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ScienceDirect
Theriogenology 70 (2008) 397–402
Theriogenology
www.theriojournal.com

Estimation of gestational age and assessment of canine fetal maturation using radiology and ultrasonography: A review

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Abstract

Since the duration of pregnancy in the bitch is relatively short, it is critical that fetuses are fully mature prior to delivery for them to survive. For breeders to be able to prepare for normal whelpings and align medical care in case of emergency, an estimated due date is necessary. In cases where ovulation timing is lacking and there is a singleton fetus or oversize fetuses, it is necessary to ascertain gestational age prior to setting the date of Cesarean section. In high-risk pregnancies, where there is poor or no ovulation timing, determination of fetal maturation and gestational age will assist in determining if pregnancy has progressed long enough to allow delivery of viable puppies. In cases where bitches are receiving supplemental progesterone for pregnancy maintenance medications must be discontinued at an appropriate time to permit delivery of viable puppies. It also allows for estimation of the likelihood of fetal survival if the pregnancy is terminated due to failing bitch health, with subsequent surgical delivery of the fetuses. Use of breeding dates alone does not provide due dates with adequate accuracy. In cases where there has been inadequate or no breeding management or ovulation timing, estimation of due date can be performed at the time of pregnancy diagnosis, or closer to term. Radiography can be used to confirm pregnancy and facilitate determination of gestational age, beginning 45 d after the LH surge. Ultrasonography can be used from 19 to 21 d after the LH surge to term to confirm pregnancy and predict gestational age, and from 25 or 26 d to term to assess fetal viability and fetal stress.

Keywords: Canine; Gestational aging; Fetal maturation; Ultrasonography; Radiology

1. Introduction

The duration of pregnancy in the bitch is relatively short, compared to other domestic species, lasting only 63 d from ovulation. Therefore, fetuses are born in an immature state and final development of most organ systems is completed in the weeks to months after birth. Consequently, considerable development of major fetal organ systems occurs during the last days of gestation, in preparation for extra-uterine survival. Failure of the fetuses to complete maturation will result in their failure to survive postpartum. Additionally, due to the nature of the canine placenta, once a fetus exceeds its due date by more than 2 d, it will demand more nutritional support than the placenta is able to provide, resulting in intrauterine fetal death. Therefore, it is critical to ensure that each fetus has attained, but not exceeded, its maximal gestational age prior to delivery.

There are several situations when assessment of gestational age and fetal maturation is necessary; most arise when there is either inadequate or no ovulation timing to allow determination of an accurate due date.

- Bitches that will be allowed to deliver naturally, but may require veterinary care during parturition. This

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0093-691X/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.theriogenology.2008.05.034

allows the breeder and veterinarian to be prepared and available for the onset of labor.

- Bitches where an elective C-section is desired, i.e. singleton fetuses, giant breed bitches with small litters and consequently very large puppies, bitches with very large litters where uterine inertia is a concern due to anticipated extended duration of labor, or bitches with a history of prior dystocia or primary uterine inertia. In singleton pregnancies, there may be inadequate cortisol release from the fetus to initiate prostaglandin $F_{2\alpha}$ production by the endometrium which causes luteolysis, which in turn initiates parturition.
- Bitches with high-risk pregnancies, including gestational diabetes mellitus or pregnancy toxemia, or in bitches requiring progesterone supplementation due to luteal failure as a result of chronic endometritis, stress, partial abortion, or idiopathic luteal insufficiency. In cases of high-risk pregnancy, the bitch is often supported as far into the pregnancy as possible, in an attempt to get fetuses to term. In some cases, it may not be possible to continue, due to failing health status of the bitch; in these cases, the fetuses may not survive if they must be delivered pre-term. In other cases of high-risk pregnancy due to chronic inflammation, concurrent pyometra, or other stressor, the bitch may be given supplemental progesterone to maintain the pregnancy until term if progesterone concentrations decline prematurely.
- Bitches that enter pre-term labor due to abnormalities or defects in the myometrium, that are due to nutritional, environmental, traumatic or inflammatory causes, or in cases where labor is interrupted through the use of tocolytic agents (terbutaline and/or progesterone).

In each of the preceding situations, there is typically no forewarning of a problem until after the bitch has been bred and confirmed pregnant. If there is no or inadequate ovulation timing, the need to determine gestational age and maturation becomes critical to a positive outcome.

Gestational length is most accurately determined by using either the LH surge or ovulation. Parturition occurs 65 ± 2 d after the LH surge, and ovulation occurs 2 d after the LH surge [1–4]. Due to the extreme variability of the bitch's estrous cycle length and receptive behavior, and the length of time sperm may survive in the bitch's reproductive tract, the use of breeding dates is not an accurate method of estimating gestational age [1,2,4]. In that regard, parturition may occur from 58 to 71 d after breeding [1,2,4,5].

This paper reviews the use of radiography and ultrasonography in the determination of gestational age and assessment of fetal maturation in the bitch. All estimations and calculations of gestational age and fetal maturation in this paper are expressed in days after the LH surge, unless otherwise noted.

2. Methods to assess fetal maturation

2.1. Radiographs

2.1.1. Factors affecting radiographic quality

Proper radiographic technique and adequate patient restraint are critical for accurate assessment [5,6]. A single lateral radiograph of the abdomen is usually sufficient to allow for pregnancy diagnosis and fetal counting. A ventrodorsal radiograph may provide additional information if gestational aging is needed, or to assess the size of the pelvic canal in relation to fetal head size [5,6]. Sometimes high fetal numbers prevent accurate visualization of the entire litter. Localization of multiple fetuses overlying each other may make it difficult to evaluate all skeletal components of each individual fetus [5,6]. Abdominal contents (food and gas) may also obscure visualization of the fetus(es) [5,6]. Varying degrees of fetal mineralization are present, depending on the day of pregnancy, which may make certain skeletal structures difficult to observe [5,6].

2.1.2. Gestational aging

Structures and when they are visible radiographically is shown (Table 1). Radiographs can help provide a rough estimate of gestational age, but are not adequate alone to determine fetal readiness for birth, because there is some overlap of radiographic detail between

Table 1
Structures that can be used to determine the stage of canine pregnancy and when they are visible radiographically [1,2,4–6]

Feature	Detection (d) after LH surge	
	Mean	Range
Spherical uterine swellings	35	31–38
Ovoid uterine swellings	41	38–44
First evidence of mineralization of the fetal skull	45	43–46
Scapula, humerus, and femur	48	46–51
Radius, ulna, and tibia	52	50–53
Pelvis and all ribs	54	53–59
Coccygeal vertebrae, fibula, calcaneus, and distal extremities	61	55–64
Teeth	61	58–63

bitches and amongst breeds [1,2,4–6]. Additionally, the fetus may be completely mineralized as early as 58 d after the LH surge, and at this stage they would not survive ex-utero [1,2,4–6].

2.1.3. Fetal death

Failure of the uterus to continue to enlarge or a rapid decrease in uterine diameter is indicative of abortion, resorption, or fetal death [5]. Fetal death can be determined if the bones of skull begin to override each other or become otherwise deformed; if there is gas accumulation within the uterus or in or around the fetus (within the blood vessels, heart, body cavities); or if there is abnormal flexion of the fetus (balling) or hyperextension of the hind limbs [5]. When gas is suspected within the uterus, it should be confirmed with a second radiographic view.

2.2. Ultrasonography

There are three major limitations of ultrasonography: (1) the quality of the machine; (2) the experience of the operator; and (3) patient factors (amount of hair, use of quality ultrasound gel, relaxation of the patient, respiratory rate and patient size). Ultrasonography can be used estimate gestational age through the use of fetal measurements and through evaluation of the progression of organ development. It may also be used to ascertain fetal viability and to detect fetal stress.

2.2.1. Gestational aging via fetal measurements

Pregnancy may be diagnosed as early as 19–21 d, at which time the conceptuses are approximately 1 cm in diameter [3,4,7,8]. Fetal heartbeats and movement may be detected as early as Day 23 [4,7,9]. Measurement to determine gestational age is more accurate when the bitch is <37 d [7,9–13]. Between Days 19 and 37, measurement of gestational sac diameter (inner chorionic cavity, ICC or outer uterine diameter, OUD) or crown-rump length (CRL) of the fetus can easily be obtained [3,7–10,12–16]. If the bitch is >37 d, fetal measurements of the biparietal diameter (BPD) and body diameter (BD) are obtained from one or more fetuses. Other rules of thumb regarding gestational aging are that body diameter exceeds head diameter by more than 2 mm between 38 and 42 d gestational age and the fetal crown-rump length first exceeds placental length between 40 and 42 d [4,7,9,10].

When measuring the gestational sacs, two transverse plane measurements should be taken at 90° angles to each other and these values averaged before using the formulas provided [11,13,15,16]. Head and body diameters are measured in the transverse plane [7,9,10,12,13,15]. When taking measurements of fetal or extra-fetal structures at least two distinct fetuses or gestational sac should be measured whenever possible and the measurements averaged before applying them to formulas [11,13,15,16]. In cases of singletons, this is of course impossible and measurement of multiple structures (i.e. ICC, OUD, CRL, BPD, or BD) may be done to increase the accuracy of any one measurement alone [13,15,16].

The deep portion of the fetal diencephalo-telencephalic vesicle (DPTV) can be visualized from Days 35 to 58, as a symmetric anechoic area viewed on sagittal midline in the fetal skull and has been used to determine gestational age [17,18]. The DPTV represents the fetal thalamus and the primordial basal nuclei [17,18]. The size of the DPTV depends on the size of the bitch and formulas for gestational aging have been determined for small- (<10 kg), medium- (11–25 kg) and large-breed (26–40 kg) bitches (Table 2) [17,18]. The accuracy of predicting parturition using DPTV measurements within 1 d of actual parturition was 40, 50, and 38% for small-, medium-, and large-breed bitches respectively, and increased to 62, 65 and 60% when the estimated due date was extended to ±2 d of actual parturition [17,18].

In early to mid-pregnancy (<37–40 d) the use of ICC was between 64 and 91% accurate (±1 d) in both small and medium breeds, and between 85 and 88% accurate in large breeds (±2 d) at predicting the day of parturition [12,13,15,16,19]. Use of the OUD was less

Table 2
Formulas for calculation of gestational age in medium- and small-breed bitches, using extra-fetal structures

ICC (medium breed)
GA = 19.66 + 6.27 × (cm) [10] or
GA = (6 × cm) + 20 [7] or
DBP = (mm − 82.13)/1.8 [15]
ICC (small breed)
DBP = (mm − 68.88)/1.53 [15]
DBP = 63.2 − (18.58 + 0.71 × mm) Maltese [16]
DBP = 63.4 − (18.92 + 0.65 × mm) Yorkshire [16]
OUD (medium breed)
GA = 17.39 + 4.98 × cm [10]
DBP = (mm − 80.78)/1.57 [15]
OUD (small breed)
DBP = (mm − 85.17)/1.83 [15]

GA: Gestational age is calculated based on days past the LH surge ±2 d; DBP: days before parturition is calculated based on a 65 ± 2 d gestation length; ICC: inner chorionic cavity; OUD: outer uterine diameter.

accurate, as it was more prone to errors in placement of the markers. Use of length of the zonary placenta and placental thickness have been proposed for measurement, but were not accurate in estimating gestational age and should not be used [13,15,16]. Use of CRL is accurate in early pregnancy, but is also prone to errors in placement of the markers, and once the fetus reaches approximately 40 d, it begins to flex substantially, rendering this measurement inaccurate [11,13,16].

In later pregnancy (>40 d), use of BPD is the most accurate measurement tool [11,13,16,19]. Biparietal diameter is more accurate than body diameter in the bitch after Day 37 [11–13,15,16]. Combining the use of BD and BPD will increase the accuracy relative to using BD alone. Care must be taken to get a mid-sagittal image and the markers placed at the parietal bones symmetrically on either side of the fetal skull. Measurement of BD should be taken at the widest portion of the fetal abdomen (level of the fetal stomach and liver). Two transverse plane measurements should be obtained at 90° angles to each other and these measurements are averaged prior to using with the formulas provided [13,16,19]. Accuracy with the use of BPD measurements within 1 d of actual parturition was 64–75% in small breeds and 65% in medium breeds; and within 2 d this increased to 85–88 and 81–86% respectively [12,13,16,19]. In cases of singletons or very small litters, BPD may be less accurate than in bitches with normal size litters [12]. Formulas for determining gestational age are shown (Tables 2 and 3).

The difference in accuracy of parturition prediction for giant or toy breeds compared with the medium breeds has been examined; it was concluded that the measurement formulas for medium-sized bitches could be used, if corrected for the extremes in size [11]. To obtain more accurate due dates, the authors recommended subtracting 2 d for giant-breed bitches and adding 1 d for small-breed bitches after gestational age was calculated [11]. After adjusting for weight, there was an accuracy of 75 and 87% for gestational aging for ±1 and ±2 d respectively for bitches evaluated by Day 30 [11]. Other formulas have since been provided for small breeds [13,15,16], but none have been proposed for giant breeds.

Some factors which may affect interpretation and accuracy of these measurements are differences in size amongst breeds (toys versus giants); litter size (singletons versus very large litters); and head shape (brachycephalic versus dolicocephalic). These factors should be taken into consideration when using fetal measurements to predict due dates. Some studies reported no effect of litter size on gestation length [11,13], whereas others reported that smaller litter size (either less than 7 puppies or less than 3 puppies) was associated with longer gestation length [12,20–23]. There may also be some breed variation with gestation length, with German Shepherd dogs [20,21] and Hound dogs [22] having shorter gestation length and West Highland White Terriers having longer gestation lengths [20,21]. In another study using Drever bitches as the model population, it was shown that once litter size exceeded the average for the breed, each additional puppy reduced gestation length by 0.25 d [23]. Conversely, when litter size was below average, 0.25 d should be added to the due date for each puppy below breed average [23].

Table 3
Formulas for calculation of gestation age in small-, medium-, and large-breed bitches, using fetal structures

CRL (medium breed)
GA = (3 × CRL) + 27 [7]
GA = 24.64 + 4.54 × cm − 0.24 × cm^2 [10]
BPD (medium breed)
GA = (15 × HD) + 20 [7]
GA = 21.08 + 14.88 × cm − 0.11 × cm^2 [10]
DBP = (mm − 29.18)/0.7 [15]
BPD (small breed)
DBP = 63.2 − (24.7 + 1.54 × mm) Maltese [16]
DBP = 63.4 − (23.89 + 1.63 × mm) Yorkshire [16]
DBP = (mm − 25.11)/0.61 [15]
BD (medium breed)
GA = (7 × BD) + 29 [7]
GA = 22.89 + 12.75 × cm − 1.17 × cm^2 [10]
BD + BPD (medium breed)
GA = (6 × BPD) + (3 × BD) + 30 [7]
DBP = 34.27 − 5.89 × BPD (cm) − 2.77 × BD (cm) [9]
DPTV (small breed)
DBP = (mm − 10.11)/0.24 [17,18]
DPTV (medium breed)
DBP = (mm – 14.15)/0.4 [17,18]
DPTV (large breed)
DBP = (mm – 10.27)/0.24 [17,18]

GA: Gestational age is calculated based on days past the LH surge ±2 d; DBP: days before parturition is calculated based on a 65 ± 2 d gestation length; CRL: crown-rump length; BPD: biparietal diameter; BD: body diameter; DPTV: deep portion of the diencephalo-telencephalic vesicle.

2.2.2. *Gestational aging via estimation of fetal maturation*

Gestational age may be estimated through the assessment of organ development [4,7,9,10,19]. Serial examination may provide more accurate information than a single examination. The embryo is first noted

within the gestational sac by Days 25 or 26 [4,7,9,10,19]; it appears to be oblong and rests adjacent to the wall of the uterus. The heartbeat is first visible at 25–26 d [4,7,9,10,19]. At Days 27–28, the embryo moves away from the endometrial wall and appears to be suspended by the fetal membranes, with the yolk sac being the larger of the two cavities [4,7,9,10,19]. The placenta can be seen as early as Days 26–27 as a distinct structure lining the uterus; it becomes zonary in appearance by Days 29–31, and the edges curl inward by Days 32–34 [4,7,9,10,19]. The embryo is located dependently in the chorionic cavity by Days 29–33, and the yolk sac takes on a tubular appearance at this time as it shrinks away [4,7,9,10,19]. The bladder is first visible between 35 and 39 d; the stomach between 36 and 39 d; the kidneys and eyes between 39 and 47 d; and the intestine between 57 and 63 d [4,7,9,10,19]. The lungs become more hyperechoic than the liver between 38 and 42 d and the liver becomes more hyperechoic than the other abdominal organs between 39 and 47 d [4,7,9,10,19].

The kidneys mature ultrasonographically as pregnancy progresses, initially being hypoechoic compared to other abdominal contents and having prominent anechoic pelves [4,7,10]. The renal cortex is differentiated from the medulla as maturation continues and the pelves are less dilated [7]. The medulla and renal pelvis become increasingly prominent until term [7]. The intestinal mucosa is first evident as a hypoechoic lumen with a hyperechoic muscular layer at ~58–60 d, with further increases in prominence and thickness until term [4,7,10]. Peristalsis becomes evident between 62 and 64 d. Panting may make visualization of peristalsis more difficult.

The combined use of these developmental changes along with fetal and extra-fetal measurements provides more accurate estimation about gestational aging than the use of either modality alone [10,19].

2.2.3. Assessment of fetal stress

Ultrasonography is also routinely used to assess fetal stress. Fetal heart rate is an excellent indicator of fetal stress. Normally, fetal heart rates are 2–3 times that of the bitch (220–240 bpm) [7,24–26]. In one study, heart rates between 180 and 220 bpm were considered indicative of slight fetal stress, whereas rates consistently <180 bpm were considered indicative of severe fetal distress due to hypoxia [26]. In other studies, fetal heart rates consistently <140–160 bpm were considered indicative of sustained fetal stress due to hypoxia [7,24,25]. Intermittent uterine contractions over a fetus can cause temporary, substantial reductions in fetal heart rate, but it should return to a normal rate within 1–2 min and remain within the normal range if there is no fetal stress.

Fetal stress can also be assessed by examining the fetal fluids and feto-placental units. Increases in the echodensity of the fetal fluids may indicate passage of meconium [26] or hemorrhage into the fetal fluids due to premature placental separation. Intrauterine growth retardation may be documented if abdomimal:biparietal diameter ratios are <2 from Day 48 to birth [26]. Puppies with low abdominal:biparietal diameter ratios tend to weigh <20% of the average birth weight for the breed and are at risk for early neonatal loss [26].

An increase or decrease in the volume of fluid surrounding a fetus may indicate rupture of one or both fetal membranes, abnormalities of placental function or abnormalities of fetal swallowing and waste fluid disposition. It is important to remember that as the gestation progresses, the amount of fluid surrounding the fetus decreases as the fetus itself enlarges. Detachment of the placenta may be partial or complete, and fetuses generally do not survive for long after the placenta begins to detach. Edema or thickening of the placenta may indicate abnormalities or alterations of blood flow, diminished ability of the placenta to drain fetal waste fluids properly, or placentitis. In the author's experience, the normal canine placenta does not exceed 1.2 cm at its centermost point at any stage of pregnancy, regardless of breed.

3. Conclusions

There is no substitute for accurate ovulation timing at the onset of the estrous cycle. Determination of the time of the LH surge (81% accuracy ±1 d), day of ovulation, or Day 1 of diestrus, remain the most accurate methods to determine fetal readiness for birth. While we do have means to assess gestational age and fetal viability beyond ovulation timing, none of the methods currently available are completely accurate for determining fetal readiness for birth, or the bitch's actual due date. If the day of the LH surge is not known, the next most accurate means of predicting parturition date is measurement of extra-fetal structures (ICC) by ultrasound [19]. Addition of measurement of fetal structures (BPD, BD, DPTV) and assessment of organ development by ultrasonography used in combination with ICC measurement, coupled with the use of radiography, can help increase the accuracy of due date estimation by increasing the available database of information.

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