



31st World Small Animal Veterinary Congress
October 11-14, 2006, Prague, Czech Republic



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Recent Advances in Canine Female Reproduction

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THE REPRODUCTIVE CYCLE

Most bitches ovulate on day 12 of their season, therefore there is a widespread tendency of owners to assume that day 12 is the ideal breeding day for all bitches. As a matter of fact, some bitches ovulate early (such as on day 8, or 6 or even 4 from the onset of proestrus) while others may ovulate as late as day 17, 19 or 22-24. One should never assume that a given bitch will ovulate on day 12 unless proven. Managing a canine breeding requires the client taking the bitch to the veterinary clinic as soon as the first signs of proestrus are displayed (vulvar discharge, male attractiveness) for a first check, and then coming back every 2-3 days to monitor how quickly the female is progressing towards ovulation through vaginal smears and serum progesterone assays. Vaginoscopy and ovarian ultrasound can be very helpful clinical tools in identifying and monitoring the ovulation process. When the first day of ovulation is identified, there are still a few days to achieve a breeding, thanks to longevity of canine oocytes (4-6 days following ovulation).

Timing ovulation: Canine proestrus and oestrus last on average 9 days each with ovulation taking place 3 days after onset of oestrus (or day 12 after onset of proestrus). However ovulation can occur as early as 5 days or as late as 27 after onset of proestrus. Therefore, it is very important to check the female's behavior, perform vaginal smears every 2-3 days starting on the first day of proestrus in order to catch early ovulators, and draw blood samples to measure progesterone once behavior and/or vaginal smear indicate oestrus. Oestrus is indicated by acceptance of the male or by a degree of vaginal cornification of $\geq 70\%$. Serum progesterone has a concentration of (values are approximate) 2.0-3.0 ng/ml on the day of the peak of luteinizing hormone (LH), 4.0-10.0 ng/ml on the day of ovulation, 10-25 ng/ml during the 2 days following ovulation, which is when oocytes are reaching maturity in the ampulae of the oviducts and fertilizations are taking place. Ovarian structures can be visualized 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 luteinized) and on appearance of an hypoechogenic area at the periphery of the ovary representing follicular fluid accumulation within the ovarian bursa.

Performing vaginal cytology as well as checking the bitch's behavior to look for onset of male receptivity are the 2 most practical ways of determining the best time for breeding. Owners should be instructed to bring their bitch to a male dog to check her behavior regularly as soon as possible after proestrus onset as well as to have a vaginal smear taken from the veterinarian every 2-3 days. Breeding should be performed as soon as the bitch stands and/or as soon as her smear is fully cornified, in order not to miss early ovulators. However, behavior does not always correlate with vaginal cytology: some bitches will not stand to be mounted even though their smear is fully cornified. Provided that vaginal abnormalities (strictures, bands of tissue, hymen) are ruled out, serum P4 as well as using different male dogs (to rule out male preference) are helpful in such cases, although some bitches become receptive to mating only in mid to late oestrus. Ovulation should always be timed using serum P4 assay every 2-3 days and the bitch should be bred when a high P4 value is observed (>5.0 ng/ml). Vaginal cytology should also be used to confirm serum P4 data (serum P4 kits which use a semi-quantitative colorimetric system are only 80% accurate) during and (most importantly) after breeding until the first day of cytological dioestrus (D1) is identified, which occurs 6-8 days after ovulation.

THE CANINE PREGNANCY

Gestation length in the dog varies considerably depending on whether it is calculated from a single breeding (57-72 days), from an LH surge (64-66 days), from ovulation (62-64 days) or from the onset of cytological dioestrus (D1--56-58 days). The relative timing of the most important events in the canine pregnancy is shown in Table 1. Events relative to the most important hormones have been studied mostly in Beagles. Data on other breeds are lacking and extrapolating results from breed to breed may not necessarily be correct at all times.

Table 1. Some of the most relevant reproductive event of the canine pregnancy timed as relative to the LH peak, ovulation and onset of cytological dioestrus (D1).

Adapted from Concannon and Lein (1989)

<i>Reproductive event</i>	<i>Days from the LH peak</i>	<i>Days from ovulation</i>	<i>Days from D1</i>
Onset of proestrus	-25 to -3	variable	variable
Vaginal cornification ¹	-1 to +7	-4 to +4	-10 to -2
Onset of oestrus	-4 to +5	-7 to +2	-13 to -4
LH peak	0	-2 to -3	-8 to -9
Maximum fertility	-1 to +6	-3 to +4	-3 to -10
Ovulation ²	+2 to +3	0	-5 to -7
Fertilization	+4 to +6	+2 to +4	-2 to -5
D1	+8 to +9	+5 to +7	0
Behavioral dioestrus	+10 to +14	+7 to +11	+1 to +5
Zygotes enter uterus	+11 to +12	+8 to +9	+2 to +3
Attachment	+16 to +18	+13 to +15	+7 to +9
Ultrasound diagnosis possible	+19 to +22	+16 to +19	+10 to +13
Abdominal palpation possible	+20 to +25	+17 to +22	+11 to +16
Foetal heartbeats	+22 to +25	+17 to +22	+11 to +16
Pregnancy anemia	+25 to +30	+22 to +27	+16 to +21
Radiopaque foetal skeleton	+44 to +46	+41 to +43	+35 to +37
Luteolysis and hypothermia	+63 to +65	+60 to +62	+55 to +57
Parturition	+64 to +66	+62 to +64	+56 to +58

1. This is intended as "Maximum" vaginal cornification, or $\geq 50\%$ of keratinized cells on a vaginal smear

2. Primary oocytes

Implantation: Canine embryos enter the uterus as zygotes or morulae around day 8-9 after ovulation. For the first 1-2 days they move actively up and down the uterine horn in which they arrived, and then for the remaining 2 days they migrate to and from the opposite horn mixing with the other embryos. Implantation starts around day 13-15 after ovulation. The canine placenta is endotheliochorial and zonary, with blood accumulation and extravasation in the marginal areas; blood components are observed as green and brown borders of the placenta and are thought to be important for foetal nutrition. Because of the type of placentation, only 5-10% of the total immunoglobulin provided by the bitch is transferred through the endotheliochorial placenta to the pup. Therefore, the majority of passive immunity is derived through colostrum.

Acute phase proteins such as C-reactive proteins, haptoglobin, acid glycoprotein, ceruloplasmin and fibrinogen increase as soon as placentation starts, due to the inflammatory reaction that takes place at the endometrial level when the trophoblast starts eroding it. Fibrinogen is produced by the liver and is found at values of 100-150 mg/dl in normal dogs, and rises to values of 250-300 mg/dl around day 25-28 post-ovulation. A value of 300 mg/dl at 28 days is considered 100% accurate in diagnosing pregnancy in the bitch. Haptoglobin and ceruloplasmin are bound to iron and haemoglobin, respectively. Haptoglobin is normally found at values of 35-50 mg/dl, and in pregnant animals, increases to values of 75-100 mg/dl around day 18-20 after ovulation. In healthy females, an increase in acute phase proteins is considered a good indirect indicator of implantation, and can be used in practice as an aid in pregnancy diagnosis (or to rule out pregnancy). Obviously, a pre-breeding sample must be drawn to make the test accurate; also, a rise in acute phase proteins could be due to a variety of other factors associated to inflammatory reaction anywhere in the organism, including endometrial inflammation due to a pyometra.

HORMONES OF PREGNANCY

Maintenance of pregnancy in the bitch depends on ovarian secretion of progesterone for the entire length of gestation. The ovaries are the only source of progesterone, as demonstrated by the fact that abortion inevitably follows ovariectomy at any stage of gestation. The canine feto-placental unit has the possibility to metabolize small amounts of exogenous progesterone, but no progesterone-synthesizing activity has been demonstrated. Corpora lutea secrete progesterone based on stimuli provided for by the pituitary, initially with Luteinising Hormone (LH), then with LH and prolactin. Administration of anti-LH compounds during the first half of pregnancy or prolactin-lowering drugs during the second half of pregnancy causes luteolysis and abortion. The luteotrophic action of LH is present at all stages of the canine gestation, even towards the end of pregnancy when

serum progesterone concentrations start to decline. Such a decline is probably due to luteolytic factors which override the luteotrophic action of LH itself.

Prolactin increases during the second half of dioestrus, after day 25 post-LH peak, and reaches concentrations which are much higher in pregnant than in non pregnant bitches. Prolactin-lowering drugs such as cabergoline, bromocriptine and metergoline cause a dose-dependant decrease in serum prolactin as well as progesterone concentrations. Serum LH does not always decrease following treatment with an antiprolactinic drug, therefore its mechanism of action in supporting progesterone secretion from the corpora lutea remains not totally clear. Prolactin concentrations are low (≤ 2.0 ng/ml) during the second half of dioestrus in non-pregnant bitches, perhaps increasing in those bitches demonstrating clinical signs of pseudopregnancy. Prolactin values tend to increase during the last week of pregnancy to about 40 ng/ml, to reach values of approximately 100 ng/ml during the last 1-2 days prior to whelping, peaking at values slightly above 100 ng/ml during the 1-2 days after parturition. Prolactin increases in response to suckling by pups.

In the bitch there is no regulatory effect of the endometrium on luteal function as is known in other species. Hysterectomized bitches cycle normally and have normal luteal phases. In both species prostaglandin F2a is secreted by the endometrium, and its absence in hysterectomized bitches does not have any effect on duration of dioestrus. Prostaglandin F2a administered in luteolytic doses will cause a decrease in serum progesterone such as what happens prior to parturition. Progesterone concentrations are similar in pregnant and non-pregnant diestrous bitches. Therefore, assaying progesterone cannot be used as test for diagnosing pregnancy. Differences in serum progesterone concentrations between pregnant and non-pregnant beagle dogs have been observed, although these are not significant and therefore of no practical use. A peak in serum progesterone concentration in the bitch occurs around the 3rd week of pregnancy to levels of 15-80 ng/ml, after which P4 levels fluctuate between 10 and 20 ng/ml until the end of pregnancy or until day 50-80 in non-pregnant bitches. The relative amount of progesterone secreted by pregnant bitches with a large litter has never been compared to the amount secreted by bitches with a small litter, although a difference is likely to occur depending on the number of active corpora lutea present. The number of corpora lutea should be equal to the number of foetuses except if foetal loss or embryo splitting (resulting in birth of twins) occur. The lack of clinical data on serum progesterone concentrations in pregnant bitches of breeds other than the Beagles as well as in middle age to older bitches makes it difficult to manage cases of infertility, especially cases of hypoluteoidism when exogenous progesterone supplementation is necessary.

Relaxin is a key hormone of pregnancy both in the bitch and queen, as it rises between the third and fourth week of gestation, peaks at 4-6 ng/ml in the bitch (6-8 ng/ml in the queen) 2-3 weeks prior to parturition and remains at high levels throughout the early puerperium. Relaxin is non-detectable in males, in non pregnant females and during stages other than pregnancy or the puerperium. It remains high in pregnant bitches ovariectomized and treated with progesterone, and is produced by the uterus as it becomes rapidly undetectable after hysterectomy. Relaxin concentrations may vary in bitches of different breeds, as Labrador retrievers were observed to have higher levels than Beagles. Commercial kits for rapid determination of canine relaxin as a pregnancy test have developed over the last decade, but have not been very successful as the time at which pregnancy could be diagnosed was not early enough to give an advantage over the use of ultrasound. Still, if available a relaxin kit can be of help to establish if foetal death and resorption have occurred in a risk pregnancy.

CARE OF THE PREGNANT FEMALE

Good pregnancy management starts before the beginning of proestrus, at a time when vaccination or immunization protocols should be checked and updated. In proestrus health status should be assessed with a clinical exam which should include palpation of the mammary glands as well as of the abdominal organs. In bitches with a history of infertility a blood sample should be drawn to perform a complete blood count and serum biochemistry in order to assess baseline values of blood cells and serum proteins/enzymes. Pregnancy status should be assessed during the 5th week in normal, healthy females, while it should be anticipated to the 4th week in females with a history of infertility, as progesterone supplementation may be needed already at implantation time. Also, if not pregnant a bitch with a history of cystic endometrial hyperplasia might be treated with prostaglandins or with a progesterone antagonist to spare her uterus from an excessive progestational stimulation. Pregnant females should not be exposed to foreign animal as they can be potential source of infection with viruses against which the female may not be immunized. A pregnant female displaying signs of disease should be seen by a veterinarian as soon as possible. If possible, anything that can be performed at the animal's premises such as clinical exam, sample collection (feces, urine, blood etc.) or remote myometrial monitoring should be done there rather than bringing the female to the veterinary clinic.

Pregnant bitches should do a moderate physical exercise to maintain a good body condition and muscle tone, which will help having a normal parturition process. Vaccinations should be avoided unless it is specifically requested by the vaccination protocol that the injection be done during pregnancy, such as with the Canine Herpes Virus (CHV) vaccine, which should be administered at the beginning of proestrus and then again during the second month of pregnancy. A good maintenance diet should be fed from week 1 through 5. The commonest mistake made by owners at this time, whether they prepare home made food or not, is overfeeding because they think that increased food intake is necessary already at an early stage of pregnancy. However, food intake should not be increased during the initial stages of pregnancy only less than 1/3 of foetal growth occurs during the first 2/3 of gestation. Foetal size increases rapidly during the last 1/3 of pregnancy. Therefore, during the 6th week the amount of food should be increased of about 30%. During the last 1-2 weeks of gestation the female should be gradually switched to a growth/lactation type diet, which should be maintained also during lactation.

Whenever litter size is large (and especially in small size bitches), the enlarged uterus will compress the stomach thus making ingestion of large quantities of food difficult. Therefore, a very concentrated food (≥ 3.6 kcal/kg food dry matter) should be used in small amounts to be fed several times daily to compensate for reduced stomach capacity. In pregnant bitches, transient periods of reduced appetite may be observed especially during or just before labor stage 1 as well as during the expulsion phase. Periods of anorexia earlier in pregnancy should be looked at with caution especially if the pregnant female stops eating completely for more than half a day, as severe ketoacidosis leading to pregnancy toxemia can be induced experimentally in the bitch by fasting. Periods of reduced food intake are described in pregnant bitches around the 3rd week of pregnancy, a time when implantation is taking place.

In the bitch, calcium requirements increase in late gestation and during lactation. However, such increased requirements do not justify providing extra amounts of calcium and vitamin D in the diet, as this has been associated in other species with a higher risk of developing hypocalcemia and eclampsia during early lactation. Similar studies have not been conducted in the bitch. Considering the scarcity of data and the fact that bitches of different breeds might have different requirements in terms of mineral supplementation, there is no need to add calcium or vitamin D especially if a properly balanced diet is used. Also, in the bovine a low blood pH has been associated with a reduced ability of parathyroid hormone to stimulate bone calcium release, and the use of blood acidifiers such as dietary anions reduces the incidence of milk fever. The relationship between blood pH and bone metabolism has not been studied in the bitch.

From day 54-55 on owners should be instructed to measure rectal temperature on their bitches at least 3 times daily and plot the temperature data on graph paper. A clinical exam during the last week of pregnancy, including an X-ray of the abdomen, is advisable in bitches with a history of dystocia, or if the owner is particularly anxious to know about litter size in case it is her/his first time in assisting parturition of a bitch or a queen.

USING DRUGS IN PREGNANCY

The most delicate period of the canine pregnancy is the first month during which organogenesis takes place. Prior to day 20-22 following ovulation (when implantation occurs and placental development starts) canine embryos are surrounded by "uterine milk", a protein endometrial secretion which is in homeostatic equilibrium with the blood compartment, i.e., any substance that arrives in the bloodstream reaches the endometrium. Therefore, use of any substance during this time carries the potential risk of harming foetal development even though there is no risk associated for the mother. After placental development foetuses become more resistant to toxic insults. Although no real "placental barrier" exists, most substances cannot reach the placental circulation unless they are present in high concentration and for a long time in the bloodstream. However, any drug that reaches the foetal circulation must be metabolised by the foetal kidney (in carnivores the foetal liver is not metabolically active) which in itself might threaten foetal survival. Aspirin, dexamethasone, bromocriptine, carbaryl, estradiol benzoate and cypionate, prostaglandin F_{2a} and antiestrogen drugs are widely described as capable of causing embryonic/foetal death in the dog. The effect of various drugs on the canine pregnancy is reported in details by Papich (1989). Table 2 shows a brief summary of drugs which have been either tested in pregnant dogs and proven safe or used in pregnant laboratory animals and pregnant women without any side effect.

Table 2. Drugs which have been either tested in pregnant dogs and cats and proven safe or used in pregnant laboratory animals and pregnant women without any side effect.

Category of drugs	Active principles safe for use in pregnant bitches
Antibiotics	Ampicillin, amoxicillin, carbenicillin, cephalosporins, clindamycin, cloxa- and dicloxacillin, hetacillin, lincomycin, neomycin, oxacillin, penicillin G, ticarcillin
Antimicrotics	Miconazole (for topical use only)
Antiparasitics	Diethylcarbamazine, fenbendazole, mebendazole, ivermectin, piperazine, praziquantel, bunamidine, pyrantel, thenium
Anticancer drugs	None
Anesthetics	Lidocaine, naloxone
Gastrointestinal drugs	Antacids, sucralfate
Cardiovascular drugs	Digitalis
Anticonvulsivant drugs	None
Muscle relaxants	None
Endocrine drugs	None

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