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Parturition prediction and timing of canine pregnancy

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Abstract

An accurate method of predicting the date of parturition in the bitch is clinically useful to minimize or prevent reproductive losses by timely intervention. Similarly, an accurate method of timing canine ovulation and gestation is critical for development of assisted reproductive technologies, e.g. estrous synchronization and embryo transfer. This review discusses present methods for accurately timing canine gestational age and outlines their use in clinical management of high-risk pregnancies and embryo transfer research.

Keywords

Parturition; LH peak; Progesterone; Ultrasonography; Embryo transfer

1. Introduction

Accurate prediction of the date of parturition in the bitch is clinically useful to prevent or minimize reproductive losses by timely intervention. For example, an accurate method of predicting parturition date is necessary to plan an elective Cesarean section. Intervening when the pregnancy is full term can reduce losses of offspring from bitches having obstructions of the pelvis or vagina, histories of primary or secondary uterine inertia, or prolonged parturition with resultant puppy mortality. For bitches with histories of pyometra, abortion, embryonic reabsorption, or insufficient luteal phase, accurate assessment of gestational age can assist in therapeutic decision making. Finally, progress in assisted reproductive techniques in this species, such as estrous synchronization and embryo transfer, requires accurate prediction of ovulation, gestational age, and parturition date.

In other species, estrus onset or insemination dates are used to predict parturition dates. However, in the domestic dog, these are not sufficiently accurate. Several studies have shown that there is little correlation between the onset of estrus and the time of ovulation [1-5]. Thus the onset of estrus, even as detected by cytology, is not an accurate predictor of ovulation or parturition dates. Fortunately, two breeding management methods that are commonly used in clinical practice, determining the timing of ovulation by serial measurements of serum progesterone concentrations and transabdominal ultrasonography for pregnancy diagnosis, can be adjusted to accurately predict the date of parturition [6,7]. This review discusses present methods of parturition prediction and outlines their use in managing high-risk pregnancies and in embryo transfer research.

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2. Overview of current parturition prediction methods

The duration of canine gestation, as timed from the preovulatory serum LH peak, is 65 ± 1 d [8]. However full-term gestation, calculated from insemination, is reported to range from 57 to 72 d [8]. The difference between these measurements was attributed to the potential 6-d viability of sperm in the female reproductive tract and the long period of receptivity in the bitch. Gestation length as measured from the first day of diestrus based on vaginal cytology [3], has a large range (51 to 60 d), although 80% of bitches in that study gave birth on the 57th day of diestrus (Table 1). Based on these and other studies, the key to timing the duration of canine gestation was neither insemination date nor estrus onset, but rather the preovulatory LH surge and concomitant increase in serum progesterone concentrations [9]. As reported previously [6,7], both measurement of preovulatory serum progesterone concentrations (progesterone method), or fetal measurements made with transabdominal ultrasonography (ultrasound method), provided a more accurate estimate (65 ± 2 d, prediction accuracy 90 and 87%, respectively) than other methods. Based on these studies, we recommend the use of serial *preovulatory* serum progesterone measurements to estimate the day of the LH peak (Day 0), followed by transabdominal ultrasonography for confirmation. If preovulatory progesterone measurements were not available, the ultrasound method provided an accurate estimate of parturition date if the examination was performed by day 39 and the gestational age was corrected for the nonpregnant body weight of the dam. However, the most accurate prediction was obtained when the ultrasound examination was conducted at day 30 [7].

The preovulatory progesterone measurement method was based upon the finding (Fig. 1) that the peak in serum LH (i.e. day 0) is followed by ovulation in approximately 2 d (i.e. day 2 [8]). In normal canine cycles, there is an abrupt rise in serum progesterone concentrations *coincident* with this preovulatory LH peak. Because serum progesterone concentrations are cheaper and easier to measure than serum LH concentrations, the coincident rise in progesterone is routinely used to estimate day 0 and to plan insemination.

3. Prediction of parturition date by measuring preovulatory serum progesterone concentrations

A retrospective study evaluated pregnancies of 63 bitches (19 breeds) that delivered without assistance [6]. Blood samples were collected every other day, beginning 1 to 2 d from proestrus onset, and progesterone concentrations were measured quantitatively with either a chemiluminescent immunoassay (CLIA), or radioimmunoassay (RIA). In that study, day 0 was defined as the first day that serum progesterone was ≥ 1.5 ng/mL and was followed by a progesterone concentration ≥ 3.0 ng/mL on the next day sampled. Parturition date was estimated as 65 d from day 0 and compared to the actual parturition date, defined as the day that the first pup was spontaneously delivered. For analysis, cases were grouped according to nonpregnant body weight of the bitch and litter size (total number of pups born).

Mean gestation length (and parturition date prediction) were not significantly affected by body weight of the bitch nor by litter size. The overall accuracy of predicting parturition date was 67% for the day 65 ± 1 d prediction, 90% for the day 65 ± 2 d prediction, and 100% for the day 65 ± 3 d prediction. Importantly, there were significant differences among bitches in the progesterone rise after day 1 (Table 2). Specifically, the rise after day 1 in small (<9 kg) and large bitches (>20 kg to <40 kg) was significantly different from those of medium (9 to 20 kg) and giant (>40 kg) bitches (Fig. 2). Thus, measurement of *preovulatory* serum progesterone concentrations to detect the initial progesterone rise were essential for accurate estimate of parturition date. Furthermore, retrospective estimates of day 0 or the time of ovulation (day 2), based upon serum progesterone concentrations collected after day 1, were likely to be inaccurate.

4. Prediction of parturition date by fetal measurements (transabdominal ultrasonography)

A related retrospective study evaluated 83 pregnant bitches (32 breeds, 107 examinations) between 20 and 62 d of gestation that later delivered without assistance [7]. Some of these bitches were the same as those in the progesterone study above; cases were also grouped according to nonpregnant body weight of the bitch and litter size. To estimate gestational age on the day of ultrasound examination, fetal characteristics were measured by one ultrasonographer (either during the examination or from tapes made during the examinations). Parturition was predicted at day 65 and compared to the actual parturition date (as defined above).

An accurate prediction of parturition date was obtained when ultrasonography was performed at $\leq$ day 39 of gestation, with the most accurate prediction when the examination was done at day 30. To accurately estimate gestational age, at least two measurements (e.g. fetal crown rump length and body diameter) were needed on ≥ 2 fetuses. However, prediction was inaccurate when made from fetal measurements in late gestation ($>$ day 39).

Similar to the use of progesterone concentrations, the accuracy of parturition date prediction was not significantly affected by litter size. However, prediction accuracy was significantly affected by nonpregnant body weight of the dam. Specifically, a correction factor was required for small bitches (<9 kg) and giant bitches (>40 kg). In previous studies, fetal growth was linear from days 17 to 30 and subsequently became exponential [10]. The studies of Kutzler et al [6,7] confirmed that there was no difference in gestation length in bitches of different body weight, despite large differences in fetal growth rates in late gestation that were correlated to maternal body weight. Thus after day 30, fetuses of small bitches (<9 kg) grew slower, and fetuses of giant bitches (>40 kg) grew faster, than those of medium or large bitches. When corrected for body weight of the dam, the overall accuracy for parturition date prediction by the ultrasound method was 75% for the Day 65 $\pm$ 1 d prediction, 87% for the Day 65 $\pm$ 2 d prediction, and 100% for the Day 65 $\pm$ 3 d prediction.

5. Recommendations for using the progesterone and ultrasound methods together

There was a strong correlation between the progesterone and ultrasound methods of parturition prediction [6,7], and it is convenient to use them together. To obtain the most information from these methods, we recommend the following:

- *Quantitative* measurement of serum progesterone concentrations (CLIA or RIA), validated for canine samples, is necessary. If CLIA is used, serum separator or clot activator tubes should *not* be used, as they affect accuracy. Blood should be collected in a plain clot tube, refrigerated, and serum separated within 12 h.
- Beginning in early *proestrus*, serum progesterone is measured at least every other day until day 0, i.e. the day that the initial rise in progesterone is identified (usually requires sampling for 7 to 10 d). Vaginal cytology is also obtained on days that progesterone was sampled (Table 2).
- The increase in serum progesterone is confirmed by collecting blood samples every other day until progesterone is ≥ 5 ng/mL.
- Timing from day 0, the bitch is inseminated with fresh semen between days 3 and 6.
- Between days 28 and 30 (timed from the preovulatory progesterone measurements), transabdominal ultrasonography is performed, with fetal crown rump length and body

diameter measured on ≥ 2 fetuses and gestational age estimated [11,12]. The gestational age estimate on the day of the ultrasound examination is corrected according to nonpregnant body weight of the dam: add 1 d for bitches < 9 kg and subtract 2 d for bitches > 40 kg (no correction factor required for dams between 9 and 40 kg). Furthermore, evaluate litter size, embryo viability, and reabsorption. In nonpregnant bitches, measure serum progesterone concentrations to rule out luteal insufficiency.

- Parturition date is estimated as day 65 ± 2 d (90% accuracy by progesterone, 87% accuracy by ultrasound) or day 65 ± 3 d (100% accuracy, either method).
- In the last week of pregnancy, monitor the bitch's rectal temperature once or twice daily, examine the vulva for discharge, and determine whether milk can be expressed from the mammary glands. Record whether the bitch is eating, nesting, or showing signs of restlessness [13].
- As the date of parturition approaches, the following are indications for fetal monitoring with transabdominal ultrasonography:
 - Failure to initiate parturition as expected
 - Unusual vaginal discharge
 - Vague signs of illness
 - Delay in parturition after delivery of part of a litter

6. Use of serum progesterone concentrations to estimate day 0 and parturition prediction in clinical practice and research

For at-risk pregnancies in a clinical setting, one author (VMW) has used parturition prediction to schedule the staff and the client for monitoring pregnancy in the bitch and to prepare for the likelihood of intervention at day 65 ± 2 d. In addition to monitoring the rectal temperature and other signs of impending parturition (anorexia, nesting, lactation), serum progesterone concentrations may also be monitored [13]. Although none of these characteristics is very accurate by itself in predicting parturition, monitoring this combination is often helpful. Monitoring by transabdominal ultrasonography is useful as day 65 approaches; it can confirm fetal viability and has occasionally detected gross fetal anatomical abnormalities that would interfere with parturition or fetal viability. At term, normal fetal heart rates (determined with ultrasonography) were approximately 200 beats/min. Although the critical rate indicative of fetal compromise has yet to be determined, in cases under one author's (VMW) care, Cesarean sections have been performed as soon as possible when consistently slow fetal heart rates (< 100 to 150) were present. These pups were viable at delivery, although some were meconium stained [13]. Frequent ultrasound examinations may be necessary for early detection of fetal compromise; however, it is unclear how frequently they should be performed to ensure timely intervention. In clinical cases of elective Cesarean section (author VMW) and in our research, these were performed no earlier than day 65. It is not known whether pups delivered prior to day 65 would be viable.

In our canine embryo transfer research, we are currently using the progesterone method to time insemination, gestation and embryo collection, predict parturition date, and schedule elective Cesarean section (day 65). Results presented here are part of this work in progress (Kim, Meyers-Wallen, and Travis, unpublished). Others have also performed canine embryo transfer using various methods [14-18]. All donor and recipient bitches in our studies to date were laboratory beagles and the sires were Labrador retrievers or Labrador mixed-breed dogs. First, daily monitoring of serum progesterone concentrations identified bitches in synchronized

estrus (based upon day 0). Second, it allowed timing of insemination and subsequently embryo removal on the appropriate days. Third, in our studies, the parturition prediction from progesterone measurements was confirmed by measuring fetuses during the ultrasound examination (scheduled at days 28 to 30). Finally, having identified when the pregnancy will be full term, elective Cesarean section was performed on day 65. To date, we have delivered 5 embryo transfer pups from 4 recipients using these methods. Examples of progesterone profiles from two donor-recipient pairs are shown in Fig. 3 (Kim, Meyers-Wallen, and Travis, unpublished). Bitches in which day 0 occurred ≤ 1 d apart were paired as donor and recipient. The donors were inseminated, the recipients were not. Serum progesterone concentrations in bitches synchronized by mibolerone withdrawal were similar to those of untreated bitches housed with the treatment group (Fig. 4). Estrus in other bitches was induced with deslorelin implants.

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Fig. 1.

Changes in serum concentrations of estrogen (E), progesterone (P) and LH, and changes in vaginal cytology during proestrus, estrus, and early diestrus in the bitch. Serum progesterone concentration increased on the day that serum LH peaked (Day 0). Ovulation occurred on Day 2, the ovulated primary oocytes mature, and breeding is recommended between Days 3 and 6. Regarding vaginal cytology, noncornified epithelial cells (P = parabasal, I = intermediate) decreased and cornified epithelial cells (S = superficial, A = anuclear squames) increased during proestrus. Cytologic estrus was defined by $\geq 90\%$ cornified epithelial cells in the vaginal smear. The first day of diestrus (Day 1) was determined by vaginal cytology as the first day that cornified cells decreased to $< 50\%$ of the epithelial cells in the vaginal smear. (Adapted from Concannon PW et al: The ovarian cycle of the bitch: Plasma estrogen, LH and progesterone. Biol Reprod 13:118, 1975, with permission).

Fig. 2.

Serum progesterone concentrations in bitches, categorized according to their body weight. The initial rise in serum progesterone concentrations (day 0) was defined as the first day that progesterone was ≥ 1.5 ng/mL. Mean progesterone concentrations were not significantly different among groups before day 1; however after day 1, there was a significant difference in the slope of the progesterone rise. (Modified from Kutzler et al *Theriogenology* 2003;60:1187-1196, with permission).

Fig. 3.

Preovulatory serum progesterone concentrations in two donor and recipient pairs in which live pups resulted from embryo transfer. Donors and recipients were paired when day 0 (progesterone concentrations 1.5 to 2.5 ng/mL; indicated by * on sample collection day), was synchronized within 1 d. Estrus cycles in Pair 1 were synchronized after mibolerone withdrawal, whereas deslorelin implants were used to induce estrus in Pair 6.

Fig. 4. Serum progesterone (P4) concentrations during proestrus and estrus, normalized to the estimated day 0, for bitches in an embryo transfer study. Bitches synchronized by mibolerone withdrawal (M1-M4) and untreated controls (C1-3) were housed together. Sampling was initially conducted every other day, then daily after serum progesterone concentrations rose to ≥ 0.4 ng/mL. Progesterone concentrations were similar in treated and control bitches up to day 1; however, they diverged after day 1, as previously documented in normal bitches (Fig. 2).

Table 1

Accuracy of predicting canine parturition using various methods. The first day of diestrus in vaginal cytology (Day 1) was reported in Holst and Phemister (Am J Vet Res 1974;35:401-406). Studies by Kutzler et al (Theriogenology 2003;60:1187-1196 and 1309-1317) described the methods to estimate day 0 from preovulatory serum progesterone (P4) measurements and transabdominal ultrasonography, respectively.

From	Parturition date	Accuracy	Requirements
Mating date	57-72 d		
Vaginal cytology (Day 1)	Days 51-60		Sampling to Day 1 (postbreeding)
Transabdominal ultrasonogram	day 65+/- 2 d	87%	Fetal dimensions (corrected for BW)
Estimate day 0 from preov P4	day 65+/- 2 d	90%	Quantitative P4 assay

Preovulatory serum progesterone concentrations in bitches, classified by nonpregnant maternal body weight. At day 0, the mean progesterone concentration for all groups was 2.02 ±0.18 ng/mL (in box). Note that the mean ±/− 3 SD at day 0 is 1.48–2.56 ng/mL. There was little variation in progesterone concentrations among groups before day 1, but significant variation in the progesterone rise after day 1, as the SD (in box) is ten times larger after day 1. (From Kutzler et al Theriogenology 2003;60:1187–1196, with permission).

Table 2

Maternal body weight (kg)	Mean serum progesterone concentrations on days from LH Peak										No. cycles	No. bitches
	−3	−2	−1	0	1	2	3					
<9	0.92	0.78	1.46	2.14	3.46	11.86	15.13				10	8
9 to 20	0.85	0.84	1.26	1.79	3.1	4.1	5				7	6
20 to 40	0.53	0.68	1.01	2.18	3.38	5.18	8.27				52	40
>40	0.57	0.61	0.96	1.97	3.99	4.62	6.98				14	10
All groups	0.72	0.73	1.17	2.02	3.48	6.44	8.84				83	64
SD												