

## Estimation of Gestational Age by Measurement of Deep Portion of Telencephalic Vesicle in Pekingese Fetus

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### ABSTRACT

This study was performed to establish a new parameter for estimating gestational age and predicting parturition day by ultrasonographic measurement of deep portion of telencephalic vesicle (DPTV) diameter in small dogs. Fetal head diameter (HD) and DPTV diameter were measured in 15 pregnant Pekingese bitches, from Day 15 to the parturition day, and evaluated the correlation between gestational age. HD was measured from day 29 of pregnancy to parturition day and increased from  $4.9 \pm 2$  mm to  $25.5 \pm 0.7$  mm. Especially, from day 38 of pregnancy to parturition day, HD uniformly increased about 0.6 mm per day and was significantly and linearly relative to gestational age during this period ( $r^2 > 0.99$ ). DPTV diameter was measured from day 35 to day 60 of pregnancy and increased from  $3.2 \pm 0.9$  mm to  $11.5 \pm 0.7$  mm. Especially from day 38 to day 60 of pregnancy, DPTV diameter uniformly increased about 1 mm per 3 days and was significantly and linearly relative to gestational age during this period ( $r^2 > 0.99$ ). In conclusion, DPTV diameter could to be a useful parameter for the estimation of gestational age and the prediction of parturition day when used alone or in combination with HD during the second half of pregnancy.

(Key words : head diameter, deep portion of telencephalic vesicle, gestational age, pekingese)

### INTRODUCTION

Accurate estimation of gestational age is useful in preparing parturition, treatment of dystocia and planning of newborn management (Lenard *et al.*, 2007). Especially, it can be a useful tool for scheduling elective cesarean section in the case of uncertain mating times (Kutzler *et al.*, 2003).

In small animals, ultrasonography is used for determining the number of conceptus (England and Allen, 1992), monitoring of fetal development and pregnancy failure (England and Russo, 2006; Zambelli *et al.*, 2002; Son *et al.*, 2001; Nautrup *et al.*, 2000; Moriyashi *et al.*, 1996), uterine examination after parturition (Pharr and Post, 1992), reproductive examination and detection of ovulation (Boyd *et al.*, 1993), estimating the gestational age and predicting the parturition day by measurements of gestational structures (Kim *et al.*, 2004; Kutzler *et al.*, 2003; Son *et al.*, 2001; Luvoni and Grioni, 2000; Moriyashi *et al.*, 1996; Yeager *et al.*, 1992; England and Allen, 1990).

In dogs, it was reported that there was a significant correlation between gestational age and extra-fetal structures such as outer uterine diameter (OUD), inner chorionic cavity diameter (ICCD) and length of placenta (PL), and fetal structure such

as fetal crown-rump length (CRL), fetal head diameter (HD) and fetal body diameter (BD) measured by ultrasonography (Kutzler *et al.*, 2003; Yeager *et al.*, 1992). According to Son *et al.* (1997), inner chorionic cavity diameter showed the best correlation to gestational age before day 37 of pregnancy and fetal head diameter showed the best correlation to gestational age from day 38 of pregnancy to parturition day.

Recently, DPTV has been studied to establish a new indicator for estimating gestational age and predicting the parturition day (Beccaglia and Luvoni, 2004). DPTV represented by thalamus and basal nuclei primordia, was visualized from day 30 to day 8 before parturition as a symmetric anechoic area around the falx cerebri with clearly defined margins in longitudinal section (Beccaglia and Luvoni, 2004).

Furthermore, according to Beccaglia and Luvoni (2004) and Beccaglia *et al.* (2008), the accuracy of prediction of the parturition day by ultrasonographic evaluation of DPTV diameter during pregnancy was significantly less reliable in comparison with ICCD and HD. But DPTV diameter measurements could be usefully combined with HD measurements in order to increase prediction reliability during the second half of pregnancy.

However, there is no accurate parameter for estimating ges-

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tational age and predicting the parturition day by measurement of DPTV diameter in canine up to now.

Therefore, this study was performed to establish a parameter for estimating gestational age by ultrasonographic measurement of DPTV diameter in small dogs.

## MATERIALS AND METHODS

### 1. Experimental Animals

15 Pekingese dogs were aged 1 to 5 years old, weighing 4.1 to 6.3 kg. They were housed individually, fed a standard commercial dog food twice daily and water was available *ad libitum*. All dogs were observed twice daily for swelling of the vulva and the presence of a vaginal discharge, which was considered as the onset of proestrus.

The bitches were mated with same kind male at estrus according to results of vaginal cytological examination. They became pregnant and whelped a total of 63 pups, 2~6 pups each.

Serial ultrasonographic examinations were performed on all fetus to estimate the gestation age by measurements of gestational structures.

### 2. The Estimation of Optimal Mating Time and Ovulation Time

To estimate the optimal mating time, from the onset of proestrus to the onset of anestrus, vaginal smears were performed every day. Vaginal cytological examinations were performed as described by Schutte (1967) and estimating the optimal mating time was performed when the cornification index is  $\geq 90\%$ , as described by Kim *et al.* (2000). To estimate the ovulation time, from the onset of proestrus to the onset of anestrus, blood samples were collected every day via cephalic veinpuncture placed immediately in chilled EDTA-coated tubes, and centrifuged for 10 min at 3,000 g. The plasma was stored at  $-20^{\circ}\text{C}$  until analysis.

The plasma progesterone concentrations were evaluated using 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.* (2000).

The time of ovulation was estimated based on the day when plasma progesterone level first increased above 4.0 ng/ml, as described by Kim *et al.* (2000) and Wallace *et al.* (1992). And the ovulation day was designated the first day of pregnancy (day 0).

### 3. Ultrasonographic Examination

Serial ultrasonographic examinations were performed daily from day 15 of pregnancy to parturition day. All dogs were examined using real-time B-mode ultrasonography in dorsal recumbency. Hair was clipped with acoustic gel applied on the abdomen. Ultrasonographic examinations were performed using LOGIQ 7 (GE Medical System Co, USA) with a 3.5 $^{\circ}\text{C}$ , 8 $^{\circ}\text{C}$ , 10.1 MHz transducer. Gestational structures were measured in all fetus and all diameters were described in millimeters.

### 4. Gestational Structure Measurements

Gestational structures were measured according to the following methods, as described by Son *et al.* (1997), Beccaglia and Luvoni (2004).

#### 1) Fetal Head Diameter (HD)

HD was measured as the largest cross-sectional diameter of the head before the formation fetal skull and the biparietal diameter when this structure was well identified in longitudinal section from day 29 of pregnancy to parturition day. 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 (Fig. 1 A).

#### 2) Deep Portion of Telencephalic Vesicle Diameter (DPTV)

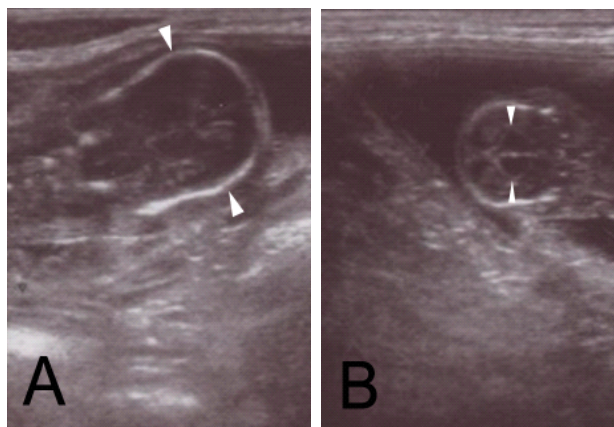


Fig. 1. Ultrasonography of the fetal structures in pregnant Pekingese bitches. (A) Longitudinal image of the fetal head was shown in the axis of symmetry, falx cerebri. The fetal head diameter was marked by white arrowheads. (B) Longitudinal image of the deep portion of telencephalic vesicle. The deep portion of telencephalic vesicle was marked by white arrowheads.

DPTV was measured when the fetal skull and the falx cerebri were identified in longitudinal section. Maximum diameter of symmetric anechoic area around the falx cerebri was measured from day 35 to day 60 of pregnancy (Fig. 1 B).

5. Statistical Analysis

For each fetal structure, the mean and SD were calculated at gestational age. HD and DPTV diameter were explored with regression analysis by Pearson's correlation procedure of SAS.

RESULTS

1. The Growth Curve of Pregnancy Structures

To examine the development of pregnancy structures relative to gestational age, the growth of HD and DPTV were measured and the results were presented.

2. Fetal Head Diameter (HD)

HD was measured from day 29 of pregnancy to parturition day and increased from  $4.9 \pm 2$  mm (mean  $\pm$  S.D.) to  $25.5 \pm 0.7$  mm (Fig. 2). Especially, from day 38 of pregnancy to parturition day, HD uniformly increased about 0.6 mm per day and was significantly and linearly relative to gestational age during this period ( $r^2 > 0.99$ , Table 1).

3. Deep Portion of Telencephalic Vesicle Diameter (DPTV)

DPTV diameter was measured from day 35 to day 60 of pregnancy and increased from  $3.2 \pm 0.9$  mm (mean  $\pm$  S.D.) to

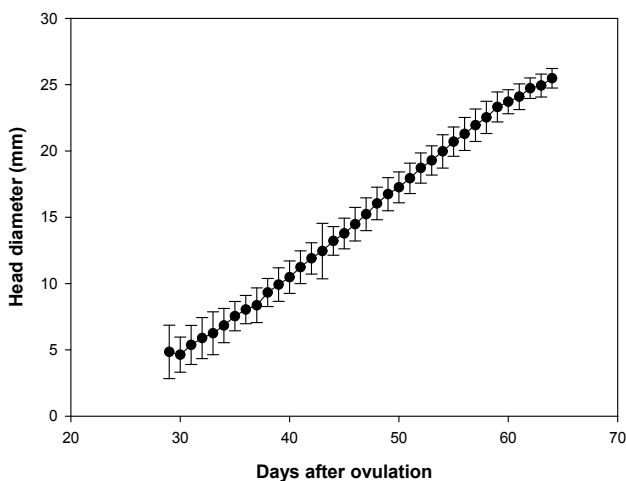


Fig. 2. The growth curve of fetal head diameter (HD) in Pekingese fetus (Mean  $\pm$  S.D.).

Table 1. The coefficients of correlation for the fetal structures to gestational age in Pekingese bitches

Gestational structure	Gestational age	$r^2$
Fetal structure	HD <sup>a</sup>	0.9974
	DPTV <sup>b</sup>	0.9914

$p < 0.001$ .

<sup>a</sup> Head diameter

<sup>b</sup> Deep portion of telencephalic vesicle

$11.5 \pm 0.7$  mm (Fig. 3). Especially, from day 38 to day 60 of pregnancy, deep portion of telencephalic vesicle diameter uniformly increased about 1 mm per 3 days and was significantly and linearly relative to gestational age during this period ( $r^2 > 0.99$ , Table 1).

DISCUSSION

Accurate estimation of gestational age using ultrasonography can be useful in predicting the parturition day, preparing parturition, managing pregnant bitches and planning cesarean section when dystocia (Lenard *et al.*, 2007; Kutzler *et al.*, 2003).

The pregnancy structures used in the estimation of gestational age by ultrasonographic evaluation are OUD, ICCD, PL, BD, HD, CRL and DPTV (Beccaglia *et al.*, 2008; Oh *et al.*, 2008; Beccaglia and Luvoni, 2004; Son *et al.*, 2001; Luvoni

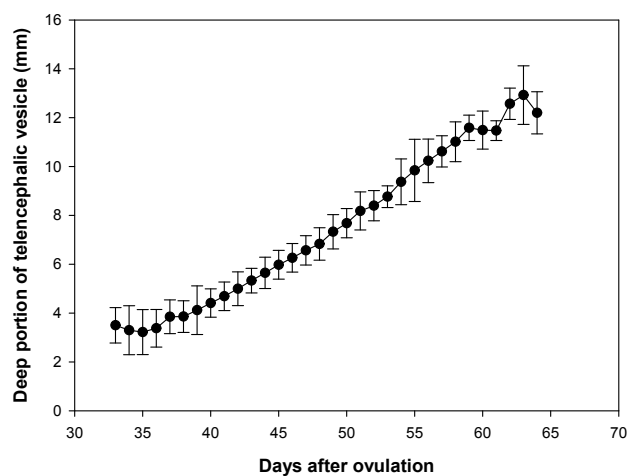


Fig. 3. The growth curve of deep portion of telencephalic vesicle (DPTV) in Pekingese fetus (Mean  $\pm$  S.D.).

and Grioni, 2000; Yeager *et al.*, 1992). Of these pregnancy structures, ICCD before day 37 of pregnancy and HD from day 38 of pregnancy to parturition day were the most accurate and mainly used for estimating gestational age and predicting parturition day (Son *et al.*, 2001; Yeager *et al.*, 1992).

In this study, HD uniformly increased about 0.6 mm per day and the growth rate is almost constant in each dog from day 38 of pregnancy to parturition day, in agreement with the results of Son *et al.* (2001). Furthermore, the measurement of HD was easy and accurate because it was easy to distinguish HD and the surrounding pregnancy structures, and HD was significantly and linearly relative to gestational age during this period ( $r^2 > 0.99$ ).

DPTV diameter was possible to measure day 35 of pregnancy and especially, from day 38 to day 60 of pregnancy, DPTV diameter uniformly increased about 1mm per 3 days. In common with HD, DPTV diameter was easy to measure because of a well-defined border between anechoic area and the peripheral structures when the fetal skull was symmetrically around the falx cerebri in longitudinal section. And DPTV diameter was significantly and linearly relative to gestational age during this period ( $r^2 > 0.99$ ). However, it was difficult to visualize DPTV as ossification of fetal skull was almost completed after day 60 of pregnancy.

Beccaglia and Luvoni (2004), and Beccaglia *et al.* (2008) reported that the correlation between DPTV diameter and gestational age was not significant in small ( $r^2 = 0.70$ ) and large ( $r^2 = 0.74$ ) size bitches, but a comparatively high regression coefficient ( $r^2 = 0.91$ ) was observed in medium size bitches when the pregnant bitches were classified in to three groups according to size and gestational age was estimated by measurements of DPTV diameter. In this study, in contrast with Beccaglia and Luvoni (2004), there was a high correlation between DPTV diameter and gestation age when measuring DPTV in small size dog.

In conclusion, DPTV was confirmed as a useful parameter for estimating gestational age and predicting parturition day in late pregnancy. Especially, DPTV diameter can be made an accurate estimation of gestational age and prediction of parturition day when used alone or in combination with HD during the second half of pregnancy.

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