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Canine Pregnancy: Predicting Parturition and Timing Events of Gestation

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Introduction

Many aspects of canine pregnancy are unique among veterinary domestic species. Therefore, an understanding of the time course and clinical correlates of ovulation, fertilization, embryo and fetal development, and pregnancy specific changes in maternal physiology is essential when providing clinical services such as breeding management and monitoring of pregnancy [1-4]. It is also important for decision-making in cases of pregnancy failure, elective caesarian section and dystocia. One important fact is that gestation length and events of gestation are very repeatable and predicable when viewed in relation to the time of ovulation or the preceding the luteinizing hormone (LH) surge (Table 1 and 2). That is true, despite the fact that the normal interval from breeding to whelping can range from 55 to 70 days.

Fertilization and Gestation Length

The reason why gestation length in dogs is relatively consistent when measured from the day of ovulation but highly variable when measured from the day of breeding is partly understood. In the vast majority of bitches parturition occurs 64, 65 or 66 days after the ovulatory surge in LH [1]. The latter represents the acute release of LH from the pituitary in late proestrus or early estrus. The LH surge triggers the event of ovulation. Since the day of the LH surge can be measured or estimated with reasonable accuracy, timing events from that day, and using it as the reference point (Day 0) can be helpful. A 64 - 66 day gestation length measured from the LH surge to parturition is the same as a 62, 63 or 64 day interval between ovulation and parturition, since ovulation has been estimated to occur 2 days after the surge in LH [4].

In contrast, using the day of mating as a reference point, as observed in cases of just a single mating or insemination, parturition can occur as early as 56 days later and as late as 68 days later. Similarly, a large variation in apparent gestation length can be encountered when counting from the first of multiple matings or the last of multiple matings, the extremes encountered differing by 2 weeks. For instance, if a bitch is held for an aggressive stud dog and forced to mate starting 3 - 5 days before the LH surge, the interval from first mating to whelping may be as long as 69 - 70 days. And, in rare instances where a bitch is still fertile 9 or 10 days after the LH surge and is bred then, the interval from mating to whelping can be as short as 55 or 56 days.

Part of the explanation is that dog sperm may, in some instances, survive in the bitch's tract for up to 7 or even 9 days and still remain viable in terms of being able to achieve fertilization and result in pregnancy. It is possible that in dogs as in other species, many sperm die or loose fertility after 1 or 2 days. However, the number that retain fertility for 2 days is sufficiently high in dogs that fertility and fecundity are not affected by matings on the day of the LH surge, 2 days before ovulation. Thus, in such pregnancies, the sperm survived 2 days before potentially penetrating the oocyte, and the chromatin had to survive another 2 to 3 days to function as a male pronucleus which fuses with the female pronucleus to form the 1-cell zygote. Fertility declines with matings earlier than the day of the LH surge (i.e. mating 3 or more days before ovulation).

However, litters have occasionally been obtained from forced matings, matings by aggressive males, and artificial inseminations of fresh semen as early as 3 - 5 days before the LH surge. Sperm deposition in such cases was 5 to 7 days before ovulation and at least 7 to 9 days before oocyte maturation. Another part of the explanation for the large variation encountered in apparent gestation lengths lies with the timing of egg maturation in this species. In dogs (and foxes), unlike most other species, the eggs are still immature when they are ovulated (i.e., they are still primary oocytes) and they do not complete meiosis and become secondary (mature) oocytes until probably 2.5 to 3 days after ovulation. An egg must be a mature, secondary oocyte containing a "female" pronucleus before the "male" pronucleus of a sperm can fuse with it to complete the process of fertilization by forming the nucleus of the new one-cell embryo. In early-mated bitches, a sperm probably penetrates each egg shortly after it is ovulated, but the male pronucleus once formed has to wait for the egg to mature. In late-bred bitches, the female pronucleus of the matured egg is ready to fuse with the pronucleus of a sperm that subsequently penetrates as soon as the male-pronucleus is formed.
The interval of nearly 3 days required for oocyte maturation after ovulation has been estimated in at least two ways. One is based on estimating how long after ovulation that matings from different males can still result in pups with different sires. Another is estimation of the time after ovulation at which mating with short-lived frozen-thawed sperm results in pregnancies. Because of this phenomenon of "delayed" oocyte maturation, bitches can readily give birth to litters with multiple sires when there are matings by different males before ovulation.

Timing of Fertilization, Fertility and Fecundity

Thus, it appears that fertilization to the point of nuclear fusion can be accomplished no earlier than about 3 days after ovulation (and, thus, 5 days after the LH surge). Following maturation of the egg to secondary-oocyte status, the fertile life span of an unfertilized egg may be only 1 or 2 days in some instances, since fertility declines if matings are delayed until 4 and 5 days after ovulation (i.e., 6 and 7 days after the LH surge). That is, both litter size and pregnancy rate decline when mating occurs more than 2 days after the maturation of the oocyte. Thus, with a narrow 2-day window for optimal fertilization to occur, it is reasonable that gestation length is consistent when measured relative to the day of the LH surge, or to the day of ovulation. However, some bitches may have one or more fertile oocytes survive to as late as 7 or even 8 days after ovulation which corresponds to 9 or 10 days after the LH surge. While fertility is typically low with matings this late, when pregnancy does occur the gestation length is usually the same that as in other bitches, i.e., with parturition occurring at 64 - 66 days after the LH surge (and 62 - 64 days after ovulation). The above scenario is the basis of well documented cases of bitches with exceptionally short apparent gestation lengths, giving birth to litters as "early" as 55 to 56 days after breeding. Why true gestation length is not always obviously longer in these "late-bred" bitches is not clear, but there are two likely reasons. First, there is as yet unpublished evidence that eggs fertilized 2 days after maturation divide slightly faster than eggs penetrated by sperm before maturation. (Tsutsui, 1999, personal communication) Second, it is likely that the timing of implantation is in part related to a sequence of events regulated by the timing of the changes in serum concentrations of estrogen and progesterone. These do not differ with the time of mating or fertilization or early embryo cleavage rate. It is likely then that there is a very narrow window of time in whh the uterus is receptive for implantation. Implantation is estimated to occur at Day 22 - 23 after the LH surge [6]. In some instances of a very late mating, there are anecdotal reports that, because of the resulting small litter size, the fetal signal for parturition is weak, and parturition may be delayed for 1 - 2 days, with an apparent increase in gestation length. However, documented evidence for this has not been published.
It is clinically useful to consider that gestation length in bitches is in most cases 64 - 66 days, when measured as the interval from LH surge to parturition. However, it is important to realize that intervals of 63 and 67 days have been seen in some normal, uncomplicated pregnancies and should not be considered out of the ordinary. Furthermore, there can be error of up to 1 days in estimating the day of the LH surge. Nevertheless, estimating the day of whelping as 65 days after the estimated day of the LH surge can be helpful to dog owners and aid in scheduling whelping management services. Timing the major event of pregnancy from the estimated day of the LH surge can also aid in pregnancy testing and pregnancy management services (Tables 1 and 2).

Table 1. Events and clinical correlates of canine pregnancy through the time of implantation and pregnancy detection, aligned to days from pre ovulatory LH surge.

Days	Events and changes in parameters
-25 to -3	Onset of proestrus (heat) - average Day -9
-3 to + 6	Onset of estrus behavior - average Day 0 to 1
-3 to +8	First acceptance of intromission and mating - average Day 1
-3	First day a single mating has significant fertility
0	Pre ovulatory LH surge - time of major increase in serum LH
0	Increase in progesterone from levels of 0.3 - 08 ng to levels of 0.9 to 3.0 ng/ml
0	Onset of peak fertility for single matings by high-fertility studs
2	Ovulation at 38 - 58 h after LH surge
3	Primary oocyte(s) in oviduct. Potential penetration by sperm
4	Oocytes presumably still without polar body or female pronucleus
5	Maturation of oocytes in distal oviduct.. Fertilization completed if already bred
6	Bred: 1-2 cell embryo. Non-bred: mature oocytes still fertile
7	Bred: 2 cell embryo. Non-bred: viability of some oocytes declines or lost
8	Bred: 4 cell embryo. Non-bred: late mating results in small or no litter
9	Bred early: 4-8 cell embryo. Bred later: 4-8 cell embryo. Mating rarely fertile
10	Oviductal embryos: 8-16 cells
11	Oviductal embryos: 16 -32 cell morulae
12	Morulae inside zona pellucida found in uterine horns
13	Intra-uterine migration of blastocysts between horns
14	Migration within uterus continues
15	Ultrasound (U/S) does not detect any difference due to pregnancy
16	Enlargement of embryos and thinning of zona pellucida
17	Blastocyst enlargement continues. Migration stops
18	Zona enclosed blastocyst in > 1 mm diameter uterine vesicle. U/S detectable
19	Uterine vesicle visible on U/S. Embryo + zona pellucida. Mucoid coat
20	Embryo expansion in >2 mm x 3-6 mm uterine vesicle. Zona absent. Thin coat
21	Blastocysts touch, but are still unattached to, endometrium. Cannot be flushed
22	Uterine swellings grossly visible by d 21-23. Embryo attached. Invasion begins
23	Placental trophectoderm invasion of endometrium continues. U/S detects embryo.
24	Heart beats may be visible on U/S. Palpable 1 cm uterine swellings
25	U/S detection of heart beat
26	Rises in serum relaxin and acute phase proteins (fibrinogen) in some bitches
28	U/S detects zonary placental mass. Relaxin typically detectable

Table 2. Events and clinical correlates of canine pregnancy from implantation to parturition, aligned to days from pre ovulatory LH surge.

Days	Events and changes in parameters
22	Uterine swellings grossly visible by d 21-23. Embryo attached. Invasion begins
23	Placental trophectoderm invasion of endometrium continues. U/S detects embryo
24	Heart beats may be visible on U/S. Palpable 1 cm uterine swellings
25	U/S detection of heart beat with high-resolution equipment
26	Rises in serum relaxin and acute phase proteins (fibrinogen) in some bitches
28	U/S detects zonary placental mass. Relaxin typically detectable. Heart beats clear
30	Palpable, distinct 3 cm uterine swellings. Easy palpation. Prolactin increases
32	Increased prolactin levels detectable
34	Maternal anemia typically evident
36	Palpation yields less-distinct uterine masses. U/S detection of fetal limb buds
38	Embryo still shorter than placental girdle
42	Embryo starts to become longer than placental girdle
46	X-ray first detects skull and spine. Obvious increase in mammary development
50	Acute phase protein levels near peak
54	X-ray may detect limbs and pelvis
56	Teeth still not visible on X-ray
58	X-ray readily detects limbs and pelvis; possibly teeth
60	X-ray readily detects teeth by now or next day. Progesterone above 3 ng/ml
62	Progesterone begins to decline. Nesting, restlessness begins over next 2-4 days
63	Early parturition / short gestation, but not abnormal
64	Early parturition / normal gestation. Progesterone below 2 ng/ml 12-24 h pre-partum
65	Mean parturition date. Predicted whelping date
66	Late parturition / normal range
67	Very late parturition, but not abnormal absent signs of dystocia
68	Over-due if normal signs of nesting and whelping are absent

Major Events of Pregnancy

Some of the major events of pregnancy in the dog include the following, based on previous reviews and reports [1-8]. Entry of embryo into uterus around Day 11; implantation around Day 22 - 23; secretion of relaxin by the placenta by Day 24 - 28 and through term; increased secretion of prolactin by Day 30 and through term and lactation; a physiological normocytic anemia evident by Day 30 or 35, and maximal (with PCV reduced to 30 - 40%) at term; slightly increased secretion of progesterone from Day 30 through term, probably due to the increase in prolactin secretion (since prolactin is luteotrophic); a simultaneous increase in metabolism and fecal excretion of progesterone such that serum progesterone concentrations do not rise much higher than in nonpregnant bitches; an acute pre-partum rise in prostaglandins to luteolytic concentrations and a resulting rapid decline in progesterone concentrations during the 24 h pre-partum; a corresponding pre-partum behavior of nesting, digging, social withdrawal, defensiveness, and, also a corresponding drop in

rectal temperature of 1^{o}C ; pre-partum and peri-partum discharge of normal green lochia; delivery (whelping) of pup(s) with an average litter taking 4 to 24 h.

Timing Events of Pregnancy

The time-course of events of canine pregnancy that have been carefully studied all appear to be relatively consistent among bitches and predictable when timed correctly. Timing can be accomplished based on the following, listed in the presumed order of reliability:

(1) the day of the ovulatory LH surge determined by serum LH assay;
(2) the day of the LH surge as estimated by the detection of the concomitant rise in serum progesterone by radioimmunoassay or sensitive ELISA;
(3) the day of ovulation as estimated by ultrasound;
(4) the day of LH surge and/or day of ovulation based on commercial ELISA progesterone assay;
(5) day of LH surge based on commercial urinary or serum LH assay;
(6) day of LH surge and/or ovulation based on the end-of-estrus (metestrus or diestrus) change in vaginal cytology;
(7) day of ovulation based on changes in the vaginoscopic appearance of the vaginal mucosa; or,
(8) day of ovulation based on the timing of the pre-ovulatory softening of the vulva and perineum.

Time Course of Gestational Events and Clinical Landmarks

When the day of the preovulatory LH surge has been determined directly or based on progesterone radioimmunoassay of the initial increase in progesterone in samples collected daily or more frequently, the sequence of events that has been observed (or estimated) has typically been consistent across studies, and are reviewed in tables 1 and 2. However there have been some variation and minor differences noted among studies [typically 1 - 2 days] when studies have been done using a pre-defined, absolute concentration of progesterone to estimate the time of ovulation. The day of parturition be predicted to be 65 + 1 days after the estimated day of the preovulatory surge in LH with a reasonable degree of accuracy is effort has been placed on accurate estimation of the day of the LH surge. The timing of other events are also predictable (Tables 1 and 2).

Impending Parturition and Elective Caesarian Sections

Pre-planned or elective c-sections can probably be performed safely after Day 63 after the LH surge. However, there are no clinical research reports to this effect and special concern for support of the pups is important. In other than brachycephalic breeds, waiting until Day 65 or 66 may result in spontaneous delivery and obviate the need for c-section. In brachycephalic breeds initiation of surgery before natural labor may be important, although there are no published data on the incidence of problems during natural delivery in these breeds. Elective and emergency c-section is extremely safe as performed in the United States and Canada [5]. The pup mortality was the same as, or possibly less than, that observed with natural delivery, and bitch mortality was 1%. The possible value of pre-surgical administration of dexamethasone has not been reported, but there is anecdotal evidence of its successful use in some practices. The intention is apparently to mimic the natural rise in corticosteroid that likely occurs at normal parturition. There is also anecdotal evidence that incidence of prematurity, irregularities of fetal heart rates, and neonatal deaths can be reduced by first confirming that the bitch is at term by assay of serum progesterone, but no reports have been published. It is reasonable to suggest that at the time of surgery progesterone should be low, and preferably near or below 2 ng/ml, i.e. at levels expected within 24 h before natural labor. The same ELISA progesterone kits used to monitor ovulation can be used in this regard.

Serum progesterone is at peak concentration between Days 15 and 30, and may reach peaks as high as 80 ng/ml (240 nmol/L) or as low as 15 ng/ml (45 nmol/L). In late gestation, Day 50 - 60, progesterone can be as high as 15 ng/ml (45 nmol/l) or as low as 3 ng/ml (9 nmol/L). Progesterone typically declines from 4 - 5 ng ml (12 - 15 nmol/L) to near or below 2 ng/ml (6 - 7 nmol/L) during the 24 h before the onset of labor. There is a concurrent pre-partum decline in body temperature which is most readily observed with twice daily or more frequent rectal temperature measurements started several days before parturition. Many practitioners routinely have dog owners measure and record rectal temperatures 2 or 3 times a day starting 1 week before predicted date of whelping.

Managing and Timing Pregnancies When Day of Ovulation is Unknown

The stage of pregnancy can be estimated based on several parameters. In recently bred bitches a vaginal smear can determine if the transition from estrus to metestrus(diestrus) has occurred. This transition or shift in the composition of the smear typically occurs 7 - 9 days after the LH surge, and thus about 57 days pre-partum. It is not entirely accurate in that the metestrus or diestrus shift in the smear can occur as early a Day 6 and as late as Day 11 (Concannon and Shille, unpublished observation). In early pregnancy, the size of uterine enlargements palpable per abdomen can be helpful, being typically 1 cm at Day 22 - 24, and 3 cm at Day 32, post LH surge. Ultrasound studies at know times relative to the LH surge have described several sonographic landmarks of fetal development that can be used to estimate the stage of gestation when the day of the LH surge is not known or accurately estimated. [7-10] Using ultrasound, the time of early detection of heart beat depends on instrumentation, experience and preparation of the abdomen. However they are never delectable before Day 23 and are likely to be delectable with any instrument by Day 28 - 30. The fetal length in relation to the length of the placental girdle can be determined by ultrasound and results related to Day 40 - 42, when the fetus crown-rump length becomes longer than the placental girdle. Details of other ultrasound criteria have been reported [7-10], including first detection of the fetal limb buds at Day 33 - 35; eyes, kidney and liver at Day 39 - 47; and intestine at Day 57 - 63. With radiography, the fetal skull is rarely visible before Day 45 and is almost always visible by Day 47 - 49; pelvic bones are not visible before Day 53 and are usually easily seen by Day 57; fetal teeth, not before Day 58 and usually by Day 63 [3].

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