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Breed-specific fetal biometry and factors affecting theprediction 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 (Englandet al., 1990; Kutzler et al., 2003a; Beccaglia and Luvoni,2006), mainly in small and medium sized dogs (Yeageret al., 1992; Moriyoshi et al., 1996; Luvoni and Grioni, 2000;Son et al., 2001; Kim and Son, 2007). For large-breed dogs,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; Munimet al., 2012). A great variation in size (from toy to giant)and in morphology of the head and the body (brachy-mesodolicho/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; Michelet 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 ,$\geq$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,Äì33), mid (D 34,Äì46) 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 multi-frequency 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 ±2days 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;25-29 kg; >29 kg), age (<4 years; 4-8years; >8 years)and litter size (≤3 pups; 4-8 pups; ≥9 pups). Gestational age was divided into early (D 20-33), middle (D34-46) and late pregnancy (D 47 until parturition). All ICC and BP ultrasonographic measurements were evaluated by linear regression and ANOVA to obtain an averaged regression equation to calculate the days before parturition. The effect of maternal weight and age on litter size was evaluated using Fisher's exact test. The effect of maternal weight and age, litter size and gestational age on fetal ultrasound measurements (ICC and BP) was evaluated by ANOVA. The effects of maternal weight and age, litter size and gestational age on accuracy of the parturition estimation were assessed by Pearson χ2 tests. The accuracy of ICC and BP measurements as well as the correlation between periovulatory plasma progesterone concentrations and ultrasonographic measurements was also estimated by Pearson χ2 tests. Pregnancy length was compared among different sized litters using ANOVA. Statistical significance was defined as $P < 0.05$.

3.Results

3.1. Bitches in Group A

Dogs (n = 40) belonging to Group A weighed 23-36 kg at oestrus and aged 1.5-10 years (Table 1). Bitches whelped a total of 185 pups with litter sizes ranging from one to 12 pups (Table 1). Litter size was not dependent on maternal weight at oestrus while it was inversely related to maternal age, with bitches older than 8 years whelping a lower number of puppies(2.5±0.7) than younger dogs (5.3±2.9; $P < 0.001$).

3.1.1. Fetal biometry

A total of 230 biometric measurements were performed from day 23 after estimated LH surge (D 0) to parturition, in 40 pregnant German shepherd dogs (Group A). The number of ultrasonographic measurements performed for each biometric parameter (ICC and BP) is reported in Table 2. Equations used to calculate the day of parturition based on ultrasonographic measurements are shown in Table 3. Both parameters (ICC and BP) were significantly and linearly correlated to gestational age ($P < 0.001$).

3.1.2. Factors affecting biometric parameters

Maternal weight and age were not statistically related to ICC and BP biometric measurement. ICC diameter varied significantly with litter size, with larger gestational chambers in pregnancies with few puppies (≤3 pups; $P = 0.04$).

3.2. Accuracy of predicting parturition

One hundred and three ultrasonographic examinations were additionally performed from day 23 to day 54 after LH surge on another 22 German shepherd bitches (Group B). ICC was evaluated from day 23 to the day 39 of pregnancy and BP diameter from day 40 to day 54 of pregnancy. The number of ultrasonographic measurements performed foreach biometric parameter and accuracy in relation to litter size and gestational age are reported in Table 4. The accuracy for ICC and BP in relation to litter size and gestational age at ±1 and ±2 days is also given.

3.2.1. Factors affecting accuracy of equations

Dogs belonging to Group B weighted 25-34 kg at oestrus and aged 2.5-9 years (Table 5). Bitches whelped a total of 122 pups with litter size ranging from 1 to 11 pups (Table 5). Maternal weight and age, litter size and gestational age did not affect the accuracy of parturition date prediction by ICC measurements. The prediction of whelping date by BP measurements was significantly more accurate in medium sized litters (4-8 pups) ($P = 0.003$).

3.3. Progesterone versus ultrasound evaluation

We did not record any statistical difference in the accuracy of whelping date prediction either based on ICC measurement or on periovulatory plasma progesterone concentrations. The latter was significantly more accurate than BP measurement (P = 0.001), particularly in small sized litters (≤3 pups). In such cases, pregnancy took longer time than usual (Table 6; P = 0.01).

4. Discussion

To our knowledge, this was the first ultrasonographic study on large scale in a single breed, the German shepherd dog. To estimate parturition date we developed a linear regression model based on two biometric parameters (ICC and BP) of proven accuracy (Lopate, 2008), easily recognizable and definable for a wide range of pregnancy by ultrasound, which makes it less prone to errors from the operators and less dependent on their skill than measurements of fetal crown-rump length, fetal body diameter and diencephalo-telencephalic vesicle diameter. In early pregnancy, accuracy of prediction based on ICC measurement is reported between 85 and 88% (±2 days) in large breeds (Luvoni and Grioni, 2000; Son et al., 2001; Kutzleret al, 2003a; Beccaglia and Luvoni, 2006; Lopate, 2008). We measured ICC from day 23 to day 39 of pregnancy recording a percentage higher than previously described of 81.8% and 94.5% at ±1 day and ±2 days, respectively.

In later pregnancy, BP is considered the most accurate parameters for the calculation of gestational age with a percentage between 81% and 88% (±2 days) (England et al.,1990; Luvoni and Grioni, 2000; Beccaglia and Luvoni, 2006; Lopate, 2008; Michel et al., 2011). We measured BP diameter from day 40 to day 54 of pregnancy obtaining an accuracy of 83.3% and 91.7% at ±1 day and ±2 days, respectively. Maternal weight and age as well as gestation age did not affect accuracy of predicting parturition. How-ever, when calculated by BP measurement, accuracy was significantly higher in medium (4-8 pups) compared to small (≤3 pups) sized litters. In agreement with literature (Eilts et al., 2005; Mir et al., 2011), this aspect reflects the longer duration of pregnancy in dogs with less than three pups. It is reported that fetal ultrasonographic measurement is not as accurate as plasma progesterone concentration in predicting the parturition date in dogs (Kutzler et al.,2003a,b; Lopate, 2008; Michel et al., 2011). A difference that may depend on several factors, such as prediction equations of parturition derived from bitches of differing breed conformation (Saunders, 1992). Indeed, many parameters involved in gestational age estimation are breed-dependent, such as litter size (Sokolowski, 1977; Johnston et al., 2001; Kutzler et al., 2003a,b), maternal body weight (Kutzler et al., 2003a,b) and duration of pregnancy (Okkens et al., 2001; Eilts et al., 2005; Lopate, 2008).We obtained excellent accuracy, comparable to that deriving from plasma progesterone concentration, basedon ICC measurement. However, when litter size was small (≤3pups), periovulatory progesterone concentrations were significantly more accurate for detecting whelping date than BP measurements. In the present study litter size was inversely related to maternal age, with bitches older than 8 years whelping a lower number of puppies than younger dogs. An aspect consistent with fertility reduction (conception rate and litter size) in aging dogs (Bobic Gavrilovic et al., 2008). ICC diameter varied significantly with litter size, with larger gestational chambers in pregnancies with few puppies. Accuracy of parturition date prediction by fetal biometry in polytocous species can further imply a common error, i.e. repeated measurements on the same fetuses. In fact, how many fetuses and which ones should be evaluated to obtain reliable result has not been established yet. Thus, foreach examination we measured two distant fetuses located in opposite positions within the uterus (cranial and caudal).To avoid over-estimation, Kutzler et al. (2003a) described a different approach based on the evaluation of only 2 fetuses at a time. No other previous studies reported their method of selection of the fetuses, implying that all fetuses were considered. Given that accurate prediction of parturition date is relevant to veterinarians and breeders, we speculated that specific equations from a single breed are likely to increase accuracy and to avoid morphological variables present in dogs of the same size/weight (brachycephalic, mesocephalic, dolichocephalic breeds). Moreover, ultra-sonographic biometric measurement of ICC and BP is a relatively simple and reliable technique in German shepherd dogs and a method able to standardize it to avoid repeated measurements of the same fetuses should be pursued.

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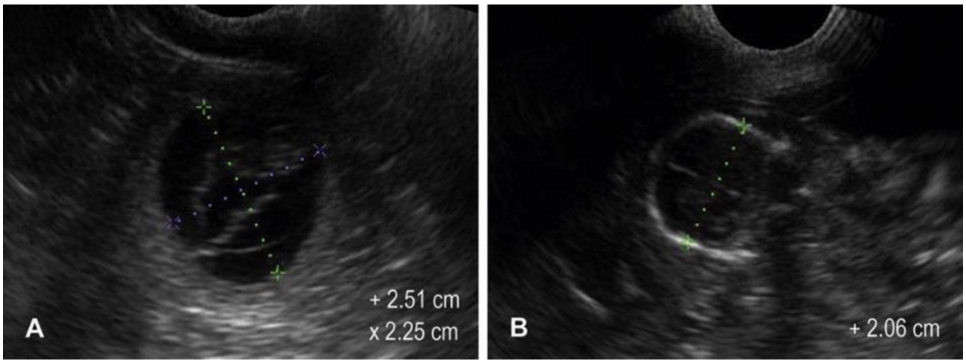

Fig.1. Ultrasonographicmeasurementsoftwodiametersoftheinnerchorioniccavity(ICC)ontransverseplaneonday30ofpregnancy(A);fetalbiparietal diameter (BP) on day 53 of pregnancy (B).

Table 1
Maternal weight and age based on litter size (Group A).

Litter size	Number of pups (mean ± SD)	Weight (kg) (mean ± SD)	Age (years) (mean ± SD)	Number of pregnancies
≤3	2.3 ± 0.6	29.9 ± 3.1	5.1 ± 2.9 [a]	17
4–8	6 ± 1.3	27.8 ± 2.6	3.6 ± 1.9[b]	19
>9	10.7 ± 1.5	28.7 ± 2.5	3.7 ± 0.8[b]	4
Overall	5.1 ± 2.9	28.7 ± 2.9	4.3 ± 2.3	40

SD: standard deviation. Different superscripts denote significant difference between litter size and maternal age $P < 0.001$

Table 2
Number of ultrasonographic biometric measurements based on litter size (Group A).

Litter size	ICC	BP
≤3	33	28
4–8	67	42
≥9	14	46
Overall	114	116

ICC: inner chorionic cavity diameter; BP: biparietal diameter.

Table 3
Equations and coefficients of correlations (r^2) for ultrasonographic biometric measurements relative to gestational age (Group A).

Equations	r^2	P
DBP = 44.76 − (4.34 × ICC)	0.81	<0.001
DBP = 38.65 − (12.86 × BP)	0.91	<0.001

DBP: days before parturition; ICC: inner chorionic cavity diameter; BP: biparietal diameter.

Table 4
Accuracy of predicting parturition based on litter size and gestational age (Group B).

Litter size	±1 day *n* (%)		±2 days *n* (%)	
	ICC[a]	BP[b]	ICC[a]	BP[c]
≤3	5/7 (71.4)	2/5 (40)	7/7 (100)	2/5 (40)
4–8	28/35 (80)	38/43 (88.4)	32/35 (91.4)	42/43 (97.7)
≥ 9	12/13 (92.3)	0/0	13/13 (100)	0/0
Overall	45/55 (81.8)	40/48 (83.3)	52/55 (94.5)	44/48 (91.7)
Gestational age	ICC	BP	ICC	BP
D 20–33	18/22 (81.8)	1/1 (100)	21/22 (95.5)	1/1 (100)
D 34–46	27/33 (81.8)	8/14 (57.1)	31/33 (93.9)	10/14 (71.4)
D 47-parturition	0/0	31/33 (93.9)	0/0	33/33 (100)
Overall	45/55 (81.8)	40/48 (83.3)	52/55 (94.5)	44/48 (91.7)

ICC: inner chorionic cavity diameter; BP: biparietal diameter; *n:* number of measurements. Different superscripts denote significant difference between ICC and BP on the same day: ab indicates $P < 0.01$; ac indicates $P < 0.001$.

Table 5

Maternal weight and age based on litter size (Group B).

Litter size	Number of pups (mean ± SD)	Weight (kg) (mean ± SD)	Age (years) (mean ± SD)	Number of pregnancies
≤3	2 ± 0.7	29.7 ± 2.7	5.3 ± 2.3	5
4–8	6.1 ± 1.6	29.2 ± 2.6	4 ± 1.7	15
>9	10 ± 1.4	30.2 ± 2.5	3.5 ± 1.4	2
Overall	5.5 ± 2.6	29.4 ± 2.5	4.3 ± 1.9	22

SD: standard deviation.

Table 6

Gestational length based on litter size (Group B).

Litter size	Days (mean ± SD)
≤3	66.6 ± 1.8[a]
4–8	65.3 ± 1.2[b]
≥9	64.3 ± 1.3[b]
Overall	65.5 ± 1.6

Gestational length was calculated from estimated LH surge (Day 0). Different superscripts denote significant difference $P = 0.01$.