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### Clinical use of fetal measurements to determine the whelping day in German shepherd breed bitches



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#### ABSTRACT

The aim of this work was to use a linear regression model previously developed in a pilot study to calculate days before parturition (DbP) using inner chorionic cavity (ICC), biparietal diameter (BPD), crown-rump length (CRL), body diameter (BD) and deep portion of telencephalic vesicle (DPTV) in German shepherd dogs (GSD) with known ovulation day and then to test that model in bitches with unknown ovulation day. In our current study, a model for GSD bitches published in a previous report, proved satisfactory for ICC [DbP =  $44.76 - (4.34 \times ICC)$ ] and BPD [DbP =  $38.65 - (12.86 \times BPD)$ ]. We therefore used their model, but developed a new one for CRL, BD and DPTV. For ICC and BPD, we tested accuracy for more than 35 days before parturition (ICC) and more than 15 days before parturition (BPD). Measurements were taken on at least two fetuses during each ultrasound recording (US) of 22 GSD bitches with known (n = 16) and unknown (n = 6) ovulation days.

The accuracy of the above model was 77–100% for ICC and 83–96% for BPD with a precision of  $\pm 1$  and  $\pm 2$  days, respectively. Accuracy increased significantly when US was performed more than 35 days before parturition for ICC and more than 15 days before parturition for BPD. BD and CRL were the most accurate parameters ( $R^2 = 0.95$  and 0.85). In bitches with unknown ovulation day, BD accuracy was 71.4–100% with a precision of  $\pm 1$  day and  $\pm 2$  days, respectively. CRL and DPTV were less accurate ( $\pm 1$  day, 60%;  $\pm 2$  days, 80% accuracy).

#### 1. Introduction

In clinical practice, determination of whelping day is increasingly requested by dog breeders, to allow careful monitoring of parturition when the history or clinical conditions of a pregnant bitch require continual assistance or veterinarian's opinion, or when an elective C-section must be planned.

Determination of gestational age and prediction of the whelping day provided by ultrasound (US) have already been variously investigated in canine species, such as assessment of first visualization of fetal and extra-fetal structures (Cartee and Rowles, 1984; Lopate, 2008). However, this method is not helpful in clinical practice due to the need to perform US examinations daily. Another approach consists of correlating the measurements of fetal and extra-fetal structures with known fetal gestational ages (Luvoni and Beccaglia, 2006; Kim and Son, 2007; Lopate, 2008). The most frequently used parameters are the crown-rump length (CRL),

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biparietal diameter (BPD), body diameter (BD) and the deep portion of telencephalic vesicle (DPTV) as fetal structures, and the inner chorionic cavity (ICC) and the outer uterine diameter (OUD) as extrafetal structures (Beccaglia et al., 2016).

As canine species have a wide range of body sizes and weights which influence the rate of fetal growth, specific formulas for ICC and BPD have been developed, providing accurate predictions in small and medium-sized bitches (Luvoni and Grioni, 2000; Son et al., 2001; Kutzler et al., 2003b). Predicting the parturition day using the same formula developed for medium-sized dogs in large and giant breeds has been reported to be accurate (Socha et al., 2015). In a recent study by Alonge et al. (2016) a particular formula was developed to determine gestational age in such breeds. A breed-type approach has also been used for the females of breeds, such as Beagles (Yeager et al., 1992), and Labrador and Golden Retrievers (England et al., 1990). A recent study (Groppetti et al., 2015) focusing on ICC and BPD linear regression models for estimating parturition date in German shepherd dogs (GSD), showed high accuracy when the effects of maternal weight, age and litter size were taken into account in relation to fetal biometry.

During the second half of pregnancy, BD and DPTV can also be easily measured (Beccaglia et al., 2008; Lopate, 2008) and many authors report that BD is positively correlated with gestational age (England et al., 1990; Yeager et al., 1992; Moriyoshi et al., 1996).

The aim of our work was to use a linear regression model previously developed in a pilot study (Milani et al., 2013) for the ICC, BPD, CRL, BD and DPTV in GSD bitches with known ovulation day and to test it in bitches with unknown ovulation day. For the purposes of this study, the model published by Groppetti et al. (2015) proved satisfactory for ICC, and BPD, therefore we used their model and developed new models for CRL, BD and DPTV.

#### 2. Materials and methods

#### 2.1. Animals

Twenty-two breeder-owned pregnant German shepherd dog (GSD) bitches, 2–7 years of age and 28–40 kg body weight were included in the study. Sixteen/22 bitches were presented to the Small Animal Reproduction Unit of the Veterinary Teaching Hospital of the University of Padova (Italy) during estrus for ovulation timing; only 6/22 bitches were examined for pregnancy diagnosis. The bitches used in this study belonged to various breeding kennels and written consent for all clinical procedures was obtained from all breeders.

To determine optimal breeding time, complete history of all bitches were collected and the animals were then clinically examined, including vaginal cytology and blood sampling for serum progesterone assays. The day of ovulation was established as the day on which progesterone range was 4–10 ng/ml (Concannon, 2011) followed by a continual and rapid increase to  $\geq$  20.0 ng/ml. Progesterone concentration was determined with a chemiluminescence method (Immulite 1000, Medical System, Genova, Italy), previously validated in the dog (Kutzler et al., 2003a). All bitches were mated 48–72 h after ovulation.

Pregnancy diagnosis was performed 21–24 days after ovulation or mating. One or more US were performed in all pregnant bitches throughout pregnancy, according to clinical indications, on a LOGIQ P5/A5 US unit equipped with a 6–10 MHz multi-frequency convex probe (General Electric<sup>\*</sup> Medical System, Solingen, Germany). All examinations of US were performed by the same operator with the dog in lateral recumbency, after hair clipping and application of contact gel. Bitches were considered pregnant when at least one gestational sac was visualized as a round anechoic structure surrounded by a thick hyper-ecogenic wall representing the uterus, and a subtle hyper-ecogenic sheet, representing the placenta (England and Russo, 2006). During US examinations, biometric measurements were carried out on at least two live fetuses (except in pregnancies with singletons), and only from fetuses with heart rates within the normal physiological range (Verstegen et al., 1993).

Whelping date and the number of fetuses born were later recorded for all bitches. For bitches with known ovulation day, only those having a pregnancy lasting 61–64 days from the estimated day of ovulation were included in this study.

#### 2.2. Parameters

The following parameters were measured: inner chorionic cavity (ICC), body diameter (BD), crown-rump length (CRL), biparietal diameter (BPD) and the deep portion of the telencephalic vesicle (DPTV) (Son et al., 2001; Kutzler et al., 2003b; Beccaglia and Luvoni, 2006; Beccaglia et al., 2008; Lopate, 2008). For all parameters, the mean of at least two measurements in each of the most caudal fetuses during each US was considered.

The entire study comprised three different experiments, designed as follows:

- Experiment 1: to verify the accuracy of the ICC and BPD linear regression formulas of Groppetti et al. (2015) to estimate parturition date in pregnant GSD bitches;
- Experiment 2: to calculate linear regression formulas for BD, CRL and DPTV to estimate the day of parturition date, as above;
- Experiment 3: to calculate the accuracy of the estimated day of parturition in GSD bitches with unknown ovulation day, according to the BD, CRL and DPTV linear regression formulas calculated in experiment 2.

#### 2.3. Statistical analysis

In experiment 1, data of ICC and BPD were used to estimate the number of days before parturition (DbP) according to the linear regression model of Groppetti et al. (2015) as follows:

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