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Predicting Whelping Day with Early Pregnancy Ultrasonography and Induction of Parturition using Mifepristone on the Expected Delivery Date in Bitches

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ABSTRACT

Background: Inducing whelping in canines is becoming an accepted practice in obstetrical management to ensure safe pregnancy outcomes, although pinpointing the exact day for induction poses challenges. The precise timing of delivery is vital for foetal survival, as the placenta's nutrient supply cannot sustain the foetus beyond the expected delivery date (EDD). Additionally, completing the full gestation term is crucial for comprehensive organ development, especially for the maturation of the lungs.

Methods: The present retrospective observational study was aimed to evaluate the accuracy of whelping day prediction by early pregnancy ultrasonography and to propose a viable whelping induction protocol with mifepristone on the EDD to improve the overall reproductive efficiency in canines.

Result: Among 115 pregnancies in Group I, 69.57% whelped exactly on the predicted day (±0), 93.04% whelped within ±1 day and 100% within ±2 days. In Group II, among 124 pregnancies 69.35% whelped on or before the EDD. Of the 38 bitches that did not whelp by the evening of EDD and which were given two doses of mifepristone, 73.68% whelped within 24 h. An additional 4 of the remaining 10 bitches whelped within 36 hours, resulting in an overall whelping induction response of 84.21% and a cumulative vaginal birth rate of 95.16%. Six bitches (4.84%) required a caesarean section (CS) 36 h post-induction. The percentages of normal whelping, dystocia and CS varied significantly across Groups I, II and III, with Group II showing a significant reduction in dystocia and caesareans, supporting the hypothesis of inducing whelping with mifepristone on the prediction day to have more vaginal births. The percentage of animals that had CS in Groups I, II and III were 14.78, 4.84 and 56.67, respectively. The perinatal mortality rates were 14.83%, 10.47% and 25.15% in Groups I, II and III, respectively, with Group II showing significantly better puppy survivability.

Key words: Mifepristone induction, Ultrasonography, Whelping prediction.

INTRODUCTION

The relatively short duration of canine pregnancy makes early and accurate pregnancy detection crucial for effective management of gestation, whelping and neonatal care. Transabdominal ultrasonography is an excellent tool for pregnancy diagnosis, assessment of foetal growth rate, development of foetal organs, viability of conceptuses and prediction of day of whelping. It is also valuable in predicting the survival chances of neonates after whelping and planning a caesarean section. Although significant development of most foetal organs occurs in the final days of pregnancy, puppies are born in an immature state, with many organs completing their development in the early neonatal period. Intrauterine death can occur if the foetus exceeds its due date by even one or two days, as the canine placenta cannot meet the increased nutritional demands of the foetus. Therefore, effective management of canine pregnancy involves ensuring that the conceptuses reach, but do not surpass, their optimal gestational age (GA) at the time of whelping.

England (1998) observed that the canine embryos grew at a linear rate of 1 mm in diameter per day from 17 to 30 days after LH surge and that after 30 days the growth was exponential. An accurate prediction of whelping date could be made by ultrasonographic measurement of inner chorionic cavity (ICC) diameter irrespective of litter size and maternal body weight, provided that correction factors were made for small and giant breeds (Kutzler *et al*., 2003a, 2003b). Beccaglia and Luvoni (2006, 2012) demonstrated that a similar accuracy (P>0.05) was obtained by measurement of ICC at weeks 4 and 5 of pregnancy (± 1d, 81% vs. 67.7%; ±2d, 93.1% vs. 85.9%) using Luvoni and Grioni (2000) equations. Groppetti *et al.* (2015) demonstrated that a breed-specific linear expression model for estimating parturition date in German Shepherd dogs based on ICC had higher accuracy than previously reported (94.4%). In another study in Toy Poodle bitches, Xavier *et al.* (2024)

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reported a strong correlation (r = 0.97 and R^2 = 0.93, p<001) between BPD and DBP. However, considering the total number of different breeds of dogs, the practicability of breed-specific formulae for all breeds of dogs would be a cumbersome option.

Simon (2015) compared prediction accuracy between GA≤30 d and GA=31-37 d and suggested that at ± 2 d, 100% of bitches whelped when predictions were based on GA≤30 d, while only 81.82% of bitches whelped at ±2 d when predictions were based on GA=31-37 d. Simon *et al.* (2017) assessed the clinical efficacy of mifepristone in inducing whelping in advanced pregnant bitches with a response rate of 95.83%.

The gestation length from pre-ovulatory LH surge in canines has been defined to be 65±1 day (Concannon and Rendano, 1983). Elective C-section has been suggested, when prepartum progesterone (P4) drop has occurred, as a safe method (even when LH surge is unknown) ensuing puppy survivability (Smith, 2007). However, this involves a larger number of hospital visits and expenses for the breeder since a series of daily P4 evaluations have to be performed. Induction of whelping with antiprogestins like aglepristone (Fieni *et al.,* 2001) or mifepristone (Simon *et al.,* 2017) in dogs in advanced pregnancy has been proposed to be a good alternative to surgical management. Agleprisone when used in planned C-sections, none of the puppies showed any external signs of prematurity (Roos *et al*., 2018).

Many experienced breeders and even novice breeders are opting for a safer whelping induction protocol with reasonable live births so that caring for the dam and puppies becomes easier and one can avoid post-operative care and more visits to the veterinarians. Moreover, such a protocol would conserve the breeding efficacy of bitches in terms of more whelping and litter size when compared to consecutive C-sections.

The overall objective of this study was to assess the accuracy of the prediction of whelping day based on early pregnancy ultrasonography (Simon, 2015) and to suggest a viable whelping induction protocol with mifepristone on the expected delivery date (EDD) if needed. The data obtained concerning normal whelping (eutocia), dystocia, C-section, litter size and stillbirth were compared in different clinical situations.

MATERIALS AND METHODS

A retrospective observational study and analysis were performed during the period from June 2018 to December 2022 in 269 pregnancies of different breeds, weighing between 4 to 60 kg and between one to seven years of age under three different situations.

Group I (Preliminary study)

In this group, we have included 115 pregnancies and the study was performed from June 2018 to December 2019 to evaluate the accuracy of prediction of whelping based on combined CRL and ICC measurements during early pregnancy (before 30 days of gestation from LH surge). The study included the following breeds: Shih-Tzu (n=27), Beagle (n=18), Pug (n=16), German Shepherd (n=7), Golden Retriever (n=7), Dobermann (n=6), Dachshund (n=6), Staffordshire Bull Terrier (n=6), American Bully (n=5), Siberian Husky (n=5), Rottweiler (n=4), Labrador (n=3), Lhasa Apso (n=2), Bull Mastiff (n=1), St. Bernard (n=1) and Dalmatian (n=1). Accuracy of prediction of day of whelping based on combined ICC and CRL was assessed in this group. Descriptive statistics regarding rectal temperature and serum progesterone were assessed in at least one bitch from each breed (n=16) on ±0 day normal whelping.

Group II

In this group, we have included 124 pregnancies from January 2020 to December 2022 and those animals that did not whelp until the evening of EDD were examined clinico-gynaecologically. The study included following breeds: Beagle (n=16), Shih-Tzu (n=14), Rottweiler (n=12), Labrador (n=11), Golden Retriever (n=11), German Shepherd (n=10), Pug (n=9), Dachshund (n=7), Staffordshire Bull Terrier (n=6), American Bully (n=9), Dobermann (n=4) and Siberian Husky (n=4), Spitz (n=3), Lhasa Apso (n=3), St. Bernard (n=2), Mixed breeds (n=2) and Dalmatian (n=1). Animals included in Groups I and II were presented for exfoliative vaginal cytology (EVC) and breeding advice was given based on the anuclear cell index (England, 1992; Simon, 1997; Simon *et al*., 1998). Two natural matings were carried out in all bitches at three days intervals. Multivitamin and mineral tablets were supplemented per os to all animals from three days before first mating and continued throughout pregnancy and lactation. Similarly, per os supplementation of folic acid was made during early pregnancy, while haematenic was provided from mid-gestation. From three days prior to whelping calcium supplementation was given per os and was continued until weaning of puppies. The bitches in Groups I and II were subjected to early pregnancy diagnosis by ultrasonography on or before 24 d from the last breeding. The ultrasonographic examinations were performed with MyLab One Vet (Esaote, Italy) ultrasound machine provided with a multifrequency micro convex transducer. The frequency of the multifrequency probe was adjusted to 6 MHz for early pregnancy echobiometry. GA and EDD were calculated based on ICC and Crown rump length (CRL). ICC was recorded as the mean of two ICC diameters (a) latero-lateral ICC (b) dorso-ventral ICC (Fig 1), while CRL was measured as the total length of the embryo in a straight line from the rostral most point of the crown to the base of the tail or usually as the total length of the embryo up to GA=29 days (Fig 2). At least two gestational sacs and two CRL measurements were taken wherever possible. Animals with a gestational age of 30 days or less from the LH surge, as determined by ultrasonography, were included in Groups I and II. Animals with a GA of more than 30 days were excluded from the study to maintain prediction accuracy. The machine was equipped with in-built software for calculating GA and EDD based on combined ICC and

CRL. EVC and ultrasonography were performed by the first author for all the bitches included in Groups I and II. The following parameters were recorded for all bitches; age, prepartum body weight, date of whelping, time taken for induction, litter size, stillbirth and early neonatal mortality within 7 days. Rectal temperature, vaginoscopic examination (with a sigmoidoscope or borescope), B-mode ultrasonographic examination and per vaginal digital examination were performed in those bitches that did not whelp until the evening of EDD. Doppler ultrasonographic evaluation of foetal arteries, maternal arteries and foetal heart rate oscillations was performed to evaluate viability of foetuses and maternal perfusion (Gil *et al.,* 2014; Giannico *et al*., 2016; Simon *et al*., 2022)

In Group II when a vaginoscopic examination was performed with a sigmoidoscope/borescope, an assessment of cervical relaxation, plug lysis and the presence of a foetal bag was made. In cases where the foetus/foetal bags were presented, no medications to induce whelping were given usually. However, in other cases where no signs of relaxation were evinced or cervical plug lysis alone was noted or when a clear mucoid discharge was present without greenish colour, whelping was induced with two doses of mifepristone @ 3.5-5 mg/kg at 8 h intervals (2 or 3 doses) (Simon *et al*., 2017; Kumar *et al*., 2022). Misoprostol was advised @ 10 mcg/kg twice daily based on a vaginoscopic examination which confirmed cervical dilatation/plug lysis or after seeing a profuse vaginal discharge. The induction of whelping was attempted only in those animals with foetal heart rates (FHR) above the critical limits and foetal head diameters posing no threat for normal whelping by way of foeto-maternal disproportion. Moreover, when a puppy with a head diameter above critical limits was present as the posterior most in either of the horns, more frequent examinations were made, since that situation posed more threat than when such foetuses were in the mid to anterior part of the horns. A C-section was performed in non-responsive animals after 36h of induction.

Group III (Control group)

The control group included 30 bitches which were not presented for early pregnancy ultrasonography and the pet parents presented them after the expected whelping day calculated based on breeding dates or since the bitches were exhibiting straining due to difficulty in whelping or greenish discharge shown. The study included the following breeds: Beagle (n=6), Labrador (n=4), Pug (n=3), Rottweiler (n=3), Spitz (n=3), German Shepherd (n=2), Dachshund (n=2), American Bully (n=2), Staffordshire Bull Terrier (n=1), Golden Retriever (n=1), Mixed breeds (n=1), Shih-Tzu (n=1) and Siberian Husky (n=1). Detailed clinico-gynaecological, B mode and Doppler ultrasonographic evaluations and vaginoscopic examinations were performed in all cases. Those animals proceeding for normal parturition were allowed to whelp normally while dystocias were managed medically or by CS.

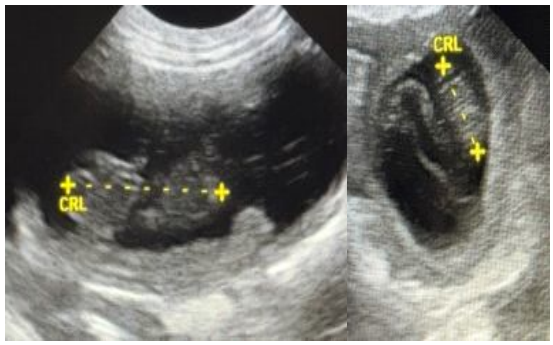
In all three groups, clinical management of dystocia was medically managed by administration of 10% calcium gluconate solution @ 0.2-0.5 mL/kg intravenously and 25% dextrose @1 mL/kg intravenously. After 15-20 minutes oxytocin 2IU was administered intramuscularly in non-responsive animals with cervical dilatation. Oxytocin was repeated at around 60 min intervals, wherever necessary (Simon *et al*., 2017). Digital manipulation or forceps delivery using whelping forceps was done in cases where it was needed. In animals not progressing normally with delivery, a CS was done under general anaesthesia using metoclopramide @ 0.3 mg/kg intramuscularly as premedication followed 15 minutes later by propofol @ 4-6 mg/kg intravenously for induction of anaesthesia. Endotracheal intubation was performed immediately with the aid of a laryngoscope to initiate isoflurane anaesthesia. Animals were further maintained using isoflurane (2-3%) (Smith, 2007; Niharika, 2014; Roos *et al*., 2018). Preoxygenation was performed in brachycephalic breeds and compromised patients.

Comparisons were made between the three groups with regard to modes of whelping, perinatal mortality (combined loss on account of stillbirths and early neonatal mortality within the first seven days of birth) (Gil, 2001), survivability of puppies (after seven days) and litter size.



(a) Latero-lateral (b) dorso-ventral.

Fig 1: ICC measured as the mean of two ICC diameters.



(a) Length of the embryo in a straight line from the rostral most point of the crown to the base of the tail (b) Up to 29 days CRL is measured as the total length of the embryo.

Fig 2: Crown-rump length.

Statistical analysis

The statistical software used for the analysis of data in the present study was SPSS version 24.0. The chi-square test was used for multiple proportions, followed by the z-test for independent proportions. A pairwise comparison was done to compare different proportions between the three groups.

RESULTS AND DISCUSSION

Group I (Preliminary study to assess the accuracy of prediction of day of whelping based on combined ICC and CRL)

Out of the selected 115 pregnancies in Group I, 69.57% whelped exactly on the day (±0) of prediction, while a cumulative total of 93.04% of bitches whelped at an accuracy of ±1d and 100% of bitches whelped at an accuracy of ±2d (Fig 3). In this group many larger breeds like Labrador, Rottweiler, St. Bernard, Bull mastiff *etc.* could not be included in the study, as even on 24th day after breeding many of these animals exceeded ultrasonographic GA of more than 30 days and in some other cases early detection of ICC and CRL was hindered by too much subcutaneous/abdominal fat deposition. However, most smaller breeds like Shihtzu, Beagle, Pug, Dachshund *etc.*, could be included in the study. Thus, more than 60% animals in Group I were smaller breeds.

The accuracy obtained in the present study was in accordance with Groppetti *et al.* (2015) and Xavier *et al.* (2024), who used breed-specific equations to get higher accuracy of prediction. This was also in agreement with Simon (2015), who suggested that the accuracy of prediction using ICC was highest when the ultrasonographic prediction was carried out before 30 days of GA from LH surge. The reason for higher prediction accuracy up to 30 days from LH surge is probably since the canine embryos grew at a linear rate of 1 mm in diameter per day from 17 to 30 days after LH surge and after 30 days the growth was exponential as reported by England (1998). However, the present study was based on ICC and CRL combined prediction. The rectal temperature on the day of whelping (±0 day) ranged from 36.22 to 38.39°C (mean 37.35°C). This was in accordance with Zonturlu *et al*. (2008), who reported that the rectal temperature was around 38.5°C during 144 h (6 d) before parturition and at the onset of whelping it decreased to 37.4°C (99.32°F).

The mean P4 concentration on the EDD in Group I was 1.73 (range 0.68-3.14) ng/mL. Similar observations were noted by Kutzler *et al*. (2003a); Kim *et al*. (2007) and Johnson (2008). The afore-mentioned authors reported that, at the time of whelping, the serum P4 concentration was below 1 ng/mL and that during the prepartum period, there was a sudden decline in the level of serum P4, dropping to less than 2 ng/mL (approximately 6.4 nmol/L) within the final 24 hours of whelping.

Group II

In Group II out of 124 pregnancies, 86 whelped on or before the EDD (69.35%) (Table 1). Among the 38 bitches that did not whelp until the evening of EDD, which were given two doses of mifepristone, 28 animals whelped within 24 h (28/38, 73.68% of whelping induction, cumulative vaginal birth being 91.24% (114/124). Among the remaining 10 bitches, 4 whelped within 36 h. Thus, the cumulative whelping induction response was 84.21% (32/38) and cumulative vaginal birth was 95.16% (118/124) and a CS was performed in 6 bitches (6/124 *i.e.*, 4.84%) 36 h after whelping induction. Simon *et al.* (2017) in an induction trial using mifepristone obtained a similar percentage of induction efficacy.

Group III

Out of 30 bitches in Group III (control group), nine (30%) whelped normally, four (13.33%) had dystocia which could be managed medically along with traction/correction, while in 17 bitches (56.67%) a CS had to be performed, of which in four cases attempts were made initially to manage them

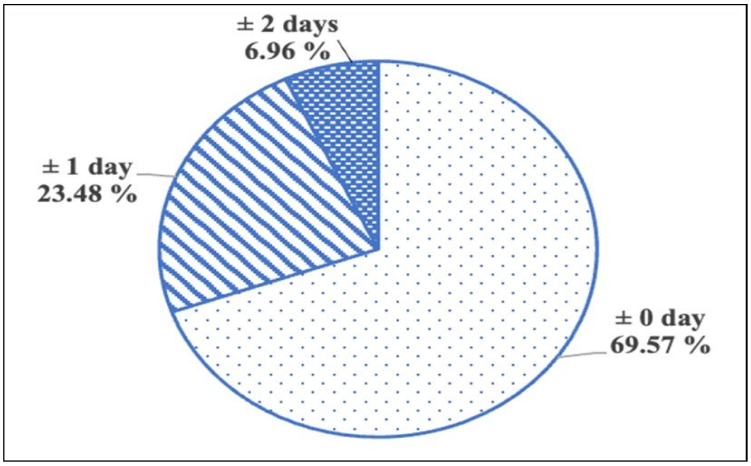


Fig 3: Accuracy of prediction of day of whelping based on combined ICC and CRL in Group I.

Table 1: Comparison of modes of parturition and overall puppy survivability and litter size in groups I, II and III.

Variables	Group I	Group II	Group III	p-value
Number of bitches/pregnancies	115	124	30	-
Total number of puppies	702	716	167	-
Average litter size	6.1 (range: 2 -13)	5.77 (range: 1-14)	5.57 (range: 1-10)	-
Puppy survivability	598 (85.19%)^{b}	641 (89.53%)^{a}	125 (74.85%)^{c}	25.10** (< 0.001)
Perinatal mortality	104 (14.83%)^{b}	75 (10.47%)^{c}	42 (25.15%)^{a}	25.10** (< 0.001)
Normal whelping	66 (57.39%)^{b}	97 (78.23%)^{a}	9 (30%)^{c}	28.10** (< 0.001)
Dystocia (medical)	32 (27.83%)	21 (16.94%)	4 (13.33%)	5.486 ^{ns} (0.064)
Percentage of CS	17 (14.78%)^{b}	6 (4.83%)^{c}	17 (56.67%)^{a}	51.26** (< 0.001)
Dystocia (medical and CS)	49 (42.61%)^{b}	27 (21.77%)^{c}	21 (70%)^{a}	28.10** (< 0.001)

** Significant at 0.01 level.

Percentage having different letters as superscripts differ significantly within a raw (between groups).

with medical support. The high percentage of animals that had to be subjected to CS in this group is in accordance with previous reports by Linde-Forsberg (2015).

Group-wise comparisons

Group-wise comparisons were made regarding modes of whelping, average litter size, puppy survivability (after seven days) and perinatal mortality (Table 1). The total number of pregnancies in Groups I, II and III were 115, 124 and 30, respectively. The percentage of animals undergoing normal whelping (vaginal births) was 57.39, 78.23 and 30 while 42.61, 21.77 and 70 per cent of bitches had dystocia (medical and CS together) in Groups I, II and III, respectively. The percentage of animals that had C sections in Groups I, II and III were 14.78, 4.84 and 56.67, respectively. The percentage of animals undergoing normal whelping, dystocia and CS showed highly significant differences between Groups I, II and III. A highly significant reduction in the number of dystocia and caesareans in Group II favours our hypothesis of induction of whelping with mifepristone on prediction day in bitches that have not whelped by then, to have more normal vaginal births. The percentage of caesarean in the whelping induction group (Group II) was significantly lower when compared to previous reports by Gil (2001) and Linde-Forsberg (2015). Many breeders prefer normal whelping with reasonable puppy survival rates, so that caring for the dam and litter becomes easier and more visits to the veterinary hospitals can be avoided. Normal delivery also ensures maximum reproductive efficiency in canines considering the higher number of whelpings, larger litter sizes in consecutive vaginal births and the absence of postoperative complications especially adhesions which may not favour further breeding. Lifetime production of puppies would be much more in bitches that have a normal whelping.

Mifepristone established safe cervical patency in the present study without any side effects as reported by Simon *et al*. (2017 and 2024). Misoprostol a synthetic analogue of prostaglandin E1 with uterotonic action (Romagnoli, 2018; Simon, 2024), increased the activity of hyaluronic acid, collagenases elastase and glycosaminoglycan in the cervix and the intracellular calcium levels in the uterus resulting in uterine contractions (Aronsson *et al*., 2004). Moreover, according to Roos *et al.* (2018) antiprogestin (single dose), when given at 60-62 d post ovulation, may help in the final maturation of the puppies by inactivating progesterone receptors and promoting a physiological P4 decrease. In singleton pregnancies as well as when the litter size is too small, whelping induction 1 or 2 d before EDD based on early ultrasound prediction, may help to have more vaginal births and prevent possible overgrowth of puppies due to prolonged gestation. Pipan *et al.* (2020) observed that puppies delivered vaginally had greater weight gains on days 2, 3 and 4 compared to those delivered *via* C-section. This advantage was attributed to the higher diversity of meconium microbiota in vaginally born puppies, which improved nutrient absorption in the gut.

A total of 702, 716 and 167 puppies were born with an average litter size of 6.1(range: 2-13), 5.77 (range: 1- 14) and 5.57 (range: 1-11), respectively in Groups I, II and III. This was in agreement with the previous finding of Simon (1997) who validated an average litter size of 6 puppies per bitch, with a range from 1 to 14 puppies, based on a study of 100 whelpings conducted as part of an extensive survey in Kerala, India.

The perinatal mortality (including stillbirth and death within seven days) was 14.83, 10.47 and 25.15 per cent, respectively in Groups I, II and III which showed highly significant statistical differences between each other (Table 1). The perinatal mortality in Groups I and II is similar to previous reports by Bowden *et al.* (1963) and Tonnessen *et al.* (2012). The perinatal mortality was lowest in Group II when compared to Group I bitches without medical induction of whelping (Table 1).

CONCLUSION

Predicting accurate whelping dates is crucial in critical management of canine pregnancies and it can be used effectively by the veterinarian to reduce the incidence of caesareans and dystocia in bitches. Among the different aids currently available for whelping day prediction, ultrasonography has gained wide acceptance since it is a

noninvasive tool. Accurate predictions and promoting more vaginal births are critical in reducing the hardships involved in the post-operative management of the dam, neonatal care and frequent hospital visits faced by pet parents. Ultrasonographic monitoring of pregnancies and induction of whelping on completion of gestational age is a feasible option with good puppy survival rates. Conservation of lifetime reproductive efficiency of the bitch is another favourable outcome of a well-structured and efficient whelping induction protocol. The results of the present study show that accurate prediction of EDD by ultrasonography and induction of whelping on the EDD can manage many canine pregnancies. A highly significant reduction in the number of dystocias and caesareans in Group II strongly supports our hypothesis that induction of whelping with mifepristone on prediction day in bitches that have not whelped by then may favour more normal vaginal births.

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Conflicts of interest

Authors have no conflict of interest to declare.

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