Review Report

Pregnancy monitoring and parturition in dogs

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Abstract

Management of term pregnant dogs is a major and frequent clinical situation in small animal reproductive veterinary practices, and can be challenging. Whether a pregnancy is planned or unplanned, these cases require careful monitoring to prevent maternal distress and neonatal loss. Reproductive system evaluation of female dogs is useful for determination of ovulation during breeding management, pregnancy diagnosis, and for timing of cesarean surgery, among other clinical scenarios. Basic understanding of female dog's reproductive physiology is mandatory to interpret relevant hormonal assays and other complementary diagnostics such as vaginal cytological evaluation and imaging (ultrasonography and radiography) for clinical decisions. This review covers physiology, diagnostics, and clinical outcomes relevant for pregnancy monitoring and parturition.

Keywords: Canine pregnancy, whelping, cesarean surgery, dystocia, neonate

Introduction

Canine reproduction management is commonly practiced by many theriogenologists and general practitioners. Knowledge of female dog's estrous cycle and pregnancy is important for clinical decisions regarding timing of parturition and plans for caesarean surgery. Additionally, canine pregnancy has a well-defined length, and neonates cannot survive if delivered 2 days before or after due date. Thus, attending veterinarians making decision for elective caesarean surgery must be confident that fetuses are mature enough to support life outside the uterus.

To best determine when a dog is ready to whelp, veterinarians must acquire as much history as possible regarding the dog's estrous cycle and breeding management. The following are important questions: 1. Was stage of estrus determined via vaginal cytology or peripheral hormone (i.e. progesterone) concentrations? 2. What was the type (i.e. live cover, artificial insemination) of breeding? and 3. Was pregnancy diagnosed prior to presentation? Prediction of parturition has the most potential for inaccuracies based on breeding dates alone. Objective of this review is to provide basic reproductive physiology and various diagnostics available to make best decisions for veterinarians practicing female dog reproductive management and plan for caesarean surgery.

Reproductive physiology

Age of sexual maturity is 6-15 months.[1] Dogs cycle regularly in intervals between estrus that ranges from 5-13 months (average 7 months for most breeds).[2] Estrous cycle stages include: proestrus, estrus, diestrus, and anestrus.

Estrous cycle

Proestrus encompasses the period before luteinizing hormone (LH) surge that lasts for 1-3 weeks (average 9 days). During proestrus, gonadotropin releasing hormone pulses from hypothalamus stimulate follicle stimulating hormone (FSH) release that leads to ovarian follicular development. Once follicles reach an appropriate size, they produce estrogen, and classical signs of proestrus are observed. These include vulvar swelling, diapedesis of endometrial red blood cells and consequent serosanguineous vulvar discharge, edema of vaginal epithelium, pheromonal secretion causing increased attraction of male dogs, and progressive cornification of vaginal epithelium.[2]

Follicular luteinization begins before ovulation with increasing peripheral progesterone concentrations as estrogen concentrations start to decrease.[3] This shift in hormonal

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concentrations induces receptivity to male dogs and the period is considered estrus. Estrus begins 0-1 days after LH surge and receptivity lasts ~ for 7 days.[2] Preovulatory LH surge coincides with doubling in progesterone concentrations (from < 1 ng/ml during anestrus and proestrus to 1-3 ng/ml at surge) and lasts 1-5 days in dogs[4,5] resulting in ovulation of matured follicles. Since dogs are polytocous, ovulation occurs over a period of 24-36 hours, starting 48-72 hours after LH surge, when peripheral progesterone concentrations are 4-10 ng/ml.[4] Clinically, lack of receptivity to male dogs is end of estrus and vaginal cytology is no longer predominantly composed of cornified epithelial cells. Cytological diestrus begins when an abrupt decrease of superficial or cornified cells from 80-100% to < 20% is detected in vaginal cytology.[6]

Dogs are unique; ovulating primary oocytes that mature in the oviduct for 48-72 hours before they are capable of fertilization. This occurs once they become secondary oocytes that have completed first stage of meiosis with first polar body extrusion.[7,8] Secondary oocytes are fertilizable until 60-108 hours after ovulation.[8] Canine sperm have prolonged longevity in the female reproductive tract, particularly after natural mating, in part because of their retention in uterine crypts and uterotubal junction, especially if fresh and unprocessed semen is used for insemination.[9] After fertilization, embryos enter the uterus between 10-12 days after LH surge. They are nourished from uterine lactotroph until implantation at ~ days 17-18 of embryonic life.[10]

Pregnancy

Pregnancy lasts for 65 ± 1 days from LH surge (or 63 ± 1 days from ovulation).[11] Canine pregnancy is maintained solely by progesterone produced from corpora lutea (CL), due to lack of placental steroid synthesis.[12,13] Although maternal recognition of pregnancy is unknown in dogs, important embryo-maternal communication exists to signal decidualization and eventually parturition cascade.[10]

After implantation, prolactin and LH secretions assume luteotropic roles, and support progesterone production from the CL. Plasma prolactin concentrations increase during second half of pregnancy, peak on the day of parturition, decrease for the next 24-48 hours, and then increase again to support lactation. During lactation, there are high plasma prolactin concentrations.[14,15] Relaxin can be detected in pregnant dogs ~ days 20-25, peaks 2-3 weeks before whelping and remains elevated until term (Figure 1). Relaxin is involved in pregnancy related remodeling of connective tissue, acting on pubic ligament, pregnant uterus and cervix, and is the endocrine marker of pregnancy in dogs. Intraluteal relaxin is also involved in regulating canine CL.[16]

Progesterone is produced by CL thecal cells throughout pregnancy and is essential for pregnancy maintenance. It increases once luteinization starts and continues after ovulation for ~ 2 weeks until it plateaus for 1-2 weeks prior to a steady decrease during remainder of pregnancy. At end of pregnancy, circulating progesterone concentrations decrease below 2 ng/ml. Estrogen is produced throughout pregnancy at low concentrations and may contribute to uterine progesterone receptors maintenance. Nonpregnant and pregnant dogs' CL express prostaglandin F2 alpha ($PGF_{2\alpha}$) receptors. In dogs, no endogenous luteolytic agents are produced by nonpregnant uterus. Regression of CL result in steady decrease in progesterone concentrations until they reach < 1 ng/ml (may be longer in nonpregnant dogs).[11] In pregnant dogs, this continuous decrease in progesterone concentrations is hastened by luteolysis due to endogenous production of $PGF_{2\alpha}$ from fetoplacental unit. This steep decrease in progesterone concentrations is observed ~ at day 60 of pregnancy. $PGF_{2\alpha}$ is also responsible for cervical relaxation and uterine contractions during parturition.[15]

Parturition

Circulating progesterone concentrations decrease to baseline 1-2 days before whelping. Maternal decidual cells express progesterone receptors and have an important role in signaling prepartum luteolysis.[16] Additionally, decrease in circulating progesterone concentrations also appear to alter vascular functions, apoptosis and modulates proinflammatory response resulting in parturition.[17] Furthermore, this is accompanied by decrease in body temperature, typically 1 °C 12-24 hours before the onset of parturition. It is important to mention that this is not observed in every dog. In a large retrospective study, only 33% of pregnant dogs had decreases in basal body temperature prior to parturition.[18] Therefore, a more complex mechanism involving multiple factors apart from progesterone may be involved.[15] Maturation of fetal pituitary-adrenal axis resulting in production of cortisol, triggering $PGF_{2\alpha}$ release and initiating parturition cascade has been suggested for many years; however studies failed to prove this theory and corticosteroids concentrations at parturition had wide inter-individual variation.[15,19] Role of exogenous corticosteroid treatment in late canine pregnancy is discussed later in this review.

Behavioral signs of parturition can precede hormonal and temperature changes with extreme nesting behavior observed 6-24 hours before whelping. Although these can give the clinician valuable information, not every dog will exhibit these signs consistently, making it difficult to rely only on clinical observations. Stage I of parturition begins with uterine contractions that may not be appreciated externally and ends when the cervix is fully dilated. Length of the canine vagina does not allow for cervical palpation. Stage II becomes evident with passage of pups through birth canal. Voluntary abdominal straining and a Ferguson's reflex should be present. Neonates may present in cranial or caudal position during normal parturition. Stage III begins after fetal expulsion and ends with expulsion of fetal membranes. Stage II and III often occur simultaneously.[20]

Pregnancy diagnosis

Duration of pregnancy is shorter in dogs compared to other species. Therefore, canine fetuses are born in an immature state and final development of most organ systems must be completed after birth. Consequently, substantial development occurs during the last days of canine pregnancy, ~ 61-63 days after ovulation. Failure of this last fetal developmental phase has poor prognosis for pups' survival. Once a fetus exceeds its due date by > 2 days, its demand for nutritional support is more than placenta is able to provide resulting in intrauterine fetal distress and eventually death.[11]

There are several reasons why determination of accurate pregnancy age is important. This is beneficial for planning prenatal care of pregnant dogs as well as organizing veterinary assistance in cases of natural whelping that may require assistance. Also, dogs that may require a planned caesarean

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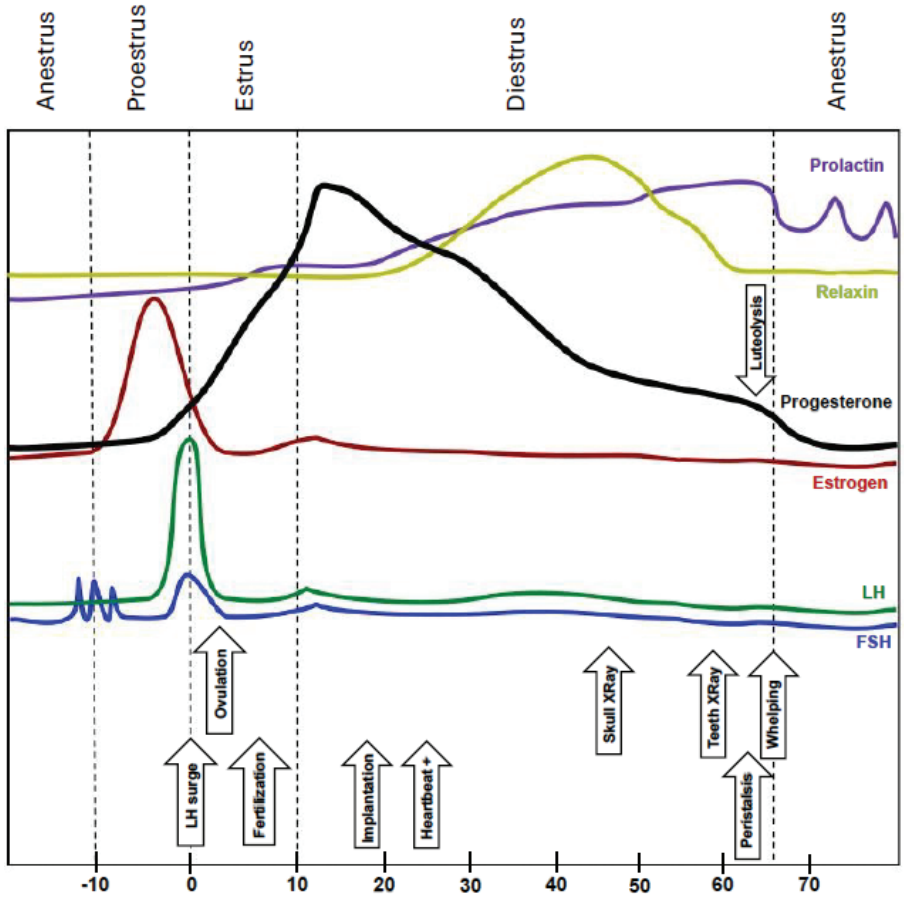

Figure. Graphic representation of canine estrous cycle, pregnancy hormones and timing of methods to determine pregnancy age; X-axis represents days from LH surge.

surgery, such as in cases of singletons, previous history of dystocia, small litters with large pups or very large litters, and high-risk pregnancies that may compromise dog's health. One of the most accurate methods to determine pregnancy age is by having a thorough breeding management and knowing the day of LH surge or ovulation.

On average, canine pregnancy lasts 65 ± 2 days from LH surge, 63 ± 2 days from beginning of ovulation[11,21] or 57 ± 3 days from the beginning of cytological diestrus. Breeding date is not accurate in determining time of whelping, and parturition can happen anywhere from 58 to 72 days after breeding. Breed variation exists regarding pregnancy duration; German shepherds, Hound dogs and Cavalier King Charles Spaniels have shorter pregnancy duration and West Highland white terriers have longer pregnancy.[22-24] We recommend researching literature while working with dog breeds that are not commonly presented in clinical practices.

Hormonal evaluation

A common approach to assess the likely day of LH surge, ovulation, and optimal fertile period is by measuring peripheral progesterone concentrations. This is often performed every other day, in combination with vaginal cytology, once serosanguineous vulvar discharge is detected. In a large study that evaluated 1,300 dogs, in 1,420 estrous cycles the mean (± standard deviation) progesterone concentrations increased from 1.7 ± 0.7 to 2.7 ± 0.6 ng/ml at LH surge and at estimated day of ovulation concentrations were from 4.8 ± 0.9 to 7.2 ± 1.3 ng/ml.[25] Ovulation occurred when progesterone concentrations were 4-10 ng/ml.[26] More than focusing on individual progesterone concentrations (since variable among dogs), a trend in increasing concentrations of progesterone should be identified to estimate the day of LH surge, ovulation, and fertile period. Assay and sample handling may affect progesterone measurement, so consistency is recommended for serial monitoring; i.e. sample collection time of day and collecting blood from a fasted or nonfasted patient. A thorough review on assay variability was recently published.[27]

Detection of LH surge by measuring peripheral concentrations is sometimes utilized in canine breeding management.[28] Radioimmunoassay has long been considered as gold standard due to its accuracy and repeatability, but due to radiation concerns, expertise required, and time to receive result makes this impractical. However, a semiquantitative enzyme linked immunosorbent assay (Witness® LH, Zoetis, USA) was developed for in-clinic use. Detection of LH surge requires daily sampling as the duration can be variable that limits this assay's utility in many clinical situations.

Radiological evaluation

Radiography is valuable to assess pregnancy age since fetal skeleton mineralizes progressively throughout pregnancy. Proper radiographic technique and patient restraint is important to obtain good quality images and commonly,

lateral view is usually sufficient for good diagnosis. Ventrodorsal view is indicated when fetomaternal mismatch is suspected; however, caution is required when placing late-term pregnant dogs in this position. Radiographs are the best method to obtain fetal count when performed between days 57-65 (reduces risk of missing a fetus) when mineralization is advanced. Mineralization of fetal skull is first visible between days 43-46 of pregnancy. Scapula, humerus, and femur are visible between days 46-51. Radius, ulna, and tibia are visible between 50 and 53 days. Pelvis and ribs are visible between days 53 and 59. Coccygeal vertebrae and distal extremities are visible between days 55-64. Teeth are the last part to mineralize and are usually visible between days 58-63.[29] Although radiographs are useful for determining fetal numbers prior to whelping and a rough estimate of pregnancy age, they have limited use as indicators of fetal maturation and readiness for birth.

Ultrasonographic evaluation

Ultrasonography has been utilized to monitor canine pregnancy and determine pregnancy age for many years. Most veterinary clinics have an ultrasound machine available and although expertise is required to become proficient in diagnosis, the non-invasiveness of this technique makes it valuable for practitioners. Repeated ultrasonography examinations of up to 3 times per day have been utilized to determine the time of ovulation.[30-32] This particular approach requires training and frequent evaluations, making it impractical for most practitioners.

Amniotic vesicles can be detected as early as day 19 as 1-2 mm rounded anechoic structures in the uterine lumen. Embryos can be first detected via ultrasonography on day 22 and heartbeat on day 24.[24] Between days 19 and 37 of pregnancy, measurement of amniotic sac diameter (inner and outer chorionic cavities) can be easily obtained, and mathematical formulas are available to determine pregnancy age.[24,33] These early pregnancy biometric indices are often more accurate than those obtained in late pregnancy. At days 27-28, embryos move away from the endometrial wall and are suspended by fetal membranes. Zonary appearance of the placenta is obvious by days 29-31 and the edges curl inward by days 32-34.[24] After day 37, several fetal morphometrics can be utilized to determine pregnancy age; e.g. biparietal diameter (BPD), body diameter (BD), deep portion of the fetal diencephalon-telencephalic vesicle (DPTV) and femur, humerus, tibia, and kidney lengths. BPD and BD remain the most accurate of all formulas available across breeds. However, practitioners should take into account the breed and dog size when using these formulas.[24]

Fetal organ development can also be used to calculate pregnancy age. Serial examinations and measurements from multiple fetuses provide more accurate information. Urinary bladder is first visible between 35-39 days, stomach between 36-39 days, kidneys between 39-47 days, and intestine between 57-63 days.[24] Fetal kidney length has been utilized to determine pregnancy age and it is most accurate between 48-52 days of pregnancy.[34] Renal cortical thickness (CT), medullary thickness (MT) and ratio of CT/MT can also be used.[35] Intestines can be visualized from days 39-43 of pregnancy; however, distinct visualization of various layers is not possible until days 48-54 and these layers become increasingly prominent until term.[24,36,37] Peristalsis becomes easily visible between 62-64 days, and it may be easier to observe in smaller breeds.[36] Panting may cause difficulties when observing peristalsis so care must be taken.[24] Once all intestinal layers are visible and peristalsis is consistently prominent on serial examinations, fetal survival ex utero is optimal; however, veterinarians must recognize that variation among individuals exist and this parameter should not be used as a sole parameter when making decisions for cesarean surgery.[24,36,37] A thorough review on pregnancy aging based on ultrasonography was published.[24]

Evaluation of fetal maturity

Changes in fetal heart rate, intestinal layer development and peristalsis, and progesterone monitoring are important to make a well-informed decision (when dogs are ready to whelp or a caesarean surgery can be scheduled). Fetal heart rate usually starts to decrease 12-36 hours prior to onset of labor as fetuses begin to meet or exceed full demands of placental function. Transient fetal bradycardia may be observed during uterine contractions. Starting to monitor dogs 2-6 days ahead of the expected parturition date usually allows for the most accurate assessment of final fetal maturation and readiness for birth and will allow steroid treatment 24 hours prior to delivery to facilitate fetal pulmonary function and thus neonatal resuscitation.[38]

Medical management before caesarean surgery

Preanesthetic considerations for term pregnant dogs are controversial. Preoxygenation and balanced hemodynamics are often performed for all presurgical veterinary patients. However, pregnant dogs have other considerations accounting for neonatal survival. Systematic review from human medical literature suggested that antenatal use of corticosteroids in term planned caesarean surgery may reduce admission to neonatal ICU due to respiratory distress syndrome; however, more evidence is needed in this area and it still remains controversial.[38,39] Betamethasone improved pulmonary capacity in canine neonates; antenatal maternal corticoid therapy in dogs improved areas of gas exchange in lungs and aided in the metabolism of pulmonary fluids rather than stimulated surfactant production.[40] Prenatal treatment with betamethasone can be adopted as clinical lung maturation protocol for pregnancies at risk in order to prevent morbidity/mortality and to increase neonatal survival.[41]

Conclusion

Determining parturition time in dogs requires a multimodal approach. Relying on a single parameter may lead to inaccurate timing of fetal maturity and readiness for delivery. Before proceeding with surgical management of dogs, a thorough history, physical examination with a vaginal digital examination, imaging, bloodwork, and determination of peripheral progesterone concentrations are helpful. If findings are indicative of fetal distress and/or fetal readiness (i.e. progesterone < 2 ng/ml), cesarean surgery may be the best option. Surgical intervention may also be the best option if dogs have signs of systemic illness, anatomical abnormalities precluding vaginal delivery or an obstructive dystocia that cannot be resolved with gentle manipulation. Planned caesarean surgery can carry an excellent prognosis if proper timing and planning is done in advance.

Conflict of interest

None to report

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