Predicting parturition in Beagles using foetal membrane measurements

By

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Declaration of originality

Full names of the student: Susan Fouché

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Declaration:

I understand what plagiarism is and am aware of the University's policy in this regard. I declare that this mini dissertation is my own original work. Where other people's work has been used (either from a printed source, Internet or any other source) it has been properly acknowledged and referenced in accordance with departmental requirements.

I have not used work previously produced by another student or any other person to hand in as my own.

I have not allowed, and will not allow, anyone to copy my work with the intention of passing it off as his or her own work.

Ethics statement

I, Susan Fouché, the author of this mini dissertation have obtained, for the research of this work, the applicable research approval required by the Faculty of Veterinary Science of the University of Pretoria's ethics and animal use committees. The approval certificate number V034-17 appears in the appendix of this mini dissertation. I further declare that I have observed the ethical standards required in terms of the University of Pretoria's *Code of ethics for researchers* and the *Policy guidelines for responsible research*.

Susan Fouché

Contents

ABBREVIATIONS USED IN TEXT

AI	Artificial insemination
ALV	Axial longitudinal view
AvLD	Average length of the avillous domes
BPD	Biparietal diameter
DCD	The day of cervical dilatation
DP	The day of parturition
DS2	The day of onset of stage two of parturition
D0	First day of cytological dioestrus
ETV	Equatorial transverse view
HYS	Height of the yolk sac
ICCD	Inner chorionic cavity diameter
LCa	Left-caudal abdomen
LCr	Left cranial abdomen
LDome	Length of the dome-shaped allantoic cavity polar to the marginal haematoma
LPZ	Length of the placental zone
NCD	The number of days to cervical dilatation
OTAU	Onderstepoort Teaching Animal Unit
OV	Outcome variable
OVAH	Onderstepoort Veterinary Academic Hospital
PV	Predictor variable
RCa	Right caudal abdomen
RCr	Right cranial abdomen
SD	Standard deviation
SEM	Standard error of the mean

TFD Total fluid diameter

TCD Time of cervical dilatation, as first seen with eight to 10-hourly vaginal speculum examinations

TS2 Time of onset of tenesmus or stage two of parturition as observed during continuous observation once cervical dilatation was first noticed

WYS Width of the yolk sac

YD Yolks sac diameter

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1. INTRODUCTION

Dog breeders invest a lot of time and money into breeding efforts and it is important for veterinarians to assist clients in achieving a successful outcome of a breeding attempt. Being able to predict when a bitch is likely to whelp will assist the owner in managing the pre-parturient bitch optimally. It will reduce the time of observation until the time of onset of parturition is noted, will help to assess when intervention is needed and assist the veterinarian in managing high-risk pregnancies with a possible elective caesarean section as an intervention to prevent foetal compromise and death.

Many methods have been investigated to predict the interval to parturition from a key event which occurred during the oestrous cycle of the bitch or during pregnancy or during the last few days of gestation. I will list a few predictors in this introduction and discuss them briefly to highlight their benefits and, or, limitations.

1.1. Predictors during the oestrous period of the bitch

1.1.1. Vaginal cytology

Vaginal cytology is a diagnostic tool used by veterinarians to determine in which stage of her oestrous cycle a bitch is in when she is examined. The day of onset of cytological dioestrus is a useful method to predict the likely day of parturition as shown by De Cramer and Nöthling (2017) when they found that all of the 242 bitches used in their study whelped within three days of the predicted day of parturition. To identify the day of onset of cytological dioestrus, vaginal smears will have to be made daily—especially towards the end of the oestrus. Although it is cost effective, some owners might not have the time to take the bitch to the veterinarian every day, might live far from the veterinary practice or the bitch might not have the temperament to be admitted as an in-house patient.

1.1.2. Hormone levels in the serum or blood plasma

1.1.2.1. Luteinizing hormone (LH)

The plasma or serum levels of LH could help predict when a bitch is likely to whelp. Concannon et al. (1983) collected blood samples daily at nine to 15 hour intervals (or more frequently during proestrus and oestrus), tested the serum for LH levels, and found that bitches whelped from 64 to 66 days after the LH peak was reached. The mean gestation length from the LH peak to the day of parturition was 65.1 days (SEM 0.1 days, n = 54). De Cramer and Nöthling (2017) predicted the interval to parturition from the first or only day of the LH surge and found that all but one of their 24 bitches whelped within two days of the predicted day of parturition. The plasma half-life of LH is 12 hours in the bovine and 3.5 hours in the guinea pig (McDermott et al., 1981). Concannon et al. (1977) collected blood three times per day (0800, 1600 and 2400 h) to identify the LH peak. Regular blood samples will therefore need to be collected from a bitch which might be impractical. Luteinizing hormone assays are not readily available in all countries, and although the LH surge could be related to the oestrous behaviour of bitches (Concannon et al., 1977), many samples may have to be tested to identify the LH peak, which might have cost implications for the owner.

1.1.2.2. Progesterone

Various studies related the LH surge or peak to the levels of progesterone in serum or plasma. Concannon et al. (1975) found the mean progesterone in plasma at the time of the LH peak to be 5.09 nmol/L (SEM 0.64 nmol/L, n = 20). In a later study Kutzler and Mohammed et al. (2003) used a predetermined serum progesterone level of 4.77 nmol/L, which they designated as the day of the LH surge, to retrospectively calculate the gestation length in their bitches. The mean level over all bitches on that day was 6.42 nmol/L (n=63). They found that all 63 bitches in their study whelped between 62 and 68 days from when the progesterone levels in serum first rose above 4.77 nmol/L. De Cramer and Nöthling (2017) found approximately the same when each of the 24 bitches in their study whelped between 62 and 67 days from the day when the plasma progesterone levels first rose above 6 nmol/L.

Progesterone levels in serum could also be related to the time of ovulation. Fontbonne (2008) found that ovulation could be related to a progesterone level of 15.9 nmol/L in plasma—18 of 20 bitches ovulated (as estimated by ultrasound) less than 24 hours after this level was reached. De Cramer and Nöthling (2017) used this value to predict the interval from when plasma progesterone first rose above 16 nmol/L to the day of parturition. All 24 bitches in their study whelped between 60 and 64 days after the plasma progesterone levels rose above 16 nmol/L for the first time. As for estimating the day of the LH surge, daily blood samples will have to be collected to identify the days on which the critical progesterone levels in serum or plasma are reached. The cost of testing multiple samples might be a limiting factor.

1.1.3. Diagnostic imaging

1.1.3.1. The use of ultrasound to identify the day of ovulation and predicting the day of parturition

Many studies aimed to identify the day of ovulation via serial ultrasonographic evaluations of the ovaries. England and Allen (1989) noted that pre- and post-ovulatory follicles are grossly similar in appearance on abdominal ultrasound and on ultrasonographic examination after removal of the ovaries via laparotomy. Hase et al. (2000) could only identify the day of ovulation in six of their 11 bitches. The uncertainty of actual occurrence of ovulation and the frequency of ultrasonographic evaluations—serial ultrasonographic evaluations are needed at 12 hour intervals (Fontbonne, 2008) or three times per day (Hase et al., 2000) from when follicles are detected for the first time—are limiting factors for using ultrasonographic identification of the day of ovulation as a predictor for the day of parturition.

1.2. Predictors during the first two trimesters of pregnancy

Many bitches are presented for pregnancy diagnoses and estimation of gestational age of the foetuses without any knowledge of when key events occurred during oestrus. In such cases it would be of value to use information obtained during different stages of pregnancy to help predict when a bitch is likely to whelp.

1.2.1. Diagnostic imaging

1.2.1.1. The use of ultrasound to detect pregnancy

Since the introduction of ultrasound, this modality has been used to diagnose pregnancy in the bitch. Bondestam et al. (1983, 1984) found that ultrasound was an accurate method to detect pregnancy. Yeager and Concannon (1990) related pregnancy detection to a key event which occurred during the oestrous cycle of the bitch. They found that pregnancy could be detected for the first time 17 to 20 days after the LH surge was identified.

1.2.1.2. The use of ultrasound to estimate the age of foetuses

England et al. (1990) used ultrasound to determine when certain structures of a conceptus could be identified for the first time after the end of oestrus in Labrador Retrievers, Golden Retrievers and their crosses. This information would only indicate that the gestational age is further than a certain day but it will not aid in predicting the day likely of parturition.

1.2.1.3. The use of ultrasound to predict the day of parturition

England et al. (1990) and Luvoni and Grinoni (2000) measured different parts of conceptuses to develop a prediction table or a formula to estimate the interval from the day the measurements were taken to the day of parturition. England et al. (1990) used Labrador Retrievers, Golden Retrievers and their crosses (22 to 30 kg, n = 50). Luvoni and Grinoni (2000) used small (5 to 6 kg, n = 3) and medium-sized bitches (16 to 24 kg, n = 8). The accuracy in predicting the day of parturition varied. The table of England et al. (1990), using combined measurements of the foetal trunk diameter and biparietal diameter, predicted the gestational age in 95% of foetuses within 2.84 days. Using the diameter of the gestational sac of each conceptus during early pregnancy (42 to 21 days before parturition), Luvoni and Grinoni (2000) predicted the day of parturition to within one day in 10 of 11 small bitches as well as in 10 of 11 medium-size bitches. When Luvoni and Grinoni (2000) used the biparietal diameter of foetuses during late pregnancy (37 days to one day before parturition) they predicted the day of parturition to within one day in 15 of 22 small bitches and in17 of 24 medium-size bitches.

The studies by England et al. (1990) and Luvoni and Grinoni (2000) were either performed on bitches of different weight categories or specific breeds which implies that a specific formula or table, as reported in these studies, could only be applied to a bitch if she falls into a defined weight category or if she is of a certain breed.

1.3. Predictors during the last trimester of pregnancy

Predictors during the last trimester and especially during the last few days are important when no predictive information was recorded during the oestrous cycle of the bitch, or during the first two trimesters of pregnancy. These late term predictors could aid in refining the estimations to days before parturition as calculated from the occurrence of previous key events. Some of these predictors have the power of negative predictive value—predicting a time period when a bitch is unlikely to whelp. This fact could be helpful in scheduling intense observation for parturition by the owner or veterinarian.

1.3.1. The value of radiographs to estimate gestational age of foetuses and predict the day of parturition

Radiographs can be used to estimate the gestational age of foetuses. Rendano et al. (1984) showed that mineralization of different anatomical structures occurred on specific days after the LH peak. Mineralization of the spine, skull and ribs occur at between 43 and 46 days after the LH surge and caudal vertebrae are some of the last structures to mineralize at 55–64 days after the LH peak.

The limitation of this modality to predict the day of parturition is that owners might present their bitches for radiographs too soon to detect mineralization and would have to bring their bitches for subsequent radiographs at regular intervals to identify, for instance, caudal vertebrae to narrow down the interval to parturition to between 10 days and one day.

1.3.2. The value of monitoring rectal or vaginal temperature to predict the day of parturition

Concannon and Hansel (1977) related the drop in plasma progesterone levels to a drop in rectal temperature of 1.39 °C on average. A drop in rectal temperature of an average value of 1 °C started one to two days prior to onset of parturition (Tsutsui and Murata, 1982). Geiser et al. (2014) continuously measured vaginal temperatures—which is closely correlated to body or rectal temperature—and found that the drop in temperature could be as low as 0.3 °C, that parturition could occur within 24, 36 or 48 hours after the drop in temperature and that some bitches do not have a temperature drop.

The detection and use of a drop in rectal temperature is therefore unreliable as a predictor of the day of parturition.

1.3.3. The value of plasma or serum progesterone levels to predict the day of parturition

Progesterone maintains pregnancy and prepartal luteolysis brought about by prostaglandin F2α would result in a drop in plasma or serum progesterone levels (Concannon and Hansel, 1977; Veronesi et al., 2002). De Cramer and Nöthling (2018a) showed that the probability of a bitch whelping within 24 hours, when the plasma progesterone level is less than 8.7 or 3.18 nmol/L, is 99% or 100% respectively. They also showed that a bitch is unlikely to whelp within 12 hours when plasma progesterone levels are above 8.7 nmol/L.

The limiting factor for using pre-parturient progesterone levels in serum or plasma is that additional information would be needed to ensure that the bitch is presented at the correct time, or serial blood samples would have to be collected and assayed for progesterone levels, to accurately detect the drop in progesterone. The serial blood collections and assays might also have cost implications for the client.

1.4. Research motivation

The current predictors of the day of parturition are limited in the sense that many of them are breed or size specific, have cost implications for the client or require daily examinations with owner compliance as an important factor. If we can develop a predictor of the day of parturition with an accuracy of ± 2 days, which does not require knowledge of specific events that occurred during the oestrous cycle of the bitch, is not influenced by the breed or size of the bitch, does not require repeated examinations or tests, is cost effective and can be used when bitches are most likely presented for pregnancy diagnosis, we can assist breeders in achieving a favourable outcome of a breeding attempt.

1.5. Aim of the study

The aim of our study was to generate a formula which can use ultrasonographic measurements taken during the ampullary phase of gestation to predict the onset of parturition to within two days (within a five-day range).

2. LITERATURE REVIEW

2.1. The value of events during the oestrous cycle of the bitch as predictors of the day of parturition

2.1.1. Vaginal cytology

Veterinarians use many diagnostic methods as aids to determine at which stage of her reproductive cycle a bitch is. Vaginal cytology will aid in indicating when key events took place or are likely to occur. Evans and Cole (1931) divided the oestrous cycle into four stages: proestrus (6–13 days), oestrus (7–13 days), metestrus, or dioestrus as we refer to it, (80–90 days) and anoestrus—the period between the end of metestrus up to the onset of the following proestrus. They defined the onset of oestrus as the first day on which the bitch would allow coitus to proceed and the onset of dioestrus as the first day of refusal of coitus. Evans and Cole (1931) also evaluated the cytology of vaginal smears taken during the complete oestrous cycle; identified different cell types present and related the proportions of cell types to the different stages of the oestrous cycle. Cell types identified were red blood cells, leucocytes and three types of vaginal epithelial cells—i) unstained living cells with abundant vacuoles, ii) unstained living cells with no vacuoles and iii) well-staining dead (cornified) cells. Schutte (1967a) also studied the morphology of vaginal epithelial cells, evaluated the different proportions of each cell type present at different stages of the oestrous cycle of the bitch (Schutte, 1967b), and compiled and compared different indices to evaluate the cycle of bitches (Schutte, 1967c). Christie et al. (1972) later described two cell types not previously identified by Schutte and also attempted to quantify the different cell types present at different stages of the oestrous cycle. Bell et al. (1973) again studied the different proportions of different cell types present on vaginal smears of the bitch at different stages of the oestrous cycle, but it was only later that cytological changes would be related to the gestation period of bitches and used to predict the day of parturition.

2.1.2. Onset of Dioestrus

Holst and Phemister (1974) used the morphological characteristics of Schutte (1967a) and Christie et al. (1972) to study the changeover from superficial and large intermediate to small intermediate and parabasal cells and compared this change to the late oestrus period of the bitch's cycle. They determined that there was a single day during late oestrus when the cell types present on vaginal smears changed from predominantly superficial type to small intermediate and parabasal types. This day they defined as the first day of dioestrus. Eilts et al. (2005) retrospectively calculated the interval from D0 to whelping and found that bitches whelped on average 56 days (SD 2.8 days) after onset of cytological dioestrus. The range was between five days before to 12 days after the predicted day of parturition. The bitches were only monitored every second day, D0 was defined as a drop of 50% in the SCI and the day of whelping was defined as the day of onset of second stage labour. These factors could account for the wide range in expected versus real whelping dates they found. De Cramer and Nöthling (2017) compared various predictors of the day of onset of parturition—defined as the day of cervical dilatation (DCD)—and found that the mean for the interval from D0 to DCD for the 242 bitches used in their study was 56.74 days (SD 0.96 days). They showed that D0 predicted DCD with a precision of ± 1 d, ± 2 d and ± 3 d in 88%, 99% and 100% respectively in the 242 pregnancies. This indicates that the first day of dioestrus is a key event in the oestrous cycle of the bitch and it could be used as a fixed point from which prediction of onset of parturition could be estimated.

2.1.3. Hormones during the oestrous cycle as predictors of the day of parturition

Hormones, and their varying concentrations in serum or plasma during the oestrous cycle of the bitch, have been the subject of many studies. Phemister et al. (1973), Concannon et al. (1975), Wildt et al. (1978), Wildt et al. (1979) and various others evaluated the level of oestrogen, luteinizing hormone (LH) and progesterone in plasma or serum during the oestrus cycle. Some of these hormones could be used to indicate when key events happened or when they are likely to occur—including the day of parturition.

2.1.3.1. Oestrogen

Concannon et al. (1975) observed bitches for signs of proestrus (sanguineous discharge from the vulva) and collected blood samples daily during proestrus up to the end of the first week of oestrus. They determined the levels of oestrogen, LH and progesterone in plasma. Mean levels of oestrogen were elevated already at the onset of proestrus and continued rising until a rapid decline was noted during oestrus. Peak oestrogen levels were reached one to two days before or on the day that plasma LH concentrations reached peak levels. Wildt et al. (1979) later measured estrone and estradiol-17β concentrations in plasma and found (in sixteen of the 25 cycles that they assessed) a sharp drop in both coinciding with the LH surge. A large variation in the concentration of both of these hormones in plasma was noted among individual cycles. The authors speculated that biosynthesis of the two hormones (with estradiol-17β as a precursor to estrone) and variations in metabolic processes, could play a role in the varying results also obtained in other studies. Due to these variations, oestrogen levels can be used to indicate when the LH surge likely occurred, but are unlikely to be accurate in predicting the expected day of parturition.

2.1.3.2. Luteinizing hormone (LH)

Peak levels of LH in plasma are reached from two days prior to the bitch allowing coitus or even up to four days after first allowing coitus, and remain elevated for one to two days—indicating that the LH surge can last between 18 to 48 hours (Concannon et al., 1975). Wildt et al. (1978) later found the LH surge lasted between three and a half, and four days. Phemister et al. (1973) related the time of the LH surge to the time of ovulation and determined that ovulation occurs two days (SEM 0.1 days) after peak values of LH are reached in plasma. This was later confirmed by another study of Concannon et al. (1977). Wildt et al. (1978) also studied the time of ovulation in relation to LH levels in serum and found the highest incidence of ovulation occurred between 24 and 72 hours after maximal LH concentrations were reached. The LH peak was also related to onset of dioestrus—as defined by Holst and Phemister (1974)—and it was found that the LH peak occurs eight days (SEM 0.3 days) before the onset of dioestrus (Holst and Phemister, 1975).

Concannon et al. (1983) determined the interval from the day of the LH peak to the day of parturition in 290 pregnant bitches. They found the mean gestation length, measured from the LH peak, was 65.3 days (SEM 0.2 days). De Cramer and Nöthling (2017) tested the levels of LH in serum and determined the interval from the first, or only, day of the LH surge to day of cervical dilatation (DCD)—their defined day of parturition. The authors found that 29% of their bitches whelped on the predicted DCD, 75% within one day and 100% within two days from the predicted DCD. The LH peak could therefore be seen as another key event of the canine oestrous cycle assisting veterinarians in predicting the expected day of parturition.

2.1.3.3. Progesterone

In practice, monitoring plasma LH levels are impractical and many studies focused on establishing a correlation between LH and progesterone. Plasma progesterone starts to increase before the LH peak is reached and continues to increase rapidly afterwards (Concannon et al., 1975; 1977). The increase in progesterone is attributed to the early luteinization of the maturing follicles (Phemister et al., 1973; Concannon et al., 1977). Concannon et al. (1975, 1977) showed that the initial rise in plasma progesterone levels could not be dissociated from the LH surge. Plasma progesterone concentrations were evaluated in relation to the LH peak and Concannon et al. (1975) found the mean progesterone at the time of the LH peak to be 5.09 nmol/L (SEM 0.64 nmol/L) and in a later study Concannon et al. (1977) found the mean progesterone to be 8.27 nmol/L (SEM 0.95 nmol/L) at time of the LH peak. Kutzler and Mohammed et al. (2003) used serum progesterone levels to retrospectively calculate gestation length in relation to serum progesterone levels of a certain value, and also compared these results within different body weight classifications of their research dogs. They defined D0 as the day on which serum progesterone rose above 4.77 nmol/L, but found the mean progesterone level on the day it rose above 4.77 nmol/L for all body weight groups to be 6.42 nmol/L. The results showed that 67% whelped 65 ± 1 day, 90% 65 ± 2 days and 100% 65 ± 3 days from the day when plasma progesterone levels rose above 4.77 nmol/L. De Cramer and Nöthling (2017) supports this study when they found that DCD occurred in 17 of 24 bitches 65 ± 1 day, in 21 of 24 bitches 65 ± 2 days and in 100% of bitches 65 ± 3 days from the day when plasma progesterone levels rose above 6 nmol/L. Concannon et al. (1975) found no difference in maximal mean progesterone levels for pregnant versus non-pregnant bitches. The only difference they recorded was the rapid decline in progesterone prior to parturition in pregnant bitches, compared to the gradual decline in progesterone, and an extended period of plasma progesterone levels above 3.18 nmol/L in non-pregnant bitches. In the light of the above, plasma progesterone levels could be used to indicate when the key event of LH surge likely occurred, and help predict when parturition is likely to occur.

2.1.4. Ovulation

The day of ovulation in the bitch could be estimated by evaluating LH levels in plasma or serum (Phemister et al., 1973; Fontbonne, 2008), evaluating progesterone levels in plasma or serum (Phemister et al., 1973; Concannon et al., 1977; Mir et al., 2011), serial ultrasonographic evaluations of the ovaries (Silva et al., 1996; Fontbonne, 2008) or via laparoscopic evaluation of the ovaries (Wildt et al., 1977; Silva et al., 1996).

2.1.4.1. Ovulation in relation to the LH peak and progesterone levels in plasma or serum

Phemister et al. (1973) measured daily LH levels in plasma and evaluated ovaries for ovulation at specific days after bitches allowed coitus. Ovulation occurred 2 ± 0.1 days (mean ± SEM) after the LH peak. They found that the plasma progesterone levels were 18.13 ± 4.13 nmol/L (mean ± SEM) two days after the day of the LH peak—the likely day of ovulation. Concannon et al. (1977) found supporting results in their study where ovulation occurred between 44 and 50 hours after peak plasma LH levels were reached, and at the presumed time of ovulation (48h after the LH peak), the mean plasma progesterone level was 17.3 nmol/L (SEM 2.96 nmol/L).

2.1.4.2. Ovulation as observed by ultrasound or laparoscopy

Visually detecting the time of ovulation has been the objective of many studies. Indaba et al. (1984) scanned three bitches daily with a 5 MHz linear array transducer after the onset of oestrus, detected ovulation and confirmed it by laparotomy on the day of suspected ovulation. England and Allen (1989) examined bitches at various stages of the oestrous cycle using a 7.5 MHz linear array transducer and compared ultrasound images to the histological appearance of the ovary removed via laparotomy or at necropsy on the day of examination. They found that the in vitro appearance of ovaries and follicles were similar to their in vivo appearance on ultrasound and that pre- and post-ovulatory follicles are grossly similar in appearance. England and Allen (1989) noted no follicular collapse and corpora lutea still had central cavities which would make it difficult to distinguish them from pre-ovulatory follicles on ultrasound. Wallace et al. (1992) collected blood and scanned bitches daily, with a 7.5 MHz mechanical sector transducer on a built-in stand-off pad, from the onset of proestrus. They defined time of ovulation as the day on which serum progesterone reached a minimal value of 12.72 nmol/L, 24 to 72 hours after LH increased three fold from baseline values and remained elevated for 48 hours, and five to 12 days prior to the onset of cytological dioestrus (defined as the return of white blood cells to vaginal smears). Wallace et al. (1992) could not identify follicular collapse but rather took the reduction in number of follicles or disappearance of follicles previously identified on ultrasound, as evidence of ovulation. Hayer et al. (1993) could identify signs of ovulation, via ultrasonographic evaluation of the ovaries in only two of the 15 oestrous cycles they followed in 13 bitches. Silva et al. (1996) examined bitches for signs of proestrus and at the onset of proestrus they removed the left ovarian bursa via laparoscopy. They collected blood from bitches once a day, examined the ovaries daily via ultrasound (using a 7.5 MHz transducer) and performed laparoscopic examinations of the left ovary every second day from the onset of proestrus until the onset of cytological dioestrus. The LH peak (when plasma progesterone levels reached values of between 4.77 and 6.36 nmol/L) was defined as D0. They did not observe any follicular collapse via ultrasound and only one bitch developed haemorrhagic points on three follicles—suggesting ovulation—on laparoscopic examinations. Hase et al. (2000) estimated the likely day of ovulation in 11 bitches. The day of ovulation was estimated by using peak levels of LH in plasma and levels of progesterone in plasma at the time of the LH peak and two days after the LH peak. Ultrasonographic evaluations of the ovary was initially performed once daily until ovarian follicles were detected, following which the growth of the follicles were monitored and recorded three times a day. The time after which follicles began to shrink was regarded as the time of ovulation. Ovulation was detected via ultrasound in only six of the 11 bitches.

Fontbonne (2008) used a 7.5 to 10 MHz sector transducer to perform ultrasonographic evaluations of the ovaries of 15 Beagle bitches and 37 bitches from different breeds (25 pure bred bitches and four mixed breed bitches) to detect the day of ovulation. The examinations were performed twice daily with a 12 hour interval from when progesterone in plasma reached 6.36 nmol/L and, with almost complete disappearance of the previously identified follicular cavities, they could identify ovulation in only 18 of 52 bitches. In the remaining 34 bitches, irregular shaped hypo-echoic structures remained identifiable in the ovary, but they appeared smaller and irregular in shape than the previously identified pre-ovulatory follicles.

Most authors could not observe ovulation with complete certainty when using ultrasound or laparoscopy.

2.1.4.2.1. The value of identifying the day of ovulation as a predictor of the day of parturition

Having used an enzyme immunoassay (EIA), Hase et al. (2000) concluded that the day of ovulation in bitches was the first day plasma progesterone rose above 6.36 nmol/L. Using this value, Tsutsui et al. (2006) mated 36 bitches once, one to five days after ovulation, and calculated gestation length as the interval from the estimated day of ovulation to the day of parturition. Gestation length ranged from 61 to 66 days with an average of 63.9 days (SEM 0.2 days, n = 63). Due to the wide range in gestation length, the daily intensive observations needed via ultrasound and the uncertainty of whether ovulation did indeed occur, the time of ovulation could not be used to predict when a bitch is likely to whelp.

2.2. The value of breeding dates as a predictor of the day of parturition

Veterinarians would be able to predict when a bitch is likely to whelp if they have knowledge of the data discussed above—onset of dioestrus, day of LH peak or day which plasma progesterone rose above 6 nmol/L—but most bitches are presented with owners only providing dates on which a successful mating occurred (some bitches are even presented without any accurate data).

2.2.1. Behavioural oestrus

Behavioural oestrus can last from three to 21 days (Concannon et al., 1989). Tsutsui (1989) defined oestrus as the first day an bitch would allow coitus and performed laparotomies 36, 48, 60, 72, 84, or 96 hours after coitus to determine the time of ovulation. The earliest an ovulation was detected was 48 hours after a bitch first allowed coitus and there was a large variation in time of ovulation post coitus. A bitch allowing coitus could therefore not be related to a key event during the oestrous cycle. Holst and Phemister (1974) bred bitches either once only on the first day of oestrus or once only on the 7th day of oestrus. Bitches bred on the first day whelped 56.9 days (SEM 0.18 days) after onset of dioestrus and bitches bred on the 7th day whelped 56.7 days (SEM 0.41 days) after onset of dioestrus. Concannon et al. (1983) bred bitches once only, on the first day they would allow coitus, and recorded the day of parturition. The interval from mating to partition ranged from 58 to 69 days. These studies (Holst and Phemister, 1974; Concannon et al., 1983) show that the time of mating could not be related to a key event during the oestrous cycle of the bitch or to the interval to the onset of parturition. Mating dates will therefore be of little use in predicting the expected day of parturition.

2.2.2. Oocyte physiology

Primordial germ cells in the foetal ovary divide repeatedly via mitosis which is arrested soon after birth. After birth the oogonia enter the first stage of meiosis becoming primary oocytes (Stabenfeldt and Edqvist, 1993)—oogonia in the prophase of the first of two meiotic divisions. The dog ovulates primary oocytes (Evans and Cole, 1931; Holst and Phemister, 1971; Phemister et al., 1973; Concannon et al., 1989; Songsasen and Wildt, 2007; Karre et al. 2012). Holst and Phemister (1971) removed the reproductive tracts of bitches, bred only once on the first day of oestrus (defined as the first day of acceptance), on different days after breeding. They found oocytes showing evidence of maturation (formation of the first polar body) from three days post breeding. Reynaud et al. (2005) used ultrasound, examined the ovaries of bitches two to three times per day, identified follicles and determined the time of ovulation. They then removed the reproductive tracts at various times after ovulation was detected. It was found that oocytes in metaphase two of meiosis were only present from 54 h after ovulation. These studies show that oocytes need time to mature after ovulation and before fertilization can occur, but they did not evaluate the fertile lifespan of mature oocytes.

Hossein et al. (2008) assumed that ovulation occurred when serum progesterone was at or above 12.72 nmol/L and then collected oocytes at 56, 72, 84 and 96 hours after ovulation. They found 2.1 (SEM 0.8, n = 295) aged or degenerate oocytes 84 hours and 3.3 (SEM 0.8, n = 180) aged or degenerate oocytes 96 hours after ovulation. Oocytes can mature at different times after ovulation, remain viable for an extended period of time and fertilization generally occurs from 90 hours after ovulation (Reynaud et al 2005). Verstegen et al. (2001) monitored oestrous cycles, identified the LH surge and performed vagino-hysterograms to determine day of cervical closure. Prior to cervical closure they inseminated the bitches into the vagina, and after cervical closure was detected, they inseminated the bitches at predetermined times into the uterus. They found that cervical closure occurred 6.9 ± 1.1 days (mean ± SD) after the LH peak and no bitch inseminated into the vagina conceived after cervical closure. However, bitches inseminated into the uterus conceived up to 72 hours after cervical closure, indicating that oocytes may be fertilised more than 200 hours after the LH peak. The findings of Verstegen et al. (2001) was later supported by Tsutsui et al. (2009). Tsutsui et al. (2009) estimated the day of ovulation when plasma progesterone levels rose above 6.36 nmol/L with the following day's plasma progesterone level rising to 12.72 nmol/L. This estimation was later corrected to two days after the measured LH peak. They performed intra uterine artificial insemination in bitches via laparotomy (IUAI) six, seven, eight, and nine days after ovulation. All of five bitches conceived when IUAI was performed six days after ovulation compared to five of seven and three of eight bitches in which IUAI was performed seven or eight days respectively after ovulation, while no bitch conceived when IUAI was performed nine days after ovulation. The findings by Tsutsui et al. (2009) indicate that oocytes can remain viable up to 192 hours (8 days) after ovulation.

2.2.3. Fertile life of spermatozoa

The lifespan of semen fresh (and frozen) have been the subject of many studies. Doak, Hall and Dale (1967) allowed a single mating and then examined the reproductive tracts of bitches at selected intervals after copulation. They found motile sperm in high concentrations throughout the uterus up to six days (and in reduced numbers up to 11 days) after the single mating. The fertile lifespan of spermatozoa of rabbits and rats is estimated to be at least 50% of their motile lifespan (Hammond and Asdell, 1926; Merton, 1940). Hammond and Asdell (1926) showed that sperm in the reproductive tract of the male remains fertile for around 50% of their motile lifespan when they vasectomised rabbits and mated them to females at specific days after the procedure. Motile sperm were found in the reproductive tracts of females after they were mated to males that had undergone vasectomy 60 days prior to the mating, but the latest mating which resulted in a litter was 40 days after the vasectomy. Merton (1940) demonstrated the same in the female reproductive tract when they inseminated female rats at various times before ovulation. Motile sperm were found up to 13.5 hours after insemination but pregnancies only resulted when the mice were inseminated more than 18 hours after parturition with ovulation occurring 19-22 hours after parturition. They estimated that rat spermatozoa retain their fertilising ability for no longer than six hours. Holst and Phemister (1971) bred bitches to fertile males on the first day of acceptance (defined as the first day of oestrus) and thereafter to vasectomised males until refusal of coitus (defined as the end of oestrus). Bitches bred 12 days before refusal did not conceive whereas bitches bred eight days before the end of oestrus did—suggesting that sperm from a mating 12 days before refusal did not remain fertile long enough to fertilize oocytes by the time they had matured. Concannon et al. (1983) found that the earliest a single mating resulted in a pregnancy was three days before the LH peak. It could therefore be estimated that canine spermatozoa can fertilize mature oocytes up to six or seven days after introduction into the female reproductive tract.

Variation in the fertile life span of spermatozoa and oocytes, and the variation in the time of mating during the fertile period, may result in a range of 11 days in the interval between a single mating and parturition, as was experimentally shown by Concannon et al. (1983). Knowledge of the date a successful mating, or of an artificial insemination, will therefore not provide us with a predictor of when the day of parturition is likely to occur.

2.3. The value of the development of the embryo and the placenta as predictors of the day of parturition

Fertilization, when viable spermatozoa are present in the reproductive tract, can occur from 48 hours after the estimated time of ovulation—as observed via laparotomy—and zygotes at the morula stage can enter the uterus from around eight to nine days after ovulation (Tsutsui, 1975; 1989). Blastocysts (still free-floating) could be observed until 11 days after onset of D0 (Holst and Phemister, 1971) or up to 20 days after the LH peak (Thatcher et al., 1994).

Ultrasonographic identification of the gestational sac was studied by Yeager and Concannon (1990) and they could visualise the gestational sac from 17 to 20 days after the LH surge (19.1 ± 0.4 SEM, n = 9), when it was one to two millimetres in diameter. Kim and Son (2007) estimated the day of ovulation as the first day when plasma progesterone exceeded 12.72 nmol/L and performed serial ultrasonographic examinations using a 3.5 MHz convex, and 7 and 10 MHz linear transducers form 15 days after ovulation. They detected the embryonic vesicle for the first time at 18 ± 0.9 days (mean ± SD, n = 9) after ovulation—on around day 20 in relation to the LH surge. As technology of ultrasound equipment improves it may become possible to identify the gestational sac even earlier than Yeager and Concannon (1990) and Kim and Son (2007) did. The relative size and growth rate of the gestational sac or conceptus, at a specific gestational age, could aid in predicting when a bitch is likely to whelp.

The term ‘conceptus’ is used to describe the embryo, or foetus, and its associated membranes (McGeady et al., 2017). These membranes are fluid filled and, in the canine conceptus, represented by the yolk sac, amnion, chorion and allantois. The yolk sac is initially formed by endodermal cells migrating to line the blastocyst cavity and, together with ectoderm derived from the trophoblast, forms the bilaminar yolk sac. Mesodermal cells then migrate from the primitive streak to a position between the endodermal and ectodermal layers forming the trilaminar yolk sac. This yolk sac—as it is folded in under the embryonic disc by the folding of the amniotic folds—will eventually form the embryonic gut which in turn will give rise to the allantoic membrane containing the allantoic fluid. The portion of the yolk sac which remains outside the embryo is the definitive yolk sac and it communicates with the embryonic gut via the viteline duct—part of the umbilicus (McGeady et al., 2017). In canines the yolk sac contains no yolk and early embryonic survival and growth (up to around day 21 of pregnancy) is supported by the choriovitelline placenta—the union of the trilaminar yolk sac with the uterine epithelium (McGeady et al., 2017). The yolk sac remains attached to the allantochorion (the fused allantoic and chorionic membranes) at the poles of the conceptus and persists until parturition (Noden and de Lahunta, 1985). As the allantois develop from the splanchnopleure (hindgut) to grow into the extra-embryonic coelom, it will eventually completely surround the yolk sac except where the yolk sac remains attached to the allatochorion (McGeady et al., 2017). Yeager et al. (1992) performed serial ultrasonographic examinations on the uteri of Beagle bitches and noted the presence of different structures on specific days after the preovulatory LH surge. They found that the yolk sac could be identified from 25 to 28 days after the LH surge (estimated as about 17 to 20 days after the onset of cytological dioestrus). If we can identify the first time the yolk sac could be visualised, measure its diameter and possibly relate the dimensions of the yolk sac to other foetal membranes at different days of gestation it could likely aid in predicting the day of parturition.

Noden and de Lahunta (1985) define the placenta as the structure formed by the apposition of foetal membranes and maternal tissues. The placental classification is based on the degree of contact between the foetal membrane (chorion) and the maternal endometrium; whether there is loss of endometrium during attachment and gestation and if the endometrium is shed when parturition occurs; and the shape and distribution of the attachment between the extra-embryonic and maternal tissues. The dog has an endotheliochorial, deciduate, zonary placenta (Noden and de Lahunta, 1985). Due to the zonary and infiltrative character of the canine placenta, the maternal endothelial vasculature is damaged, which causes accumulation of maternal blood at the edge (margins) of the periphery of the zone of attachment. These areas of pooled blood, between the chorion and endometrium, are called marginal hematomas (Noden and de Lahunta, 1985; McGeady et al., 2017). Yeager et al. (1992) first detected—via ultrasound—the zonary placenta at 27 to 30 days after the LH surge. If one could identify and relate the length of the placental zone to the total length of the conceptus at a specific day of gestation it could be used as a predictor of the day of onset of parturition.

2.4. The value of pregnancy detection and the staging of gestation as predictors of parturition

Pregnancy could be detected via abdominal palpation, abdominal radiographs, plasma relaxin levels, acute-phase protein levels and ultrasonographic evaluation of the uterus.

2.4.1. Abdominal palpation

Abdominal palpation was initially the only method of detecting pregnancy. Hancock and Rowlands (1949) examined various bitches via palpation three times at fortnightly intervals starting three weeks after breeding was stopped. Their results were not very accurate or reliable in determining whether a bitch was pregnant or not. Later studies by Allen and Meredith (1981) and Taverne et al. (1985) compared accuracy of abdominal palpation to other methods or modalities in diagnosing pregnancy. Allen and Meredith (1981) used abdominal palpation to assess the pregnancy status of bitches. When they palpated bitches between 21 and 25 days after the first mating they could only identify 11 pregnancies in 21 bitches (52%) which eventually whelped. Palpation between 26 and 35 days after the first mating diagnosed pregnancy in only 49 of 56 bitches (87%) which eventually whelped. Eleven of 12 (92%) bitches diagnosed as not pregnant did not whelp when palpated 21 to 25 days after the first mating. Sixteen of 22 bitches (73%), when palpated 23 – 35 days after the first mating, was diagnosed as not pregnant and did not whelp. All bitches (100%) palpated from 35 days after the first mating and diagnosed as not pregnant did not whelp. Taverne et al. (1985) found the sensitivity about 89% and specificity between 90% and 93% for abdominal palpation in diagnosing pregnancy during the period 20–49 days after the first breeding. The false negatives were attributed to small litter sizes and undated moment of conception. Shille and Gontarek (1985) also found abdominal palpation less accurate and that a false negative is the most common error to make. The stage of pregnancy would also influence accuracy as Toal et al. (1986) demonstrated. Palpation of six bitches on day 21 and 22 after the first mating could only identify pregnancy in one of the four bitches which eventually whelped. Pregnancy could not be diagnosed in the other three bitches. Ten bitches were palpated between 24 and 32 days, 17 bitches between 33 and 42 days, and 22 bitches later than 43 days after the first mating. Five of five, seven of eight and all of 16 bitches, respectively, were correctly identified as pregnant. One bitch was incorrectly diagnosed as pregnant after day 43.

Although an experienced veterinary surgeon might be able to accurately diagnose pregnancy via abdominal palpation, the window period for an accurate diagnosis is limited and it could only confirm pregnancy but it will not provide any information as to the gestational age of the foetus or foetuses, or the likely day of parturition.

2.4.2. Abdominal radiographs

Rendano et al. (1984) used the day of the LH peak as day 0 of gestation. They showed that mineralized foetal skeletons can be seen on radiographs from around 22 days prior to the onset of parturition. The spine, skull and ribs were the first structures visualized at 22 days prior to parturition, the scapula, humerus and femur from 18 days prior to parturition and the radius, ulna, tibia and pelvis from 13 days prior to parturition. Caudal vertebrae and phalanges were seen for the first time on average five days (range nine to two days) prior to the onset of parturition and all six bitches used in their study whelped within eight days of foetal teeth being visible on radiographs (mean four days, range eight to three days). Radiography is the most accurate modality in predicting litter size (Toal et al., 1986). Radiographs are limited in providing information on foetal health but radiographic signs of foetal death include intravascular foetal gas—more commonly found in the foetal heart but also found in veins arteries and umbilical vessels. Other radiographic signs are intra-foetal gas, overlapping or misalignment of the cranial bones (Farrow, Morgan and Story, 1976; Kinns and Nelson, 2013) and Deuel's halo sign—the separation of the subcutaneous fat from the foetal scalp leaving a radiological identifiable space between the two structures (Borell and Fernstrom, 1957; Ohlson, 1962). The use of radiographs could therefore be used to confirm pregnancy during late gestation and could narrow down the gestational age of the foetuses by evaluating anatomical structures visualised, but will not be of any aid in accurately predicting likely day of parturition.

2.4.3. Relaxin

Relaxin is a polypeptide hormone structurally resembling insulin and insulin-like growth factors. Plasma relaxin levels were measured in pregnant, pseudo-pregnant and ovariectomized pregnant bitches of various breeds (Steinetz et al. 1987; 1989).

They determined that relaxin is not detectable in serum of pseudo-pregnant bitches, that it is present in pregnant bitches—in reduced concentrations in pregnant ovariectomized bitches—and that it is present in smaller concentrations during lactation. These findings suggest that the ovary, together with the placenta, secretes relaxin during pregnancy but that the ovary is the sole source of relaxin during lactation. Stewart et al. (1992) later determined the sequence of canine relaxin. The highest concentration was found in the placental tissue (Klonisch et al., 1999). A more recent study by Nowak et al. (2017) indicated that relaxin and its target receptors are localized in the uterus and utero-placental units, and that cytotrophoblasts are the main providers of circulating relaxin.

In view of the above, relaxin can be used to differentiate between pregnancy and pseudopregnancy, but it will be of no use to determine the stage of gestation and likely day of parturition.

2.4.4. Levels of acute phase proteins

Gentry and Liptrap (1981) conducted a study on the fibrinogen levels in dogs treated with progesterone and extrapolated this to pregnant bitches. They injected male dogs and non-oestrous adult females with progesterone and detected a rise in plasma fibrinogen levels 72 hours after the progesterone injection and it remained elevated for 78 hours. Since plasma progesterone levels are high in pregnant as well as pseudo-pregnant bitches it should follow that plasma fibrinogen levels should also increase in bitches which had not been bred. Gentry and Liptrap (1981) therefore compared the fibrinogen levels of two pregnant and one non-pregnant bitch but found no increase in plasma fibrinogen levels in the bitch which had not been bred during oestrus. This indicated that progesterone alone is not the cause of higher plasma fibrinogen levels during pregnancy. Eckersall et al. (1993) used this study to investigate another acute phase protein; C-reactive protein (CRP). They measured CRP in 15 bitches; nine pregnant, one pregnant bitch which lost the pregnancy, and five non-pregnant bitches. They retrospectively calculated day of ovulation by deducting 63 days from the day of parturition and found that CRP increased from around 20 days after ovulation in all pregnant bitches. CRP was not detected in significant amounts in non-pregnant bitches.

Although CRP levels in plasma could be used to determine whether a bitch is pregnant or not, it will not aid in predicting the estimated day of parturition.

2.4.5. Ultrasound of the uterus and its contents

Ultrasound, whether by Doppler (Helper, 1970), A-mode ultrasound (Smith and Kirk, 1975) or B-mode (real-time) ultrasound (Bondestam et al., 1983) has been used to diagnose pregnancy in the bitch. Bondestam et al. (1983) found ultrasound to be accurate in diagnosing pregnancy when they scanned bitches from 21 days after mating (n = 8). Following this study Bondestam et al. (1984) repeated the study on a larger sample size (n = 153) and confirmed that ultrasound is an accurate modality to diagnose pregnancy in bitches when they are scanned 28 days after mating or artificial inseminations. Shille and Gontarek (1985) compared palpation to ultrasound evaluation in detecting pregnancy in bitches. Their findings supported those of Bondestam et al. (1983, 1984). Shille and Gontarek (1985) also determined that the earliest a conceptus could be identified via ultrasound was about 19 days after ovulation (D0), which they assumed occurred 63 days prior to the day of parturition and which corresponds to about 13 days after the onset of cytological dioestrus. They scanned 23 Greyhound bitches using a portable ultrasound machine and a 3 MHz linear transducer. They also measured the average diameter of the gestational sac in transverse view and related these measurements to D0. The earliest they could identify a foetal heartbeat was 35 days after the assumed day of ovulation (around 29 days after the onset of cytological dioestrus) but the only critical factor, affecting the accuracy of the findings of this study, was that they scanned their bitches only once every three days after a single mating was allowed. The earliest a conceptus can be detected, when related to a key event in the oestrus cycle of the bitch, is 21 days after the LH Peak—retrospectively calculated from the day of parturition (England and Allen, 1990)—or 17 to 20 days after the measured LH surge (Yeager and Concannon 1990; Yeager et al. 1992; England and Yeager 1993). Heartbeats could be detected once the embryonic mass could be visualized or a day later—23 to 25 days after the LH surge. The growth rate of the conceptus (or gestational sac) was exponential from day 17 to day 25 and the diameter of the gestational sac seemed more predictive of the gestational age, compared to the length of the gestational sac. The identification of the early conceptus or the first time a heartbeat could be visualized will depend of the quality of the ultrasound machine and the quality and type of transducer used to scan the bitches. Yeager and Concannon (1990) scanned 16 Beagle bitches from 15 to 17 day after the estimated LH surge (when serum LH levels rose rapidly to above 6.36 nmol/L) with a 7.5 MHz transducer with a stand-off. They found that the diameter of the conceptus—from when it could be detected for the first time until the heartbeat could be identified for the first time—could be used to predict the gestational age to within ± 1–1.5 days.

The diameter of conceptus during early gestation has been used to estimated gestational age and predict the day of parturition (England and Allen, 1990; England et al., 1990; Yeager and Concannon, 1990; Yeager et al., 1992; Luvoni and Grioni, 2000). Foetal biparietal diameter has been used during mid to late pregnancy to estimate gestational age and predict the day of parturition (England et al., 1990; Yeager et al., 1992; Luvoni and Grioni, 2000; Kutzler and Yeager et al., 2003). England et al. (1990) found that the diameter of the conceptus was significantly related to gestational age, and used a combined regression formula—combining measurements of the foetal biparietal and trunk diameter—to construct a table for predicting the day of parturition. Using the table allowed England et al. (1990) to predict the day of parturition to within 2.84 days (SD of 1.96) in Labrador Retrievers, Golden Retrievers and their crosses. Yeager et al. (1992) scanned eight Beagle bitches between 20 and 60 days after the LH surge. They found there was a significant linear correlation between the inner chorionic cavity diameter (ICCD) measurements and the gestational age and that ICCD was the most accurate predictor of gestational age during early pregnancy—20 to 37 days after the LH surge. During later stages of pregnancy—days 38 to 60—the foetal head diameter or biparietal diameter (BPD) was the most accurate predictor of gestational age. Yeager et al. (1992) determined regression coefficients to estimate the gestational age and developed a table—using the first day a selected feature was visualized after the LH surge—to predict the gestational age of Beagle pregnancies. Kutzler and Yeager et al. (2003) used the data of 83 bitches of different breeds, and Lenard et al. (2007) the data of 76 bitches of different breeds to compare the predictive value of the tables constructed by England et al. (1990) and Yeager et al. (1992). Both studies concluded that the table of Yeager et al. (1992) was more accurate in predicting the day of parturition than the table of England et al. (1990).

Luvoni and Grioni (2000) used a regression analysis of measurements of the ICCD and BPD in four Golden Retriever-Irish Setter mixed breed bitches (medium size) for two consecutive pregnancies and three Jack Russel terrier bitches (small size) to create equations for each category to determine the days before parturition in either medium or small size dogs. They tested the equations for ICCD and BPD on 68 bitches and found that the ICCD predicted the day of parturition to within one day in 10 of 11 (90.9%) small and 10 of 11 (90.9%) medium sized bitches compared to biparietal diameter which had a lower accuracy of 70.8% (17 of 24) medium-sized bitches and 68.2% (15 of 22) small-sized bitches.

Various authors studied measurements in different breeds and different sized bitches (Son et al., 2001; Groppetti et al., 2015; Socha et al., 2015; Cecchetto et al., 2017) to determine whether day of parturition could be predicted across breeds using formulas developed from data collected from smaller sized breeds (Socha and Janowski, 2011; 2014). Socha et al. (2015) used the formulas developed by Luvoni and Grioni (2000) for medium sized dogs in giant breed bitches, and found the accuracy for predicting the day of parturition within ± 1 day or ± 2 days by using ICCD was 76.6% and 90% respectively. Using BPD was less accurate at 54.1% and 79.1%. Socha and Janowski (2017) evaluated the accuracy of three different formulas—those of Luvoni and Grioni (2000), Milani et al. (2013) and Groppetti et al. (2015)—for predicting the parturition date in German shepherd bitches. They found the accuracy of predicting day of parturition to within ± 2 days varied between 92.5% (Luvoni and Grioni) and 30.2% (Milani et al.) when using ICC measurements, and between 95.2% (Groppetti et al.) and 57.1% (Milani et al.) when using BPD measurements.

De Cramer and Nöthling (2018b) measured the biparietal diameter (BPD) of 208 English bulldog puppies (34 caesarean sections in 31 bitches) and 660 Boerboel puppies (78 caesarean sections in 70 bitches) starting 15 minutes after delivery. The difference in BPD among littermates varied from 0 mm to 12 mm (mean 4.99 mm, SD 2.78 mm) in 34 English bulldog litters and from 0 mm to 17.99 mm (mean 7.63 mm, SD 3.61 mm) among littermates from 78 Boerboel litters. This indicates that foetal BPD could vary significantly within a litter.

Variations in accuracy in predicting the day of parturition between different formulas used in ultrasonographic fetometry and using foetal measurements which could vary significantly between foetuses of the same litter in predictive formulas might not be accurate enough to predict when a bitch is likely to whelp.

3. Materials and Methods

This research project was approved by the Animal Ethics Committee of the University of Pretoria, project number V034-17 of which a copy appears in the appendix.

3.1. Model system

This was an observational study. We used beagle bitches from the Onderstepoort Teaching Animal Unit (OTAU) colony—since the standard breeding method used on them permitted the execution of our experimental design. The bitches were assessed throughout their oestrous cycle, bred optimally to breeding sound males and the onset of cytological dioestrus was accurately determined. They were brought to the Onderstepoort Veterinary Academic Hospital (OVAH) to confirm pregnancy. Ultrasound examinations were done daily from 17 to 32 days after the onset of cytological dioestrus (D17 to D32) to record the required measurements. Bitches were returned to the OVAH during the last week of gestation for litter size determination by means of abdominal radiographs and then remained in the OVAH for partus observation. Once the bitches were hospitalised for observation of parturition, they could be examined every eight to 12 hours as D57 approached and beyond, when needed, to record the day of cervical dilatation. Although we only used Beagle bitches in our study, we measured extra-foetal structures and related their dimensions to one another to derive relative variables that were not dependent on foetal size, with the aim of generating a model that could be used in bitches of different breeds and sizes.

3.2. Experimental design

3.2.1. Animals

Fourteen beagle bitches belonging to the Onderstepoort Teaching Animal Unit (OTAU) colony, which were destined for breeding, were used in this study. The bitches are kept in a breeding colony which is housed in kennels with concrete floors to which they are confined to during the night and during inclement weather. They have access to grass camps which they share with specific groups during the day. They are fed a well-balanced commercial diet twice a day and have free access to clean water. They are vaccinated according to prescribed schedules, dewormed on a regular basis and the Beagle interest group of the University of Pretoria, Onderstepoort assess their health status daily.

The bitches were assessed twice a week for signs of pro-oestrus. When pro-oestrus was detected the bitches underwent vaginal speculum examinations every second day until they progressed to oestrus. During oestrus the bitches were optimally bred by means of artificial insemination (AI) to a dog selected by OTAU—the dog was confirmed as being breeding sound before being used for AI.

During late oestrus vaginal speculum examinations and cytological evaluations were done daily, to accurately determine the first day of cytological dioestrus (D0). Gestational age of foetuses and parturition dates were predicted based on this date in each bitch. This date guided the researcher as to when to perform ultrasound examinations and collect the relevant data.

The ampullary phase of gestation lasts from about D17 to about D32. This is the stage of gestation when the conceptuses are still spherical or ellipsoid in appearance with empty uterus between two adjacent conceptuses, with the conceptuses not distorting or overlying one another (England et al. 1990). The bitches were examined by means of ultrasound for the first time on D13 and, if confirmed pregnant, every day until D32. Daily ultrasound measurement of the relevant lengths and diameters, as well as the day of cervical dilatation (DCD) and the day of parturition (DP) allowed the relationships between the predictor variables and the intervals to DCD and DP to be established.

Determining D0 helps to estimate when the bitches are likely to whelp (~D57). The bitches were, as part of the routine management of pregnant bitches of the colony, brought to the Onderstepoort Veterinary Academic Hospital (OVAH) for radiographs on D50 to determine litter size, and then remain in the hospital for pre-partum examinations and partus observation.

The researcher examined pre-parturient bitches every eight to 12 hours to determine and record the date and time of cervical dilatation (DCD). Once DCD was noted, final year clinic students observed the bitches and recorded the date and time of onset of second stage labour (DS2).

Data collection for study was complete once a complete set of data from the 14 bitches was collected during the mid-gestational period and DCD and DS2 had been recorded for all bitches.

3.3. Experimental procedures

3.3.1. Vaginal speculum examination

The vaginal speculums consisted of pieces of Perspex tubing 220–330 mm long with an outer diameter between eleven and 22 mm and an inner diameter between nine and 16 mm. The smaller speculums were used during the peri-oestrous period and the larger ones close to preparturion. Speculums were cleaned and sterilised in ethylene oxide gas and then aerated as part of an automated process to remove ethylene oxide gas residues. The vagina was illuminated by a cold light source shone through the speculum. Care was taken to evaluate the entire length of the vaginal mucosal folds without delay whilst slowly advancing and retracting the speculum (taking care not compress or stretch the vaginal folds) to obtain an overall impression of the appearance of the folds. The folds of the vaginal mucous membrane were described as being oedematous; shrinking rounded (still large, but shrinking with rounded profiles); shrunken angular (shrunken with the primary, as well as the secondary, folds having angular crests) or small and rounded, indicating that the bitch was in dioestrus or anoestrus (Jeffcoate & Lindsay 1989).

3.3.2. Vaginal Cytology

Cells for vaginal smears were be obtained by means of cotton-tipped ear buds fixed to long (~300 mm) stainless steel wire handles and sterilised by autoclaving. The swabs were moistened with saline. The ear bud was passed through the vaginal speculum into the cranial vagina and rotated through 360° to collect cellular material (Olson et al., 1984; Allison, 2014). Cells were then rolled onto a marked glass slide (identified with the bitch's name and the date), dried and stained with Diff Quick stain (Kyroquick, Kyron Laboratories, Benrose, Johannesburg, South Africa) (Olson et al., 1984).

The thickness of the smear was noted (thin, moderate or thick). The entire slide was examined at x 200 magnification (Olympus BH-2 bright-field microscope) to determine the predominant epithelial cell type (basal, para-basal, early intermediate, late intermediate or superficial). The superficial cell index (percentage of vaginal epithelial cells that were of the superficial type) was determined. The numbers of leukocytes and erythrocytes was subjectively appraised and recorded as none, few or many. The amount of debris was subjectively appraised and recorded as absent, slight or copious.

3.3.3. Staging of the oestrous cycle

The stage of the oestrous cycle was determined from the information gathered during vaginal speculum examination and from the vaginal smear. Bitches were judged to be ready for insemination when they were in oestrus or in late oestrus.

The bitch was assessed as being in early oestrus when the vaginal mucosal folds appeared dry, shrinking rounded or on the verge of turning shrunken angular on vaginal speculum examination. The bitch was assessed as being in late oestrus when the vaginal mucosal folds appeared dry, shrunken angular and pale pink or pale. When the folds have progressed to appear slightly moist and start changing from shrunken angular to small round folds—this would indicate that the bitch was in very late oestrus and that the onset of dioestrus was imminent. On microscopic examination of vaginal cytology smears the superficial cell index (SCI) was 100%, there were no leukocytes present and the background was clear with no debris present. In very late oestrus some leucocytes (in small numbers) might appear on the smear and the SCI might start decreasing (usually by no more than 5%) from 100%.

When the vaginal mucosal folds changed in appearance to moist, pink, small and round and the SCI for the first time decreased by more than 20% from the previous day's SCI, the bitch was judged to be on D0 and she was not inseminated again.

3.3.4. Semen collection, breeding soundness evaluation of dogs and AI of bitches

We collected semen from a dog before he was due to be used for insemination of a particular bitch. Semen was collected by teasing the dog with a bitch in oestrus, collecting the sperm-rich and post-sperm fractions separately in sterile plastic tubes which were immersed in a water jacket with the water at a starting temperature of 37 °C, which was allowed to gradually cool towards room temperature at 25–28 °C. The volume, colour and pH were recorded and smears were made from each fraction, stained in Diff Quick and examined separately. Sperm morphology was assessed on a smear of the sperm-rich fraction stained with eosin and nigrosin. Sperm concentration was not measured. All the dogs that were used for insemination yielded ejaculates with more than 80% progressively motile spermatozoa and more than 60% morphologically normal spermatozoa. Once a dog was considered as being breeding sound we collected semen in the same way for each insemination but only assessed volume, colour, consistency and motility of the sperm-rich fraction before using the ejaculate for insemination. We extended the sperm-rich fraction with prostate fluid, or occasionally we used Triladyl® extender (Minitüb GmbH, Hauptsraβe 41, Tiefenbach, Germany). Containing TRIS, citric acid, sugar, buffers, glycerol, water and antibiotics), to increase the volume of the insemination dose to ~4 ml. Bitches were inseminated intra vaginally every second day from when we estimated that the bitch was entering late oestrus until D0 was determined. A five or 10 mm syringe (Braun Injekt® Solo, 25 Tsessebe Crescent, Corporate Park South, 1685 Johannesburg, Gauteng, South Africa) was attached to a pipette (made of PVC with outer diameter of 5 mm, inner diameter of 3 mm and length of 450 mm, supplied by Kyron Laboratories, 29 Barney Road, Johannesburg, South Africa). The tip of the pipette was inserted into the semen, the semen drawn up through the pipette and suction was stopped as soon as the last of the semen volume was filling the pipette. There was no air in the pipette. The pipette was inserted into the vagina and advanced up to the cervix. We raised the bitch's hind quarters so that her stifles (in a flexed position) were higher than her withers before depositing the semen at the cervix after which we massaged the clitoris for about 10 seconds and kept her hind quarters raised for 10 minutes. The timing and method ensured that viable semen was present in the reproductive tract at the time when mature oocytes should be ready for fertilization.

3.3.5. Ultrasound identification of foetuses to examine

Examinations were performed daily from D13 (to assess pregnancy status of the bitch) to D32 but measurements were only taken and recorded from D17 to D32.

The bitch was placed in dorsal recumbence and the ventral abdomen was clipped from her xiphoid to her pubis and from her *linea alba* to two centimetres lateral of her flank folds on each side. The clipped area was maintained until the last ultrasound examination had been done to ensure optimal contact between the transducer and the skin when acoustic gel was applied.

B-mode ultrasonography was used. An optimally focused and adjusted 5 MHz or 7.5 MHz (depending on the depth and size of the object of interest) convex linear phase-array transducer connected to a Sonoline Omnia ultrasound machine was used. Firstly, pregnancy was confirmed, following which the litter size was determined or estimated.

Ultrasound examinations were performed daily and all required measurements of three or four conceptuses were recorded. If the litter consisted of three conceptuses, each conceptus was examined. If the litter consisted of four or more conceptuses, four were examined; one in the left-caudal abdomen (LCa), one in the left cranial abdomen (LCr), one in the right caudal abdomen (RCa) and one in the right cranial abdomen (RCr). Measuring conceptuses that were distant from one another avoided measuring the same conceptus more than once on the same day. All the measurements of one conceptus were completed before the next conceptus was measured, to ensure a valid data set for each conceptus. (We realise, of course, that with litters of more than four conceptuses, the four conceptuses examined were not necessarily the same conceptuses which were examined each day but the model is deemed sufficiently robust to tolerate that variation).

3.3.6. Ultrasound measurements of the conceptus in axial longitudinal view (ALV)

The following identified an axial longitudinal section through the conceptus:

ALV during the early stages:

The empty uterus was seen on each end of the conceptus.

The banana-shaped embryo (when seen) lay with its long axis perpendicular to that of the uterus.

ALV during later stages:

A perfect section though the near and far parts of the placental zone was seen with no tangential pseudo-thickening.

Marginal haematomas were symmetrical in the near and distal fields, with the opposite openings between the near and distant sections through the marginal haematomas symmetrical and maximal.

The fluid in the polar parts of the allantois were symmetrical and of maximal length.

Measurements in ALV:

Maximum straight-line length of the placental zone (LPZ). LPZ was measured from the one polar extreme of one marginal haematoma of a placental zone to that of its other marginal haematoma.

Maximum length of the dome-shaped allantoic cavity polar to the first marginal haematoma (LDome1). A line was first drawn from the polar extreme of the near section through the first marginal haematoma to that of the far section through the first haematoma. LDome1, which was the longest perpendicular measurement from this line to the first pole of the conceptus, was measured.

Maximum length of the allantoic cavity polar to the second marginal haematoma (LDome2). A line was first drawn from the polar extreme of the near section through the second marginal haematoma to that of the far section through the second haematoma. LDome2, which was the longest perpendicular measurement from this line to the second pole of the conceptus, was measured.

The average for LDome1 and LDome2 was calculated as AvLD and recorded.

If the conceptus was still small enough to completely fit onto one image in perfect ALV, all the necessary measurements could be obtained from one image, otherwise more images from a conceptus had to be used.

If the embryo (foetus) was already seen in transverse section, it was assessed whether the amnion is visible dorsal to the embryo or foetus as a closed membrane, enclosing the amniotic cavity with its fluid.

3.3.7. Ultrasound measurements with the conceptus in equatorial transverse view (ETV):

The following identified an equatorial transverse view (ETV):

The placental zone was seen as a circular echoic ring with no pseudo-thickening due to oblique sectioning.

The fluid central to the placental zone was circular.

Both the placental ring and the fluid central to it, as described in the preceding two points, were at their largest.

Later, when the yolk sac had started to elongate in a polar direction, ETV showed it at its smallest sectional surface, usually quite circular.

The early embryo (when seen) appeared as a mural echoic, banana-shaped structure, which later was more centrally positioned.

Later, the centre of the foetus was seen in perfect cross section.

Ultrasound measurements in ETV:

The diameter of the extra-foetal fluid of the conceptus (TFD), standing for Total fluid diameter—irrespective of whether the fluid is only yolk or also allantoic fluid or also amniotic fluid—was measured. (If the embryo or foetus had a mural position and it lay in the plane of section, the circular periphery of the fluid was extrapolated and measured, or the transducer moved minimally to still maintain a perfect transverse section but immediately next to the embryo or foetus). The two largest perpendicular measurements were recorded and the average thereafter determined.

Once the yolk sac could be seen as a sac distinct from either the allantois or the amnion, the inner transverse diameters of the yolk sac was measured. The method of measurement was analogous to that described for the TFD, except that the direction of the first diameter—and therefore the one perpendicular to it—was not random but predetermined: The height of the yolk sac (HYS) was measured from the embryonic (foetal) aspect of the yolk sac towards the opposite side and the width of the yolk sac (WYS) was measured as the largest diameter perpendicular to the HYS. The YD (Yolk sac Diameter) was determined as the average of HYS and WYS. (Note that before the yolk sac was seen as a sac distinct from the allantois or amnion, HYS, WYS and therefore the YD was be the same as the TFD).

If the embryo (foetus) was seen in transverse section, it was assessed whether the amnion was visible dorsal to the embryo or foetus as a closed membrane, enclosing the amniotic cavity with its fluid.

3.3.8. Identifying the onset of cervical dilatation

The pre-parturient bitches boarded in the OVAH from D50 and clinical examinations were performed on them daily, this included recording observations related to the possible onset of parturition—relaxation of the birth canal, nesting behaviour or discharges from the vulva. From D55 (or earlier when we suspected parturition was imminent) we also performed a vaginal speculum examination every eight to 12 hours. We assessed if we could visualise the cervix and, once seen, whether or not the cervix was starting to dilate. The first sign of cervical dilatation, from a few millimetres up to visualising foetal membranes in the birth canal, was considered as onset of cervical dilatation and was recorded as the onset of parturition (DCD).

3.3.9. Onset of stage two of parturition

Once cervical dilatation was recorded students observed the bitch continuously to record data, assess if parturition was progressing normally, assist if needed and inform the researcher if a possible dystocia is suspected. Stage two of parturition was recorded as the time when the bitch displayed strong abdominal contractions resulting in the birth of a puppy. If a bitch strained for longer than 30 minutes and a dystocia was diagnosed the onset of those contractions was recorded as Stage two of parturition. When a bitch delivered a puppy during the time when continuous abdominal contractions were noted for the first time, the combined result was recorded as the onset of Stage two of parturition.

3.4. Observational and analytical procedures

3.4.1. Data collection

For each bitch the microchip number, name, age, and parity were recorded on the data capture sheet. The data capture sheet recorded all procedures, results thereof and observations made in a chronological fashion. This included the time and dates of each event as they were observed.

On each day the bitch was examined clinically to confirm that she was healthy.

Critical data that were collected were:

3.4.1.1. Data collection during oestrus

1. The date of onset of pro-oestrus

2. Findings on vaginal speculum examination

3. Vaginal cytology results (thickness of smear, superficial cell index and the presence of leukocytes, red blood cells or debris)

4. AI dates

5. The name and microchip number of the male used for semen collection

6. The semen quality

7. The date of D0

3.4.1.2. Data collection each day from D13 to D32:

1. The date

2. Estimated litter size. (Only recorded until it was established whether the litter size was <4 or ≥4.) A zero was recorded if a bitch was not pregnant.

3. Identity of conceptus, based on its position (LCa, LCr, RCa or RCr)

4. For each conceptus:

LPZ

LDome1

LDome2

AvLD

TFD

HYS

WYS

YD.

Whether or not the amnion was seen as a closed and fluid-filled sac dorsal to the embryo or foetus.

3.4.1.3. Day of onset of cervical dilatation

Date and time when the cervix could first be visualised as being open.

3.4.1.4. Day and time of onset of stage two of parturition

The date (DS2) and time (TS2) of onset of tenesmus and, or, the date (DP) and time (TP) of expulsion of the first foetus, whichever was observed first if they were not observed at the same time.

3.4.1.5. Litter size, gender and health of the puppies.

Litter size was determined via abdominal radiographs on D50. The puppies were assessed by the students after birth. They recorded the litter size (although radiographs had been taken to assess this) and helped to remove the foetal membranes and clear the mucus from the nose and mouth when the bitch was too tired to do this herself. They also recorded if the puppies were regarded as dysmature, the sequence in which they were born, their gender and they examined the pups for cleft palates and *atresia ani*.

3.4.2. Data Analysis

3.4.2.1. Data preparation

We strived to design a model using the Beagle whereby we can predict the interval between the day of a single ultrasound examination and the day of parturition in bitches of different breeds and sizes. To do this the outcome variable was the number of days to cervical dilatation (NCD), where the day of cervical dilatation (DCD) marks the day of parturition.

Predictor variables were categorised as direct or relative variables. The direct variables were the total fluid diameter (TFD), length of the placental zone (LPZ), yolk sac diameter (YD) and the average length of the avillous domes (AvLD). All the direct variables were measured in millimetres. The relative variables were the total fluid diameter divided by the yolk sac diameter (TFD/YD), mean dome length divided by length of placental zone (AvLD/LPZ), length of placental zone divided by total fluid diameter (LPZ/TFD) and the mean dome length divided by total fluid diameter (AvLD/TFD). Their inverses, namely the yolk sac diameter divided by the total fluid diameter (YD/TFD), length of placental zone divided by mean dome length (LPZ/AvLD), fluid diameter divided by length of the placental zone (TFD/LPZ) and the total fluid diameter divided by the mean dome length (TFD/AvLD) were also considered.

We calculated TFD by calculating the average of the two largest perpendicular measurements as seen in equatorial transverse view (ETV). The YD was calculated as the average of the height of the yolk sac (HYS) and the width of the yolk sac (WYS) as seen in ETV. LPZ was recorded as the average of the measured LPZ in the near and far field as seen in axial longitudinal view (ALV). AvLD was calculated as the average of the lengths of the two domes (LDome1 and LDome2) as measured in ALV.

We started the data analysis by looking at scatterplots of the outcome variable against each predictor variable on its own. The scatterplots showed that the data of Smiler was obviously different to the data of the other bitches (Figures 1 and 2), therefore we excluded her data.

Using the R-squared value we assessed the fit of linear-, power-, logarithmic-, exponential- and fractional polynomial functions to describe the relationship between the outcome variable and each direct and relative predictor variable on its own. For most variables, the R^2 value for a linear function was not much lower than for more complex functions. Seeing that our aim was to keep the model as simple and practical as possible we therefore decided to use linear functions only.

An observation consisted of an ultrasound examination with all the measurements done during that examination on a particular conceptus in a particular bitch on the particular day. An observation therefore consisted of a bitch by day by conceptus combination. Each variable of an observation was recorded in a separate column and recorded in a specific row of a data file (in Excel and Stata 14) assigned to that observation.

We compiled a histogram of NCD and it showed that observations were made from as early as 46 to as late as 24 days before DCD, giving a period of 23 days. Once all data were recorded in the data file, a unique random number was allocated to each observation. All observations were then sorted by NDC and, within that, by bitch name and within that by random number. We marked two conceptuses with the smallest random numbers from the same bitch measured 24 days before DCD (DCD24) with a 1 in a newly created variable named xmodel. For DCD25 we selected the two conceptuses with the smallest random number from the next bitch, and so on. The two conceptuses from each bitch by day combination by NCD selected this way were excluded from model generation and later used to test the model we generated.

3.4.2.2. Selection of data assigned to model generation or to model testing

The observations not marked with a 1 in the newly created variable were used for model generation (xmodel = 0) and the observations marked with a 1 were used to test the model we generated (xmodel = 1).

3.4.2.3. Generating the model to predict NCD from direct variables

We calculated the average for each direct variable over all conceptuses marked for model generation for each bitch by day combination. Although, in the data file, this mean was assigned to each conceptus assigned to model generation for a particular bitch by day group, we labelled the mean once for each bitch by day combination by assigning the value of one to a variable named "avxdot_pickone" to one of the conceptuses assigned to model generation within that bitch by day combination. We used a mean only if it occurred in a row of the data file for which the column named avxdot_pickone held the value of one. We did a multiple linear regression, with NCD as outcome variable and all four direct variables (TFD, LPZ, YD and AvLD) as predictor variables. For this regression we used the mean over all the conceptuses assigned to model generation for each bitch by day combination. We used the Y intercept and the coefficients of each direct predictor variable to predict the NCD for each bitch by day combination.

3.4.2.4. Generating the model to predict NCD from relative variables

We calculated the average for each relative variable as for the direct variables. We compiled a Pearson correlation coefficient matrix for the relative predictor variables and, as expected, found high negative correlations between each relative variable and its inverse relative variable. We therefore grouped the relative variables in two groups which did not contain inverse variables. We identified two relative variables that were highly correlated but since they were not the inverse of each other we retained them as predictors for the generation of the model using relative predictor variables.

We did a linear regression with NCD as outcome variable on each of the above-created groups of relative variables (TFD/YD, AvLD/LPZ, LPZ/TFD, AvLD/TFD; and YD/TFD, LPZ/AvLD, TFD/LPZ, TFD/AvLD), using the mean over all the conceptuses assigned to model generation for each bitch by day combination. We used the Y intercept and the coefficients of each relative predictor variable in their group to predict the NCD for each bitch by day combination and selected the model with the highest R-squared value for predicting NCD with relative predictor variables.

3.4.2.5. Calculating and assessing the error in predicting NCD

For each bitch by day combination we subtracted the predicted interval to parturition, derived from the direct predictor variable model, from the observed interval to parturition to derive the error in predicting the interval to parturition. We also did the same for the relative predictor variable model. We rounded off the errors to the nearest integer.

We generated and inspected scatterplots of the error in predicting the interval to DCD from direct predictor variables for each bitch by day combination used for model generation. We did the same for the relative predictor variables. We inspected each model (based on direct and relative variables) for heteroskedasticity—that is we inspected the magnitude of residuals against different values of the interval to DCD.

We compiled bar graphs of the error in predicting the interval to DCD from direct predictor variables for each bitch by day combination used for model generation. In the same way, we compiled bar graphs of the error in predicting the interval to DCD from relative predictor variables for each bitch by day combination used for model generation.

3.4.2.6. Using the data from conceptuses assigned to model testing to assess the precision of predicting NDC

The conceptuses previously selected and marked with a 1 in the variable xmodel were used for testing the precision and accuracy of the model we generated. We calculated the average for each direct and relative variable over all conceptuses assigned to model testing and labelled them with the prefix avexone. We labelled the mean once for each bitch by day combination by assigning the value of 1 to a variable named "avxone_pickone" to one of the conceptuses assigned to model testing within that bitch by day combination. We compiled bar graphs of the error in predicting the interval to DCD from direct and relative predictor variables for each bitch by day combination used for model testing. We also generated and inspected scatterplots of the error in predicting the interval to DCD from direct and relative predictor variables for each bitch by day combination used for model testing.

3.4.2.7. Comparing the precision of the models we generated to the precision of others studies in predicting the day of parturition

In view of the results of our study we thought it appropriate to compare the results of our study to the accuracy of predicting the likely day of parturition investigated by other studies. First we evaluated each data set to assess whether its distribution was normal or not. Where necessary a few extreme values were excluded to render the data normally distributed. We used Levene's test to compare the variance in the difference between the observed date of parturition and the predicted date thereof that we found using our models to those reported in other, published, data sets as described below. We compared the variance of the differences calculated using the model we generated for the direct variables to those obtained from the interval between the day of onset of cytological dioestrus and parturition as reported by Holst and Phemister (1974) and De Cramer and Nöthling (2017). We also compared the variance of the differences calculated using the model we generated for the relative variables to those obtained from the interval between the day of mating and parturition as reported by Holst and Phemister (1974) and Concannon et al. (1983). We performed each of these comparisons first using each of the bitch by day means that had been used to derive the models for direct and relative variables. We then performed each of the above-mentioned comparisons using those 16 bitch by day means that were not used to derive our models but reserved for testing them.

4. Results

4.1. Removal of data

For some predictor variables Smiler clearly shows a pattern largely different to the pattern shown by the other bitches (see Figure 1 in respect to the direct variable, yolk sac diameter and Figure 2 in respect to the relative variables, fluid diameter divided by yolk sac diameter). Smiler was the first bitch to be examined and we ascribe the different pattern of the scatter plots to the inexperience of the researcher. Based on these patterns we decided to remove the data of Smiler from all further analyses.

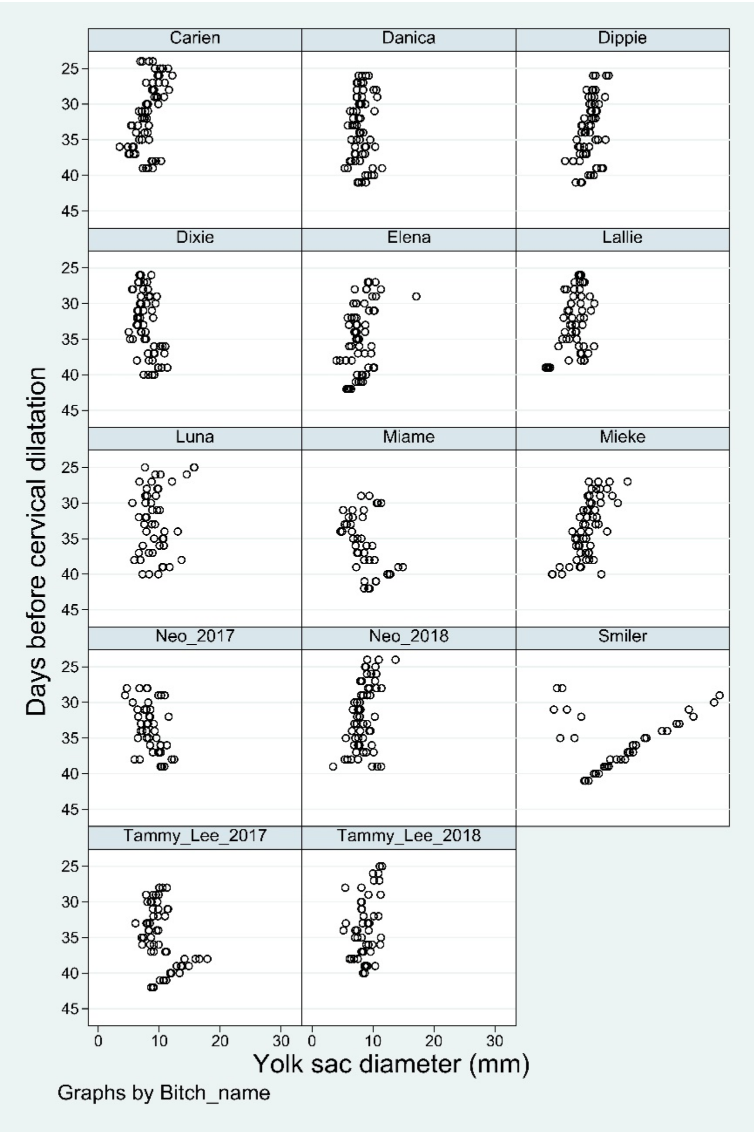

Figure 1
The relationship of the direct variable, the yolk sac diameter, to days before cervical dilatation for each bitch—with Smiler clearly showing a pattern largely different to the pattern shown by the other bitches.

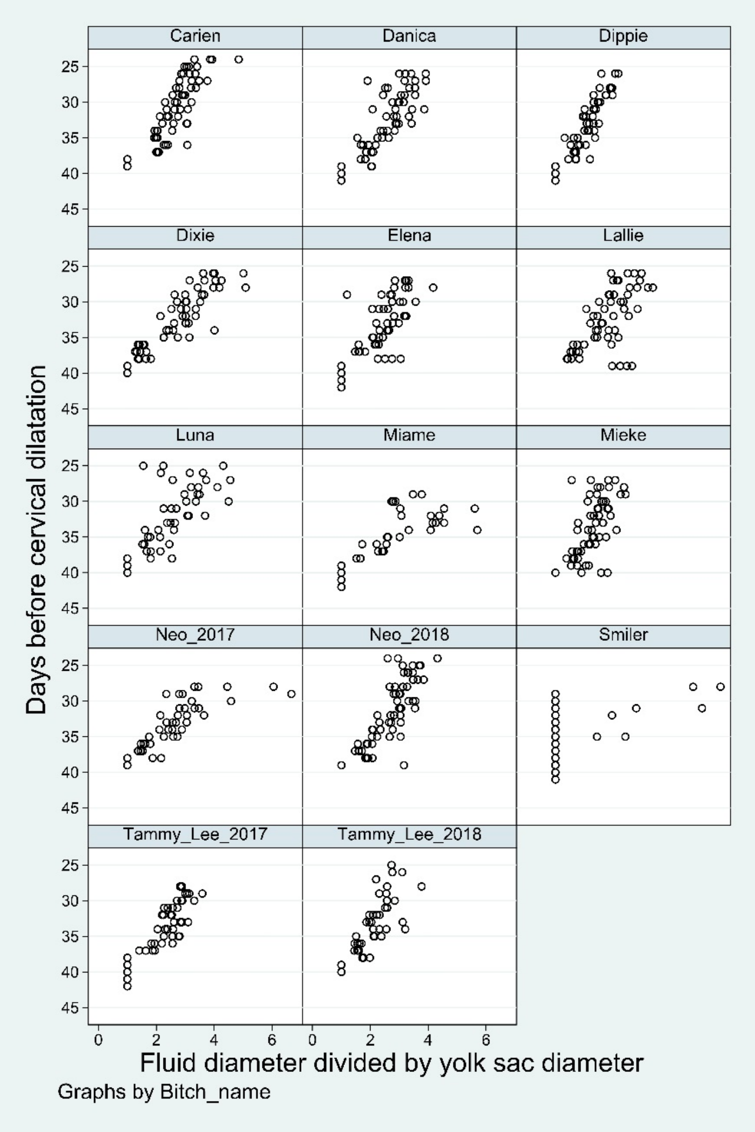

Figure 2
The relationship of the relative variable, the total fluid diameter divided by the yolk sac diameter, to days before cervical dilatation for each bitch—with Smiler clearly showing a pattern largely different to the pattern shown by the other bitches.

4.2. Interval between time of cervical dilatation (TCD) and time of stage two of parturition (TS2)

Eight of the thirteen bitches whelped naturally and five needed to have caesarean sections. One of the five bitches that needed a caesarean section did show signs of tenesmus and the onset of second stage labour but it did not progress to the birth of a puppy. The interval between TCD and TS2 for the nine bitches varied between 0.8 and 24.92 hours, with an average of 5.63 hours (SD, 7.62).

4.3. Interval between D0 and DCD

Cervical dilatation occurred in five of the 13 bitches (38%) used in our study 57 days after D0, in four of the 13 bitches (30%) 56 days after D0 and in three of the 13 bitches (23%) 58 days after D0. In one bitch cervical dilatation only occurred two days after the predicted day of parturition (D57), 59 days after D0.

4.4. Scatterplots of days before cervical dilatation on selected dimensions of foetal membranes (direct predictor variables)

There was a clear strong linear relationship between days before cervical dilatation and the direct predictor variable, fluid diameter (Figure 3), with fluid diameter increasing as the bitch approached DCD.

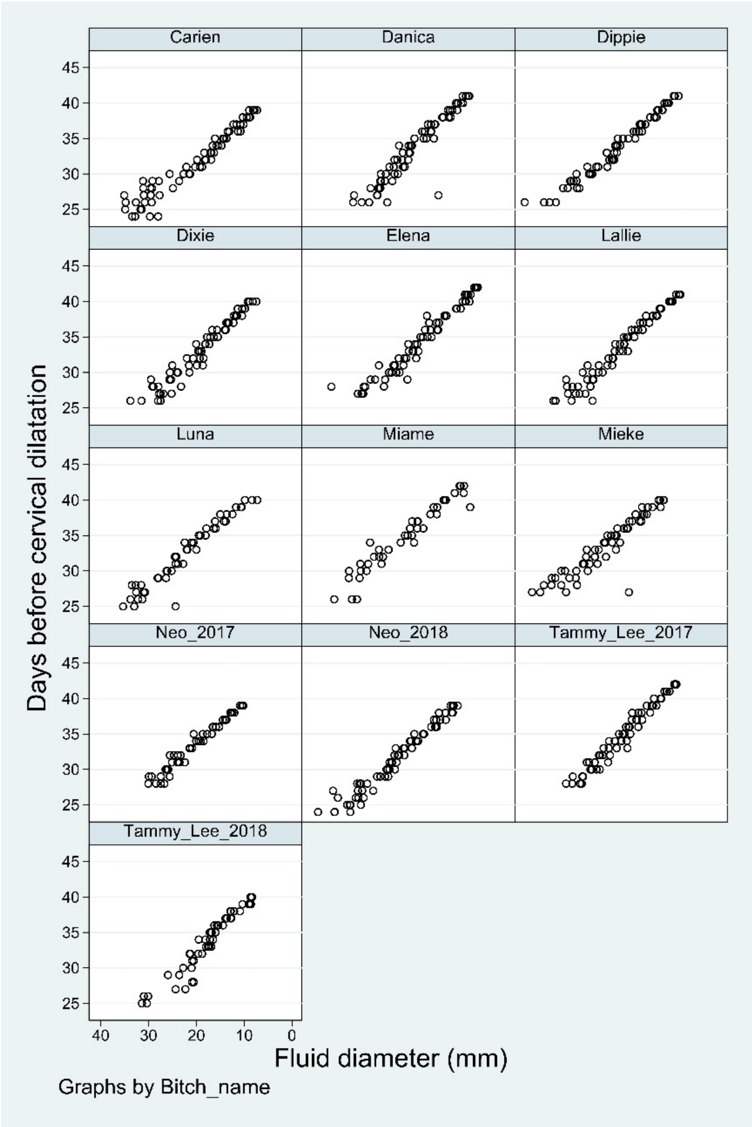

Figure 3
The relationship of the direct variable, the total fluid diameter, to days before cervical dilatation for each bitch.

The relationship between days before cervical dilatation and the direct predictor variable, yolk sac diameter, varied significantly among bitches (Figure 4). There is a fairly wide scatter in days before cervical dilatation and any particular yolk sac diameter value. There is also a fairly wide scatter in yolk sac diameter for any particular day before cervical dilatation.

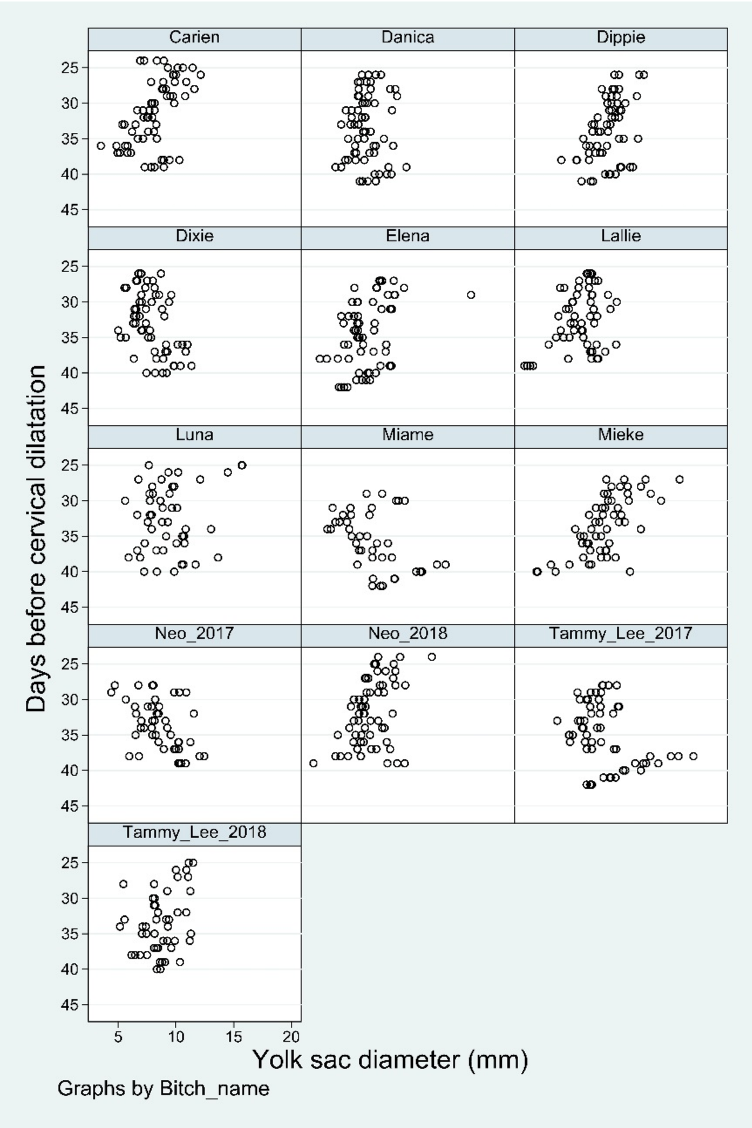

Figure 4
The relationship of the direct variable, the yolk sac diameter, to days before cervical dilatation for each bitch.

There is a similar strong relationship between days before cervical dilatation and the average length of the domes among bitches (Figure 5). The pattern of the relationship is linear from around 40 days up to about 30 days before cervical dilatation but the dome length increases more rapidly (from around day 25) as the bitch gets closer to the day of cervical dilatation.

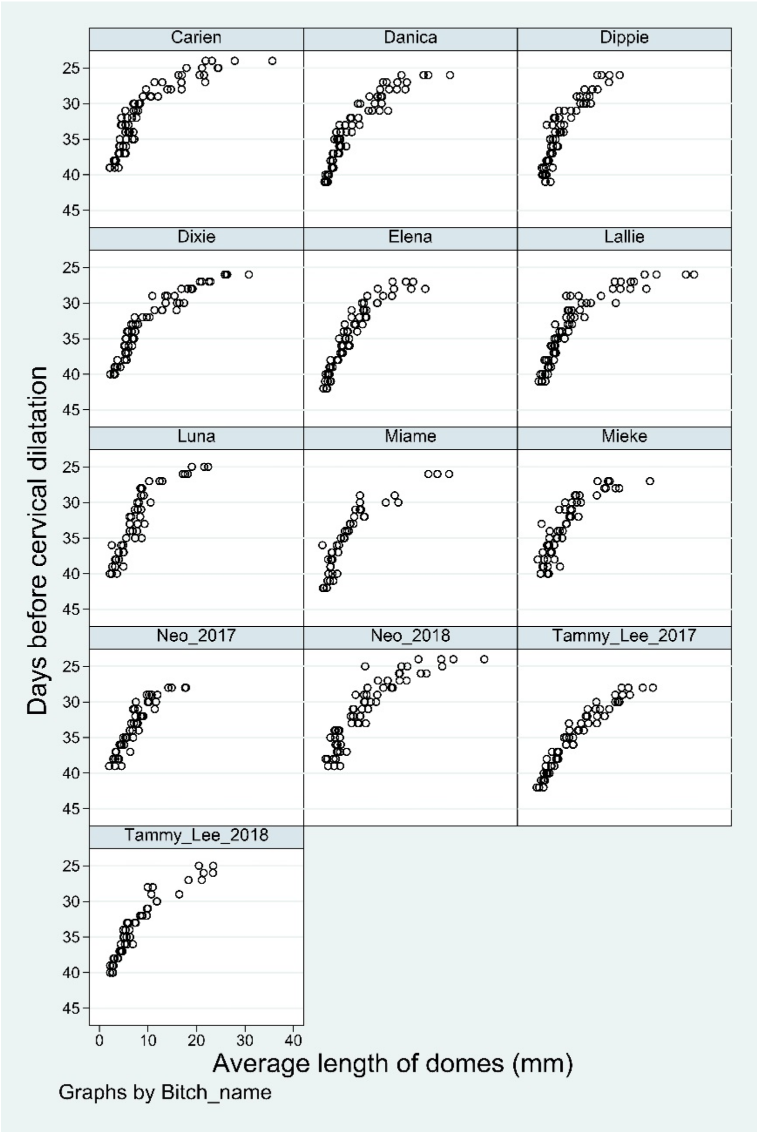

Figure 5
The relationship of the direct variable, the average length of the domes, to days before cervical dilatation for each bitch

There is a strong linear and similar relationship among bitches in respect of days before cervical dilatation and the length of the placental zone, with the length of the placental zone increasing as the bitch approaches the day of cervical dilatation (Figure 6).

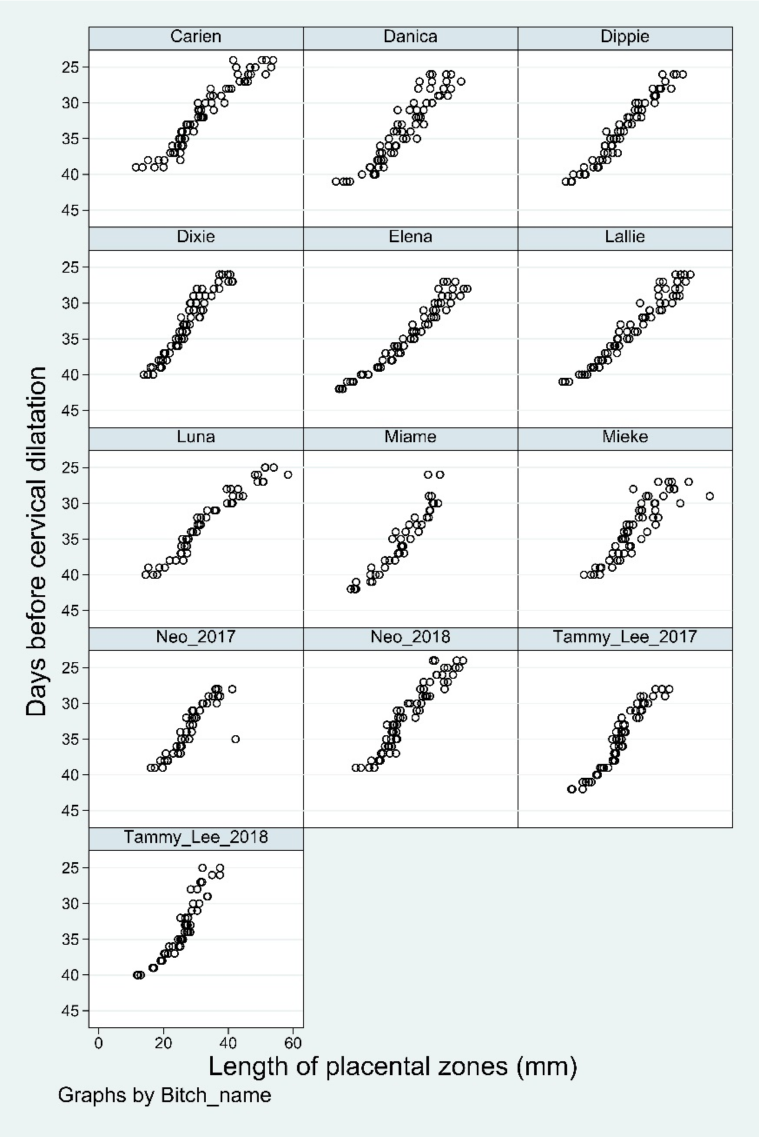

Figure 6
The relationship of the direct variable, the length of the placental zone, to days before cervical dilatation for each bitch

4.5. Scatterplots of days before cervical dilatation of each relative predictor variable

There is a linear relationship between days to cervical dilatation and the fluid diameter divided by the yolk sac diameter (Figure 7). The ratio of the fluid diameter divided by the yolk sac diameter tends to increase as DCD approaches. Although the relationship is similar among bitches it is not very strong as there is considerable scatter present.

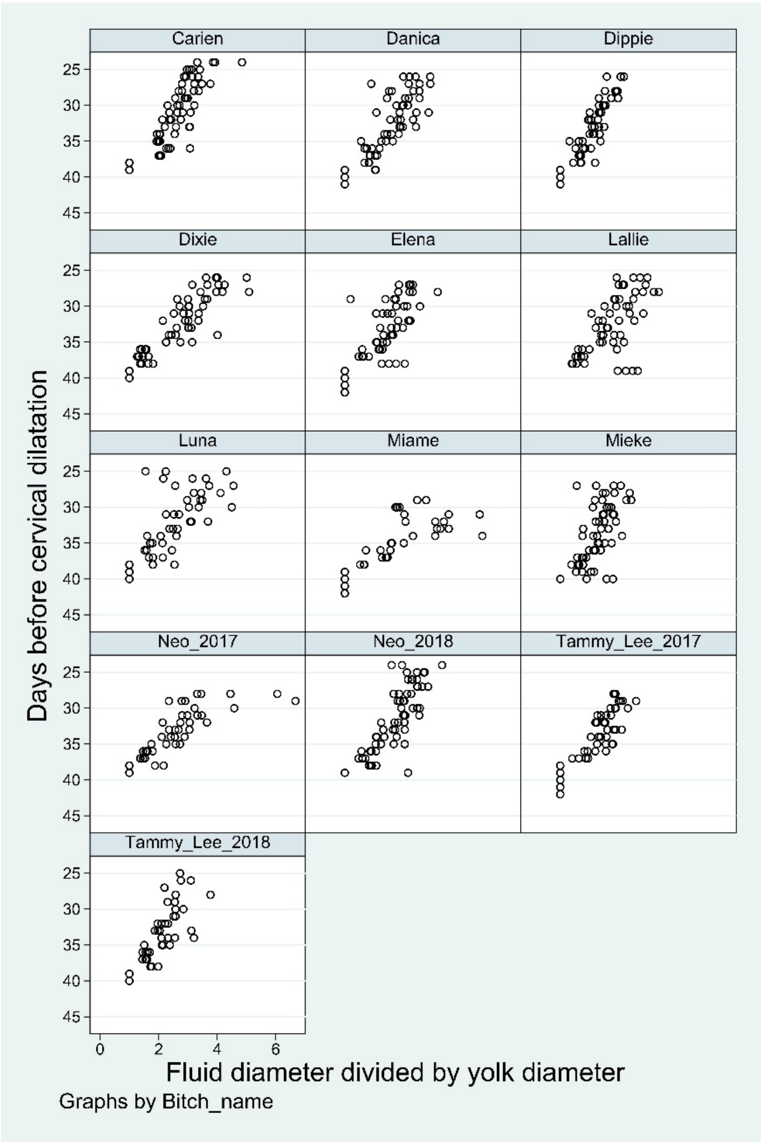

Figure 7
The relationship of the relative variable, the total fluid diameter divided by the yolk sac diameter, to days before cervical dilatation for each bitch.

There is a linear relationship, although not strong, between days before cervical dilatation and the average length of the domes divided by the length of the placental zone. The ratio increases very slightly between 40 and 30 days before cervical dilatation in most bitches but as the bitch approaches 25 days before cervical dilatation the change in ratio increases more rapidly (Figure 8).

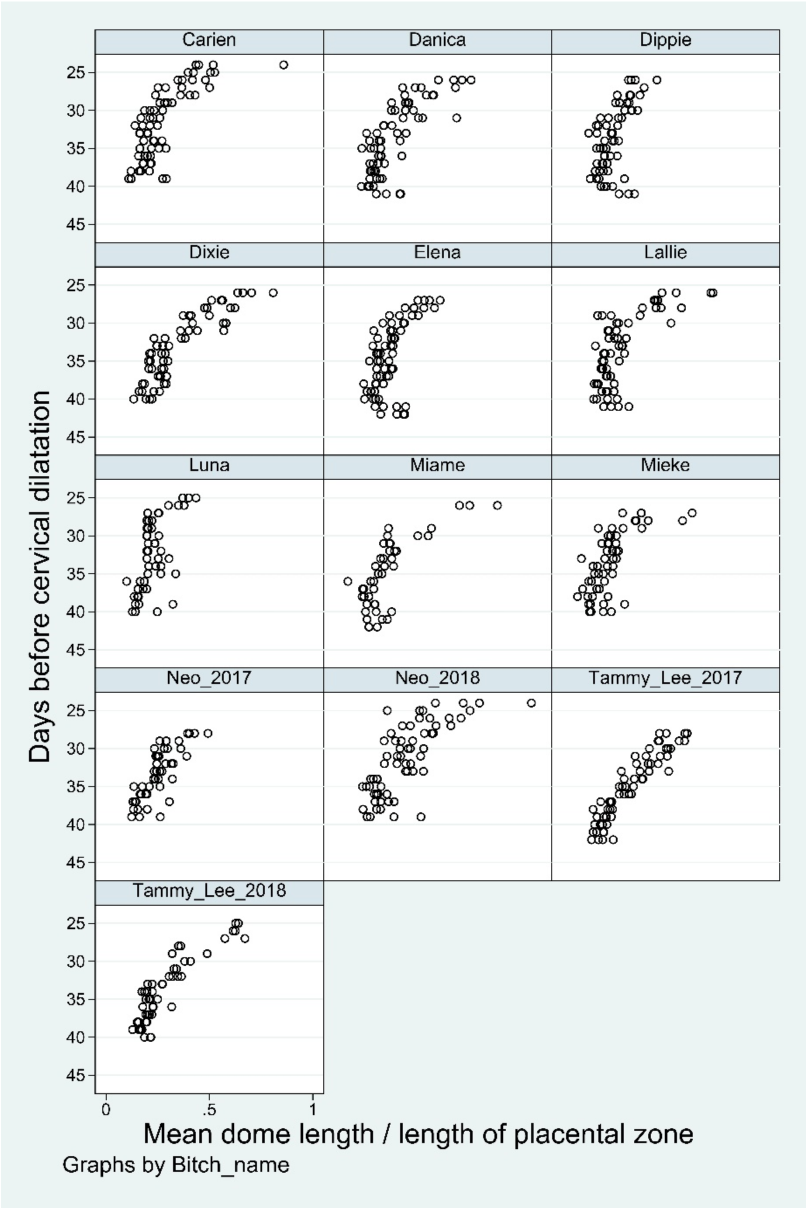

Figure 8
The relationship of the relative variable, the average length of the domes divided by the length of the placental zone, to days before cervical dilatation for each bitch

There is a weak linear relationship between days before cervical dilatation and the length of the placental zone divided by the fluid diameter (Figure 9). There is a general trend for the ratio between LPZ/TFD to decrease as the bitch approaches the day of cervical dilatation. This pattern is discernible in almost all of the bitches, but there is a variable amount of scatter which varies among the bitches.

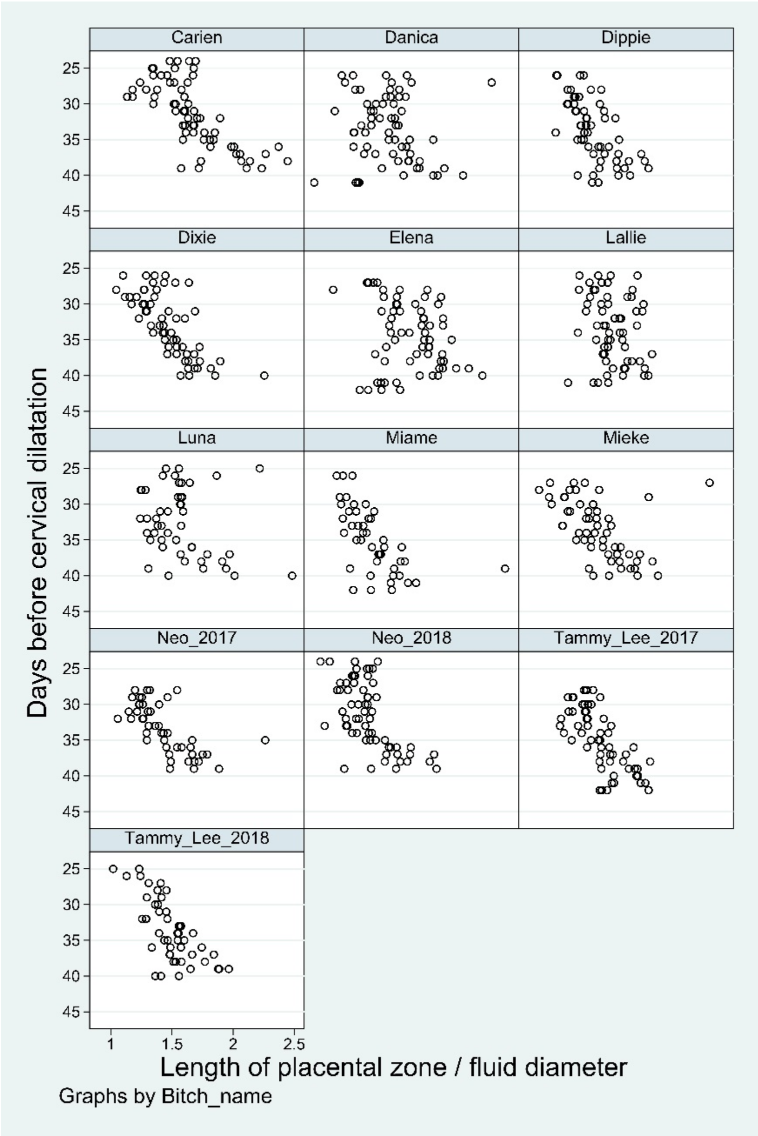

Figure 9
The relationship of the relative variable, the length of the placenta zone divided by the total fluid diameter, to days before cervical dilatation for each bitch

There is a strong linear relationship between days before cervical dilatation and the average length of the dome divided by the fluid diameter until about 30–27 d before DCD, following which the ratio starts to increase rapidly as the bitch approaches the day of cervical dilatation (Figure 10).

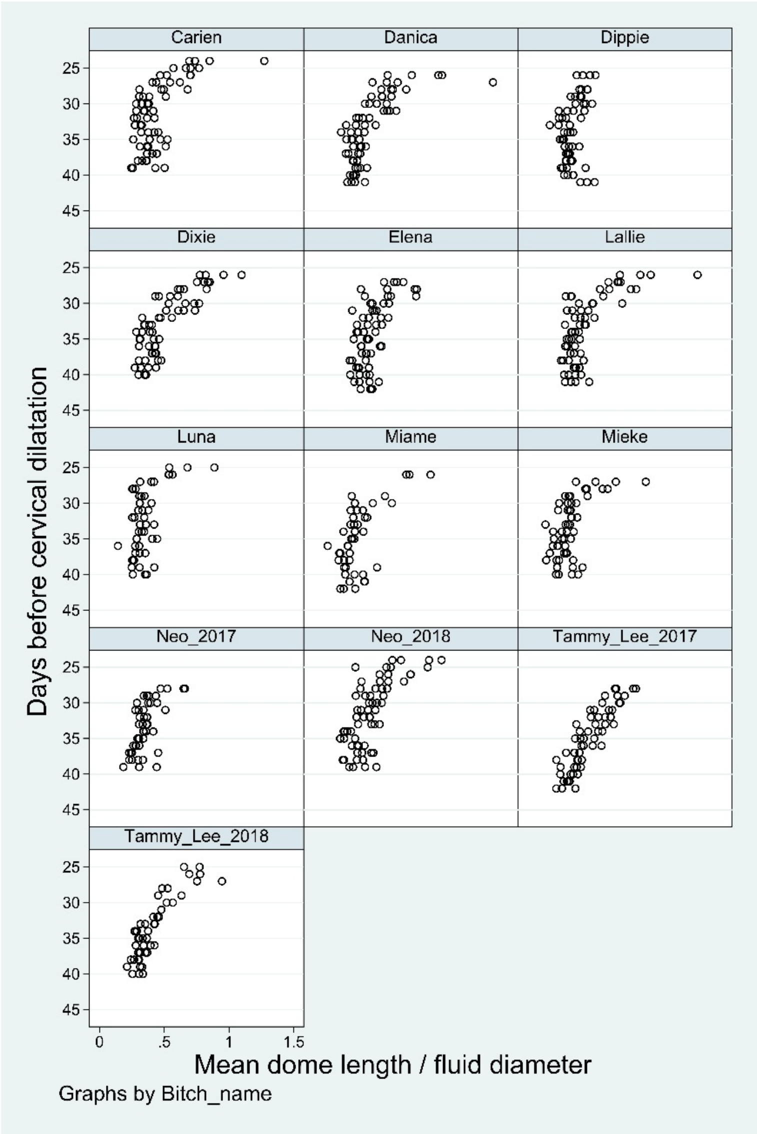

Figure 10
The relationship of the relative variable, the average length of the domes divided by the total fluid diameter, to days before cervical dilatation for each bitch

There is a linear relationship between days before cervical dilatation and the yolk sac diameter divided by the fluid diameter for most bitches (Figure 11). The ratio varies quite widely up to about 37 days before cervical dilatation, following which it starts to decrease in a linear fashion as the bitch approaches the day of cervical dilatation.

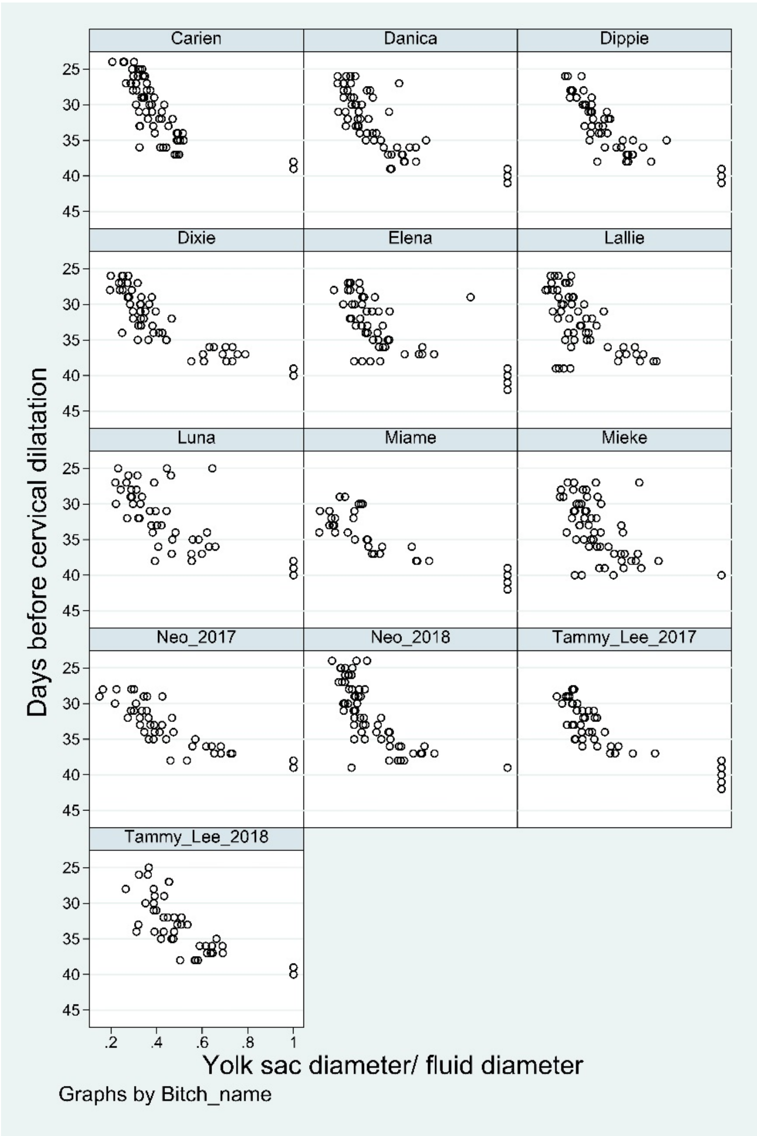

Figure 11
The relationship of the relative variable, the yolk sac diameter divided by the total fluid diameter, to days before cervical dilatation for each bitch

There is a strong linear relationship between days before cervical dilatation and the length of the placental zone divided by the average length of the domes between bitches (Figure 12). The ratio decreases steadily as the bitch approaches the day of cervical dilatation.

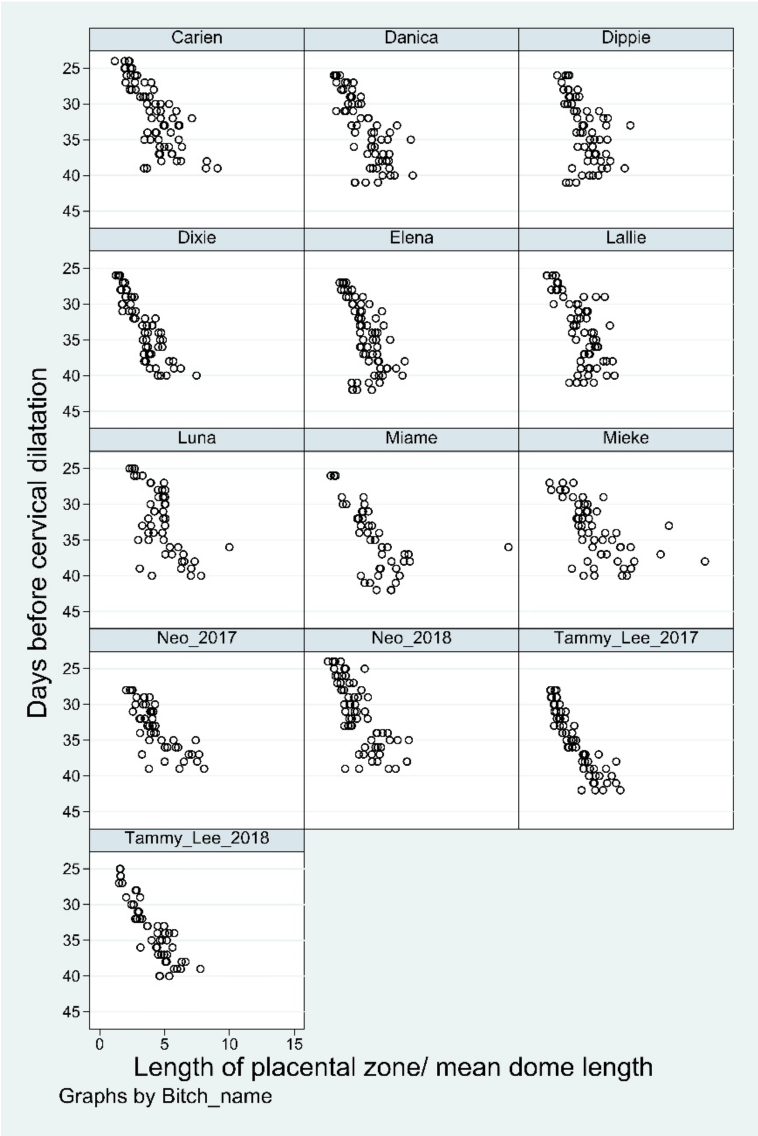

Figure 12
The relationship of the relative variable, the length of the placental zone divided by the average length of the domes, to days before cervical dilatation for each bitch

There is a linear relationship between days before cervical dilatation and the fluid diameter divided by the length of the placental zone (Figure 13). The relationship is not strong as there is a fair amount of scatter, but the general trend is that the ratio increases as the bitch approaches the day of cervical dilatation.

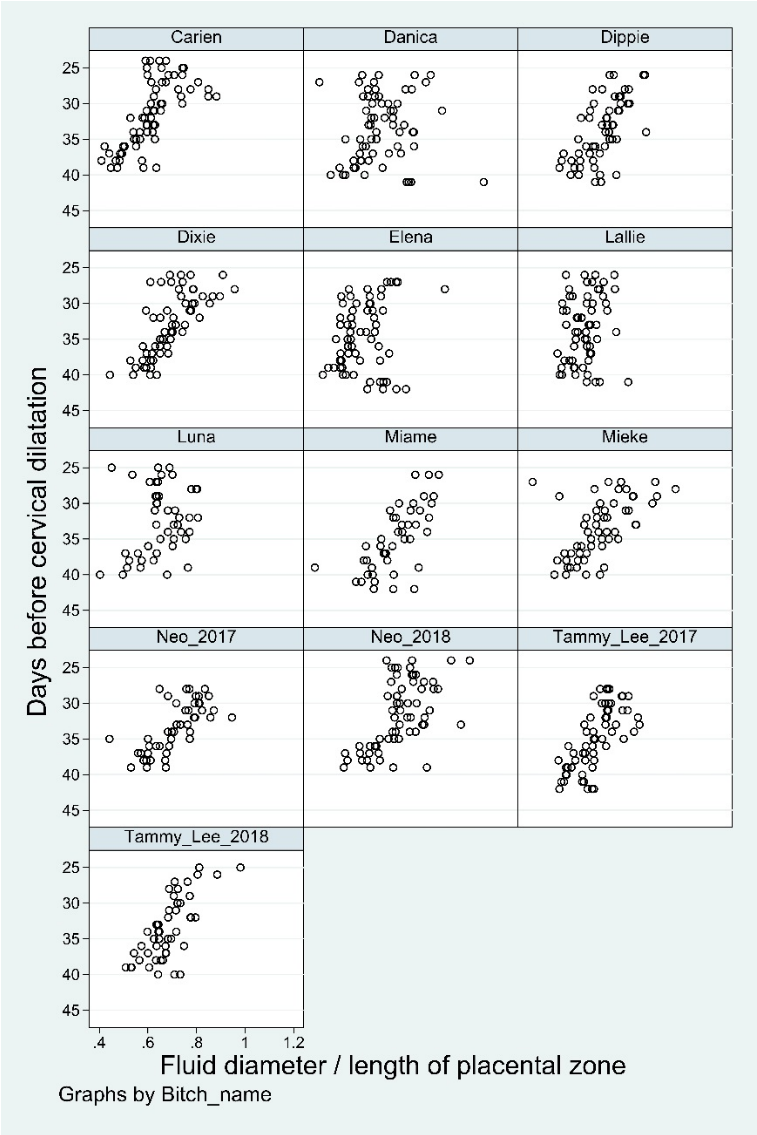

Figure 13
The relationship of the relative variable, the total fluid diameter divided by the length of the placental zone, to days before cervical dilatation for each bitch

There is a strong linear relationship between days before cervical dilatation and the fluid diameter divided by the average length of the dome (Figure 14). The ratio shows a slight but steady decrease as the bitch approaches the day of cervical dilatation.

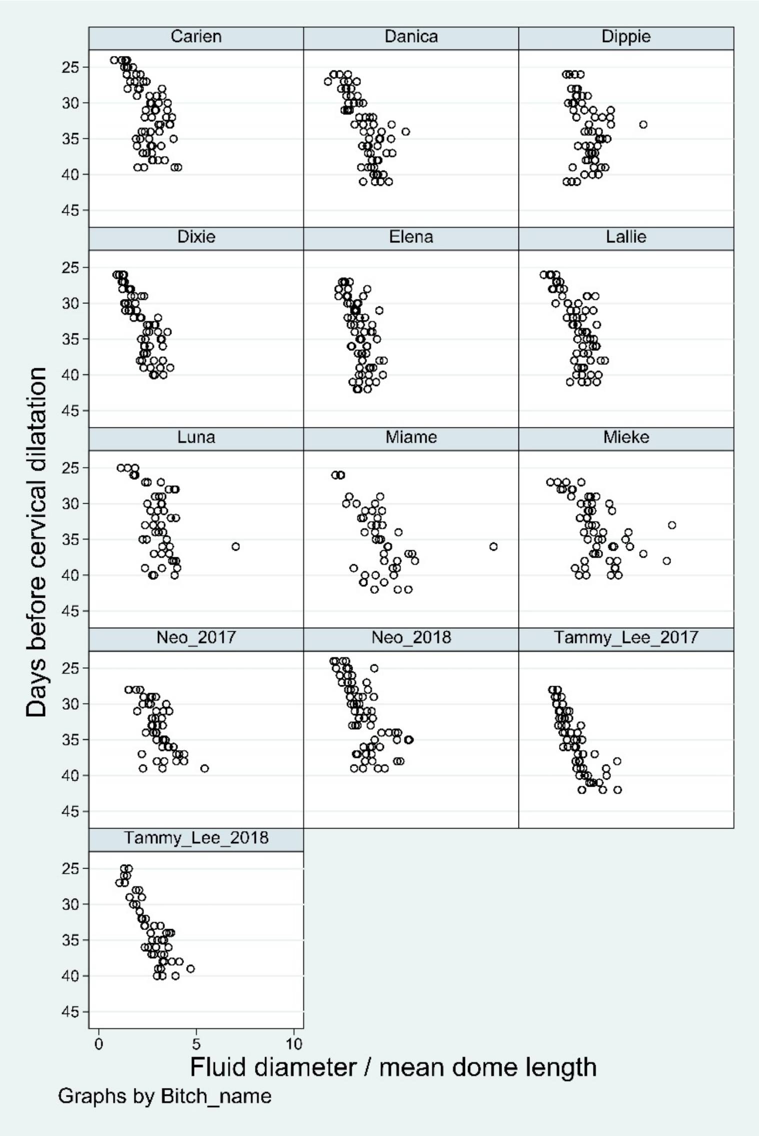

Figure 14
The relationship of the relative variable, the total fluid diameter divided by the average length of the domes, to days before cervical dilatation for each bitch

4.6. Considering days before cervical dilatation as a linear or more complex function of each predictor variable

We inspected the relationship between days before cervical dilatation (the outcome variable) and each direct and relative predictor variable, and assessed the fit of linear -, power -, logarithmic -, exponential - and fractional polynomial functions. In most cases the gain in R^2, by using functions which were more complex than the linear function, was negligible (see Figure 15 as an example). Occasionally a fairly substantial gain was achieved by using a more complex function—compare the power function and the linear function in Figure 16—but overall the gain was offset against the application of more complex functions in the generation of the regression model of days before cervical dilatation on the direct and relative predictor variables.

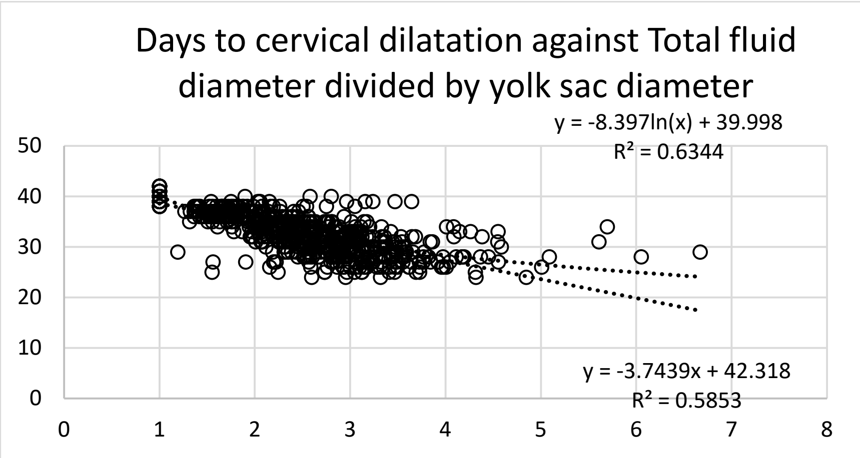

Figure 15
Scatter plots showing the gain in using a linear function compared to using a logarithmic function.

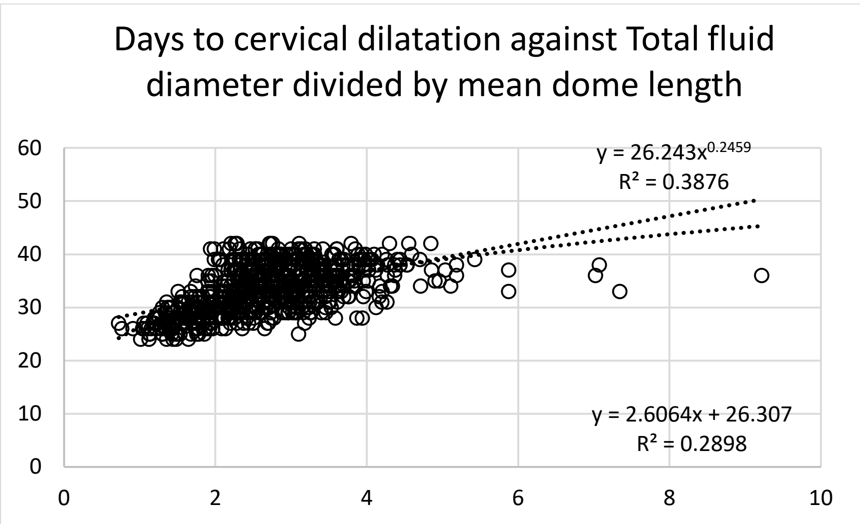

Figure 16
Scatter plots showing the gain in using a linear function compared to using a power function.

4.7. Predicting the day of cervical dilatation using selected dimensions of foetal membranes (direct variables)

The model we generated for the direct predictor variables is as follows:

$\hat{DCD}$ = 43.3440 – 0.1186(LPZ) – 0.2266(AvLD) + 0.2355(YD) – 0.3199(TFD)

With $F (4, 190) = 812.58$, $P < 0.001$ and $R^2 = 0.945$

Figure 17shows the error in estimating the interval to DCD for bitch by day combinations used for model generation when using the model for direct variables to estimate the interval to DCD. It suggests that the error in estimating the interval to cervical dilatation might increase as the day of cervical dilatation approaches. Yet, the analysis of the residuals of this model showed that there is insufficient support for the rejection of the null hypothesis that the errors were homoskedastic (P = 0.07).

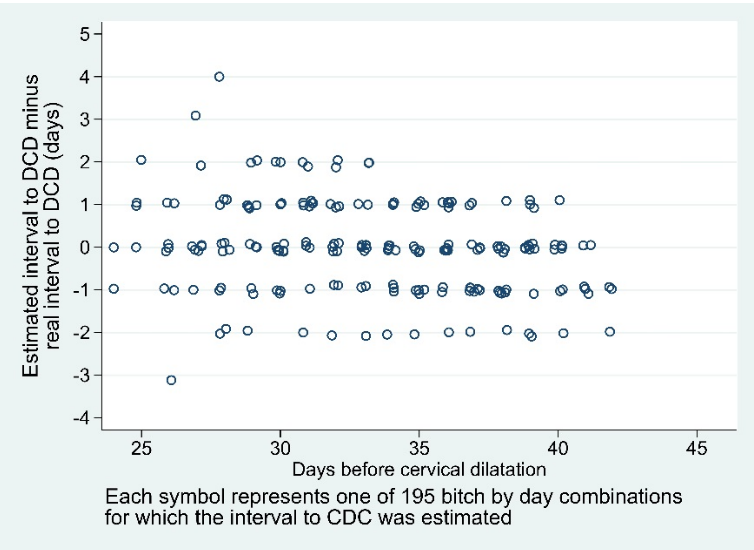

Figure 17
The distribution of error in estimating the days to cervical dilatation using the direct variables, throughout the interval of data collection, for each bitch by day combination used for model generation.

Using the model for direct predictor variables, Figure 18 shows the magnitude in error of estimating the interval to DCD for the mean value of a specific day for the bitch by day combinations marked for model generation. A positive value indicates that the interval to DCD was overestimated. Clinically, an overestimation means that the bitch would enter parturition before the estimation suggests she would. A negative value indicates that the interval to DCD was underestimated and that the bitch would only enter parturition one or more days after the estimated date. Thirty nine percent of the estimations predicted DCD accurately, 84% predicted DCD to within one day or less and 98% predicted DCD to within two days or less.

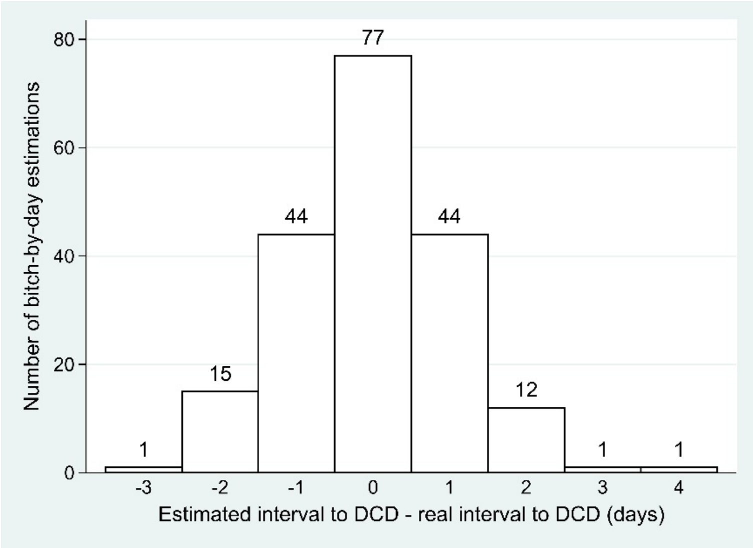

Figure 18
The magnitude in error of estimating the interval to DCD, using the model for direct predictor variables, for the mean value of a specific day for the bitch by day combinations marked for model generation.

Figure 19 shows the magnitude in error of estimating the interval to DCD, using the model for direct predictor variables, for the mean value of a specific day for the bitch by day combinations marked for model testing. A positive value indicates that the interval to DCD was overestimated and a negative value indicates that the interval was underestimated. Sixty two percent of the estimations predicted DCD correctly, 94% predicted DCD to within one day or less and only one estimation underestimated DCD with two days or less.

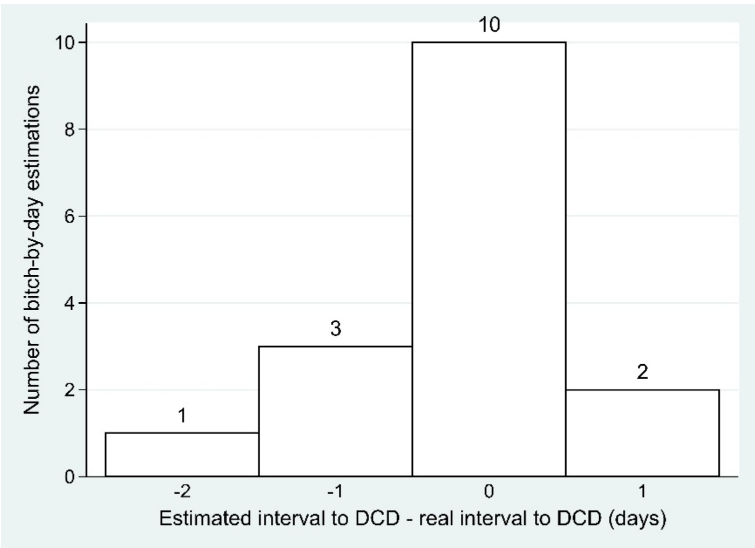

Figure 19
The magnitude in error of estimating the interval to DCD, using the model for direct predictor variables, for the mean value of a specific day for each conceptus marked for model testing.

Figure 20 shows the magnitude in error of estimating the interval to DCD, using the model for direct predictor variables, for each conceptus marked for model testing. A positive value indicates that the interval to DCD was overestimated and a negative value indicates that the interval was underestimated. Fifty two percent of the estimations predicted DCD correctly, 94% predicted DCD to within one day or less, one estimation underestimated DCD with two days and one estimation underestimated DCD with three days.

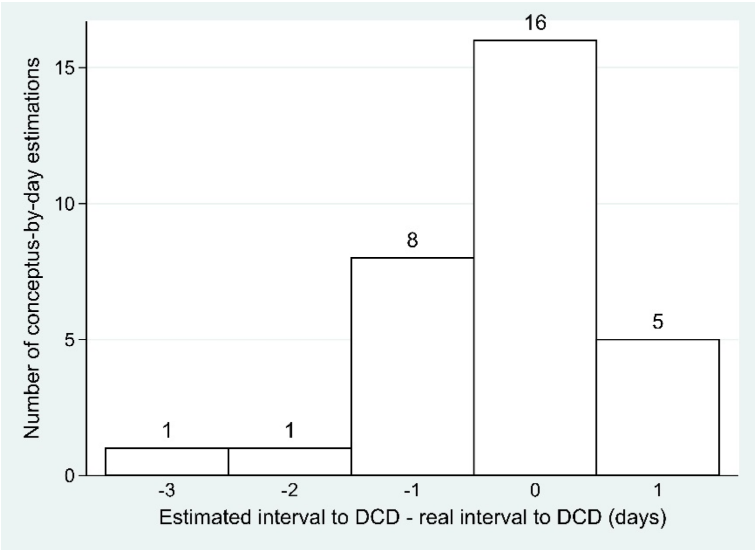

Figure 20
The magnitude in error of estimating the interval to DCD, using the model for direct predictor variables, for each conceptus marked for model testing.

4.8. Predicting the day of cervical dilatation using selected dimensions of foetal membranes relative to each other (relative predictor variables)

For the relative predictor variables AvLD/LPZ had no significant effect on DCD and it was removed from model generation.

The model we generated for the relative predicator variables is as follows:

$\hat{DCD} = 41.3575 - 12.1523(AvLD/TFD) + 2.7626(LPZ/TFD) - 2.9849(TFD/YD)$

With $F(3, 191) = 252.44$, $P < 0.001$ and $R^2 = 0.799$

Figure 21 shows the error in estimating the interval to DCD for the bitch by day combinations used for model generation when using the model for relative variables to estimate the interval to DCD. It suggests that the error in estimating the interval to cervical dilatation varies over the range of intervals to cervical dilatation. The analysis of the residuals of this model showed that there is strong support for the rejection of the null hypothesis that the errors were homoskedastic ($P < 0.001$).

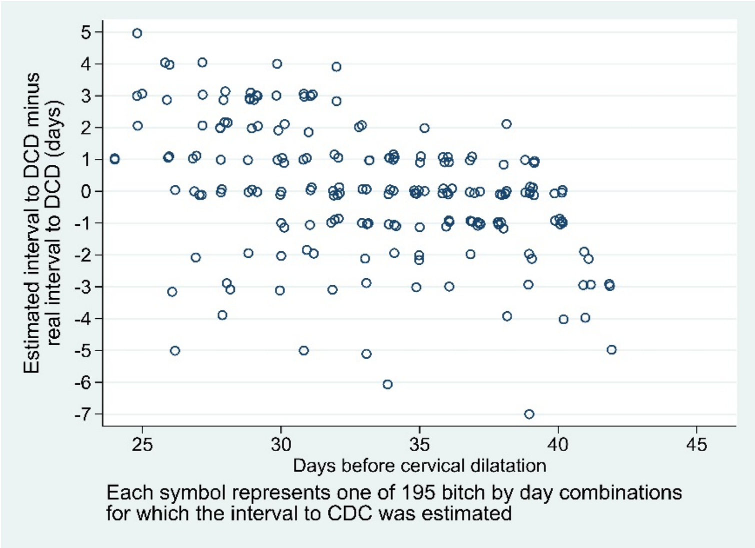

Figure 21
The distribution of error in estimating the days to cervical dilatation using the relative variables, throughout the interval of data collection, for each bitch by day combination used for model generation.

Using the model for the relative predictor variables, Figure 22 shows the magnitude in error of estimating the interval to DCD for the mean value of a specific day for the bitch by day combinations marked for model generation. A positive value indicates that the interval to DCD was overestimated and a negative value indicates that the interval was underestimated. Twenty-seven percent of estimations predicted DCD correctly, 61% predicted DCD to within one day or less and 75% within two days or less. The estimations ranged between an underestimation of seven days to an overestimation of five days.

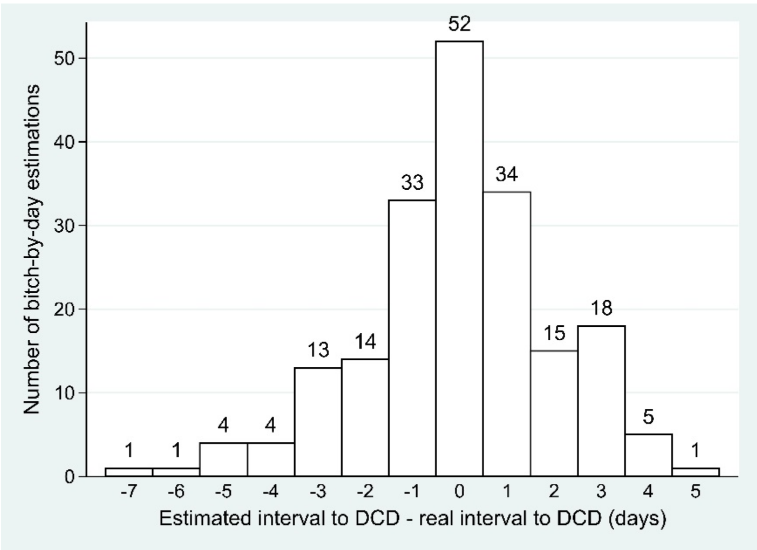

Figure 22
The magnitude in error of estimating the interval to DCD using the model for relative predictor variables, for the mean value of a specific day for the bitch by day combinations marked for model generation.

Figure 23 shows the magnitude in error of estimating the interval to DCD, using the model for relative predictor variables, for the mean value of a specific day for the bitch by day combinations marked for model testing. A positive value indicates that the interval to DCD was overestimated and a negative value indicates that the interval was underestimated. Only one estimation predicted DCD correctly, nine of the 16 estimations (56%) predicted DCD to within one day or less, 13 of the 16 estimations (81%) predicted DCD to within two days or less and all of the 16 estimations (100%) predicted DCD to within three days or less.

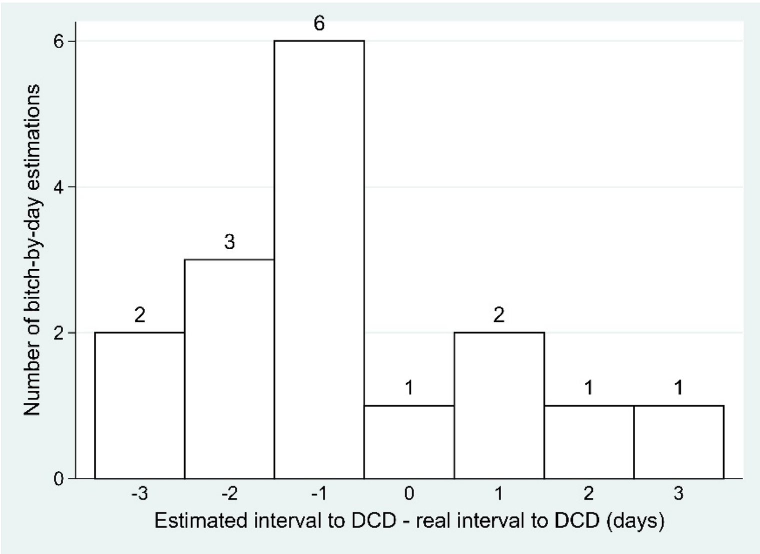

Figure 23
The magnitude in error of estimating the interval to DCD using the model for relative predictor variables, for the mean value of a specific day for the bitch by day combinations marked for model testing.

Figure 24 shows the magnitude in error of estimating the interval to DCD, using the model for relative predictor variables, for each bitch by day by conceptus marked for model testing. A positive value indicates that the interval to DCD was overestimated and a negative value indicates that the interval was underestimated. Six of the 31 estimations (19%) predicted DCD correctly, 14 of the 31 estimations (45%) predicted DCD to within one day or less, 25 of the 31 estimations (81%) predicted DCD to within two days or less and 29 of the 31 estimations (93%) predicted DCD to within three days or less. One was an overestimation of four days and another overestimation of six days.

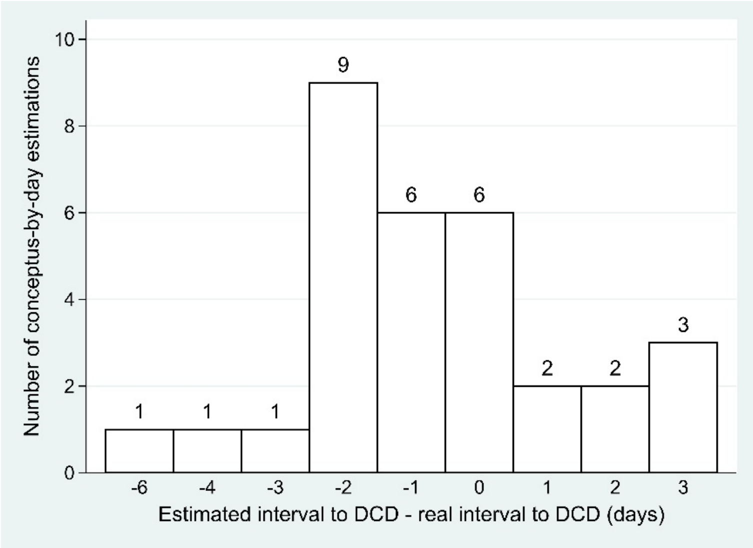

Figure 24
The magnitude in error of estimating the interval to DCD, using the model for relative predictor variables, for each conceptus marked for model testing.

4.9. The interval between closure of the amnion and the day of cervical dilatation

The predictor variable, the first time the amnion could be seen as a closed membrane over the embryo or foetus, showed that it varied between 37 and 33 days before DCD. In two bitches the amnion could be seen as a closed membrane in one or more conceptuses the day before it was noticed in all of the conceptuses. The average for this predictor variable was calculated for each bitch. This average showed that the amnion could be seen for the first time as a closed membrane in most bitches (in six of the 13 bitches—46%) 35 days before DCD. The amnion could be seen as a closed membrane 37, 36 or 34 days before DCD in two bitches each, whereas, in one bitch, it could only be seen 33 days before DCD.

4.10. The precision of the model generated in this study relative to the precision of models from other studies in predicting the day of parturition

We excluded two extreme data, which were more than three standard deviations from the mean from the 93 data of Holst and Phemister (1974). The variance in the error of estimating the day of parturition using the bitch by day means that had been used to derive our model for direct variables (n = 178) is smaller than the variance in the error in predicting the day of parturition from the onset of cytological dioestrus as reported by Holst and Phemister (1974) (n = 91, P = 0.0012). The variance was similar to the variance of De Cramer and Nöthling (2017) (n = 242, P = 0.18).

The variance of the error in estimating the day of parturition using the 16 bitch by day means that had not been used to derive our model for direct variables but were reserved for testing it is smaller than the variance in the error in predicting the day of parturition from the onset of cytological dioestrus as reported by Holst and Phemister (1974) (n = 91, P = 0.012). The variance was similar to the variance of De Cramer and Nöthling (2017) (n = 242, P = 0.33).

The variance in the error on estimating the day of parturition using the bitch by day means that had been used to derive our model for relative variables (n = 195) is smaller than the variance in the error in predicting the day of parturition from the day of the first or only mating as reported by Concannon et al. (1983) (n = 291, P < 0.001). The variance was similar to the variance of Holst and Phemister (1974) (n = 74, P = 0.08).

The variance in the error on estimating the day of parturition using the 16 bitch by day means reserved for testing our model for relative variables is smaller than the variance in the error in predicting the day of parturition from the day of the first or only mating as reported by Concannon et al. (1983) (n = 291, P = 0.016). The variance was similar to the variance of Holst and Phemister (1974) (n = 74, P = 0.31).

5. Discussion

This is the first study where the relationship of extra foetal structures in relation to one another was evaluated as predictors of the day of parturition in Beagle bitches. Previous studies have measured foetal structures, and some extra foetal structures, (England et al., 1990; Moriyoshi et al, 1996; Son et al., 2001; Alonge et al., 2016) to estimate the gestational age of foetuses and predict the day of parturition. These studies were conducted on specific breeds or on bitches within a specified weight range and would therefore be specific to those breeds or weight categories. Studies on different breeds or weight groups have attempted to determine different equations, or correction factors applied to formulas to compensate for the difference in breed and, or, weight of the bitch and the size of the embryo or foetus (Luvoni and Grioni, 2000; Son et al., 2001; Kutzler, Yeager et al., 2003; Beccaglia and Luvoni, 2004; Beccaglia et al., 2008).

In our study we evaluated the relationship dimensions of extra-foetal structures (direct variables) to the day of parturition as well as the ratio between the dimensions of these structures (relative variables) in relation to the day of parturition in an attempt to generate a formula which could be used across breeds.

5.1. Removal of data from the data analysis

The data of one bitch, Smiler, was inconsistent with the pattern shown by data of the other bitches. Scatterplots of Smiler's yolk sac diameter data showed a pattern clearly different to that of any other bitch's scatterplots (Figure 1). Smiler was the first bitch used in the study and the difference in distribution of the data was most likely due to the inexperience of the researcher. We would have retained the data if it was only a single variable which was affected but the direct variable, yolk sac diameter, would have also influenced the relative variables, for example fluid diameter divided by yolk sac diameter (Figure 2). We therefore excluded all of the data of Smiler from the data used for model generation.

5.2. Interval between time of cervical dilatation and onset of stage two of parturition

We performed vaginoscopies on pre-parturient bitches at intervals between eight and 10 hours, or more frequently when the bitches displayed signs of progressing to the first stage of parturition. The stage of dilatation of the cervix, when noted for the first time, varied between a few millimetres and complete dilatation. The onset of stage two of labour after cervical dilatation was noted, varied between 48 minutes and almost 25 hours. Since dilatation of the cervix could have occurred at any time between last examination (when the cervix was still closed) and the next examination (when it was observed as open) we assumed that the cervix could have started dilating directly after the previous examination and calculated the interval to the onset of stage two of parturition from the previous examination when the cervix was still closed. It therefore follows that the onset of stage two of parturition could vary between 8.8 (48 minutes after the examination when the cervix was open plus the interval of 8 hours form the previous examination when the cervix was still closed) and almost 25 hours after the cervical dilatation was noted for the first time.

5.3. Interval between onset of cytological dioestrus and day of onset of parturition

The onset of cytological dioestrus predicted the day of onset of parturition accurately in five of the 13 bitches (38%) in the current study, within one day in 12 of 13 bitches (92%) and within two days in all bitches. This compares favourably with the results of De Cramer and Nöthling (2017).

5.4. The more rapid extension of the domes once gestation has progressed to within 30 days of parturition

Figure 5 of his study shows that prior to about 30 days before DCD the number of days before parturition shows a strong linear relationship to the average length of the domes. Once gestation has progressed to within 30 days of parturition, the slope of the relationship decreases sharply, so that the average length of the domes increases more sharply for each day closer to parturition. This implies that the domes grow faster in length once pregnancy has progressed to within 30 d of parturition, or that the measurements are increased due to causes other than growth of the domes. Such causes may include the limited space the uterus can provide to the growing conceptuses. The villous rings of each placenta, which are presumably less expandable than other parts of the foetal membranes such as the avillous domes, may compress the growing conceptus and the fluid compartments of the foetal membranes within their confines. Such compression may result in a more rapid accumulation of fluid in the avillous domes than within the placental rings. Conceptuses may also be compressed by abdominal organs other than the uterus, or even by the adjacent uterine horn, which is enlarging due to the growing conceptuses within. Such compression may also cause the fluid to be displaced into the avillous domes, which in turn would increase the dome length. Towards the end of the ampullary phase the measurements of the avillous chorionic domes might also be affected by the neighbouring conceptus. The expansion of the domes of each conceptus and the limited space in the uterine horns will cause the chorionic domes of neighbouring conceptuses to abut each other causing distortion of the structure, or even force these domes to lie in a position adjacent to one another, thus overlying one another in the axial longitudinal view. Manual compression of the conceptus by applying pressure to the abdomen, via the ultrasound transducer in order to obtain a clear image is also a factor that must be considered. In an attempt to minimize the distortive effect of manual compression, the researcher applied as little pressure with the transducer on the abdomen when taking measurements.

The effect of the limited space in the uterus and compression of the conceptuses by one another or abdominal organs are the reasons why one needs to scan the bitches during the ampullary phase of gestation. In line with this reasoning, England et al. (1990) also found the growing conceptuses hindered their attempts at counting them accurately and visualising other foetuses clearly during late pregnancy. During the ampullary phase of gestation the conceptus, in the axial longitudinal view, will still appear ovoid and there will be empty uterus between two adjacent conceptuses. This will allow for accurate measurements to be recorded without the interference or distortion caused by neighbouring conceptuses.

During this study some bitches were examined beyond the ampullary phase and accurate measurements after the ampullary phase had ended were difficult to obtain. In the axial longitudinal view conceptuses often overlay one another—making them difficult to distinguish. They also compressed one another at the poles distorting the avillous chorionic domes. Some conceptuses we visualised were in the shape of a banana, with the length of the placental zone in the near field measuring significantly less than the length of the placental zone in the far field. The measurements of the domes had to be taken from the centre of the two opposing marginal haematomas at an angle instead of a straight line to the polar extent of the domes. In the transverse view the conceptus often did not have a circular outline and appeared to be indented at any area of the circumference by adjacent organs including the contralateral uterine horn and associated conceptuses.

5.5. Selection of conceptuses for measurement

All the principles of optimal breeding were applied to ensure that bitches were given the best opportunity to produce a large litter. This involved using breeding sound males for semen collection, monitoring the bitch's oestrous cycle accurately, performing the artificial inseminations at the appropriate times and using the correct

AI technique. We started ultrasound examinations of the bitches from thirteen days after the first day of dioestrus to assess whether they conceived and if we could count at least four conceptuses. From day 15 of dioestrus in one bitch or day 16 of dioestrus in three other bitches or day 17 of dioestrus in the remaining nine we randomly selected one conceptus in the left cranial abdomen, one in the left caudal abdomen, another in the right cranial abdomen and a fourth in the right caudal abdomen for ultrasound measurement. Each day, an ultrasound examination of a conceptus yielded a set of four measurements, consisting of the four direct variables. Each day thus yielded four sets of measurements, one from each of four conceptuses. Daily thereafter we continued selecting the conceptuses in this manner for each bitch, each day until day 28 of dioestrus (one bitch) or day 29 (two bitches) or day 31 (two bitches) or day 32 (eight bitches). The 12–16 days on which measurements were made from each of the 13 bitches yielded 211 sets of measurements, of which 195 were used for model generation and 16 for model testing. We realise that we could have measured the same or a different conceptus in a particular quadrant of the abdomen over the period in which we collected data in a bitch. We therefore anticipated that we would not follow the growth curve of the foetal membranes of a specific conceptus. The aim of the current study was to generate a model that may be used to predict the day of parturition in a bitch of any breed or size of which we have no accurate data by which to estimate the gestational age of the foetuses. The possibility of having measured different conceptuses in a particular quadrant of a bitch's abdomen would have contributed towards a model that is robust enough to compensate for genetic and individual variation of conceptuses within a litter and among bitches. Instead of daily measuring four conceptuses in each of 13 bitches on 12–16 days to yield the 195 sets of measurements for model generation, a better model would have resulted from using 195 random beagle bitches and measuring four conceptuses of each bitch once on a random day between 15 and 32 days after the onset of cytological dioestrus. Such a model would have been much more robust and resilient to the influence of genetics and the individual itself. We unfortunately did not have the time or the number of bitches to attempt conducting the study in this manner.

The relative variables should not be related to the size of the bitch or the conceptus and the change in ratio between these variables should be constant even if we did not follow the increase or decline of a specific ratio on each of the 12–16 days in the same conceptus of a specific bitch. Thus the way in which we selected the conceptuses should be mitigated to some extent by using the relative variables to generate a model to predict the day of parturition in a bitch of any breed or size.

5.6. Using the average of the four conceptuses on a specific day to generate the models

Although we measured four conceptuses every day in each bitch, we only used the average of four or two (if the other two were assigned to testing of the model) on that specific day to generate the model. If we had used the measurements of each conceptus for every day for each bitch as a separate datum in the compilation of the model, we would have artificially inflated the data to 780 sets of measurements instead of the 195 sets of means calculated over the four conceptuses of each day. The litters of the bitches had a single sire which implies that the conceptuses shared the genetic characteristics of the sire and the dam, the conceptuses were subjected to the same uterine environment influenced by the genetics, nutritional, metabolic and health status of the bitch. It would follow that the conceptuses of a bitch would then be very alike and it would be wrong to ignore the grouping effect of litter on the data and the resulting models.

5.7. The value of direct variables in predicting the day of parturition

The yolk sac diameter was not related to the interval to cervical dilatation. There was no discernible pattern within and among bitches. This could be because the conceptus was scanned in an orientation which may have affected the diameter of the structure. So, for instance, the embryo (foetus) may have compressed the yolk sack when the yolk sac was scanned from the dorsal aspect of the embryo (foetus). We took all the measurements that were needed in the equatorial transverse view at the level of the foetal heart when it could be visualized. The embryo or foetus is freely mobile within the foetal fluids and therefore the T-shaped yolk sac (Miglino et al. 2006) could be scanned through different transverse sections in different conceptuses.

Our study supports other studies which show that the growth of the foetus and or extra-foetal structures follows a linear trend (England et al., 1990; Yeager et al., 1992; Luvoni and Grioni, 2000), with the total fluid diameter, the average length of the domes and the length of the placental zone all increasing as the interval to parturition decreases.

The model we generated for direct variables—when used on bitches excluded form model generation—correctly predicted the day of parturition in 10 out of 16 estimations we calculated (62%) when we used the average of the measurements of two conceptuses on any random day. Fifteen of the 16 estimations (94%) predicted the day of parturition to within one day of the predicted day or parturition, and only one estimation underestimated the day of parturition by two days—the bitch whelped two days after our predicted day of parturition. This result compares well to using D0 (De Cramer and Nöthling, 2017), the LH peak (Concannon et al., 1983; De Cramer and Nöthling, 2017) and plasma progesterone levels (Kutzler and Mohammed et al., 2003; De Cramer and Nöthling, 2017) as predictors of the day of parturition.

5.8. The value of relative variables in predicting the day of parturition

Visual appraisal of scatterplots for individual bitches showed varying degrees of linear relationship between days before parturition (cervical dilatation) and the relative variables. The $t-$ value of the coefficient, together with the narrow margin of its 95% confidence interval, showed that relative variable fluid diameter divided by yolk sac diameter (TFD/YD) has the strongest influence on the outcome variable.

The model we generated for the relative variables in predicting the day of parturition correctly predicted the day of parturition in only one of the 16 estimations when we used the average of the measurements of two conceptuses assigned to testing the model on any random day. It predicted the day of parturition ± 1 day in nine of the 16 estimations (56%), ± 2 days in 13 of 16 estimations (81%) and ± 3 days in 100% of the estimations. The precision and accuracy resulting from the use of our formula, on the same breed used to generate the formula, compares favourably with results from Socha and Janowski (2017) where they compared the accuracy of breed specific (Milani et al., 2013; Groppetti et al., 2015) and non-specific (Luvoni and Grioni, 2000) formulas in predicting the day of parturition in German shepherd bitches. They found the formula of Luvoni and Grioni (2000) most accurate in predicting the day of parturition when using ICC measurements during early pregnancy (92% of bitches whelped within two days of the predicted day of parturition); and the formula of Groppetti et al. (2015) most accurate in predicting the day of parturition when using BP diameter during late pregnancy (95% of bitches whelped within two days of the predicted day of parturition).

5.9. The value of the closure of the amnion in predicting the day of parturition

The amnion could be seen as a closed membrane between 37 and 33 days before the onset of parturition. The results compare favourably with the study of England and Allen (1990). In two bitches the closure of the amnion was observed in some conceptuses a day before it was recorded in all four conceptuses. Detecting the amnion as a closed membrane in some but not in all of the conceptuses might have some value when we consider that the amnion is in the process of being formed. This will narrow down the time of intense observation for signs of parturition to five days—observation will be required from 20 to 24 days after the amnion was detected for the first time in some but not all of the conceptuses.

5.10. The value of our study compared to other studies in predicting the day of parturition

The model we developed for predicting the day of parturition using measurements of the direct variables, predicted the day of parturition with the same precision as the onset of cytological dioestrus does. The model we generated for predicting the day of parturition, by using measurements of the relative variables, did not predict the onset of parturition as accurately as the first or only day of mating used by Holst and Phemister (1974) did. The model generated for the relative variables did however predict the day of parturition more precisely than the first or only day of mating used by Concannon et al. (1983). The comparison of the variance of our models against the variance of other models (Holst and Phemister, 1974; Concannon et al. 1983; De Cramer and Nöthling, 2017) indicates that there is enough support to justify further studies to investigate the use of extra-foetal measurements during the ampullary phase of gestation to predict the day of parturition.

5.11. Significance of our study in general

Yeager and Concannon (1990) used 12 Beagle bitches to estimate the gestational age of the embryo during early pregnancy (the LH surge was defined as D0). They found that the gestational sac could be detected for the first time 17 to 19 days after the LH surge and that measurement of the of the gestational sac diameter predicted the day of parturition within one to 1.5 days up to day 25 after the LH surge (around 42 to 40 days prior to the day of parturition). Most owners would not present their bitches as early as seventeen to 25 days after the LH surge—around 11 to 17 days after the onset of cytological dioestrus—for pregnancy diagnosis. Owners often present their bitches for pregnancy diagnosis four weeks after mating. It is also recommended to owners to present their bitches for pregnancy diagnosis four weeks after the last breeding since bitches allowing the first or only mating on the first day of the fertile period of her oestrous cycle will be on D17 of dioestrus when she is presented 28 days later, enabling one to make a definitive diagnosis of pregnancy status on a single examination. Very rarely bitches will allow coitus one or two days after D0 and this could result in bitches being presented for pregnancy diagnosis more than 28 days after the onset of dioestrus on D29 or D30. These bitches will not be presented during the ampullary phase of gestation and the measurements will be affected by the distortive forces of neighbouring conceptuses, abdominal organs or the ultrasound transducer. The timing of weeks after the last mating is therefore highly likely to coincide with the ampullary phase and the period of gestation investigated in our research. The model we generated for the direct variables predicted the day of parturition in Beagle bitches correctly in 62% of the predictions, within one day in 94% of the predictions and within two days in 100% of the predictions. The model we generated using the direct variables is therefore of great value in predicting the interval to the day of parturition specifically in Beagle bitches.

Bitches are very often presented with breeding dates as the only guide to estimate the interval to parturition and, based on breeding dates alone, the interval to parturition could range between 58 and 71 days from the first of multiple matings (Concannon et al., 1983) or between 59 and 68 days from the first and only breeding (Holst and Phemister, 1974). This would require the owner to observe the bitch for signs of parturition for a period of 14 days. Our study, using the model generated for the relative variables, narrows down this period of observation to only seven days if parturition has to be observed with sufficient intensity to ensure the timely identification of any complications to the progression of parturition.

5.12. Further or future investigations

The accuracy and precision of our study could be improved by repeating the study on a larger population of Beagle bitches. This would be of great value for Beagle bitches in particular when using only the direct variables, as the direct variables reflect the growth rate and relative size of extra-foetal structures in the Beagle at a specific day during the ampullary phase of gestation.

In our study we used only 13 Beagle bitches to generate the data, not only to develop the model to predict the day of parturition, but also to test the models we generated. The data sets we used to test the formula was excluded from the data used to generate the model but the overlapping use of the data gathered from the same bitches, although of different conceptuses, could have biased our results when we tested the models we generated. Testing the model on different Beagle bitches or redesigning the study to include using bitches of different breeds and sizes, and conducting it on a larger sample size would overcome this bias. It would yield results which reflect the accuracy and precision of the formula when using the relative variables to predict the day of parturition in any bitch on any day during the ampullary phase of gestation.

Refinement of this model might include only using measurements taken during the ampullary phase of gestation and not thereafter, as happened on some days in the current study. Such measurements will not be affected by interference by other conceptuses, or by compression of conceptuses by abdominal organs or the ultrasound transducer which in turn will result in using more accurate measurements. If a model was developed using measurements taken strictly during the ampullary phase of gestation, and it proves more accurate and precise in predicting the day of parturition, it would restrict the period the during which a bitch may be scanned for optimal prediction of the day of parturition.

5.13. Clinical and practical use of our model by veterinarians and owners

Owners should be advised to present their bitches for pregnancy diagnosis four weeks after breeding. This will most likely ensure that the bitch is in the ampullary phase of her pregnancy which is required for the use of our model.

Veterinarians should measure at least two conceptuses and ensure that they are measuring different conceptuses during any single examination. The average of the measurements should be used in the formula to obtain the most accurate prediction.

The prediction of when the day of parturition is likely to occur is only an estimation, and intense observation of the bitch for signs of parturition should still be performed from three days prior to the predicted day of parturition until parturition is observed.

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7. Summary

Dog breeders invest a lot of time and money into breeding efforts and it is important for veterinarians to assist clients in achieving a successful outcome of a breeding attempt. Being able to predict when a bitch is likely to whelp will assist the owner in managing the pre-parturient bitch optimally—via a well-timed restricted observation period which would allow for a timely intervention, when needed, to prevent unnecessary foetal loss. Studies aimed at predicting the parturition dates in bitches used foetal measurements and applied formulas to specific breeds or bitches in predetermined weight categories. The aim of this study was to predict the parturition date in bitches based on measurements, taken during the ampullary phase of gestation, which are not influenced by the size of the foetus and therefore not by the breed and size of the sire and dam.

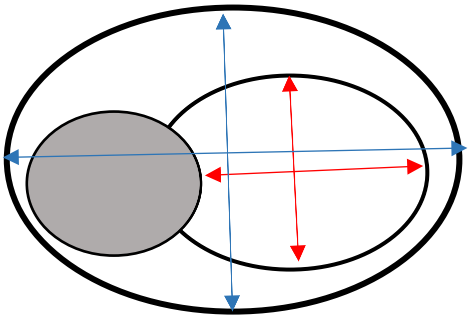
Various measurements of extrafoetal fluid compartments of the conceptus in an axial longitudinal view (ALV) and in an equatorial transverse view (ETV) were recorded daily between 17 and 32 days after the onset of cytological dioestrus. Measurements were categorised as direct variables (a measured datum) or relative variables (the relationship between two measured data). A linear function was used to generate models to predict the day of parturition using either the direct or relative predictor variables. Some of the measurements were excluded from the data used to generate the models, and these values were used to test the accuracy of the generated models in predicting the day of parturition in bitches.

This study, using the model for direct variables, predicted the day of parturition accurately in 62% of bitches, within one day in 94% of bitches and within two days in all bitches. The model for relative variable predicted the day of parturition within one day in 56% of bitches, within two days in 81% of bitches and within three days in all bitches.

8. Appendix

Schematic of ultrasound measurements recorded.

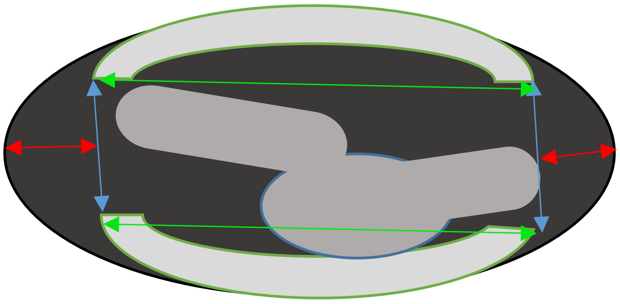
Equatorial transverse view (ETV)



TFD = average of the two perpendicular measurements

YD = average of the two perpendicular measurements (HYS and WYS)

Axial longitudinal view (ALV)



LPZ = average of the two measurements (LPZ in the near field and LPZ in the far field)

AvLD = average of the two measurements (LDome1 and LDome 2)

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Animal Ethics Committee

PROJECT TITLE	**Predicting parturition dates based on ultrasonographical measurement of selected extrafoetal fluid compartments during the ampullary phase of gestation in beagle bitches**
PROJECT NUMBER	**V034-17**
RESEARCHER/PRINCIPAL INVESTIGATOR	**Dr. S Fouche**

STUDENT NUMBER (where applicable)	**U_ 89690436**
DISSERTATION/THESIS SUBMITTED FOR	**MMedVet**

ANIMAL SPECIES	**Canine**	
NUMBER OF SAMPLES	**25**	
Approval period to use animals for research/testing purposes		**April 2017-April 2018**
SUPERVISOR	**Prof. J Nothling**	

KINDLY NOTE:

Should there be a change in the species or number of animal/s required, or the experimental procedure/s - please submit an amendment form to the UP Animal Ethics Committee for approval before commencing with the experiment

APPROVED	Date 9 May 2017
CHAIRMAN: UP Animal Ethics Committee	Signature

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