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# Breed-specific fetal biometry and factors affecting the prediction of whelping date in the German shepherd dog

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ABSTRACT

To date many studies have been published about predicting parturition by ultrasonographic fetal measurements in the bitch. Given that accuracy in such prediction is a key point for clinicians and breeders, formulas to calculate the whelping date were mainly obtained from small and medium sized dogs, which means poor accuracy when applied to large or giant breeds.

Based on the evidence that ethnicity significantly affects fetal biometry in humans, this study aimed at developing a breed-specific linear regression model for estimating parturition date in the German shepherd dog. For this purpose, serial ultrasonographic measurements of the inner chorionic cavity diameter (ICC) and the fetal biparietal diameter (BP) were collected in 40 pregnant German shepherd bitches. The quality of the regression models for estimating parturition date was further verified in 22 other pregnant German shepherd bitches. Accuracy related to the prediction of parturition date was higher than previously reported: 94.5% and 91.7% within ±2 days interval based on ICC and BP measurements, respectively. Additional investigation was performed on the effects of maternal weight, age and litter size in relation to fetal biometry and to accuracy of parturition estimation. Moreover, the study included a comparison between hormonal and fetal ultrasound (ICC and BP) measurements connected to the estimation of whelping date.

We suggest that specific equations from a single breed are likely to offer excellent accuracy, comparable to that of periovulatory progesteronemia, in parturition prediction and to avoid morphological variables present in dogs of different breeds even with the same size/weight.



## 1. Introduction

The prediction of parturition is a veterinary service increasingly required by owners and breeders. In fact, knowing a litter whelping date is essential to schedule a C-section and to better manage new-born puppies. Ultrasonographic biometry allows both fetal viability assessment and gestational age estimation (Yeager et al., 1992) and represents an important diagnostic tool mostly if mating is unknown and peri-ovulatory hormonal monitoring is not available. To date, many multi-breed models to calculate whelping date have been described (England et al., 1990; Kutzler et al., 2003a; Beccaglia and Luvoni, 2006), mainly in small and medium sized dogs (Yeager et al., 1992; Moriyoshi et al., 1996; Luvoni and Grioni, 2000; Son et al., 2001; Kim and Son, 2007). For large-breed dogs,

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no specific formulas are given but the ones regarding the diencephalo-telencephalic vesicle measurement, whose clinical use is limited by low accuracy of prediction parturition (62% within ±2 days) and by a short gestational period of detection (35th to the 58th day of pregnancy) (Beccaglia et al., 2008; Michel et al., 2011). And when applied to large-giant breeds, formulas derived from small-medium sized dog show poor accuracy (Lopate, 2008).

In humans, ethnicity significantly affects fetal biometry (Jacquemyn et al., 2000), therefore different growth charts based on phenotypes are used for dating pregnancy in women (Davis et al., 1993; Shipp et al., 2001; Munim et al., 2012). A great variation in size (from toy to giant) and in morphology of the head and the body (brachy-meso dolicho/morphous) is even more evident among canine breeds than in human of different ethnicity. For example, breeds such as Greyhound, Basset hound, English bulldog and German shepherd dog, are almost the same weight but differ highly in height and morphology. Based on what is evident in humans, we speculated that breed-dependent morphology rather than canine size/weight can affect fetal measurements and consequently, estimation of gestational age. Thus, the purpose of this study was to design a German shepherd-specific linear regression model of practical clinical use to better estimate the date of pregnancy. Data were collected by ultrasonographic biometric measurements of ICC and BP in 40 pregnant bitches (Group A). Further, accuracy of these prediction formulas was verified in 22 other pregnant bitches (Group B). Which factors could affect pregnancy length in dog is still a controversial issue (Kutzler et al., 2003a; Eilts et al., 2005; Michel et al., 2011; Mir et al., 2011). We hypothesized that, in dogs from the same breed, gestational duration and, as a consequence, fetal biometric measurements and accuracy of related equations were not influenced by maternal weight and age, while being inversely proportional to the litter size.

It is known that whelping date and pregnancy length can be accurately calculated by periovulatory progesterone measurement (Kutzler et al., 2003b). Thus, to further verify the clinical efficacy of our formulas, we compared accuracy of predicting parturition by both hormonal (periovulatory progesteronemia) and fetal ultrasound measurements (ICC and BP).

## 2. Materials and methods

### 2.1. Animals

A total of 62 German shepherd bitches from different kennels in northern Italy were enrolled in this study. Bitches were under investigation at the Reproduction Unit of Università degli Studi di Milano from January 2008 to April 2013. All animals underwent an accurate anamnestic and clinical assessment. Weight, age, date of delivery and number of pups were recorded for all bitches.

### 2.2. Evaluation of the reproductive cycle

Reproductive cycle was monitored in all bitches by vaginal cytology and plasma progesterone measurement in order to deduce LH surge and optimal time for mating (Concannon et al., 1977; Concannon and Rendano, 1983; Johnston et al., 2001; Kutzler et al., 2003a; Michel et al., 2011). Only bitches whose initial progesterone sample was <2 ng/mL were included in the study. The day of LH peak was regarded as the first day when the serum progesterone was ≥2 ng/mL (Concannon et al., 1977; Concannon and Rendano, 1983; Johnston et al., 2001; Kutzler et al., 2003b; Michel et al., 2011). Gestational age was calculated from the estimated LH surge (D 0) and parturition expected to occur 65 days after (Michel et al., 2011). Plasma progesterone concentration was determined using a quantitative test based on ELFA technique (Enzyme Linked Fluorescent Assay; MiniVidas, bioMérieux). The assay combines an enzyme immunoassay competition method with a final fluorescent detection (Brugger et al., 2011).

### 2.3. Ultrasonographic biometry

With the aim to develop a linear regression model suitable to estimate the date of pregnancy, 40 bitches (Group A) were examined at least on three occasions: early (D 20–33), mid (D 34–46) and late pregnancy (D 47 until parturition). Further, 22 additional German shepherd bitches (Group B) were scanned throughout their pregnancy to evaluate accuracy of implied equations.

We measured two fetuses during each examination, except in cases of singleton. The fetuses selected for measurements were the ones that were located most cranially and caudally within the uterus.

Ultrasound examinations were performed by a SonoAce 8000 SE (Medison) equipped with a micro-convex multifrequency probe (5.5–6.5–7.5 MHz). The same operator carried out all ultrasonographic exams with dogs in standing position or lateral recumbency. Hair clipping was not performed to keep the competitive show career of dogs under investigation.

ICC was calculated as the average of two diameters of the inner circumference of the chorionic cavity (Son et al., 2001). BP was the distance between the parietal bones when these structures were arranged in the true longitudinal place (Son et al., 2001). ICC (Fig. 1A) was evaluated from day 23 to day 37 of pregnancy, and BP diameter (Fig. 1B) from day 43 of pregnancy to parturition.

### 2.4. Accuracy of parturition date prediction

Accuracy of prediction was stated as the percentage of expected parturition dates occurring ±1 day and ±2 days from actual parturition dates in the bitches from Group B ($n = 22$) based on ICC and BP ultrasonographic measurements and on periovulatory plasma progesterone concentrations.

### 2.5. Statistical analysis

All data were analyzed using a commercial statistical program (IBM SPSS 21.0 for Windows, IBM SPSS, Armonk, New York, USA). Descriptive statistics were expressed as mean ± SD. For statistical purposes dogs were stratified in three groups according to their weight (<25 kg;