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Understanding And Monitoring Canine Pregnancy

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Background. The current expectation of breeding management clients includes supplemental services including client education and evaluation of breeding outcome or pregnancy, including pregnancy detection, confirmation of normal gestation, estimation of litter size, and accurate estimation of day of gestation. These all require - as do clinical evaluations of bitches presented for health reasons during suspected or confirmed pregnancy, provision of pregnancy termination service for bitches with unwanted pregnancies, and the management of bitches with a history of pregnancy failure or abnormal pregnancies- a working knowledge of the biology and clinical progress of normal canine pregnancy and of potential important diagnostic and therapeutic modalities applicable to pregnant bitches. The following is a brief review of some of the important elements and issues, albeit excluding dystocia and mammary function.

Timing of Events and Consistent Gestation Length. Ovulation occurs approximately 48-60 h (2 days) after the preovulatory LH surge. The LH surge (the central critical reproductive event, Day 0) may occur any time from late proestrus to mid-estrus based on observed behavior changes; on average, it is at 1 day after estrus-behavior onset. Gestation length is mistakenly thought to be quite variable because pregnancy can result in intervals from a single mating to partition that range in the extreme from 55 to 68 days; the intervals counting from the first or last of multiple matings can be even longer or shorter, respectively, i.e. 50 or 70 days. Parturition, however, consistently occurs 64-66 days after the preovulatory LH surge in over 95% of normal pregnancies (Concannon, 2003). The discrepancy is due to several phenomena: oocyte maturation does not occur until 2.5 to 3 days after ovulation; the mature oocyte may remain fertile for up to 5-6 days in the extreme, i.e. until 10-11 days after LH surge; sperm may remain fertile for up to 8 days in the female tract; some bitches may abnormally allow mating 5 days before ovulation, others may abnormally refuse to allow mating until 5 days after ovulation; thus, single matings can be fertile from 5 days before ovulation to as late as 8 days after ovulation (i.e., days – 3 to + 10 from LH surge). However, the typical bitch has a limited peak fertile period from Day 0 to Day 5 or 6 after the LH surge, with oocytes apparently dying rapidly 1-2 days after oocyte-maturation and with cervical closure occurring between Day 6 and 9 after the LH surge further reducing fertility even when the oocytes retain their viability beyond that time. In cases where sperm are weak or compromised either naturally or due to methodologies of artificial insemination, the fertile period in an individual instance may be limited to a 1-2 days period. Ideally, breeding management techniques will have estimated the time of the LH surge based on one or more parameters including: timing of Day –1 to +1 increases in vulval and vaginal-mucosal edema; timing of Day 6-9 metestrus/diestrus change in vaginal cytology; and timing of Day-0 rapid preovulatory rise in serum progesterone as well as any acute change in behavior. Such timing can not only provide for accurate prediction of the date of a Day 64-66 parturition, but also permits establishing preferable times for pregnancy testing, for monitoring of normal fetal developmental changes, and of data collection in the monitoring of pregnancies considered high-risk based on prior pregnancy failures.

Implantation. Permanent apposition of embryonic trophoblast and the uterine implantation-sites occurs around Day 16-18, after a Day 10-11 entry into the uterus and 6-7 days of intra-uterine and trans-cornual migration of blastocysts. Actual implantation by trophoblast-cell invasion is estimated to occur at Day 21.

Landmark Events Assessed By Ultrasound. Using 5-7 MHz ultrasonographic (U/S) equipment, fluid filled pregnancy-associated uterine vesicles may be obvious at 1-2 mm diameter as early as day 18-20 but may not be clear until 3 mm diameter at day 21. The fetal mass within the vesicle may be visible by day 20-21, but may not be clear until Day 23. The beating fetal heart may be seen as a distinct flickering by day 23-24, but may not be easily detected until day 25. U/S equipment of greater or lesser resolution may detect these and other landmark states or events earlier or later, respectively. Changes in embryo diameter, crown rump length, biparietal head diameter, and other parameters have been well characterized, at least for research dogs. These data have also been successfully applied to estimating gestational age clinically on many breeds.
Endocrine Changes and Progesterone Assays. Progesterone concentrations increase throughout estrus, peak at 15-90 ng/ml some time between Day 15 and 25, and slowly decline thereafter, in both pregnancy and non-pregnant bitches. Progesterone assessment around Day 30 can be useful; in the absence of a positive pregnancy diagnosis, progesterone can confirm whether or not the bitches failed to ovulate or to normal luteal function; progesterone can detect the possible onset of luteal insufficiency associated with low progesterone levels. In such problem cases, progesterone assays at 5-7 days intervals can be useful in monitoring the pregnancy; values above 5 ng/ml are assuring to some extent; those below 4-5 ng/ml any time prior to day 55 may indicate luteal insufficiency. Such insufficiency is difficult to define; research bitches have maintained normal pregnancies despite spontaneous or drug-induced declines in progesterone to or near non-detectable levels for several days; anecdotally, some clinical cases with progesterone at 2-3 ng/ml at day 50 have produced normal litters in the absence of treatment while others have not.

In non-pregnant bitches progesterone declines to under 1 ng/ml some time between Days 55 and 90, often between Days 60 and 70. In pregnancy, progesterone typically remains distinctly elevated above 3-5 ng/ml until Day 62-63 and 1-3 days prepartum, at which time the mechanism of parturition causes a luteolytic rise in plasma prostaglandin-F2alpha (PGF) and a decline in progesterone to < 1-2 ng/ml. Progesterone assays can be also used in late gestation to help determine the timing of elective c-sections in pregnancies where Day 0 was not determined with sufficient accuracy (e.g., waiting no longer than when progesterone declines to 2 ng/ml or undergoes an abrupt decline to 2-3 ng/ml over a 24-36 hr period). Estrogen assays are of little value, in that many assays have limited application to canine samples; levels are variable and provide no useful information not available otherwise. Relaxin levels in the bitch increase between Day 27 and 30, peak by Day 40-50, are elevated but declining towards term, and fall at parturition to reach non-detectable levels within 1-6 weeks. Relaxin assays would be a means to monitor the placental function and pregnancy beyond initial pregnancy detection, but the magnitude of decline that would warrant intervention is not known. Pregnancy termination could be followed by some secretion of relaxin for a week or more if some placental tissue is retained, as may occur postpartum. Relaxin in dogs is mainly or entirely of placental origin; detectable relaxin postpartum is presumably due to retained placental (trophoblast) tissue. Prolactin. PRL concentrations increase above those in most non-pregnant bitches at or shortly before day 30; whether or not the increase is in response to the pregnancy specific elevation is not known. PRL concentrations peak near term, and are as high or higher during much of lactation, following a transient postpartum decline. PRL elevations are not pregnancy specific because they can also occur in no pregnant bitches showing symptoms of overt (clinical) pseudopregnancy. Pregnancy causes insulin resistance, possibly more so than the nonpregnant luteal phase; insulin
requirements of diabetic bitches become increased, and pre-diabetic bitches may become hyperglycemic.

Pregnancy Maintenance and Termination. The only hormone required to maintain pregnancy in dogs is progesterone, based on pregnancies so maintained in ovariolectomized bitches. Normal estrogen concentrations may however be important for full mammary development and lactation. The only source of progesterone in dogs is ovarian (corpora luteal). Pregnancy specific increases in ovarian progesterone secretion from Day 30 to near-term are not obvious in plasma levels because of increased hemo-dilution and increased metabolism by the uterus, placenta and mammary; however, they normally ensure that peripheral concentrations are maintained at or above the minimum required for pregnancy maintenance. Luteal progesterone production requires both LH and prolactin; at any time suppression of either hormone diminishes or terminates progesterone secretion. Administration of an anti-progestin (e.g., aglepristone, 10 mg/kg/d for 2 days) is the preferred mode of pregnancy termination more available and affordable, as it is effective without side effects at any time. PGF (30-50 ug/kg, b.i.d. or t.i.d.) or PGF-agonist (Cloprostenol 2.5 ug/kg, every 48 hours) formulations after day 25 are luteolytic and abortifacient when given “to effect” as confirmed by ultrasound. Dopamine agonists such as bromocriptine (100ug/kg q.d. or b.i.d, to effect) or cabergoline (5 ug/kg/d, to effect) given orally after day 30 can cause complete luteolysis and terminate pregnancy but are more effective in combination with low-moderate doses of PGF. Dexamethasone (0.2 mg/kg, b.i.d., p.o.) for 9.5 days or longer also terminates canine pregnancy after day 30; however, the immunosuppressive doses also cause adrenal suppression for 2-3 weeks in otherwise healthy bitches.

Altered Clinical Parameters. Hematocrits normally decline after Day 25, are typically below 40 in late gestation and may reach values as low as 31 at term; normal non-pregnant values are not re-attained until 1.5 to 3 months post partum; This physiological, normo-chronic normocytic anemia appears primarily due to increased plasma volume; it potentially facilitates circulation through placental vessels. As in humans there is also likely to be an associated but normal thrombocytopenia, total protein, oncotic pressure decreases in albumin, total calcium remains in plasma. Plasma concentrations of the hepatic acute phase proteins (APP) fibrinogen and c-reactive protein increase following implantation, are evident by Day 30, peak at Day 45-55, decrease but remain elevated to term, and decline rapidly to normal post-partum. The APP increases are distinct despite the concurrent dilution effect of increased plasma volume; although not truly pregnancy specific, they have been used as the basis of marketed canine pregnancy tests. Elevated APP levels are seen in most instances of inflammation. APP levels may also increase with pregnancy may represent a similar response to placentation. The induced increase in fibrinogen is compensated by a simultaneous increase in local fibrinolytic activity as reflected in increased serum fibrinogen degradation products. A 40 percent average increase in food consumption accompanies a 20 to 55 percent increase in body weight, and requires corresponding increased food supply. Calcium or vitamin D supplementation is contraindicated it can complicate normal calcium regulation and increase the increase risk of periarticular hypocalcaemia.

Resorptions and Abortions. Spontaneous resorption of partial or entire litters can occur with little or no evidence or side effects between days 28 and 36 in apparently healthy and otherwise normal pregnant bitches, as shown in several U/S studies. Resorptions and abortions after day 36 with some related vaginal discharges can occur without detection when bitches lick away or ingest discharged materials. Resorption of one or more conceptuses likely occurs in 10-15 % of pregnancies. Many instances of resorption are proceeded by early embryonic development retarded in relation to the expected time course. U/S exams for pregnancy diagnosis performed by day 28 can detect bitches that resorb entire litters but would otherwise be presumed to have failed to become pregnant. Any relationship to delayed breeding time, stress, or uterine status is not known. Gross abortions after day 40 are less common and more likely to represent disease or infection, and merit evaluation of brucella, herpesvirus, or other suspected agents. Abortion may be accompanied by a large decline in progesterone, and absent other obvious causes, insufficient luteal function has been suspected or assumed. Whether low progesterone is a primary cause or simply a consequence of the abortion in any individual case is difficult to determine. While not well documented, abortion due to a non-endocrine cause would be expected to result in a decline in progesterone due to a loss of the gestational stimulus for increased prolactin and its luteotrophic effects; and, due to a uterine release of PGF. Documentation of insufficient luteal function would require detection of decreased progesterone prior to the pregnancy loss. Anecdotaly, progesterone supplementation has been “successful” in pregnancies in bitches that previously failed to maintain pregnancy.

Progestosterone Supplementation. Supplementation can be considered in previously problematic bitches if progesterone falls below 5 ng/ml before day 55 or declines more rapidly than expected in mid-gestation. Some progestins are inappropriate (e.g., medroxyprogesterone and megestrol can cause masculinization of female fetuses), Progesterone supplementation protocols have included the following: (1) progesterone in oil, i.m., 2 mg/kg, every 72 h. (2) altrenogest, daily, p.o., 0.088 mg/kg (0.2 cc/10 lb using Regumate ©, Hoechst-Roussel). Micronized natural progesterone marketed in capsules for humans has been given orally 1-2 times per day at doses of 5-10 mg/kg (i.e., 100 mg capsules in 10-20 kg dogs) and the efficacy monitored by progesterone assays. Natural progesterone requires frequent administration but has the advantage that withdrawal can be done rapidly. Whether reported successes have been incidental or the result of treatment is not known. Therapy must be discontinued to permit or promote normal whelping.
Excessive exogenous progesterone or other progestin can compromise normal parturition and can result in dead puppies that must be recovered by c'section. Oral progestin therapy should be discontinued 2 days prior to the estimated day of parturition. Serial injections of synthetic preparations should be discontinued early enough for the progestin to decline to non-effective concentrations by the expected day of parturition. A mistimed or long-acting treatment can potentially be overcome by administration of otherwise abortifacient doses (or higher doses) of an antiprogestin, where available.

**Normal parturition.** As in other species it appears that near-term fetal maturation, including hypothalamic changes in CRF and other peptides, pituitary ACTH secretion, and fetal-adrenal steroidogenesis results in a surge in corticosteroid activity that initiates the rapid positive feedback mechanism for parturition. Unlike in many species, there is no large increase in estrogen pre-term, and as in primates the cortisol may act to stimulate endometrial and placental production of PGF and release, possibly by acting locally as a progesterone antagonist. Elevated PGF has direct luteolytic effects causing an abrupt decline in progesterone that in turn increases the extent of utero/placental PGF release and consequently a more accelerated luteolysis and decline in progesterone. PGF stimulates uterine contraction. Declining progesterone causes placental separation and increases myometrial sensitivities to PGF and to oxytocin. Uterine contractions and fetal impingement on the cervix stimulate oxytocin secretion. The initial sharp decline in progesterone from 5-10 ng/ml to 2-3 ng/ml is associated with stage 1 labor (vocalization, nesting behavior); stage 2 labors occur following the continued progesterone decline below 2 ng/ml. Normal intervals between consecutive deliveries of 100% term newborn puppies range from 20 min to 4 h or more. The prepartum decline in progesterone is presumably the cause of the acute rise in prolactin immediately prepartum. It also dis-inhibits the suppressive effects of progesterone on lactation and thus initiates lactation. And, it terminates ongoing hyperthermic effects of elevated progesterone on hypothalamic thermoregulation, causing an acute, transient hypothermia. Temperature declines 1°C or more during the 12-24 h prepartum. Rectal temperature can be monitored twice daily starting at estimated Day 60 by owners who wish to be involved.

**Fetal Viability.** Fetal HR can be variable but should remain above 200. U/S detection of fetal HR below 180 is indicative of fetal distress, is often accompanied by fetal bowel movements, and can be the basis of elective intervention in near-term bitches. U/S can also reveal fetal overgrowth or growth retardation, and other abnormalities including hydrocephalus, fetal anasarca, herniation of the ventral abdominal wall. Such cases can then benefit form intervention. Uterine contraction activity has also been monitored with commercial devices designed for that purpose. Monitoring of fetal and uterine activity near term in conjunction with intervention c'sections when indicated resulted in a decline neonatal mortality from 9 to 3% of pups in one study. There are no confirmed markers of placental function; relaxin tends to decline in late gestation and is only a qualitative and not a quantitative marker of placental function.

**Interventions.** These can include planned c'sections (e.g., in brachycephalic breeds), therapeutic c'sections in response to fetal distress, prolonged gestation or maternal dystocia, and potentially antiprogestin-induced parturition. Election of c'section may involve a brachycephalic breed with history of dystocia, or a bitch with over-grown fetuses, a history of dystocia, or excessive early neonatal loss of pups. In the absence of signs of dystocia, prolonged gestation should not be presumed based on breeding records alone. Monitoring and/or examining for the expected prepartum decline in progesterone can be an important tool in managing possibly-prolonged gestation and in timing elective c'sections, especially in bitches in which the day of day of the preovulatory LH surge was not estimated with accuracy. Recent and ongoing clinical studies suggest that the outcome of c'sections can be improved by providing bitches access to 100% oxygen gas before as well as during surgery to improve fetal surfactant and decrease neonatal growth retardation, and other abnormalities including hydrocephalus, fetal anasarca, herniation of the ventral abdominal wall. Such cases can then benefit from intervention. Uterine contraction activity has also been monitored with commercial devices designed for that purpose. Monitoring of fetal and uterine activity near term in conjunction with intervention c'sections when indicated resulted in a decline neonatal mortality from 9 to 3% of pups in one study. There are no confirmed markers of placental function; relaxin tends to decline in late gestation and is only a qualitative and not a quantitative marker of placental function.

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