 to 7 layers of parabasal and intermediate cells covered by 4 to 6 layers of keratinized cells.¹⁶ Because the swab collects cells from the lumen, only these keratinized cells will be collected. Superficial cells and anuclear squames are the keratinized or cornified cell types. Superficial cells are misshapen and angular and have a pyknotic nucleus (**Fig. 2**). Anuclear squames are superficial cells with no visible nucleus (see **Fig. 2**).

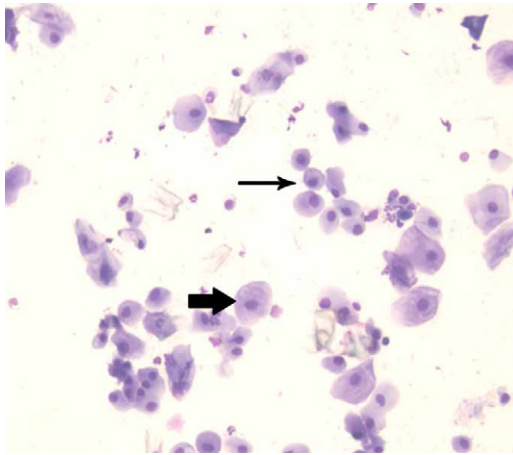


Fig. 1. Early proestrus vaginal cytology from a bitch. Note parabasal epithelial cell (*small arrow*) and intermediate epithelial cell (*large arrow*).

During proestrus, percentage cornified cells increases gradually and the number of polymorphonuclear cells (PMNs) decrease. RBCs may be present throughout. Estrus is defined cytologically as presence of 100% cornified cells types with at least 50% of those being anuclear squames (**Fig. 3**). No PMNs are present and there are variable numbers of RBCs and bacteria. Ovulation occurs on the second day of estrus in the average bitch, but this timing is widely variable and should not be relied upon for prospective timing of ovulation day, since peak in cornification can occur anywhere from 5 days before to 1 day after the LH peak.³ Six days after ovulation, the cornified cell layers are abruptly sloughed off, such that noncornified epithelial cells are recovered on cytology. Many PMNs are present in the first couple of days after this physiologic inflammatory event (**Fig. 4**). Identification of onset of diestrus can be used to retrospectively define ovulation day. Whelping date can be projected as 56 to 58 days from diestrus onset or from 62 to 64 days from ovulation.

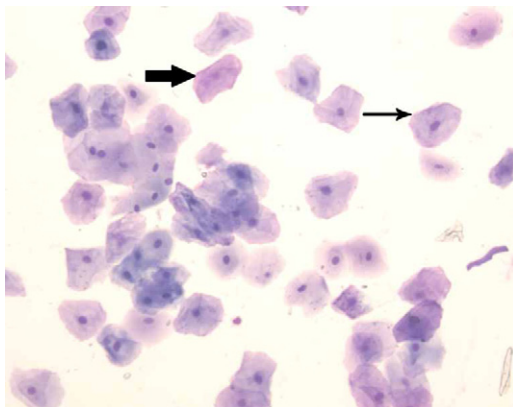


Fig. 2. Late proestrus vaginal cytology from a bitch. Note superficial epithelial cell (*small arrow*) and anuclear squamous epithelial cell (*large arrow*).

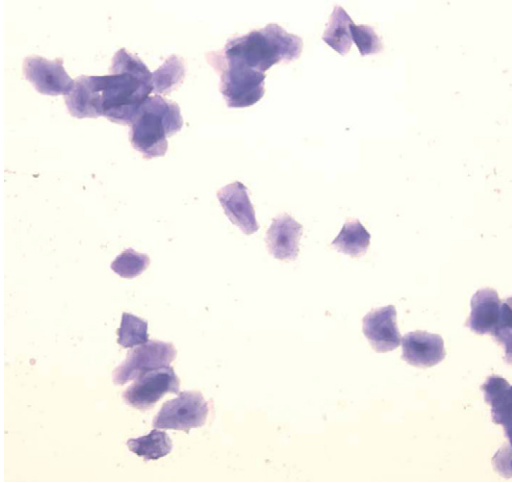


Fig. 3. Estrus vaginal cytology from a bitch. Note predominance of cells with no visible nucleus (anuclear squame cells).

Ultrasound of the Ovaries

Follicles can be seen on transabdominal ultrasound in bitches by experienced operators with good equipment, especially with serial evaluations of a given bitch. Ovulation is not readily defined by changes in sonographic appearance as follicles do not collapse at the time of ovulation and the CLs often have a cystic center.¹⁵ This technique is not commonly used in clinical practice for determining stage of cycle in the bitch.

Hormone Assays

LH is the stimulus for ovulation in bitches. It is secreted pulsatilely, with a large, single peak associated with decline in serum estrogen concentrations in late proestrus or

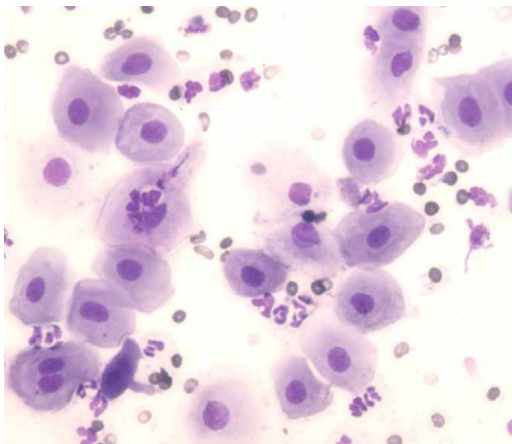


Fig. 4. Early diestrus vaginal cytology from a bitch. Note noncornified epithelial cells and presence of PMNs.

estrus. Ovulation occurs 36 to 50 hours later.¹ Direct measurement of LH is the most definitive diagnostic test available. Unfortunately, commercial LH assays are not readily available for the bitch and turn-around time makes their use impractical for clinical cases. In-house LH assays are intermittently available. Because duration of the LH peak is relatively short, daily testing is recommended; this is problematic for some owners due to logistics and cost of daily visits to the veterinarian for venipuncture and processing of samples.¹⁵ Some veterinarians will circumvent this by drawing blood for progesterone, as described next, freezing extra serum, and then running LH assays on select samples, based on progesterone-based predictions of days of the LH peak and ovulation. In-house LH assays do not provide quantitative measurement of serum LH concentration but only differentiate low from high ($<$ or $>$ 1 ng/mL [3.1 nmol/L]).

Progesterone is the hormone assay most commonly used for assessment of ovulation date in bitches. Because bitches undergo preovulatory luteinization, rise in serum progesterone concentrations can be used to infer date of the LH peak and prospectively predict ovulation day. In general, progesterone concentration on the day of the LH peak will be about 2.0 ng/mL and on ovulation day from 4 to 10 ng/mL.¹⁷ Some veterinarians prefer not to look at individual values but instead watch for a sudden increase in progesterone concentration by 3 ng/mL or more from one day to the next, denoting that as ovulation day. Some denote the first day progesterone concentration is 5 ng/mL or greater as ovulation day.³ Finally, some veterinarians will look for an absolute value of progesterone at the time of breeding, with anecdotal reports of breeding optimized when progesterone is greater than 10 ng/mL or when progesterone is 15 ng/mL. The author is unaware of scientific studies documenting clinical significance of the latter method and prefers to use values to identify LH surge and ovulation day and to base timing of breeding on those values. It is generally accepted that one should not base all of one's decisions on a single blood sample as there is great variability between bitches. Collection of multiple blood samples over proestrus and estrus are required if any accuracy is expected in determining ovulation day using progesterone assay.

Progesterone can be measured using in-house assays or commercial assays. Commercial laboratories usually use either radioimmunoassay (RIA) or chemiluminescence assay (CA). These assays are quantitative. One study comparing RIA to CA demonstrated good correlation between the two when used to assay aliquots from the same sample. Progesterone concentrations in those samples assayed using CA were consistently higher by 0.69 ng/mL on average, a value those authors did not consider biologically significant.¹⁸ It is valuable to note this difference in values by assay used, especially if samples are being run by more than one laboratory as a bitch is moved across the country for breeding. In-house assays are enzyme-linked immunosorbent assays (ELISAs); those available in the United States are semiquantitative, with various shades of color corresponding to ranges of serum progesterone concentration. Semiquantitative ELISA is less accurate than either RIA or CA. It is reported that ELISAs are inaccurate 85% to 89% of the time, with errors most commonly due to low values being misread as high.^{19,20} Because the ELISA is semiquantitative, samples must be collected and assayed more frequently than with RIA or CA to ensure identification of changes in progesterone concentration significant for breeding timing. The primary advantage of ELISA assay is quick turn-around time as it can be run in-house.

The author prefers to evaluate bitches about day 4 after proestrus onset. If vaginal cytology is at least 60% cornified, blood is drawn for progesterone assay by RIA or CA. Samples are drawn every 3 to 5 days until values are suggestive of ovulation

(4–10 ng/mL). Sampling may continue past this time to ensure continuing increase in progesterone; one should never manage breeding based on only one progesterone value.²¹ For natural service and artificial insemination (AI) with fresh semen or chilled semen, optimal breeding day is 2 days postovulation. Ideally, the bitch is bred at least twice, 2 and 4 days after ovulation. For frozen/thawed semen, which has decreased viability and so must be introduced when all ova are mature, insemination should take place 4 to 5 days after ovulation. Insemination too late after ovulation with any kind of semen is associated with decreased conception rate and increased embryo resorption, perhaps due to aging of DNA in the ova and subsequent errors in transcription or to asynchrony between embryologic development and the intrauterine environment.²²

Miscellaneous Diagnostic Tests

Vaginoscopy can be used to gauge changes in the vaginal mucosa related to elevated circulating concentrations of estrogen. During anestrus, the vaginal epithelium is thin and the vasculature more readily visible, such that the mucosa is translucent red to pink and smooth. During proestrus, as estrogen stimulates vaginal edema and cornification, the mucosa will change from pink and billowy to white and sharp-edged. The sharp-edged, or crenated appearance, with subsequent loss of edema and wrinkling of vaginal folds, occurs approximately 2 days before ovulation.¹⁵ This technique cannot be used alone to prospectively define day of ovulation.

Other measures that have been investigated include changes in electrical resistance across the vaginal mucosa, changes in glucose concentrations in vaginal fluid, change in progesterone concentrations in saliva, and ferning, or crystallization of vaginal fluid or saliva across glass slides (M.V. Root Kustritz and R. Davies, unpublished observations, 2003).^{15,23} Although some correlations have been noted between these changes and physiologic events, none of the latter three techniques are sufficient to prospectively determine ovulation day.

ESTRUS INDUCTION

Because bitches cycle so infrequently compared to other species, there is great interest in inducing heat in this species. Induction of estrus may be used to treat pathologic anestrus, to make the bitch available for a given stud dog, to manage birth of pups at an optimal time of year, to ensure continuity of litter production for a breeding colony, to create reproductively similar dogs for research, to synchronize recipients for embryo transfer, or to teach canine reproduction.⁷ No drugs are approved for estrus induction in bitches in the United States.

Bitches are more likely to respond to any estrus induction protocol if they are nearer the time of spontaneous proestrus onset.²⁴ This most likely is due to necessary changes in endocrinology in anestrus and also may be due to need for endometrial repair after the previous cycle.⁷ The common therapies used for estrus induction are listed in **Table 2**.

General Management

It has been well demonstrated that bitches housed together will cycle together. This is called the *dormitory effect* and most likely is pheromonally based.²⁵ Bitches to be induced into proestrus are housed in close proximity with cycling bitches. This is noninvasive and inexpensive but consistency of this technique has not been reported. Other factors to consider when inducing estrus, especially in those bitches with pathologic anestrus, are general health and activity level. Very active bitches, such as hunting or show bitches, may not cycle due to alterations in body fat and associated

Drug Type	Regimen(s) Described	General Success	General Concerns
Estrogen	DES; 5 mg once daily per os for 6–9 d or until proestrus induced	Few studies but good success reported, anecdotal reports variable	Split heat, lack of documentation about possible toxicity with repeated use
GnRH agonist	1. Ovuplant; 2.1 mg implant SQ 2. BioRelease deslorelin; 1.5 mg SQ	1. Good 2. Variable	1. Premature luteal failure, project variably available 2. Variable response
Dopamine agonist	Cabergoline Dostinex; 5 μ g once daily per os for 30–40 days or until proestrus induced	Good	Expensive, difficult to dose for small bitches

changes in gonadotropin secretion. Bitches with systemic disease, such as hyperadrenocorticism, also may fail to cycle. Finally, some suggest that bitches with hypothyroidism may cycle less frequently. For this reason, it is valuable to perform a complete physical examination and routine blood work and to talk to the owner about management and activity level of the bitch before inducing estrus with any drug regimen.

Gonadotropins

The pituitary gonadotropins LH and FSH induce spontaneous proestrus, so one could hypothesize that treatment with these hormones could readily induce estrus. Unfortunately, protocols with these drugs have not been demonstrated to be successful. Treatment failure is associated with luteinization of follicles and ovulation failure, failure of implantation, and a shortened luteal phase.³ Acute allergic response to LH was reported in 2 bitches.⁷ A commercially available swine product (PG600; Intervet Schering-Plough, Summit, NJ, USA) contains 80 IU of equine chorionic gonadotropin (eCG) and 40 IU of human chorionic gonadotropin (hCG) per mL. Both eCG and hCG variably bind and activate LH and FSH receptors in bitches. In one study, injection of 5 mL of PG600 induced proestrus in 17 of 19 bitches and caused ovulation in 8 of 19; pregnancy rate was not reported.⁷ Problems with use of PG600 for estrus induction in bitches include unpredictability of response, potential for allergic reactions to the large proteins contained in the product, and premature luteal failure.⁷

Estrogen

Estrogen priming occurs late in anestrus, making the ovary more responsive to pituitary gonadotropins. The goal of using estrogen for estrus induction is to increase responsiveness of the bitch to endogenous gonadotropins. Treatment with several different forms of estrogen has been described.⁷ The most readily available regimen

described is the use of diethylstilbestrol (DES; 5 mg per os once daily for 6–9 days or until onset of proestrus). Success rate in one study of 5 dogs was 100% for estrus induction, ovulation, and pregnancy in those bitches.⁷ The author has had some bitches respond with a split heat, where they show signs of proestrus, go out of heat without ovulating, and then have a spontaneous heat within 4 to 6 weeks. Bitches have been successfully bred on that subsequent heat. There is nothing in the literature describing possible dangers of repeating treatment with DES to induce subsequent heat cycles; concerns about bone marrow suppression often are expressed anecdotally.

GnRH Agonists

GnRH agonists work by mimicking the normal increase in GnRH stimulation of gonadotropin secretion. GnRH is secreted pulsatilely and early work mimicked this pulsatile release by use of subcutaneous osmotic pumps. This technique, while successful, is not practical in clinics. Sustained administration of GnRH has been reported successful with some formulations. Concerns include failure to stimulate an adequate LH surge at the end of proestrus and premature luteal failure with prolonged administration.⁷ Synthetic GnRH analogues vary in potency and efficacy.⁷ Estrus induction is more successful in bitches with serum progesterone concentrations of <5 ng/mL.²⁶ GnRH agonists may be available either as subcutaneous implants or as depot injection preparations. The implant most commonly described for use in dogs is Ovuplant (Ayerst Laboratories, Guelph, Ontario, Canada), a product original designed for use in horses. A 2.1-mg implant is placed in the subcutaneous space, often in the vestibular mucosa just within the vulvar lips. Placement of the implant in an area from which it can be removed may be desirable.²⁷ Reported success rate for induction of proestrus within 2 to 9 days was 100%, with pregnancy rates varying from 40 to 67%.⁷ This product is variably available in the United States. An injectable preparation is more readily available (BioRelease deslorelin; BET Pharmacy, Lexington, KY, USA). Subcutaneous injection of 1.5 mg one time was associated with variable success in induction of proestrus, with reported rates varying from 0 to 60%, and pregnancy rate also varying from 0 to 60%.⁷

Cabergoline

Cabergoline and bromocriptine are dopamine agonists that cause a decrease in serum prolactin concentrations and may be used to induce estrus in bitches. Bromocriptine is a human product and will not be described in detail. Cabergoline is a veterinary product (Dostinex; Pfizer, New York, NY, USA). The effect of cabergoline for estrus induction most likely is associated with its role as a dopamine agonist rather than in association with decline in prolactin.^{7,14}

The standard dose regimen used for cabergoline is 5 $\mu\text{g}/\text{kg}/\text{day}$ until proestrus is induced or for 30 to 40 days. A lower-dose regimen (0.6 $\mu\text{g}/\text{kg}/\text{day}$) was shown to be equally successful for estrus induction in one study.²⁸ The drug is available as a 0.5-mg tablet, which makes dosing difficult for small dogs. Dissolution in distilled water at room temperature to form a 10 $\mu\text{g}/\text{mL}$ solution is described; this must be prepared daily and used within 15 minutes of preparation.⁷ Compounding by dissolution into 1% acetic acid may create a more stable product.²⁹ Time until proestrus onset varies from 4 to 48 days.²⁸ Reported success rate for induction of proestrus is 80 to 100% and for pregnancy is 60% to 100%.⁷ One reported side effect is change in coat color or texture.⁷ This should be reversible as the hair follicles go through their normal cycle but will be of significant concern to owners of show bitches.

ESTRUS SUPPRESSION

Estrus suppression most commonly is effected in dogs in the United States by ovariectomy. Gonadectomy is an effective and irreversible form of estrus control that may not be suitable in all situations and is associated with some detriments.³⁰ Similarly, immunologic means of contraception are being investigated for temporary or permanent estrus suppression in bitches.³¹ This discussion will revolve around drug-based shorter-term estrus suppression in bitches.

Progestins

Progesterone-based products suppress estrus by negative feedback to the pituitary suppressing follicular development and subsequent secretion of estrogen, FSH, and LH.³² Natural products exert significant progestogenic effects and are not commonly used. Synthetic forms of progesterone that have been used include megestrol acetate, medroxyprogesterone acetate, and proligestone. Megestrol acetate is administered at a low dose (0.55 mg/kg per os for 32 days) during anestrus or at a high dose (2.2 mg/kg per os for 8 days) during the first 3 days of proestrus. If used properly during anestrus, return to subsequent proestrus will be postponed for about 3 months. If used properly during proestrus, physical manifestations of proestrus and estrus, and breeding behavior will subside within days and the bitch will not ovulate on that cycle. Megestrol acetate was approved for use in bitches for estrus suppression but the commercial product is no longer available. Veterinarians can call prescriptions for megestrol acetate into human pharmacies. Medroxyprogesterone acetate is an injectable synthetic progestin. Severity of side effects and need for frequent readministration make this a less widely used drug. Proligestone is a synthetic progestin with few progestational properties, making it a more desirable injectable product. It is not available in the United States.

There are many reported side effects associated with use of progestins for estrus suppression in dogs. Synthetic products vary in their progestational properties.³² It has been reported that if used as directed by the manufacturer, incidence of pyometra after treatment with megestrol acetate is about 0.8%.³¹ Endometrial changes may be minimized by using drugs with less progestogenic activity and ensuring estrogen priming has not occurred.³²

Progestins also may stimulate secretion of growth hormone with subsequent acromegaly, suppress the adrenal cortex, and suppress responsiveness to insulin, with subsequent diabetes mellitus.^{32,33} Increased appetite and weight gain commonly are reported. Mammary stimulation with development of mammary nodules or neoplasia also has been reported.³ Progestins may be teratogenic if administered to bitches early in pregnancy. Finally, localized reactions with hair loss and change in hair color have been reported in some bitches after use of injectable progestins.³²

Androgens

Testosterone has never been approved for estrus suppression in bitches in the United States. Side effects include masculinization, clitoral hypertrophy, and aggression. Concerns have been expressed about long-term suppression of estrous activity in bitches treated with testosterone. Research results disagree as to whether treatment with testosterone interferes with subsequent ability to induce estrus in bitches.³⁴

Mibolerone is a synthetic weak androgen that was approved for use in bitches for estrus suppression but is no longer available as a commercial product. The chemical may be available through compounding pharmacies. Therapy must be instituted at

least 30 days before onset of the next proestrus. Dose varies with size of the dog, with dogs weighing less than 12 kg receiving 30 μg daily per os, those weighing 12 to 23 kg receiving 60 μg , those weighing 23 to 45 kg receiving μg mcg, and those weighing greater than 45 kg and all German shepherd dogs and their crosses receiving 180 μg daily. The drug is given continuously for up to 2 years and return to estrus after withdrawal of the drug averages about 70 days. Side effects include clitoral hypertrophy, exudation of creamy vulvar discharge, musky body odor and mounting behavior, and epiphora. This drug should not be used in Bedlington terriers.

GnRH Agonists and Antagonists

GnRH agonists suppress estrus by downregulation of hypothalamic and pituitary function. In postpubertal bitches with serum progesterone concentration less than 5 ng/mL, estrus may be induced first; this may be minimized by treating within 60 days of an ovulatory estrus, within 7 days of whelping, or following 7 days of progestogen therapy.^{26,31} Treatment in prepubertal bitches is associated with prolonged estrus suppression.²⁶ Estrus suppression with subcutaneous implants containing either 4.7 or 9.4 mg of the GnRH agonist deslorelin suppressed estrus in 6 of 10 bitches in one study. Lower-dose implants must be replaced about every 4.5 months and higher dose implants more frequently than annually to be effective. No local side effects were noted.³⁵

GnRH antagonists act by blocking effect of GnRH at the pituitary. Acyline is a drug that has been used to suppress estrus when implanted subcutaneously within the first 3 days of proestrus, with decrease in estrus signs within about 3 days and lack of ovulation on that cycle. Bitches returned to proestrus 20 to 25 days later.³⁶ Although this drug is not available in the United States, it would be most useful for bitches requiring very short-term suppression of estrus for travel or show purposes.

SUMMARY

Knowledge of the underlying endocrinology of the canine estrous cycle permits veterinarians to make the best possible recommendations to clients regarding management of their bitch's estrous cycle. Breeding management requires assay of progesterone to determine ovulation day. Estrus induction and suppression can be managed through drug or management schemes, with the client's understanding that no perfect protocols exist that would permit veterinarians to manipulate timing of estrus and ovulation with great accuracy.

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