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ORIGINAL ARTICLE

DETERMINATION OF GESTATION TIME IN DOGS BY MEASUREMENT OF FOETAL SIZE

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Ethical considerations: When reporting experiments on animals Observation of the ARRIVE guidelines 2.0: Updated guidelines for reporting animal research, published in July 14, 2020 (https://doi.org/10.1371/journal.pbio.3000410), is applied. The authors ensure that all procedures were performed in compliance with the guidelines for animal care of their institutions or with national/international guidelines.

ABSTRACT

Establishing the gestation period in pregnant dogs is important to minimize neonatal loss and complications. This study aimed to predict the day of parturition using ultrasound to measure the inner chorionic cavity (ICC), biparietal parameter (BP) and crown to rump length (CRL) in 10 pregnant dogs. The measurements were taken once between days 21–34 of gestation. The results were inserted into various formulas to calculate days before parturition (DBP) and compared with actual parturition dates. The ICC measurement, using Luvoni and Grioni's and Groppetti et al.'s formulas, showed the highest accuracy (94 %). The study enhances methods for predicting parturition in dogs, improving prenatal care and reducing neonatal losses.

Key words: dog; foetal size; formula; gestation

INTRODUCTION

Determination of gestation time in pregnant bitches is crucial just as in humans, because the pregnancy is occuring during a short period of time. It is critical for the foetuses to be fully developed before parturition, to prevent and reduce the loss of neonates. An approximate due date is not only necessary for prevention of neonatal mortality, but also for planning an eventual caesarean section in case of high-risk pregnancies, or in case of oversized foetuses. The determination of gestation not only allows delivery of viable puppies, it also encourages health of the pregnant bitch [9]. This approach enables the scheduling of a planned caesarean section and also provides veterinarians with the ability to predict the day of parturition, even when the mating date is unknown. As a result, this technique improves the management of reproductive health for animals [19].

Different methods can be used to predict the time of parturition, however a combination of these methods is the key for a precise determination. Ultrasonographic examination can be performed during the first appearance of embryonic or foetal structures, radiographical examination can be used during the later stages of pregnancy, once bones are formed. During these examinations, different structures are observed in relation to the day of gestation. Ultrasonography is the most frequent and most accurate method used for the measurement of the foetuses and extra foetal structures during pregnancy. Determination of the due date after measurement of the embryos or foetuses with ultrasound is done by using special formulas. The formulas are dependent on the parameters measured by the ultrasound, but also other important factors must be taken into consideration, such as the size of the bitch. Apart from radiographic and USG examinations, hormonal assays can be performed, not only to determine the parturition date but also to determine the state of ovulation [1].

The aim of this study was to evaluate the precision of formulas suggested by Beccaglia et al. [1], Luvoni and Grioni [12], Milani et al. [13], Groppetti et al. [5], and Luvoni and Beccaglia [11], utilizing measurements of three different ultrasonographic parameters: inner chorionic cavity (ICC) measurement, crown-to-rump length (CRL) measurement and biparietal (BP) parameter. A comparison was conducted between the accuracy determined by these formulas and the actual length of gestation, with the objective of evaluating their efficacy in predicting parturition.

This suggests that employing the parameter closer to the latter stages of gestation enhances the precision of predicting the parturition.

Factors influencing gestational length in dogs

The accuracy of predicting the parturition ultimately based on the date of mating may be compromised, as a result of the variability of the gestational length among individual dogs [11]. Causes behind the variability of gestational length in bitches can be different.

Mating can take place as early as three days before ovulation [23]. However, once ovulation has taken place, the oocyst must undergo maturation to reach the suitable stage for fertilization; this process typically takes 48–72 hours [17]. It is also important to consider the fact that the spermatozoa can survive in the uterus for up to 9 days in comparison to other domestic animals. This prolonged viability of the spermatozoa can facilitate a successful fertilization of the oocyte, even in cases where natural mating has occurred 9 days before the actual ovulation, ultimately resulting in pregnancy and production of a litter [15].

It has also been demonstrated that there is a clear correlation between the litter size and gestational length [18]. In litters consisting of four puppies or fewer, there is typically an observation of prolonged gestation time, however in litters with four or more puppies, the gestational length decreases [2].

Additionally, it is important to take into consideration that gestational length and even pregnancy termination can be influenced by various factors beyond normal physiological conditions, including infections as Canine herpes virus and Brucellosis [24]. Canine herpesvirus-1 (CHV-1) is common in Europe [25] and is a virus transmitted through oronasal secretions or by venereal transmission in adult dogs, however transplacental transmission affects the foetuses and may cause embryonic resorption, abortion and stillbirth [16]. Canine Brucellosis is caused by *Brucella canis*, and is a zoonotic bacterial disease which is associated with reproductive failure in dogs characterized by abortions and infertility [6].

During the breeding period, the luteinizing hormone (LH) surge and increase in progesterone levels can offer information on the gestational length in the bitch. However, once the pregnancy is confirmed, ultrasonography stands out as the most effective tool for predicting the date of parturition [11].

Ultrasonographic examination and the appearances

Pregnancy in the bitch can be diagnosed as early as on day 18–20 through a ultrasonographic examination, which is considered being the most accurate method today [7]. This technique is particularly helpful in the assessment of gestational stage when the date of ovulation is unknown [1].

The first appearing structure that can be seen during an ultrasonographic examination after mating is the gestational sac. The gestational sac is seen on the ultrasound as an anechogenic structure as early as on day 18 after ovulation has taken place [1]. As the pregnancy progresses, the diameter of the gestational sacs increases; initially these structures are spherical and maintain the spherical shape until approximately day 25 prior to parturition. However,

if the gestational sac with the conceptus appears flattened upon the ultrasonographic examination, it indicates that the gestational age is greater than 25 days before parturition [3].

On day 23 after the ovulation, an embryo can be seen with a heartbeat inside the chorionic cavity [1]. The size of the embryo at this stage is approximately 1 cm in diameter [9]. Later, between days 27–31, the embryo changes in shape and becomes a bipolar structure with limb buds which can be detected upon ultrasonography [1].

Between days 23–26, the zonary placenta can be observed as a cylindrical structure [8].

When it comes to the detection and presence of organs, the first abdominal viscera to appear is the stomach and the urinary bladder between days 29–33. Approximately around the same time, the skeleton can be visualized in the form of a hyperechoic structure between days 29–33 after ovulation [1]. The first ossification is seen on the mandible, maxilla, frontal lobe and the clavicle, followed by ossification of the palatine, incisive, zygomatic and nasal bones around day 33 of gestation, as well as soft ossification of several long bones including humerus, radius and femur among others, can be observed [14]. Only a few days later, between day 32–34, the foetus starts to move and this can be seen on during the ultrasonographic examination [1].

Abdomen and the thorax become properly visible between days 34–36, the liver and lungs can be seen around this time as well. However, the lungs appear more hyperechoic than the liver which is a hypoechoic structure in comparison to the rest of the abdomen. Around day 41–43, the kidneys start to be visible [1].

Between days 48–57 of pregnancy lungs become more mature and the foetus should theoretically be ready for the extra-uterine life; further development of the lungs take place during the neonatal period [19].

The last organ to be seen on the ultrasound is said to be the bowel, which does not appear until the end of the pregnancy around days 57–63 of gestation [1]. Detection of the bowel around day 57 marks the end of the foetal organogenesis [4]. Between day 58 and 62 of gestation, all layers of the intestinal wall become visible and gastrointestinal peristalsis can also be seen [19]. However, the intestine surpasses the available room within the abdominal cavity around day 30, and protrude into the umbilical stalk. This phenomenon is referred to as physiological herniation [14].

Predictive tools for parturition date estimate

Precisely predicting the day or parturition based on the time of mating is not possible. Parturition prediction can be achieved through various methods, including hormonal and/or cytological approaches. However, one of the drawbacks of these methods is their expenses [20]. By measuring the extra foetal and foetal parameters with the help of ultrasound, prediction of whelping date can be determined, even if the day of ovulation is unknown. During initial stages of pregnancy, between day 19 and 37 after the LH peak, measurement of the Inner chorionic cavity (ICC) and foetal Crown-to-rump length (CRL) can be obtained [1]. Meanwhile, it is suggested that the measurement of the biparietal parameter (BP), should be obtained during the second half of pregnancy, around 25 days before whelping. This is because ossification is more clearer, making measurements more precise [21].

Measurements of the Inner chorionic cavity (ICC)

The measurement of the ICC involves taking the average of two diameters of measurement of the ICC, each measured at a 90 degree angle [11]. It has been suggested that the measurement of the ICC should be performed during the first half of the gestation, 42 to 30 days before whelping to obtain the highest accuracy [21].

Specific formulas for measurement of the ICC have been developed for dogs up to 10 kg, and for dogs with a weight of 11–25 kg. For giant breeds with a weight of above 40 kg, formula for medium sized dogs should be used, however with a deviation factor of -2 days. The precise prediction of parturition can be determined when the size-specific formulas for small and medium sized breeds are applied:

ICC in small sized bitches: DBP = (mm – 68.68)/1.53

ICC in medium sized bitches: DBP = (mm – 82.13)/1.8 [1]

In addition to formulas provided by Beccaglia et al. [1], other formulas for the measurement of the ICC in dogs have been suggested by Luvoni and Grioni [12], Milani et al. [13], and Groppetti et al. [5].

Luvoni and Grioni: DBP = 45.628–0.556 x ICC (mm)

Milani et al.: DBP = 48.121–0.5237 x ICC (mm)

Groppetti et al.: DBP = 44.76–0.434 x ICC (mm) [22]

Measurements of the foetal Crown-to-rump length (CRL)

The CRL is measured as the distance from the most rostral point of the crown to the caudal edge of the perineum [10]. This parameter can be obtained during the first half of pregnancy [1]. Equation for the prediction of gestational age is:

Days after LH peak = 24.64 + 4.54 x cm – 0.24 x cm^2 [11]

Measurements of the foetal Biparietal parameter (BP)

The BP is determined by measuring the distance between the two parietal bones [10].

Formulas for the measurement of the BP are also based on the weight of the bitch, for small breeds (up to 10 kg) and for medium-sized breeds (11–25 kg). For dogs above 40 kg of weight, formula for medium sized breeds is used with a deviation factor of -2 days [1]. Measurement of this parameter is the most accurate to predict the day of parturition during the later stages of pregnancy [11]:

BP in small sized bitches: DBP = (mm – 25.11)/0.6

BP in medium sized bitches: DBP = (mm – 29.18)/0.7 [1]

MATERIALS AND METHODS

The research sample consisted of 10 bitches of different breeds, and different age categories ranging from 2–6 years of age. All females that participated in this study had a weight between 10 kg–40 kg. Certain criteria had to be met for the females to participate in this study, including the exact date of mating (which was determined by a progesterone test) and exact day of parturition to know the exact duration of the pregnancy. All patients were admitted to the Small Animal Clinic of the University of Veterinary Medicine and Pharmacy in Košice, for the purpose of pregnancy diagnosis. Females comprising the research sample underwent a complex clinical and gynaecological examination, before the actual ultrasonographic examination was conducted. The ultrasonographic examination was performed by the ultrasound ALOKA ProSound Alpha 6 using a linear probe up to 16 MHz. All examinations were performed with the consent and authorization of the owners. The ultrasonographic examination was performed in a latero-lateral position between days 21–34 of gestation. In the research, three key parameters were used to obtain the results including the measurement of the Inner chorionic cavity (ICC), Biparietal parameter (BP) and Crown-to-rump length (CRL). The measurements were either obtained during the actual examination of the patients, through the utilization of the electronic callipers integrated in the ultrasound, or after the examination by using the same method and performing measurements on saved prints. All examinations were performed under controlled conditions to ensure accuracy and consistency of the results. Furthermore, the results of the measurements were inserted to formulas suggested by Beccaglia et al. [ICC in medium sized bitches: DBP = (mm – 82,13)/1,8 and BP in medium size bitches: DBP = (mm – 29,18)/0,7)], Luvoni and Grioni, Milani et al. and Groppetti et al., to determine the days before parturition (DBP) based on the measurement of the Intra chorionic cavity (ICC) [1], [11], [13]. Measurements of the ICC were performed across two axes to obtain an average value, which was then inserted into the formula to obtain DBP. Accuracy of the formulas was assessed by comparing the results of the formulas with reality. Beccaglia et al.'s formula for medium sized breeds was used to obtain DBP based on BP and Luvoni and Beccaglia's formula was used to obtain DBP based on the measurement of CRL [1], [11]. Furthermore, the determination of gestation time was assessed by using another formula suggested by Beccaglia et al. This mathematical formula was based on the measurement of the Biparietal parameter (BP) of the head of the foetus to determine DBP [1]. Formula for the measurement of medium sized bitches was used to obtain results. In this study, the CRL parameter was determined by using the formula suggested by Luvoni and Beccaglia to obtain days after LH peak [11].

After calculating the days after LH peak, the results were converted into DBP to obtain the results of all measurements in the same values. The objective of using these parameters was to gain an insight into the developmental stages of canine gestation and their relationship to foetal size and days before parturition.

Ultrasonographic parameters

In the research, three key parameters were used to obtain the results including the measurement of the Inner chorionic cavity (ICC), Biparietal parameter (BP) and Crown-to-rump length (CRL). The mentioned parameters were selected due to their significance in the foetal development, and they were measured by the using ultrasound imagining techniques. The objective of using these parameters was to gain an insight into the developmental stages

of canine gestation and their relationship to foetal size and days before parturition.

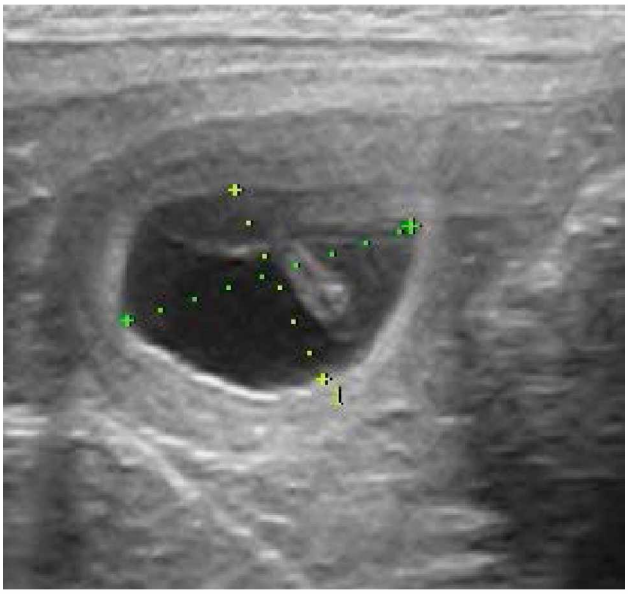

Fig. 1. Measurement of the Intra chorionic cavity (ICC).
The figure represents the ICC measurement, which refers to the space within the chorionic sac, where the embryo develops. Measurement of the longitudinal and transversal axis of the ICC was performed to obtain an average size in mm.

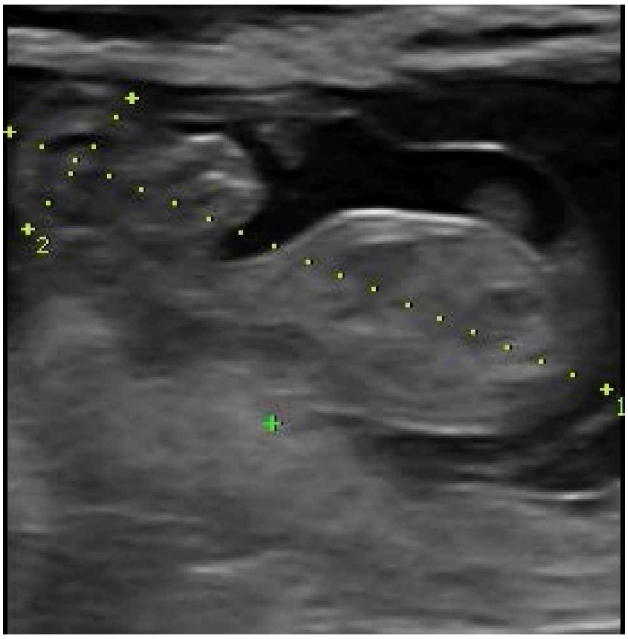

Fig. 2. Measurement of the Crown-to-rump length (1) and measurement of the Biparietal parameter (2).
The figure represents the measurements of CRL of the foetus by measuring the highest (crown of the head) and the lowest point of the body (crown) and the BP measuring the distance between the parietal bones.

Data analysis

Data analysis was performed by the utilization of four pre-existing mathematical formulas suggested by Beccaglia et al. [1] (ICC in medium sized bitches), Luvoni and Grioni [12], Milani et al. [13] and Groppetti et al. [5] to determine the days before parturition (DBP) based on the measurement of the Intra chorionic cavity (ICC).

Measurements of the ICC were performed across two axes to obtain an average value, which was then inserted into the formula to obtain DBP. Accuracy of the formulas was assessed by comparing the results of the formulas with reality.

Table 1. Formulas used in the research to obtain DBP by the use of ICC.

Beccaglia et al.	DBP = (mm – 82,13) / 1,8
Luvoni and Grioni	DBP = 45,628 – 0,556 x ICC (mm)
Milan et al.	DBP = 48,121 – 0,5237 x ICC (mm)
Groppetti et al.	DBP = 44,76 – 0,434 x ICC (mm)

Source: Alexandra Dohnal, 2024

Furthermore, the determination of gestation time was assessed by using another formula suggested by Beccaglia et al. [1]. This mathematical formula was based on the measurement of the Biparietal parameter (BP) of the head of the foetus to determine DBP. Formula for the measurement of medium sized bitches was used to obtain results.

Table 2. Formula used in the research to obtain DBP by the use of BP.

Beccaglia	DBP = (mm – 29,18) / 0,7

Source: Alexandra Dohnal, 2024

Finally, in this study, the CRL parameter was determined by using the formula suggested by Luvoni and Beccaglia (2006) to obtain Days after LH peak.

After calculating the days after LH peak, the results were converted into DBP to obtain the results of all measurements in the same values.

Table 3. Formula to obtain Days after LH peak by the use of CRL.

Luvoni and Beccaglia	Days after LH peak = 24,64 + 4,54 x cm – 0,24 x cm^2

Source: Alexandra Dohnal, 2024

RESULTS

By obtaining three different measurements of the Inner chorionic cavity (ICC), Biparietal parameter (BP) and the

Crown-to-rump length (CRL) through USG in pregnant bitches, we obtained an average size of the parameters. This allowed us to gather valuable information about the foetal development and the stage of pregnancy. Once the measurements were obtained from the USG examination, the values could be inserted into the various mathematical formulas which were designed specifically for assessment of the stage of pregnancy in the bitch based on the mentioned parameters.

Formula suggested by Groppetti et al. [5] based on the measurement of ICC, predicted the exact date of parturition in two of the ten females that participated in the study, which aligned with the actual date of delivery provided by the owner. Luvoni and Grioni's formula, which was based on the measurement of ICC as well, predicted the exact date of parturition in one of the ten females [12]. Overall average accuracy rate of these two formulas was 94 %.

Furthermore, Luvoni and Beccaglia's [11] formula for prediction of parturition based on the measurement of CRL accurately forecasted the precise delivery date in one out of the ten patients, with an average accuracy rate of 88 % [11]. However, Beccaglia's [1] formula for prediction of parturition based on the measurement of the BP did not align with the actual delivery dates in any of the cases measured. It's worth noting that this measurement was only taken in six out of the ten females, and the average accuracy rate was 85 %.

After calculation of the results obtained from the examination, with the help of the mathematical formulas suggested by Beccaglia et al. [1], Luvoni and Grioni [12], Milani et al. [13], Groppetti et al.[5] and Luvoni and Beccaglia [11], we were able to assess the days before parturition (DBP) and we could further compare the accuracy of the formulas with the actual DBP, based on the known reproductive history and day of parturition of the females.

An average of the days before parturition (DBP) was calculated based on the formulas to compare them with the average DBP based on the actual date of parturition; this to obtain the average accuracy in %.

Patient information

The following tables provide a summary of data on pregnant bitches, measured parameters, and calculations of days before parturition (DBP) using mathematical formulas by Beccaglia et al. [1], Luvoni and Grioni [12], Milani et al. [13], Groppetti et al.[5] and Luvoni and Beccaglia [11]. The tables include identification numbers and breed information of the patients, as well as recorded values of the Inner chorionic cavity (ICC), Biparietal parameter (BP), and Crown-rump length (CRL) at different stages of pregnancy. Based on these measurements, formulas from various authors were applied to predict the length of gestation, and the accuracy of these predictions was compared with the actual date of parturition. The results offer an overview of the reliability of different methods in determining the expected delivery date in bitches.

DISCUSSION

The data that were presented in the work results, give us an insight into the determination of DBP in pregnant bitches and thereby making it possible to predict the day of parturition. By the use of various mathematical formulas based on the ICC measurement, BP parameter and CRL measurement, we could determine the DBP.

Every female DBP based on ICC were calculated by using four different formulas suggested by different authors including Beccaglia et al. [1], Groppetti et al. [5] Luvoni and Grioni [12], and Milani et al. [13]. After obtaining results from each formula, we could compare them to the actual DBP using the exact known day of mating and the exact day of whelping.

In females where the BP was measurable, we used Beccaglia's formula for medium sized breeds to obtain results, as all of the dogs weighted between 10 kg and 40 kg. However, it is important to note that the BP was not measurable in all patients. This was primarily due to the fact that the examination was performed at an early stage of gestation, where obtaining precise measurements of this parameter was not possible. As a result of this, our analysis was limited to the 6 females in whom we could measure this parameter.

The CRL parameter could be measured in all 10 females, and was calculated by the formula suggested by Luvoni and Beccaglia [11], to obtain Days after LH peak, which were further converted into DBP.

However, obtaining precise measurements of this parameter was in some of the cases challenging, particular-

Table 4. Identification number, breed of patients and length of pregnancy.

Patient ID	Breed	Actual length of pregnancy (given in days)
1	Tatra hound	62
2	German shepherd	60
3	Golden retriever	66
4	Czechoslovak wolfdog	62
5	Sheltie	61
6	Rhodesian ridgeback	63
7	Bavarian mountain dog	62
8	Kooikerhondje	63
9	Hovawart	63
10	Boxer	61

Source: Alexandra Dohnal, 2024

Table 5. Day of gestation, measurement of the ICC, CRL and BP in mm.

Patient ID	Day of gestation	ICC (mm) average	CRL (mm) average	BP (mm) average
1	23	6,57 mm	7,0 mm	-
2	21	14,9 mm	9,5 mm	-
3	26	22,9 mm	25,8 mm	7,5 mm
4	21	8,4 mm	4,5 mm	-
5	26	20,1 mm	12,6 mm	5,5 mm
6	25	14,15 mm	8,6 mm	4,5 mm
7	21	9,3 mm	5,2 mm	14,0 mm
8	34	29,6 mm	24,7 mm	8,2 mm
9	21	22,7 mm	8,2 mm	-
10	21	24,35 mm	22,8 mm	6,5 mm

Source: Alexandra Dohnal, 2024

Days before parturition based on measurement of the ICC

Table 6. Calculation of DBP based on the size of the ICC according to Beccaglia, Groppetti et al., Luvoni and Grioni and Milani et al.

Patient ID	DBP based on ICC (Beccaglia)	DBP based on ICC (Groppetti et al.)	DBP based on ICC (Luvoni and Grioni)	DBP based on ICC (Milani et al.)	Actual DBP based on actual date of birth
1	42d	42d	42d	44d	39d
2	37d	38d	37d	40d	39d
3	32d	34d	33d	36d	40d
4	40d	41d	40d	43d	41d
5	34d	36d	34d	37d	35d
6	37d	38d	37d	40d	38d
7	40d	40d	40d	43d	41d
8	30d	31d	29d	32d	29d
9	33d	34d	33d	36d	28d
10	32d	34d	32d	35d	33d

d - days of pregnancy
Source: Alexandra Dohnal, 2024

Table 7. Accuracy rate of Beccaglia formula, accuracy rate of Groppetti formula, accuracy rate of Luvoni formula, accuracy rate of Milani formula and average accuracy of each formula expressed in % based on the measurement of ICC

Patient ID	Accuracy Beccaglia	Accuracy Groppetti et al.	Accuracy Luvoni & Grioni	Accuracy Milani et al.
1	92 %	92 %	92 %	87 %
2	95 %	97 %	95 %	97 %
3	80 %	85 %	83 %	90 %
4	98 %	100 %	98 %	95 %
5	97 %	97 %	97 %	94 %
6	97 %	100 %	97 %	95 %
7	98 %	98 %	98 %	95 %
8	97 %	93 %	100 %	90 %
9	82 %	79 %	82 %	71 %
10	97 %	97 %	97 %	94 %
Average accuracy	93 %	94 %	94 %	91 %

Source: Alexandra Dohnal, 2024

Days before parturition based on measurement of the BP

Table 8. Calculation of DBP based on the size of the BP parameter according to Beccaglia et al.

Patient ID	DBP based on BP (Beccaglia et al.)	Actual DBP based on actual date of birth	Accuracy Beccaglia et al.
3	31d	40d	78 %
5	34d	35d	97 %
6	35d	38d	92 %
7	21d	41d	51 %
8	30d	29d	97 %
10	32d	33d	97 %
Average accuracy			85 %

d - days of pregnancy
Source: Alexandra Dohnal, 2024

Days before parturition based on measurement of the CRL

Table 9. Calculation of "Days after LH peak" by Luvoni and Beccaglia based on the size of CRL, converted values to DBP and actual DBP based on the known date of birth. (Luvoni and Beccaglia, 2006)

Patient ID	Days after LH peak (Luvoni and Beccaglia	Converted values to DBP	Actual DBP based on actual date of birth	Accuracy
1	27d	35d	39d	90 %
2	28d	32d	39d	82 %
3	34d	32d	40d	90 %
4	26d	36d	41d	88 %
5	31d	30d	35d	86 %
6	28d	35d	38d	92 %
7	26d	36d	41d	88 %
8	34d	29d	29d	100 %
9	28d	35d	28d	75 %
10	33d	28d	33d	85 %
Average accuracy				88 %

d - days of pregnancy
Source: Alexandra Dohnal, 2024

ly during the later stages of gestation, as the entire foetus could not be completely fitted within a single frame.

Accuracy of DBP prediction based on measurement of the ICC

Following the measurements of the ICC, we proceeded to calculate the average accuracy rate of the formulas proposed by the different authors, aiming to compare them with the actual DBP based on the exact date of mating.

The analysis demonstrated that the formulas suggested by Luvoni and Grioni [12] and Groppetti et al. [5] were the most accurate. Both formulas exhibited an average accuracy rate of 94 %, which suggests that they are particularly reliable in estimating the DBP and prediction of parturition. It is however essential to take into consideration the fact that all four formulas yielded an average accuracy rate exceeding 90 %, where formula suggested by Beccaglia [1] yielded an average accuracy of 93 % and formula by Milani et al. [13] yielded an average accuracy of 91 %.

The high average accuracy rates of all the formulas, surpassing the 90 % threshold, may be attributed to the fact that the parameters were measured during the first half of gestation (between day 21 and 34 of pregnancy), which lasted till day 37 as suggested by Beccaglia et al. [1]. Beccaglia et al. [1] also states that the most accurate prediction based on the ICC parameter can be obtained during this time of gestation, which has been confirmed by our results.

It is further suggested by Luvoni [10] that the most accurate prediction can be done, when the bitch is examined between day 20–30 or day 31–43 of gestation.

Measurement of particularly patient number 8 was performed during a later stage of gestation (on day 34), in comparison to the other patients. A 100 % accuracy rate was calculated using the formula proposed by Luvoni and Grioni [12], which suggest that this statement is correct.

However, patient number 9 exhibited the lowest accuracy rates across all of the formulas, varying from 71–82 %, despite the fact that the measurement was obtained during the first half of pregnancy, on day 21, as suggested by the previously mentioned authors. These lowered accuracy rates may be attributed to several factors including the litter size, as suggested by Eilts et al. [2], but also other variations in the canine physiology. Other factors that may have lowered the accuracy include subjective interpretation and difficulty in obtaining precise measurements of this parameter. However, it seems less likely that the accuracy rates stem directly from the formulas themselves, given the fact that all formulas exhibited lower accuracy rate. Therefore, it is more probable that the discrepancies arise either from the measurement technique or inherited physiological variations of the dog itself, in this particular patient.

Accuracy of DBP prediction based on measurement of the foetal CRL

Another method employed in this study to determine the DBP and compare them with the reality, was the measurement of the foetal CRL, followed by the use of formula proposed by Luvoni and Beccaglia [11]. The accuracy rate of this parameter ranged between 75–100 % with an average accuracy rate of 88 %. Once again, it was suggested by different authors including Beccaglia et al. [1] that this measurement should be obtained during the first half of pregnancy. However, Siena and Milani [19] states that the measurement of the CRL should not be performed earlier than day 26, and later than day 45 of pregnancy, as the body of the foetus undergoes a change and the measurement is less accurate. Siena and Milani [19] further states that the optimal time for the CRL measurement is on day 30 of gestation. This statement finds support in our study as evidenced by the lowest accuracy rates observed in Table 9.

Patient number 9, where the measurement of CRL was conducted on day 21 of gestation, exhibits an accuracy rate of 75 %. Additionally patient number 2, also measured on day 21 of gestation, exhibits the second lowest accuracy at 82 %. This statement can also be viewed from the other perspective, where the patient number 8, who was measured on day 34 of gestation, shows a 100 % accuracy rate, followed by patient number 6, measured on day 25 of gestation, exhibiting the second highest accuracy rate of 92 %. Based on our observation, it appears that the accuracy of predictions increases as we approach day 30 of pregnancy. Despite this, there are patients in our study who do not support this statement. A comparison of patient number 3 and patient number 5 can be done, where both were measured on day 26 of pregnancy, however a difference was observed in these patients, where number 3 yielded a higher accuracy by 4 % (90 %). This lower accuracy rate can once again be attributed to different factors including the

litter size and physiological differences among dogs, maybe even breed and size, as patient 3 is a Golden Retriever and patient 5 is a Sheltie.

In general, the lower average accuracy rate of 88 % in our study may not only be attributed to the timing of the measurement before day 30 of pregnancy, several other factors may have contributed to this trend. For instance, the positioning and movement of the foetus during the ultrasonographic examination can significantly affect the clarity and precision of the measurement of the CRL. Additionally, during the second half of gestation, after day 37, it may become more challenging to capture the entire foetus in a single frame due to its larger size and increased mobility. Hence, the timing timing of measurement of this parameter is crucial.

Accuracy of DBP prediction based on measurement of the foetal BP

The third and final method utilized in this study for determining the DBP and subsequently comparing it with the actual DBP involved the assessment of the BP.

Measurement of the BP parameter was conducted in only 6 out of the 10 females participating in this study and a 85 % average accuracy rate was calculated. This limitation was a result of the fact that the ultrasonographic examinations of the bitches were performed during an early stage of pregnancy, which made it challenging to obtain this parameter. Furthermore, even among patients in whom the measurement was performed, its precision was compromised, as indicated by the results in Table 8. In comparison to the two other parameters used in this study, which each yielded at least one 100 % match with the actual DBP, the Biparietal parameter did not achieve a single instance of perfect alignment with reality. We believe that this discrepancy primarily stems from the fact that the measurements were obtained early in gestation, spanning from day 21 to day 34 as indicated in Table 5.

According to Siena and Milani [19], the optimal time for obtaining the Biparietal parameter is from day 30 of gestation, with even better results anticipated if the measurement is acquired from day 35 and later, when the parietal bones are more visible.

In our study, a relatively clear trend can be seen which indicates that the accuracy of the parameter is higher as the gestational length progresses. This suggests that measurements obtained closer to day 30 of pregnancy yield more accurate results. This phenomenon can particularly be seen in patient number 5, 6 and 8, where the accuracy rate is above 90 %. However, in two of the patients (7, 10), BP was measured on day 21 of gestation. In patient 7, a 51 % accuracy was calculated, and in patient 10, a 97 % accuracy rate was calculated despite the fact that the measurement was performed early during the pregnancy. This suggests that a larger sample size would enhance the objectivity and, more importantly, the accuracy of the study. However, our study was limited in this regard, particularly concerning the Biparietal parameter, as it was only measured in 6 out of the 10 dogs.

Our study further indicates the importance of utilizing the BP later in gestation, aligning with the recommendations of previously mentioned authors. This suggests that employing the parameter closer to the latter stages of gestation enhances the precision of predicting the parturition.

CONCLUSION

In conclusion, this study investigated the accuracy of formulas proposed by Beccaglia et al., Luvoni and Grioni, Milani et al., Groppetti et al., and Luvoni and Beccaglia for estimating DBP in dogs, utilizing three different parameters including the Inner chorionic cavity (ICC), foetal Crown-to-rump length (CRL) and the Biparietal parameter (BP) obtained through ultrasonographic examination.

Our analysis revealed that the measurement of the Inner chorionic cavity (ICC) achieved the highest accuracy rate when utilizing the formulas of Luvoni and Grioni, and Groppetti et al., exhibiting a 94 % accuracy rate. Following were the formulas proposed by Beccaglia et al. with a 93 % accuracy rate and Milani et al. with a 91 % accuracy rate. These findings suggest that measuring the Inner chorionic cavity during the early stages of pregnancy can serve as a reliable method for predicting parturition.

Furthermore, our study revealed that the CRL measurement exhibited an accuracy rate of 88 %, slightly lower than that of the ICC. Importantly, our findings highlighted the significance of timing in obtaining accurate measurements. Specifically, measurements taken closer to day 30 of pregnancy demonstrated a higher accuracy rate, aligning with recommendations from Siena and Milani.

Lastly, the BP demonstrated an average accuracy rate of 85 %, which was the lowest among the three parameters. This may be attributed to the fact that the majority of measurements were obtained before day 30 of gestation, when the parietal bones were less visible. Additionally, the lower accuracy rate could be influenced by the limited amount of patients, as we performed this measurement in only 6 out of the 10 females. Notably, accuracy tended to increase in patients closer to the day 30 of gestation.

Ultimately, our study highlights that the most accurate prediction of parturition can be determined by the measurement of the Inner chorionic cavity (ICC) during early gestation. Furthermore, our findings emphasize the importance of timing in obtaining accurate measurements, especially with regards to the BP and CRL, aligning with recommendations from prior research. Further investigation into refining measurement techniques could benefit from considering variables such as weight categories, breed of the dogs and size of the litter. By exploring these factors, we may gain a deeper insight into how variations in canine physiology influence the accuracy of prenatal measurements and their predictive value for parturition. Such a comprehensive approach could contribute significantly to enhancing the precision and reliability of early gestational assessments in dogs.

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CONFLICT OF INTEREST

We declare that all listed authors are without a Conflict of Interest.

DATA AVAILABILITY STATEMENT

The raw data of this article will be made available by the authors, without undue reservation.

ETHICAL STATEMENT

This study did not require any Ethical approval.

AUTHORS CONTRIBUTIONS

Ľ. H.: Conceived and designed the study, developed the research methodology, supervised the project execution, contributed to defining the research objectives and study parameters; N. V.: performed ultrasonographic examinations, collected measurements (ICC, BP, CRL), ensured data accuracy and consistency, assisted in refining the methodology based on initial findings; A. D.: conducted statistical analyses, compared predictive formulas with actual parturition dates, interpreted the accuracy of different methods, played a key role in data processing and validation; G. K.: conducted literature review, provided background context, contributed to the interpretation of findings, assisted in drafting the introduction and discussion sections; A. V.: drafted the manuscript, ensured scientific rigor, clarity, and coherence, provided insights into improving data presentation and refining conclusions; S. H.: critical revision of the manuscript, final editing, supervision, project administration, coordinated team efforts, provided final approval for manuscript submission and managed funding acquisition. All authors contributed significantly to the study and approved the final version of the manuscript.

GENERATIVE AI STATEMENT

The authors declare that no Gen AI was used in the creation of this manuscript.

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