J Vet Clin 25(6) : 501-505 (2008)

Journal of
Veterinary Clinics
www.ksvc.or.kr

Estimation of Gestational Age by Measurement of Fetal and Extra-fetal Structures in Miniature Schnauzer Bitches

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(Accepted: July 11, 2008)

Abstract : Serial ultrasonographic examinations were performed on 9 Miniature Schnauzer bitches from day 15 of gestation until parturition to determine the ultrasonographic measurement of gestational structures. Ovulation was designated the day that plasma progesterone concentration exceeded 4.0 ng/ml (day 0). Extra-fetal structures were measured from day 17 or 18 to 60. Outer uterine diameter (OUD) increased from 6.50 ± 1.06 mm (Mean ± SD) to 50.89 ± 5.62 mm, inner chorionic cavity diameter (ICCD) increased from 2.10 ± 0.15 mm to 37.15 ± 4.36 mm, and length of placenta (PL) increased from 7.50 ± 1.41 mm to 40.62 ± 3.27 mm. OUD and ICCD were significantly and linearly relative to gestational age especially through day 37, whereas PL was not significantly relative to gestational age. Of the extra-fetal structures, ICCD was the most accurate for estimation of gestational age before day 38 of gestation. Fetal structures increased at a linear rate to parturition. Fetal crown-rump length (CRL) increased from 2.55 ± 0.07 mm on day 22 to 85.25 ± 9.89 mm on day 48. Fetal head diameter (HD) increased from 3.43 ± 0.64 mm on day 27 to 25.06 ± 0.41 mm on day 63. Fetal body diameter (BD) increased from 5.96 ± 0.84 mm on day 30 to 43.76 ± 3.36 mm on day 63. Of the fetal structures, HD was the most accurate for estimation of gestational age after day 38 to parturition.

Key words **:** gestational age, Miniature Schnauzer bitches, gestational structures, ultrasonographic measurement.

Introduction

An essential requirement for predicting gestational age in the bitch is ovulation determination as a constant, short and easily identifiable occurrence during estrus. By defining gestational age as the time interval between ovulation and examination, ultrasonographic measurements of pregnancy features is accurate.

Numerous ultrasonographic measurements are applied in human medicine, such as cephalometry, thoracometry and abdominometry, as well as measuring the long limb bone (4). In human medicine, guidelines have been established for obstetric ultrasound examination (7). Most biometric parameters should be evaluated with caution. Errors occur and are difficult to avoid. Errors can be systemic or incidental and can occur anywhere within the triad of machine, patient and sonographer. Therefore, in human medicine, these guidelines discuss equipment, documentation, and first-, second-, and third-trimester criteria.

In veterinary medicine, false measurement in dog are caused by the usual influences, such as image quality, bladder filling, and individual growth variations but they are also caused by breed-related size and shape difference (10).

According to Yeager *et al.*(15), chorionic cavity diameter, outer uterine diameter, and placental length each increased at a linear rate through day 37 after LH surge in Beagle bitches. Of the extra-fetal structures, chorionic cavity diameter was the most accurate for estimation of gestational age. Crown-rump length, body diameter, and head diameter each increased at a linear rate. Of fetal structures, the head diameter was the most predictor of gestational age from day 38 to 60 after LH surge.

It was reported that growth curves of pregnancy structures was related to the estimation of gestational age and prediction of parturition day in each dog breed, that is, Retriever (2), Beagle(15), Korea Jin-do (14), Maltese and Yorkshire terrier (13) and Shih-tzu(5), respectively.

The present study, in Miniature Schnauzer bitches, was designed to estimate of gestational age by measurement of fetal and extra-fetal structures using ultrasonography.

Materials and Methods

Experimental animals

Nine healthy Miniature Schnauzer dogs were aged 1 to 3 years old, weighing 5.0 to 6.9 kg. They were housed in individual in indoor-outdoor runs, fed a standard commercial dog food twice daily, and water was available ad libitum. Each dog was examined twice daily for swelling of the vulva and the presence of a vaginal discharge, which considered to signify the onset of proestrus.

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They were mated, pregnant and whelped 2-6 pups each. Serial ultrasonographic examinations were performed on total 34 litters to determine gestational age by using ultrasonographic measurement of the fetal and extra-fetal structures.

The estimation of optimal mating time and ovulation time

1) Vaginal cytology

To estimate optimal mating time, serial vaginal smears were performed from the onset of proestrus to the onset of diestrus. The mating were performed when the cornification index is≥90%, as described by Kim *et al*.(6).

2) Plasma progesterone concentration measurement

Ovulation (day 0) was estimated by measuring the plasma progesterone concentration daily from the onset of proestrus to the onset of diestrus using a ^{125}I radioimmunoassay (RIA) previously validated for fertility breeding management (12).

Blood samples were collected via cephalic venipuncture placed immediately in chilled EDTA-coated tubes and centrifuged for 10 min at 3,000 g, plasma was stored at –25°C until analysis.

Plasma progesterone concentrations were determined in duplicate with a commercial progesterone kit (Progesterone-Coat-A-Count; Diagnostic Products Corporation, USA) by Gamma counter (EG & G Wallace, Finland), as described by Kim *et al*.(6).

Ovulation was estimated to occur when plasma progesterone concentration exceeded 4.0 ng/ml, as described by Kim *et al*.(6). And ovulation was designated the first day of gestation (day 0).

Ultrasonographic examination

Serial ultrasonographic examinations were performed daily from 15 days of gestation until parturition. All dogs were examined using real-time B-mode ultrasonography in dorsal recumbency.

Ultrasonographic examinations were performed using LOGIQ 7 (GE Medical system Co, USA) with 3.5Convex, 7Linear, 10Linear MHz transducer on 34 litters from 9 Miniature Schnauzer. Measurements were undertaken from all conceptuses. All diameters were described in millimeters.

Extra-fetal measurement.

Extra-fetal structures were examined according to following methods.

1) Outer uterine diameter (OUD): OUD was measured outer diameter of uterine horn at implantation sites to day 17 from day 60. It was calculated to be the mean value of the dorsal to ventral and medial to lateral diameters.

2) Inner chorionic cavity diameter (ICCD): ICCD was measured at the location of the zonary placenta once it was detectable from day 17 to day 60. It was calculated to be the mean value of the dorsal to ventral and medial to lateral diameters.

3) Length of placenta (PL): The length of chorionic cavity was measured until zonary placenta was recognizable, after that time, length of zonary placenta was measured from day 21 to day 55.

Fetal measurement. Fetal structures were examined according to following methods.

1) Fetal crown-rump length (CRL): CRL was measured that the distance of embryo until fetal structures was distinct, after that time, the distance of the skull to the caudal edge to the perineum from day 20 to day 47.

2) Fetal head diameter (HD): HD was measured as the largest cross-sectional diameter of the head or the biparietal diameter when this structure was well identified in longitudinal section. The image quality was initially assessed by symmetry of the section and later in pregnancy by the central location of an echogenic line produced by the falx cerebri in the fetal head.

3) Fetal body diameter (BD): BD was measured as the largest cross-sectional diameter of the body of the fetus at the level of the liver and stomach in the thoracic region when these could be identified.

Statistical analysis

For each pregnancy structure, the mean and SD were cal-

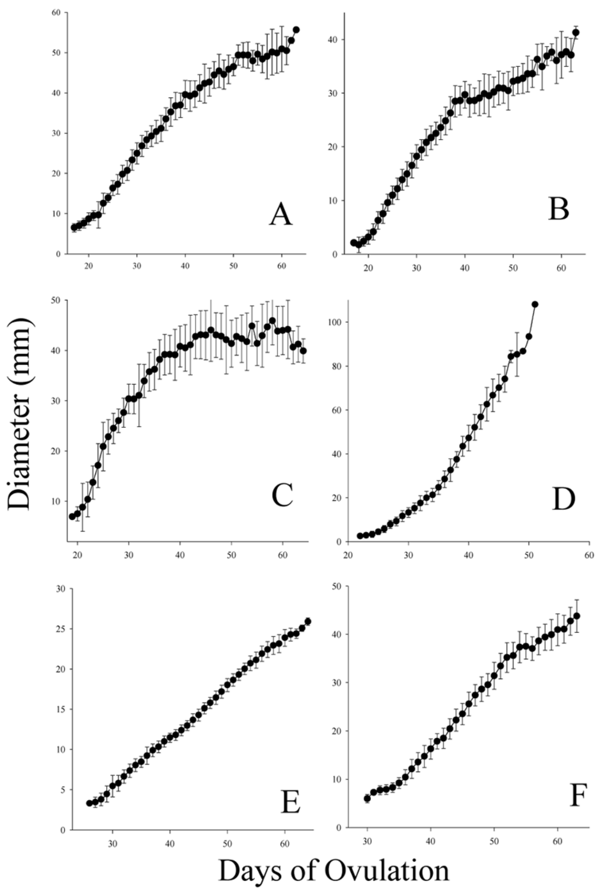

Fig 1. The growth curves of outer uterine diameter (A), inner chorionic diameter (B), length of placenta (C), fetal crown-rump length (D), fetal head diameter (E), and fetal body diameter (F) in Miniature Schnauzer bitches (Mean ± SD).

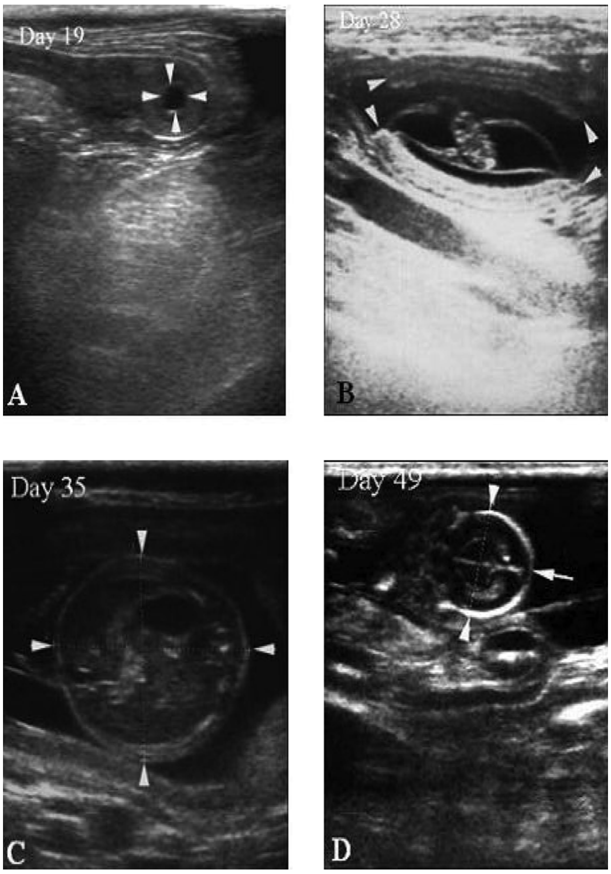

Fig 2. Ultrasonogram of extra-fetal and fetal structures in pregnant Miniature Schnauzer bitches. A: Transverse image of gestational sac at day 19. The outer uterine diameter and inner chorionic cavity diameter can be measured. B: Longitudinal image of gestational sac with zonary placenta, tubular shape of yolk sac membrane and embryo at day 28. Length of placenta and fetal crown-rump length can be measured. C: Transverse image of fetal body with anechoic stomach and hypoechoic liver at day 35. Fetal body diameter was marked by white arrowheads. D: Longitudinal image of fetal head (fetal head diameter was marked by white arrowheads) was showed in the axis of symmetry, falx cerebri (white arrow) at day 49.

culated at gestational days. All of the measurements were explored with regression analysis by Pearson's correlation procedure of SAS.

Results

The gestational lengths timed from the day of ovulation were 63.0 ± 1.7 (Mean ± SD, range: 61-65) days in Miniature Schnauzer bitches.

Extra-fetal measurements

OUD was larger at implantation sites than between implantation sites from the beginning of the study on day 17-19. OUD increased from 6.50 ± 4.06 mm (Mean ± SD) on day 17 to 50.89 ± 5.62 mm on day 60 (Fig 1A).

Table 1. The coefficients of correlation for extra-fetal structures relative at the gestational age ($p < 0.001$)

gestational features	OUD*	ICCD**	PL***
	r^2	r^2	r^2
Day 17 to 38	0.9390	0.9611	0.8170
Day 38 to parturition	0.5482	0.8644	0.6040
Day 17 to parturition	0.9115	0.8644	0.0232

*Outer uterine diameter
**Inner chorionic cavity diameter
***Length of placenta

Table 2. The coefficients of correlation for fetal structures relative at the gestational age ($p < 0.001$)

gestational features	CRL*	HD**	BD***
	r^2	r^2	r^2
Day 38 to parturition	0.8779	0.9846	0.9521
Day 27 to parturition	0.9219****	0.9787	0.9463

*Crown-rump length
**Head diameter
***Body diameter
****means r2 value of CRL on day 22 to 48

ICCD increased from 2.01 ± 0.15 mm on day 17 to 37.15 ± 4.36 mm on day 60 (Fig 1B, Fig 2A). Before day 38, ICCD showed that it had very linear growth (1.3 mm/d). Through day 17-38, there was significant linear correlation between gestational age and OUD and ICCD, respectively. After day 39, these structures were irregular shape and grew slowly.

On day 18, the placenta was first detected as an echogenic layer and zonary placenta are completely established on day 23-26. The PL from 18 to 60 increased from 7.50 ± 1.41 mm to 40.62 ± 3.27 mm. The growth of this structure was not significantly and linearly related on gestational age before 35 and, after that time, was not considered for regression analysis because of the irregular growth (Fig 1, 2)

Through day 17-38, there was significant linear correlation between gestational age and OUD and ICCD (Table 1). The r^2 values were > 0.9 during this period, which indicates that > 90% of the variability of gestational age was explained by these measurement. But PL was not significantly related to gestational age compared with those of other extra-fetal structures. After day 38, OUD and PL were not considered for regression analysis because they had a marked decrease in growth rate or no growth. As shown Table 1, the r^2 value of ICCD was higher than that of OUD. Of the extra-fetal structures, ICCD showed the best correlation to gestational age before day 38 of gestation (Table 1).

Fetal measurements

CRL increased from 2.55 ± 0.07 mm on day 22 to 85.25 ± 9.89 mm on day 48. After day 48, CRL cannot be measured because movement and flexion of fetus (Fig 1D). HD increased from 3.43 ± 0.64 mm on day 27 to 25.06 ± 0.41 mm on day 63 (Fig lE, Fig 2D). BD increased from 5.96 ± 0.84 mm on day 30 to 43.76 ± 3.36 mm on day 63(Fig 1F, Fig 2C).

All of the fetal measurements were considered for regression analysis (Table 2). There were significant correlations between gestational age and these 3 measurements. From day 22 to parturition day, the r^2 values for all measurements were > 0.9.

Of fetal structures, HD increased at an almost fixed growth rate (0.6 mm/d) and the regression was significantly and linearly related to gestational age on day 38 to parturition day (Table 2).

Discussion

Ovulation in the bitch occurs 1 to 3 days after LH surge. Estrus begins a day or two before LH surge and lasts 5 to 9 days. During estrus, breeding may occur once or on multiple occasions. Canine sperm remain fertile for at least 4 to 6 days (3). Therefore, the exact time of conception and gestational or fetal age is really unknown.

The evaluation of gestational age can be estimated by obtaining measurements of developing fetal and extra-fetal structures by ultrasonographic examination.

In this study, there was a high correlation between size of gestational structures and time after ovulation as shown by their r^2 values (Table 1 and 2). Of the development of extra-fetal structures (Fig 1A, 1B, and 1C), OUD, ICCD and PL were measured until day 60. OUD and ICCD were significantly and linearly related to gestational age before day 38. Especially, from its first detection at day 17 to day 38, ICCD with clearly defined margins was easier to measure. After day 39, the uterus was converted, irregular shape in the cross-section and grew slowly.

These results are similar to the previous study by Son *et al.*(13) that contradict the work of Yeager *et al.*(15), who suggested there was significant linear correlation between gestational age and all extra-fetal structures. The regression of placental length was not significantly related to gestational age compared with those of OUD and ICCD before day 38.

And according to Nyland and Matton (11), ICCD was measured up until approximately day 40 after LH surge in the dog and about 3 cm in diameter after which HD and BD are easier to determine accurately.

In this study, ultrasonographic measurement of extra-fetal structures could be done until day 60 owing to the new ultrasonographic equipment. But, as shown in Table 1, the r2 value of ICCD on day 17 to 38 was 0.9611, whereas that on 38 to parturition was 0.8644. The ICCD of day 38 was approximately 3 cm.

Therefore, ICCD on day 17 to 38 was the most accurate to the estimation of gestational age (5, 8, 13-15).

Of the development of fetal structures, CRL was highly relative to gestational age on day 22 to 48. CRL is also useful in early to mid-gestation for predicting gestational age (10), the r2 of that on day 22 to 48 was 0.9219. But after day 35 (approximately 2.5 cm in length), very rapid growth of fetus was observed and after day 48 (approximately 8 cm in length), it cannot be measured movement and flexion of fetus. Moreover, CRL that exceeds the size of the probe image field make this measurement difficult to obtain.

BD and HD increased at an almost fixed rate and the regression was significantly and linearly related to gestational age on day 38 to parturition, as in the previous studies (2, 9, 13-15). After day 38, HD was easier to measure than other fetal structures whereas BD was difficult to measure as fetus grew because the fetus was close to the wall of chorionic cavity, and so fetal body was difficult to differentiate from the wall of chorionic cavity. The r^2 value of HD (0.9846) on day 38 to parturition was higher than that of BD (0.9521).

For this reason, HD was the most accurate measurement for estimation of gestational age from day 38 to parturition (5, 8, 9, 13, 15).

Therefore, by ultrasonographic measurement of ICCD of the extra-fetal structures and HD of the fetal structures can be made an accurate estimation of gestational age and prediction of parturition day in early and late pregnancy, respectively.

Besides, by England and Russo (1), prediction of impending embryonic resorption could be achieved by comparison of pregnant structures and stage of development with adjacent conceptuses, or by measuring specific characteristics and estimating the rate of growth by performing a subsequent examination several days later. Thus, ultrasonographic measurements of gestational structures can be reliable means of an evaluation of normal fetal growth.

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