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Original article

Effectiveness of determining the parturition date in bitches using the ultrasonographic fetometry as compared to hormonal and cytological methods

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Abstract

In clinical practice, the parturition date in bitches is calculated on the basis of the established ovulation date, using determination of the level of LH and P4. Additionally cytological examination of vaginal smears makes it possible to establish the onset of diestrus, from which the delivery occurs after about 57 days. The latest method used in bitches for this purpose is foetal biometry. Measurements of the inner chorionic cavity diameter (ICC) and biparietal diameter (BP) with formulas published by Luvoni and Grioni have been popularized and the most practically useful. In a group of six bitches the effectiveness obtained with the use of ICC was 66.67% with an accuracy of 1 day and 100% with an accuracy of 2 days. BP measurements gave better results, as the accuracy was respectively 83.33% and 100%. The effectiveness of predicting the delivery date on the base of method consisting in determining the LH surge was 66.67% with an accuracy of 1 day and 100% with an accuracy of 2 days. The best effectiveness was obtained while using the method based on P4 level determination (100% both with an accuracy of 1 day, as well as of 2 days). The lowest accuracy was obtained while using the cytological method. Generally, a comparison of the methods used for predicting the date of delivery in bitches confirms practical usefulness of fetometry. Its effectiveness was not lower than traditional methods used so far, while its advantage lies in the ease of performance and the long term of application during gestation. However, full confirmation of the practical usefulness of this method requires further research on a larger number of animals.

Key words: bitch, pregnancy, parturition, fetometry

Introduction

Pregnancy in the bitch, as compared to females of other species of animals, is short, since it lasts about 9 weeks. Its length, usually calculated as a interval from mating till delivery, ranges from 57 to 72 days (Concannon et al. 1983, 1989, Luvoni and Grioni 2000, Kutzler et al. 2003a, Eilts et al. 2005, Johnson 2008, Socha et al. 2008), which is related to the specificity of fertilization in this species (Concannon et al. 1975, Concannon et al. 1983, Concannon et al. 1989, Hoffmann et al. 1996).

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However, the opinion prevails that real gestational length in the bitch, related to the process of fertilization, is constant and amounts to about 65 ± 1 days as of the pre-ovulation surge of luteinizing hormone (LH). This phenomenon is also correlated with an increase in progesterone concentration (P4) to about 2 ng/ml (Concannon et al. 1975, Concannon et al. 1989, Luvoni and Grioni 2000, Root Kustritz 2001, Luvoni and Beccaglia 2006, Rota et al. 2007). It has been proven that ovulation, which lasts on average 12 hours, occurs after about 2 days of the described hormonal changes, while the delivery usually starts after 63 ± 1 days as of this process (Concannon et al. 1975, Concannon et al. 1989, England 1996, Kutzler et al. 2003a, Eilts et al. 2005, Luvoni and Beccaglia 2006). However, it seems that hormonal fluctuations in individual animals, as well as the breed, can affect the length of pregnancy (Okkens et al. 2001, Son et al. 2001, Eilts et al. 2005, Luvoni and Beccaglia 2006).

In clinical practice, the parturition date is calculated on the basis of the established ovulation date, using for this purpose determination of the level of luteinizing hormone or progesterone (England 1996, Kutzler et al. 2003a, Chapwanya et al. 2008). Additionally, cytological examinations of vaginal smears are used, making it possible to establish the onset of *diestrus*, from which the deliveries should occur after about 57 days (Holst and Phemister 1974, Niżański et al. 2003). As results from literature reports, none of the current methods of predicting the delivery date in bitches is fully efficient. They are additionally limited by certain restricted periods of application during the heat, inconvenience of performance and interpretation difficulties (Holst and Phemister 1974, Kutzler et al. 2003a, Luvoni and Beccaglia 2006).

The lack of information concerning the expected date of delivery makes it difficult to provide veterinary assistance in the optimal time for the bitch and the pups (Root Kustritz 2005, Luvoni and Beccaglia 2006, Kim et al. 2007). It is particularly important to avoid premature Caesarean sections in view of the immaturity of newborns delivered in this manner (Root Kustritz 2005, Johnson 2008, Socha et al. 2008). The problem of well-timed performance of the Caesarean section also arises in case of difficult deliveries in brachycephalic breeds, in bitches with damage of the pelvic canal bones and in singleton pregnancies beyond term (Concannon et al. 1989, Yeager et al. 1992, Eilts et al. 2005, Luvoni and Beccaglia 2006, Socha et al. 2008).

The need to solve the above-mentioned problems has provided an impulse for further research on the improved control of pregnancy in bitches to make it possible to predict the parturition date (England and Allen 1990, England et al. 1990, Luvoni and Grioni 2000, Luvoni and Beccaglia 2006). The latest method used in bitches for this purpose is foetal biometry, consisting of measuring the size of selected anatomic structures and parts of the foetal body. In the next step, after applying appropriate mathematical formulas taking into account the body weight of the examined animals, the expected delivery date is calculated (England et al. 1990, Luvoni and Grioni 2000, Son et al. 2001, Kutzler et al. 2003b, Beccaglia and Luvoni 2006, Luvoni and Beccaglia 2006, Kim and Son 2007, Lopate 2008). The above-described method is based on the assumption that the development of individual parts and organs of the foetus body during the pregnancy is linear and proportional (Nyland and Matton 1995, Moriyoshi et al. 1996, Luvoni and Grioni 2000, Son et al. 2001, Lenard et al. 2007). Previously published studies evaluated the usefulness of different biometric indicators, focusing on dogs of small and medium body weight (Yeager et al. 1992, Son et al. 2001, Kutzler et al. 2003b, Beccaglia and Luvoni 2006, Kim and Son 2007, Beccaglia et al. 2008, Lopate 2008). However, as a result of the current research, measurements of the inner chorionic cavity diameter (ICC) and biparietal diameter (BP) (Luvoni and Grioni 2000, Beccaglia and Luvoni 2006, Luvoni and Beccaglia 2006, Socha and Janowski 2009), have been popularized as the most practically useful. It should be added that this issue has not yet been fully explained and former research has revealed the variable effectiveness of foetal biometry (Beccaglia and Luvoni 2006, Lenard et al. 2007, Lopate 2008, Socha and Janowski 2011).

For these reasons, the aim of the study was to determine the accuracy of predicting the parturition date by ultrasonographic fetometry in comparison with the effectiveness obtained by previously established methods.

Materials and Methods

Experimental design

The study was performed on six clinically healthy, sexually mature bitches aged 3-4 years, crossbreds, with an average body weight of 15.5 kg (7-24 kg). Mating was performed during the period of controlled ovulation, between day 10 and 14 of heat by artificial insemination with fresh semen collected from dogs with a body weight similar to the body weight of the bitches. In all bitches, during *proestrus*, *estrus* and at the onset of *diestrus*, blood samples were taken twice a day (at about 8.00 a.m. and 8.00 p.m.) to determine the level of progesterone and luteinizing hormone. Additionally, vaginal smears were taken and cytologically assessed once a day, following classical diagnostic criteria. After confirming pregnancy in the fourth

week after insemination, animals were subjected to regular ultrasound examinations in order to obtain biometric data. In the gestational sac stadium (from about day 20 to 35 after ovulation), the inner chorionic cavity diameter (ICC) was measured and in the second half of the pregnancy, diameter of parietal bones of foetuses (BP) were measured according to the technique described by Luvoni and Grioni (2000). At the gestational sac stage, the examinations were performed every day and, after this time, every 2-3 days. The animals were treated and examined in accordance with the rules approved by the Local Ethics Commission (Ethic Commission Opinion No 1/2009)

Hormone assays

Progesterone level in blood plasma was determined using the radioimmunological method with extraction, according to Hoffmann et al. (1977) and the level of the luteinizing hormone in blood plasma was established with the use of a sandwich-type immunoenzymatic test (LH-Detect, Repropharm, Nouzilly, France).

The date of LH surge was considered to be the day in which this hormone reached its maximum value and the beginning of ovulation was determined at the moment when P4 exceeded 3.6 ng/ml (Luvoni and Beccaglia 2006). The expected delivery date was determined by adding 65 days to the LH surge date and 63 days to the ovulation date (Luvoni and Beccaglia 2006).

Cytological examination

In cytological examinations, vaginal smear preparations were stained using Shorr's method in Kubick's modification (Niżański et al. 2003). The evaluation of preparations taken to determine the first day of *diestrus* (IDD), commonly-known methods of cytological assessment (Niżański et al. 2003) were used while assessing the index of akaryotic cells, the presence of metestrum cells and leucocytes. The parturition date was calculated in this method by adding 57 days to the cytological ascertainment of *diestrus* (Holst and Phemister 1974, Niżański et al. 2003, Luvoni and Beccaglia 2006).

Ultrasonographic examination and measurements

Ultrasonographic examination of pregnant bitches was carried out with the use of Pie Medical 260 scanner with a convex probe 5-7.5 MHz (Maastricht, The Netherlands). The examinations performed during the experiment were based on standards commonly applied in veterinary ultrasonography. The examinations were made through the abdomen integument, along the white line, with the animal standing or lying down. Depending on the pregnancy stage, proper anatomic structures were identified and measurements of inner chorionic cavity diameter (ICC) or biparietal diameter (BP) were measured (Luvoni and Grioni 2000, Luvoni and Beccaglia 2006).

The results of ICC and BP measurements were recorded in millimetres, after which the delivery date was calculated with Luvoni-Grioni formulas (Luvoni and Grioni 2000, Beccaglia and Luvoni 2006, Luvoni and Beccaglia 2006), taking into consideration the body weight of the experimental bitch. The following formulas were used:

- small bitches (≤ 10 kg);
  - ICC: number of days before parturition (negative value) = (mm – 68.68)/1.53,
  - BP: number of days before parturition (negative value) = (mm – 25.11)/0.61
- medium bitches (10-25 kg);
  - ICC: number of days before parturition (negative value) = (mm – 82.13)/1.8
  - BP: number of days before parturition (negative value) = (mm – 29.18)/0.7

The study involved comparison of the differences between the predicted and the actual delivery date with reference to all prediction methods applied. The percentage of correct results with an accuracy of 1 or 2 days was determined, where the accuracy stands for the absolute value of the difference.

Results

The effectiveness obtained with the use of ICC measurements with an accuracy of 1 day was 66.67%, while the 100% correctness was achieved with an accuracy of 2 days. BP measurements gave better results, as the accuracy was respectively 83.33% and 100%. For ICC, the mean difference between the predicted and the actual parturition date ranged from -2 days to -1 day, while when applying BP it was between -2 and +1. The mean accuracy in case of ICC was between 2 days and 1 day, while with the application of BP the value of this indicator ranged from 3 days to 1 day. The effectiveness of predicting the delivery date on the basis of the method consisting in determining the LH surge was 66.67% with an accuracy of 1 day and 100% with an accuracy of 2 days (Table 1). Differences between the predicted and the actual date ranged from -2 days (bitch 3 and 5) to +1 day (bitch 4). In the remainders, no differences were observed be-

Table 1. Effectiveness of determining the parturition date in bitches using hormonal, cytological methods and ultrasonographic fetometry.

Animal's number	Delivery date	LH			P4			IDD			ICC	BP
		examination date	predicted date	D	examination date	predicted date	D	examination date	predicted date	D	MD	MD
1	02.01.09	29.10.08	02.01.09	0	31.10.08	02.01.09	0	07.11.08	03.01.09	1	-1	0
2	17.03.09	11.01.09	17.03.09	0	12.01.09	16.03.09	-1	18.01.09	16.03.09	-1	-2	-2
3	29.03.09	21.01.09	27.03.09	-2	24.01. 09	28.03.09	-1	30.01.09	28.03.09	-1	-1	1
4	17.09.09	15.07.09	18.09.09	1	16.07.09	17.09.09	0	25.07.09	20.09.09	3	-1	-1
5	07.02.10	02.12.09	05.02.10	-2	05.12.09	06.02.10	-1	09.12.09	04.02.10	-3	-2	-1
6	22.05.09	18.03.09	22.05.09	0	20.03.09	22.05.09	0	28.03.09	24.05.09	2	-1	-1
Percentage (%) of results with the accuracy ± 1				66.67			100.00			50.00	66.67	83.33
Percentage (%) of results with the accuracy ± 2				100.00			100.00			66.67	100.00	100.00

LH – luteinizing hormone, P4 – progesterone, IDD – first day of *diestrus,* ICC – inner chorionic cavity diameter, BP – biparietal diameter, D – difference, MD – mean difference

tween predicted and the actual delivery date. The method based on P4 level determination in the periovulatory period, both with an accuracy of 1 day, as well as of 2 days, revealed 100% effectiveness (Table 1). In the case of three bitches, the predicted parturition date turned out to be premature by only 1 day, but in the other three animals, this date coincided with the actual date of delivery. The lowest effectiveness was obtained while using the cytological method. Assuming a precision of 1 day, its accuracy was only 50%, while at a precision of 2 days, it increased to 66.67%, which was still lower than the results obtained while using other methods (Table 1). In two bitches (bitch 4 and 5), the difference between the predicted date and the actual date was 3 days (both *in plus* and *in minus*) and in one case (bitch 6), this error amounted to 2 days; only in three bitches (bitches 1, 2 and 3) this difference was only 1 day.

Discussion

The main aim of the study was to assess the usefulness of prediction the parturition date using ultrasonographic fetometry. The results obtained in this part of the research were good or even better than those obtained by other authors. Kutzler et al. (2003b) while applying the method combined several other parameters obtained 63% effectiveness with an accuracy of 1 day. However, those authors did not take into consideration the body mass of the examined dogs. On the other hand, Kim et al. (2007) reported the higher effectiveness of the fetometric method, amounting to 75% with an accuracy of 1 day, using simultaneously measurements of the diameter of the foetus body and its length. Nevertheless, at the accuracy of 2 days, those authors obtained a result of 87%, which is lower than the 100% accuracy obtained in the present study. Similarly, Lenard et al. (2007), using the measurement of two parameters in early and late pregnancy, obtained a result of 70.8% with an accuracy of 1 day and 86.1% with an accuracy of 2 days. Undoubtedly, the simultaneous use of two parameters in those studies improved the effectiveness of predicting the date of delivery. On the other hand, Yeager et al. (1992), examining only Beagle bitches, obtained a high effectiveness of 74.6% with an accuracy of 1 day and 91.9% with an accuracy of 2 days. This could indicate that the effectiveness of the method may depend on the breed homogeneity of the examined population as well. Own results also correspond to those obtained by Beccaglia and Luvoni (2006). Those authors, taking into account an accuracy of 1 day, obtained an effectiveness between 69.5% for BP to 76.8% for ICC and at the accuracy of 2 days, 85% and 86.3%, respectively.

The authors of all cited publications unanimously emphasize the higher effectiveness of measurements in early pregnancy (ICC), in comparison to results obtained in the third trimester (BP) (England et al. 1990, Yeager et al. 1992, Luvoni and Grioni 2000, Son et al. 2001, Kutzler et al. 2003 b, Beccaglia and Luvoni 2006, Luvoni and Beccaglia 2006, Kim and Son 2007, Socha and Janowski 2010, Socha and Janowski 2011). However, this dependency was not confirmed in the present study. The research on this topic requires a larger study population, which would allow better assessment of the usefulness of the examined indicators. However, the analysis of the usefulness of both parameters used in own research is promising and

encourages further ultrasonographic research on the development of pregnancy and the use of fetometry for prediction of the delivery date. Such research should particularly include individual and population studies on the organogenesis of morphological structures assessed in ultrasonographical biometry. An additional practical advantage of this method is the possibility of its application with high effectiveness for almost the entire duration of the gestation, from implantation to the delivery. This is confirmed both in own research, as well as in the literature cited (Luvoni and Grioni 2000, Son et al. 2001, Beccaglia and Luvoni 2006, Luvoni and Beccaglia 2006, Socha and Janowski 2010).

The present study additionally compared the effectiveness of predicting the delivery date in bitches using the already established methods. Three of them, i.e. determination of the parturition date on the basis of the level of LH or P4 and the cytological onset of *diestrus*, have been known for long time (Holst and Phemister 1974, Concannon et al. 1989, England 1996, Kutzler et al. 2003 a, Eilts et al. 2005, Luvoni and Beccaglia 2006, Chapwanya et al. 2008). Those methods have been so far commonly regarded as practically useful, but in most studies, the authors did not consider the clinical accuracy. On the other hand, own research revealed varied precision levels of studied methods ranging from 50% to 100% while assuming an accuracy of 1 day and 66.67% – 100% with the accuracy of 2 days. It was also demonstrated that the best results of delivery prediction were obtained while determining the ovulation date using P4 measurements and the worst while determining the onset of *diestrus* by the cytological method. Intermediate results were obtained while establishing ovulation time on the basis of LH determinations. They were similar to the results of the biometric method, which had a much broader scope of application.

It is difficult to explicitly refer to some results obtained by hormonal and cytological methods, since the available literature lacks studies comparing them on the same population of dogs. In own research, 100% accuracy obtained by determining the progesterone level proved to be surprisingly good, since other authors applying the same method obtained worse results. Kutzler et al. (2003a), while assuming the criterion of 1 day accuracy, reached only 67% effectiveness. Only at the accuracy of 3 days those authors obtained 100% effectiveness. However, it should be emphasized that 1-2 day accuracy in predicting the parturition date, although not ideal, seems to be sufficient in veterinary practice for supervising deliveries (Nyland and Matton 1995, Root Kustritz 2005, Kim and Son 2007, Kim et al. 2007, Johnson 2008, Lopate 2008, Socha et al. 2008). This results from the fact that parturition in bitches, generally, lasts relatively long, and additionally, there are high individual differences of its duration. Due to this we assumed two levels of accuracy in own research, which is consistent with the opinions of other authors (Luvoni and Grioni 2000, Kutzler et al. 2003b, Beccaglia and Luvoni 2006, Luvoni and Beccaglia 2006, Lopate 2008). The method of predicting the delivery date on the basis of periovulatory measurement of the progesterone level is commonly considered to be highly practical and recommendable (England 1996, Root Kustritz 2001, Kutzler et al. 2003a, Luvoni and Beccaglia 2006). Its unquestionable advantages include high stability of secretion of this hormone, high resistance of progesterone to biochemical decomposition and its specific prognostic importance for the ovulation process (Concannon et al. 1975, Luvoni and Beccaglia 2006, Rota et al. 2007, Chapwanya et al. 2008). Easy determination of this hormone is also an evident advantage of the method (Conannon et al. 1975, Rota et al. 2007, Chapwanya et al. 2008). On the other hand, its disadvantages include the extremely short time in which blood samples should be taken, around mating. Also, the necessity of frequent sampling, which involves the need for frequent visits at the clinic, is a significant impediment (Concannon et al. 1975, England 1996, Hoffmann et al. 1996, Luvoni and Beccaglia 2006, Rota et al. 2007, Chapwanya et al. 2008).

In the current study the accuracy of measurement, and calculation of the parturition date with the use of the LH secretion was surprisingly poorer than results obtained by other authors (Concannon et al. 1975, Nishiyama et al. 1999, Root Kustritz 2001, Luvoni and Beccaglia 2006). This method was effective only in 66.67%, at the assumption of 1 day accuracy. On the other hand, assuming the accuracy of 2 days, the present results and results described in the literature (Root Kustritz 2001, Luvoni and Beccaglia 2006) were similar reaching even 100%. Despite initially lower effectiveness, prediction of parturition with the use of LH levels, in combination with its clinical symptoms, made it possible to efficiently supervise deliveries in all of the controlled cases. Nevertheless, it should be added that pulsating LH secretion is characterized by a high individual variability (Concannon et al. 1975, Hoffmann et al. 1996, Root Kustritz 2001, Luvoni and Beccaglia 2006), which probably also occurred in own research in case of some experimental animals (No. 3 and 4). A drawback of the described method is the difficulty of luteinizing hormone determination, a problem which is much higher than in the case of progesterone. This refers both to the need for taking blood samples

more frequently (at least every 12 hours) and to the low stability of LH in the blood samples taken, requiring the application of enzymatic blockers. Additionally, due to the species; specificity of this hormone, its determination is possible only in specialised laboratories. Altogether, this reduces the practical usefulness of this method and is the reason why delivery day calculation on the basis of LH determination is performed in research studies rather than in veterinary practice (Nyland and Matton 1995, Root Kustritz 2001, Luvoni and Beccaglia 2006).

Cytological determination of the onset of *diestrus* proved to be the least useful of the tested methods (50% – 67% effectiveness). This result is somewhat surprising in view of the fact that this method is frequently recommended in textbooks manuals, without any explicit reservations concerning its accuracy (Holst and Phemister 1974, Niżański et al. 2003). However, a more detailed analysis of experimental studies shows that cytological determination of the delivery date in the research of other authors, was also related to lower effectiveness as expected (Holst and Phemister 1974, Luvoni and Beccaglia 2006). Those studies demonstrated that 80% bitches gave birth on the expected date, 57 ± 1 days as of the beginning of *diestrus*. The existence of discrepancies concerning delivery date prediction is emphasized, which can reach up to several days. According to that research, the delivery can occur after 51-60 days as of the first day of *diestrus* (Holst and Phemister 1974, Niżański et al. 2003, Luvoni and Beccaglia 2006). Such high differences may result from an individual course of cytological changes in individual animals, different frequency of smear taking, as well as lack of standardization of staining techniques and subjective assessments of microscopic images of cytological changes (Holst and Phemister 1974, Niżański et al. 2003). Also the need for taking samples at least once a day is emphasized, which causes certain inconvenience for the animal owners (Holst and Phemister 1974, Niżański et al. 2003). A relatively low effectiveness of the cytological method for determining the parturition date shows that it should be used only as an auxiliary element (Holst and Phemister 1974, Luvoni and Grioni 2000, Niżański et al. 2003, Luvoni and Beccaglia 2006, Lopate et al. 2008).

Generally, a comparison of the methods used for predicting the date of delivery in bitches confirms practical usefulness of fetometry. Its effectiveness was not lower than traditional methods used so far, while its advantages are the ease of performance and the long term of application during gestation. However, full confirmation of the practical usefulness of this method requires further research on a larger animal group.

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