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Ultrasonographic and progesterone changes during Days 21 to 63 of pregnancy in queens

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ABSTRACT

Ultrasonography has been used to diagnose and monitor pregnancy. However, in the queen, most of ultrasonographic and endocrinological studies have been performed using small number of observations during limited periods of pregnancy. The aim of this study was to derive equations to predict the gestation age and parturition time using ultrasonographic embryo fetus measurements and serum progesterone (P_4) concentration measurements. Mixed-breed queens (n = 16), aged between 24 and 36 months and weighing between 2 and 4 kg, were daily monitored by ultrasonography since 21 days after the first mating to parturition. Gestational sac (GS) was measured from longitudinal (length [LEN], anterior–posterior [ATP]) and transverse images (width [WID]), GS volume was calculated by the prolate ellipse formula, and GS diameter was calculated by orthogonal measurements. Fetal measurements included crown–rump length (CRL), head diameter (HD), and body diameter (BD). Gestational sac, fetal measurements, and serum P_4 concentration were recorded and analyzed by ANOVA. Correlation and linear regression analyses were performed and equations were derived to estimate predicted values and 95% confidence interval for GS parameters and P_4 concentrations from 21 to 63 days after the first mating and to estimate predicted values and 95% confidence interval for fetal parameters from Day 35 to 63 of gestation. The average concentrations of serum P_4 concentration from Day 22 to 47 of gestation remained between 32.27 ± 4.25 and 16.25 ± 2.45 ng/mL. After that, a gradual decline occurs reaching a concentration of 2.99 ± 1.29 ng/mL 1 day before parturition. A positive and significant correlation between the ultrasonographic measurements (LEN, ATP, WID, GS volume and diameter, uterine wall thickness, CRL, HD, and BD) with number of days after the first mating was observed ($P < 0.001$). We observed a positive and significant correlation between GS measurements (LEN, ATP, and WID) and between fetal measurements (CRL, HD, and BD) and a negative and significant correlation between serum P_4 concentration with GS (LEN, ATP, and WID), uterine wall thickness, and fetal (CRL, HD, and BD) measurements. In addition, there was a positive and significant correlation between serum P_4 concentrations with days after the first mating to parturition. In conclusion, the equations derived from this study will be useful for pregnancy monitoring and for estimating pregnancy age in queens from Day 21 until parturition for animals with similar weight and age.

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1. Introduction

Pregnancy diagnosis and estimation of gestational age are commonly requested in small animal reproduction practice. Ultrasonographic measurement of embryo/fetal development allows early identification of pregnancy abnormalities in clinical practice. Because the duration of pregnancy in the queen is relatively short, it is critical that fetuses are mature enough to survive before delivery [1–4].

Progesterone (P_4) is essential for pregnancy development in domestic mammals [5]. Plasma P_4 concentration in the pregnant queen increases from baseline to more than 2 ng/mL starting 1 to 2 days after ovulation. Plasma P_4 concentration in the pregnant queen then continues to increase, to a peak concentration of 15 to 30 ng/mL at 25 to 30 days of pregnancy, after which, it slowly declines throughout the rest of pregnancy. In the queen, the CL produces P_4 during 40 to 50 days of pregnancy [6]. Recent studies confirm that in pregnant queens, the placenta is an additional source of P_4. Therefore, it should be considered as an important endocrine organ supporting feline pregnancy [7].

Ultrasonography is a noninvasive technique that permits an accurate diagnosis of pregnancy and allows serial evaluation of the developing embryo/fetus and extrafetal structures [8]. Fetal development progresses rapidly from Day 30, allowing recognition of organogenesis by ultrasonography. Thus, different organs could be recognized at different gestational ages by ultrasound and deviation from normal development could be promptly identified [8,9]. Breed differences in fetal size are not as pronounced in the cat as in the dog [10].

Gestational age is an important piece of information in many clinical situations. In cases where breeding dates are lacking and there is a singleton fetus or oversize fetuses, it is necessary to calculate gestational age before setting the date of cesarean section to assure a high kitten survival rate. In high-risk pregnancies, where there is poor or no ovulation timing, determination of fetal maturation and gestational age will assist in determining whether pregnancy has progressed long enough to allow delivery of viable kittens. In cases where queens are receiving supplemental P_4 for pregnancy maintenance, medications need to be discontinued at an appropriate time before parturition to permit delivery of viable kittens [11].

Pregnancy can be diagnosed by ultrasonography as early as 10 days after mating [8,9,12]. Although several researchers have done many ultrasonography studies on queen pregnancy, to our knowledge, there are no thorough studies during the last two-thirds of gestation [10,12–18]. In 1986, Davidson et al. [10] described a considerable variability in the diameters of the gestational sacs (GSs) and the lengths of fetal poles among individual fetuses within a litter, as well as between litters [10]. In 1990, Beck et al. [13] formulated fetal growth curves using head and body diameter (HD and BD, respectively) and tested the curves using them to predict parturition dates in queens with unknown breeding dates. Zambelli et al. [12] observed a high correlation between the fetal manual measurement and the ultrasonographic measurements of the external diameter of the GS and the length of the embryos/fetuses during the first 30 days of gestation. In addition, 2 years later, Zambelli et al. [14] found a correlation between fetal measurements and gestational age during the second half of pregnancy.

Recent studies have investigated whether the accuracy of parturition date prediction is affected by the week of pregnancy when ultrasonographic examination is performed [16,17]. Beccaglia and Luvoni [17] showed that prediction of parturition date obtained by measurement of inner chorionic cavity and biparietal measurements was equally accurate in predicting parturition date at week 5 of gestation. More recently, Brito et al. [18] observed that all GS and fetal measurements increased as gestational age advanced [18]. Similarly, Gatel et al. [15] found that biparietal diameter and femoral length increase linearly during pregnancy [15].

Most of these ultrasonographic studies on feline pregnancy have been performed using small number of pregnancies and observations were made during a limited period of pregnancy [10,12–14,18].

Thus, ultrasonographic and endocrinological studies throughout feline pregnancy to assess embryo/fetal development and hormonal fluctuations could be important for pregnancy monitoring and early medical intervention. Therefore, the aim of this study was to derive equations to predict the gestation age and parturition time using ultrasonographic embryo/fetus measurements and serum P_4 concentrations measurements. The hypothesis was that gestational age could be calculated from embryo/fetus measurements and serum P_4 concentration by use of the regression polynomial equations. These equations could be used to predict the gestation age when breeding data is unavailable, to determine whether normal embryo/fetal development took place by comparing the observed measurement to the predicted or expected measurement based on a given breeding date, and to determine whether measured serum P_4 concentration is within the range of those expected in a normal pregnancy.

2. Materials and methods

Mixed-breed queens (n = 16), aged between 24 and 36 months and weighing between 2 and 4 kg, were used in an experimental group. In addition, two 3-year-old intact tomcats were used for breeding. There are no family ties between animals used in the study. The queens were housed in individual stainless steel cages and were fed commercial cat food (Fit 32; Royal Canin, Buenos Aires, Argentina) and water *ad libitum*. A physical examination of all animals included in the study was performed once a week. The toms were housed separately from queens and fed the same diet. All animals were maintained in a controlled environment with artificial incandescent illumination (14 hours of daily bright light [19]). Animal care, housing, and experimentation complied with International Guiding Principles for Biomedical Research Involving Animals (1985) [20]. The queens were observed on a daily basis to detect estrous behavior, and receptivity to the male and vaginal cytology was performed daily to detect cytologic estrus. After the detection of estrous behavior of the queen, a vaginal cytology was performed, and later, the male and female were put together to observe female receptivity to the male; each queen was placed with the tom for 48 hours. First mating was documented, and pregnancies were confirmed by abdominal palpation and transabdominal ultrasonographic examination using an ultrasound scanner equipped with a 5/7.5/10-MHz linear transducer (DP-6600Vet, Mindray, Nanshan, China) 20 days after the first mating [8]. Ultrasonographic examinations were performed by García Mitacek M.C. Blood samples were taken from the jugular vein from 21 days after the first mating to parturition to measure serum P_4 concentrations. All the blood samples were centrifuged and stored at −20 °C until P_4 was measured by solid RIA using I^{125} (Coat-A-Count, progesterone; Diagnostic Products Corporation, Los Angeles, CA, USA). The intra-assay coefficients of variation for high-pool (6.4 ng/mL) and low-pool P_4 (0.9 ng/mL) were 5.0% and 4.1%, respectively.

When pregnancies were confirmed, daily ultrasound examinations were made from 21 days after the first mating to parturition. Food and water were not restricted before the ultrasound examination. The queens, were not sedated or anesthetized, were held by a nurse on the examination table, and transabdominal ultrasonographic examinations were performed with the queens positioned in dorsal recumbency. Before the first ultrasonographic examination and every 5 days, the hair on the ventral abdomen was clipped. Acoustic coupling gel was applied to the skin of the ventral abdomen. The abdomen was scanned just cranial to the pubis, where the bladder and colon were identified as landmarks for the uterus. Once located, the uterine body, bifurcation, and horns were scanned in a transverse and in a sagittal plane. Dimensional measurements of GS were recorded from 21 days after the first mating to parturition, and fetal measurements were recorded from 35 days after the first mating. All measurements were made with electronic calipers from appropriate frozen images. At each examination, two sets of images of GS and fetal measurements were recorded for each queen, regardless of the number of fetuses present.

During each ultrasound examination, GSs were measured from longitudinal and transverse images. From longitudinal images, the length (LEN, mm) and anterior–posterior (ATP, mm) dimensions of the GS were measured. The LEN of the GS have been measured in the sector image field. From transverse images, the width (WID, mm) dimension of the GS was measured. The GS volume (GSV, mm^3) was calculated by the prolate ellipse formula: $[4/3\pi \times (GSLEN/2) \times (GSATP/2) \times (GSWID/2)]$, and GS diameter (GSD, mm) was calculated averaging three orthogonal measurements made from frozen images. In addition, the uterine wall thickness (UWT; Fig. 1A) was measured. The developing placenta can be recognized within the gestational chamber as two hyperechoic lines separated by a hypoechoic line since Day 16 of pregnancy. This stratified appearance is maintained throughout pregnancy. From Day 25, it is possible to distinguish the zonary nature of the placenta which occupies the entire surface of the gestational chamber with the exception of two poles. The structure of the placenta does not change in ultrasonographic appearance until the end of pregnancy [8]. Therefore, we were able to measure the UWT excluding the placenta in our work [21,22].

Fetal measurements included crown–rump length (CRL), HD, and BD. The CRL length was measured along the longest axis of the fetus. For this study, fetal CRL measurements were made on frozen images including the fetus using electronic calipers. The greatest length was measured by placing the calipers from the top of the head (crown) to the bottom of the buttocks (rump), and the maximal straight line was observed from 35 days after the first mating to the parturition. Crown–rump length values measured on two satisfactory images. During the early gestational period, the longest linear measurement was taken. Later, CRL measurements have been performed when the fetus has assumed its natural curvature. In addition, in our study, a single operator has performed the ultrasound studies to reduce interobserver error. Transverse HD was measured from a dorsal plane image. Image quality of HD was initially assessed by a symmetric frontal scanning of the skull with an evident *falx cere*bri. The BD was measured from a transverse image of the abdomen at the level of the stomach (Fig. 1B) [9].

2.1. Statistical analysis

Correlation analysis between the mean values of the ultrasonographic measurement of the GS (LEN, ATP, WID, GSV, and GSD), UWT, and fetal (CRL, HD, and BD) with the days after the first mating was performed using the CORR procedure from SAS 9.1. In addition, linear regression analysis using ultrasonographic measurements as dependent variables and days after the first mating and days before parturition as independent variable was performed using the REG procedure from SAS 9.1 [23]. Polynomial models for each response variable studied were manually selected on the basis of the maximum R-squared (r^2) goodness of fit method. Serum concentrations of P_4 were analyzed by ANOVA with the MIXED procedure of SAS 9.1 [23] as repeated measures. The model included the random effect of queen. The results of the linear regression analyses are presented as scattered plots with observed and expected measurements (mm) with 95% confidence interval (CI) and results from the ANOVA as the least-square mean ± standard error of the mean. Significance was defined as $P < 0.05$. We used both measurements in the statistical analysis (duplicates). We had two measurements influenced our prediction making them more accurate because we increased the degrees of freedom of the stat analysis.

The accuracy of the prediction (0, ±1, ±2, ±3 days) using GSV, GSD, HD, and BD measurements was analyzed retrospectively on the gestational period basis (from week 3 to 9 of pregnancy) in pregnant queens. The mean value of the data was calculated, and the predicted day of parturition was obtained by the using the equations previously described in Table 1. Mean percent of accuracy at 0, ±1, ±2, or ±3 days was analyzed by the chi-square test, and the level of significance was considered at $P < 0.05$. Statistical analysis was performed by García Mitacek M.C. and de la Sota R.L.

3. Results

All queens (n = 16) were pregnant in the first estrous recorded and gave birth after a normal gestation period

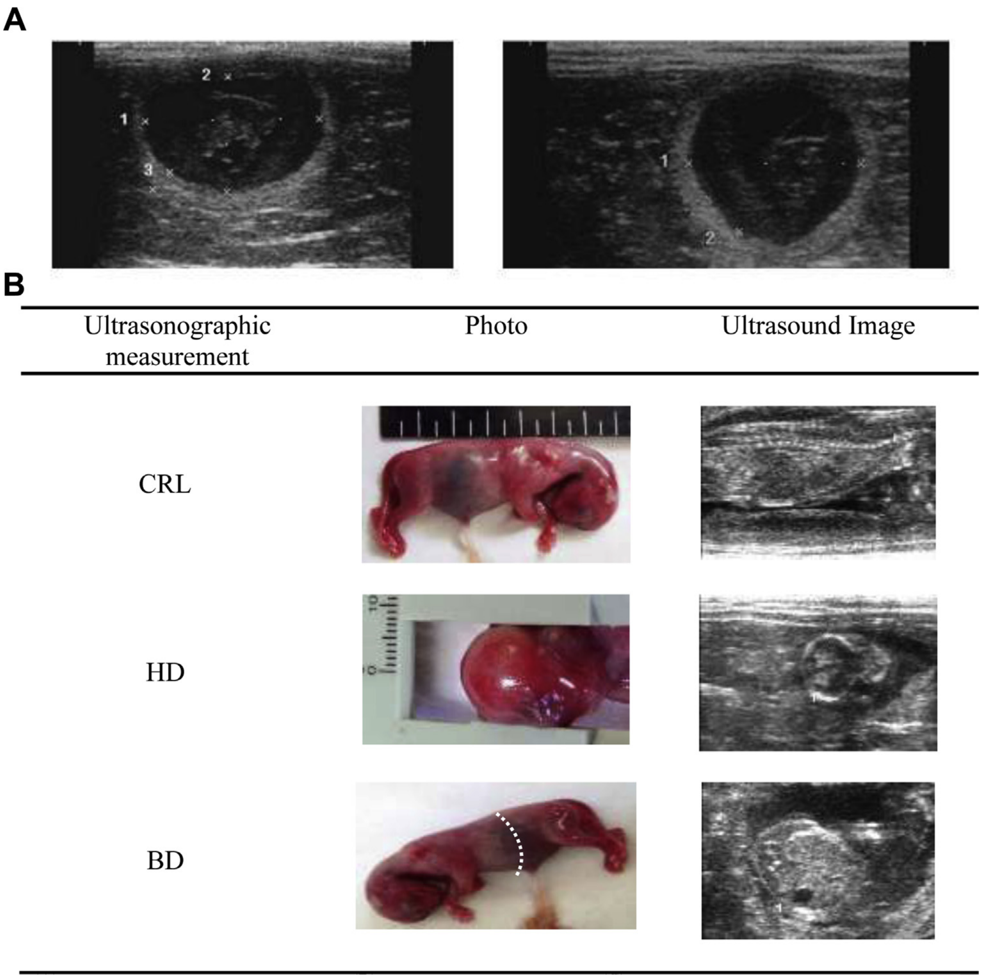

Fig. 1. (A) Day 21 after the first mating, ultrasound and image section of gestational sac (GS). Longitudinal dimensions of GS: caliper 1, length (mm); caliper 2, anterior–posterior (mm). Transverse dimensions of GS: caliper 1, width (mm); caliper 2, uterine wall thickness (mm). Ultrasound scanner with a 7.5-MHz linear transducer. (B) Day 40 after the first mating, photo and ultrasound image with a 7.5-MHz linear transducer. BD, body diameter; CRL, crown–rump length; HD, head diameter.

(64.8 ± 0.53 days) to normal kittens. The litter size was 3.1 ± 0.23 kittens, and 100% of animals were weaned.

Serum P_4 concentrations were analyzed from 21 days after the first mating to parturition. Maximal value of serum P_4 concentration on Day 21 after the first mating was 35.52 ± 6.14 ng/mL. From Day 22 to 47 of gestation, average concentrations remained elevated between 32.27 ± 4.25 and 16.25 ± 2.45 ng/mL (Fig. 2). A gradual decline in serum P_4 concentration started on Day 48 after the first mating and continued through parturition reaching a concentration of 2.99 ± 1.29 ng/mL 1 day before parturition (Fig. 3). Therefore, the decrease between Days 45 and 46 was not significant, whereas the decrease from Day 48 onward was significant.

A positive and significant correlation between the ultrasonographic measurements (LEN, ATP, WID, GSV, GSD, UWT, CRL, HD, and BD) with the number of days after the first mating was observed ($P < 0.001$; Table 1). All the ultrasonographic measurements were explained by polynomial functions with an r^2 greater than 0.70, with the exception of UWT which r^2 was greater than 0.45 (Table 1). The relationship between the observed measurements of LEN, ATP, WID, GSD, GSV, UWT, CRL, HD, and BD and the predicted values from equations in Table 1 are shown in Figure 4A–C, Figure 5A–C, and Figure 6A–C. Furthermore, we observed a positive and significant correlation between GS measurements (LEN, ATP, and WID) and a positive and significant correlation between fetal measurements (CRL, HD, and BD; Table 2). On the contrary, there was a negative and significant correlation between serum P_4 concentration with GS (LEN, ATP, and WID), UWT, and fetal (CRL, HD, and BD) measurements (Table 2). In addition, there was a positive and significant correlation between serum P_4 concentrations with days after the first mating to parturition. We found the following: $r^2 = 0.87$ ($P < 0.001$) for P_4 during days of gestation and $r^2 = 0.75$ ($P < 0.009$) for P_4 1 day before parturition. The correlation models were as follows: $[40.525 - (0.312 \times DG) - (0.00423 \times DG^2)]$ for P_4 during days of gestation (DG) and $[0.954 - (3.025 \times DBP) - (0.359 \times DBP^2) - (0.0138 \times DBP^3)]$ for P_4 1 day before parturition (DPB).

Table 1
Multiple regression analysis of the parameters evaluated during ultrasound examination from 21 to 63 days after the first mating in mixed-breed queens (n = 16).

Ultrasonographic measurements	Days	r^2	P	Coefficients	Inverse function of the formula
LEN	AFM	0.89	<0.001	$-54.3419 + (4.0349 \times DAM) + (-0.0327 \times DAM^2)$	$= 4.0349 - [(4.0349)^2 - 4 \times -0.0327\ (mm + 54.3419)]^{1/2}/2 \times -0.0327$
	BP	0.85	<0.001	$69.4510 + (-0.1755 \times DBP) + (-0.0308 \times DBP^2)$	$= -0.1755 + [(-0.1755)^2 + 4 \times -0.0308\ (mm - 69.4510)]^{1/2}/2 \times -0.0308$
ATP	AFM	0.71	<0.001	$-4.0705 + (0.9974 \times DAM) + (-0.00704 \times DAM^2)$	$= 0.9974 - [(0.9974)^2 - 4 \times -0.00704\ (mm + 4.0705)]^{1/2}/2 \times -0.00704$
	BP	0.69	<0.001	$30.7210 + (0.0659 \times DBP) + (-0.0071 \times DBP^2)$	$= 0.0659 + [(-0.0659)^2 + 4 \times -0.0071\ (mm - 30.7210)]^{1/2}/2 \times -0.0071$
WID	AFM	0.85	<0.001	$-24.0533 + (2.3234 \times DAM) + (-0.0207 \times DAM^2)$	$= 2.3234 - [(2.3234)^2 - 4 \times -0.0207\ (mm + 24.0533)]^{1/2}/2 \times -0.0207$
	BP	0.81	<0.001	$39.5162 + (-0.3333 \times DBP) + (-0.0195 \times DBP^2)$	$= -0.3333 + [(-0.3333)^2 + 4 \times -0.0195\ (mm - 39.5162)]^{1/2}/2 \times -0.0195$
GSV	AFM	0.86	<0.001	$-26912.0000 + (1283.9921 \times DAM)$	$= (mm + 26912.0000)/1283.9921$
	BP	0.83	<0.001	$55361 + (1237.0462 \times DBP)$	$= (mm - 55361)/1237.0462$
GSD	AFM	0.93	<0.001	$-27.5108 + (2.4548 \times DAM) - (0.02022 \times DAM^2)$	$= 2.4548 - [(2.4548)^2 - 4 \times -0.02022\ (mm + 27.5108)]^{1/2}/2 \times -0.02022$
	BP	0.89	<0.001	$46.4584 + (-0.1605 \times DBP) + (-0.0194 \times DBP^2)$	$= -0.1605+[(-0.1605)^2 + 4 \times -0.0194\ (mm - 46.4584)]^{1/2}/2 \times -0.0194$
UWT	AFM	0.46	<0.001	$-1.7281 + (0.2463 \times DAM) + (-0.00241 \times DAM^2)$	$= 0.2463 - [(0.2463)^2 - 4 \times -0.00241\ (mm + 1.7281)]^{1/2}/2 \times -0.00241$
	BP	0.42	<0.001	$4.1538 + (-0.0589 \times DBP) + (-0.0021 \times DBP^2)$	$= -0.0589 + [(-0.0589)^2 + 4 \times -0.0021\ (mm - 4.1538)]^{1/2}/2 \times -0.0021$
CRL	AFM	0.70	<0.001	$-71.0221 + (3.9710 \times DAM) - (0.02968 \times DAM^2)$	$= 3.9710 - [(3.9710)^2 - 4 \times -0.02968\ (mm + 71.0221)]^{1/2}/2 \times -0.02968$
	BP	0.70	<0.001	$61.5707 + (0.2154 \times DBP) + (-0.0238 \times DBP^2)$	$= 0.2154 + [(-0.2154)^2 + 4 \times -0.0238\ (mm - 61.5707)]^{1/2}/2 \times -0.0238$
HD	AFM	0.94	<0.001	$-5.8136 + (0.4539 \times DAM)$	$= (mm + 5.8136)/0.4539$
	BP	0.92	<0.001	$23.0844 + (0.4200 \times DBP)$	$= (mm - 23.0844)/0.4200$
BD	AFM	0.77	<0.001	$-46.9206 + (2.1614 \times DAM) - (0.0143 \times DAM^2)$	$= 2.1614 - [(2.1614)^2 - 4 \times -0.0143\ (mm + 46.9206)]^{1/2}/2 \times -0.0143$
	BP	0.74	<0.001	$35.1042 + (0.7060 \times DBP)$	$= (mm - 35.1042)/0.7060$

r^2 represents the coefficient of determination.
All measurements in mm.
Abbreviations: AFM, after first mating; ATP, anterior–posterior; BD, body diameter; BP, before parturition; CRL, crown–rump length; DAM, day after first mating; DBP, day before parturition; GSD, gestational sac diameter; GSV, gestational sac volume; HD, head diameter; LEN, length; P, probability; UWT, uterine wall thickness; WID, width.

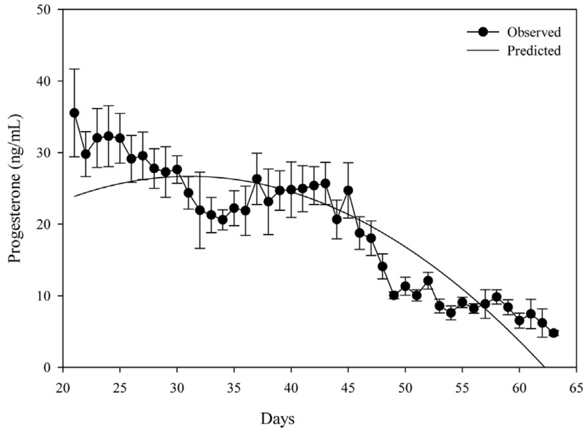

Fig. 2. The least-square mean ± standard error of the mean of progesterone (ng/mL) from 21 to 63 days after the first mating in mixed-breed queens (n = 16).

Similar results were obtained when days before parturition rather days after first mating was used as an independent variable in the linear regression model. However, the coefficient of determination (r^2) was always higher when days after first mating rather than days before parturition was used as an independent variable (Table 1).

Table 1 depicts multiple regression analysis of the parameters evaluated during ultrasound examination. Table 3 depicts the predicted values and 95% CI for GS parameters from 21 to 63 days after the first mating derived from polynomial equations shown in Table 1. In addition, Table 4 depicts the predicted values and 95% CI for fetal parameters from 35 to 63 days after the first mating derived from polynomial equations shown in Table 1.

The equations obtained allowed to predict GS measurements, fetal measurements, and P_4 concentrations with 95% CI using any given day after 21 days from the first mating. The prediction was considered accurate when the difference between actual and predicted parturition dates was within 0, ±1, ±2, or ±3 days (Table 5).

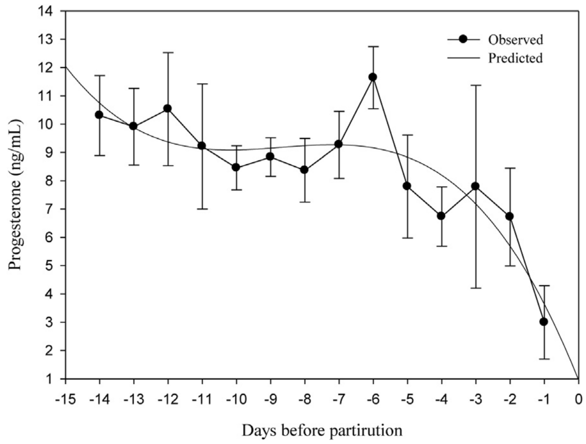

Fig. 3. The least-square mean ± standard error of the mean of progesterone (ng/mL) from 1 to 14 days before parturition in mixed-breed queens (n = 16).

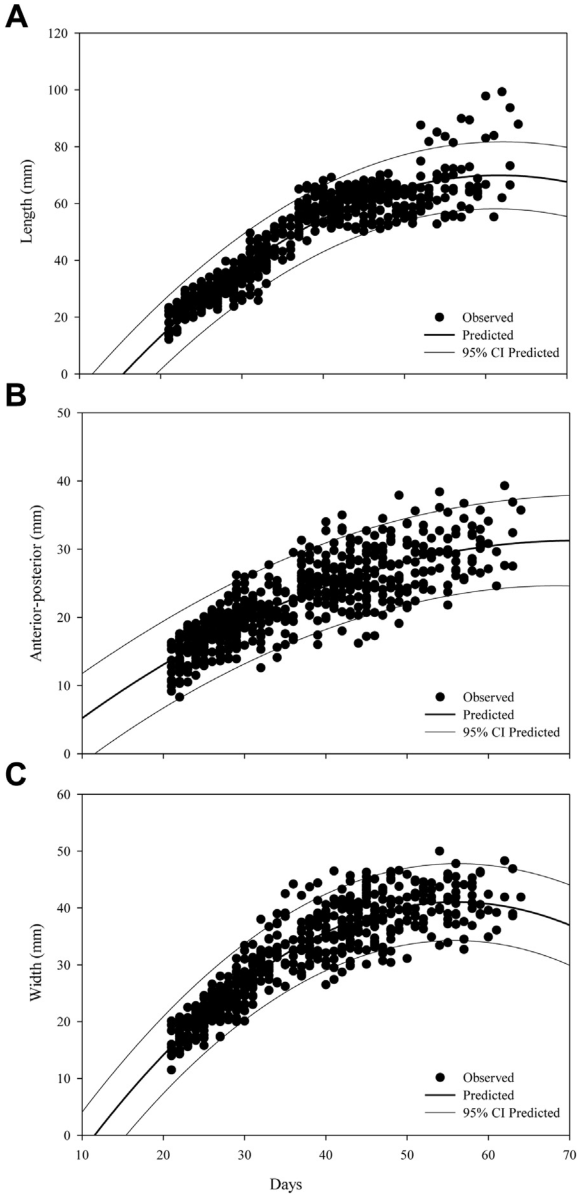

Fig. 4. Multiple regression analysis of gestational sac length (A, mm), anterior–posterior (B, mm), and width (mm, C) of gestational sac with days after the first mating using a second-degree polynomial model (A–C) in mixed-breed queens (n = 16). CI, confidence interval.

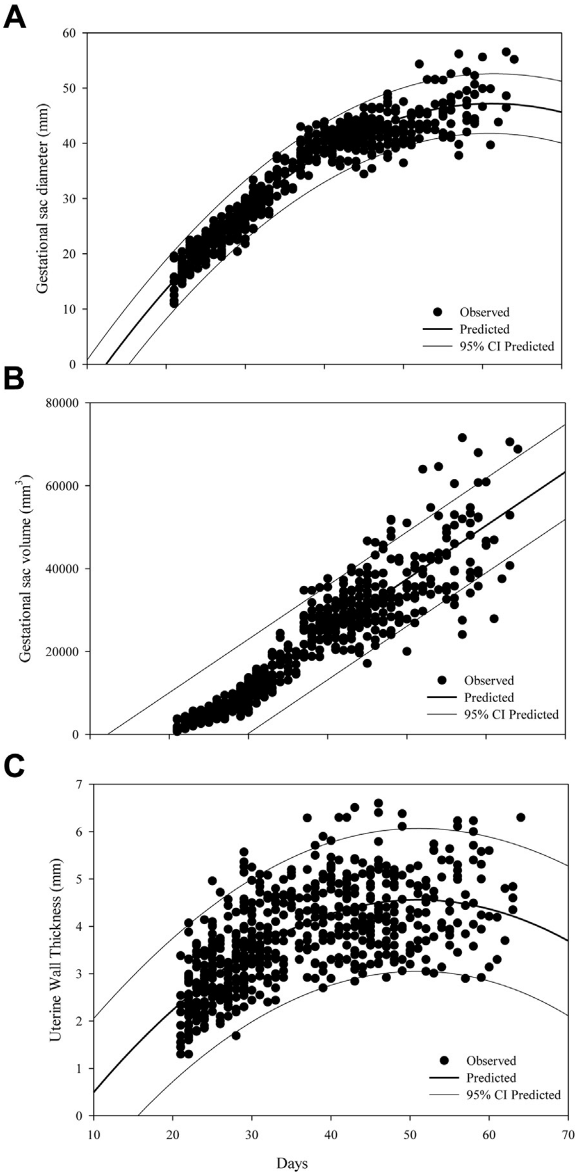

Fig. 5. Multiple regression analysis of gestational sac diameter (mm, A), gestational sac volume (mm^3, B), and uterine wall thickness (mm, C) with days after the first mating using first (B) and second (A, C) degrees of polynomial model in mixed-breed queens (n = 16). CI, confidence interval.

4. Discussion

Our result show a maximal value of serum P_4 on Day 21 after the first mating; after which, it slowly declines throughout the rest of pregnancy. These findings are in agreement with the results obtained by Verhage et al. [24] who found the peak of plasma P_4 concentration on Day 21 after the first mating and a gradual decrease through parturition. Similarly, Schmidt et al. [25] founded a peak of serum P_4 concentration on Day 23 of gestation and a gradual decline in mean serum P_4 initiated on Day 44 and continued through parturition.

Ultrasonography has been used to diagnose early pregnancy in the queen [26]. Breed differences in fetal size are not as pronounced in the cat as in the dog [10]. Hence, ultrasonographic measurements could be useful to estimate gestational age in almost every breed of queen and to confirm that measurements are within the expected range to a given day of gestation. Gatel et al. [15] observed that the time to parturition increased more the queen weighed before mating, suggesting that the queens' size influenced gestational length. In addition, the time to parturition was shorter when the queen was older [15]. In contrast, in our study, no significant relationship

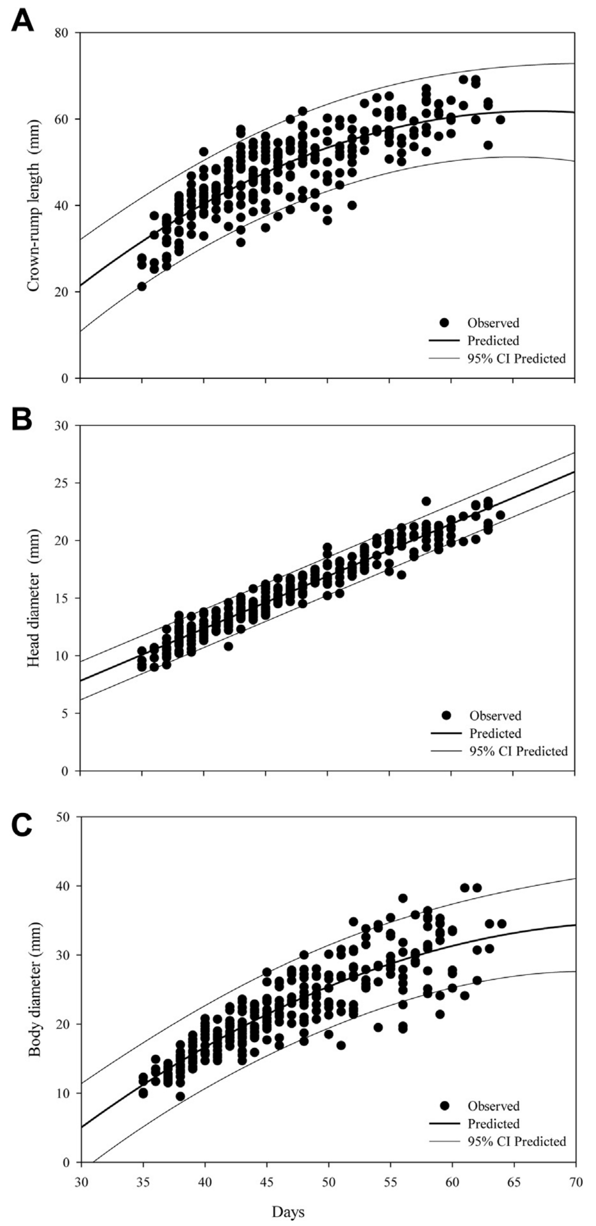

Fig. 6. Multiple regression analysis of crown–rump length (mm, A), head diameter (mm, B), and body diameter (mm, C) with days after the first mating using a using first (B) and second (A, C) degrees of polynomial model in mixed-breed queens (n = 16). CI, confidence interval.

between the queen's weight and age and gestation length because the animals used in our work had similar weight and age.

According to our objective, we derived a set of equations to predict ultrasonographic GS (LEN, ATP, WID, GSV, GSD, and UWT) and fetal measurements (CRL, HD, and BD) and P_4 concentrations from 21 days after the first mating to 1 day before parturition. Using these equations, two-way entry tables (Tables 3 and 4) were derived that allow predicting GS and fetal measurements and P_4 concentrations with 95% CI using any day given after the first mating

Table 2
Pearson correlation coefficients and probability values of ultrasonographic measurements and serum progesterone concentrations during 21 to 63 days after the first mating in mixed-breed queens (n = 16).

r/P	LEN	ATP	WID	UWT	CRL	HD	BD	P_4
LEN	1.00	0.80/<0.01	0.88/<0.01	0.67<0.01	0.65/<0.01	0.57/<0.017	0.56/<0.01	−0.50/<0. 01
ATP		1.00	0.72/<0.01	0.55/<0.01	0.39/<0.01	0.47/<0.01	0.48/<0.01	−0.52/<0.01
WID			1.00	0.67/<0.01	0.40/<0.01	0.47/<0.01	0.42/<0.01	−0.46/<0.01
UWT				1.00	0.14/0.01	0.10/0.07	0.07/0.20	−0.36/<0.01
CRL					1.00	0.82/<0.01	0.80/<0.01	−0.43/<0.01
HD						1.00	0.87/<0.01	−0.58/<0.01
BD							1.00	−0.54/<0.01
P_4								1.00

Abbreviations: ATP, anterior–posterior; BD, body diameter; CRL, crown–rump length; HD, head diameter; LEN, length; P_4, progesterone; r/P, correlation coefficient/probability; UWT, uterine wall thickness; WID, width.

Table 3
Predicted values and 95% confidence interval (CI) of gestational sac parameters and serum progesterone concentration from 21 to 63 days after the first mating in queens (n = 16) drawn from regression equations using polynomial equations shown in Tables 1 and 3.

DG	Ultrasound gestational sac measurements and serum progesterone concentrations													
	LEN		ATP		WID		GSV		GSD		UWT		P_4	
	Predicted	95% CI	Predicted	95% CI	Predicted	95% CI	Predicted	95% CI	Predicted	95% CI	Predicted	95% CI	Predicted	95% CI
21	16.0	15.0–17.0	13.8	13.1–14.3	15.6	14.9–16.3	1.0	0.3–1.7	15.2	14.6–15.6	2.4	2.2–2.5	32.1	28.4–34.6
22	18.6	17.6–19.6	14.4	13.9–15.0	17.0	16.4–17.6	1.2	0.6–2.1	16.8	16.2–17.1	2.5	2.3–2.6	31.6	28.5–33.8
23	21.2	20.2–22.0	15.1	14.6–15.6	18.4	17.9–18.9	2.5	1.8–3.3	18.3	17.8–18.7	2.7	2.5–2.7	31.1	28.5–33.3
24	23.7	22.8–24.5	15.8	15.3–16.2	19.8	19.3–20.2	3.8	3.2–4.6	19.8	19.3–20.1	2.8	2.6–2.9	30.6	28.5–32.5
25	26.1	25.3–26.8	16.5	16.0–16.8	21.0	20.6–21.5	5.1	4.5–5.8	21.3	20.8–21.5	2.9	2.8–3.0	30.1	28.5–32.3
26	28.5	27.7–29.1	17.1	16.7–17.4	22.4	21.9–22.7	6.4	5.8–7.1	22.7	22.3–22.9	3.0	2.9–3.1	29.6	28.3–31.9
27	30.8	30.0–31.3	17.7	17.3–18.0	23.6	23.2–23.9	7.7	7.1–8.3	24.0	23.7–24.3	3.2	3.0–3.2	29.0	28.1–31.4
28	33.0	32.3–33.6	18.3	18.0–18.6	24.8	24.4–25.0	9.0	8.4–9.6	25.4	25.1–25.6	3.3	3.2–3.5	28.5	27.9–30.9
29	35.2	34.5–35.7	18.9	18.6–19.2	25.9	25.5–26.3	10.3	9.7–10.3	26.7	26.4–26.9	3.4	3.3–3.4	27.9	27.6–30.5
30	37.3	36.6–37.8	19.5	19.2–19.8	27.0	26.5–27.3	11.5	11.0–12.1	27.9	27.6–28.2	3.5	3.4–3.5	27.4	27.3–30.0
31	39.3	38.6–39.8	20.1	19.8–20.4	28.0	27.7–28.3	12.8	12.3–13.4	29.1	28.9–29.4	3.6	3.5–3.6	28.2	26.9–29.6
32	41.3	40.6–41.8	20.6	20.3–20.9	29.1	28.7–29.4	14.1	13.6–14.6	30.3	30.0–30.6	3.7	3.6–3.7	27.8	26.5–29.1
33	43.2	42.5–43.7	21.2	20.8–21.5	30.0	29.6–30.4	15.4	14.9–15.9	31.5	31.2–31.7	3.8	3.6–3.7	27.3	26.0–28.6
34	45.0	44.3–45.6	21.7	21.3–22.0	31.0	30.6–31.3	16.7	16.2–17.2	32.6	32.2–32.8	3.9	3.7–3.9	26.8	25.5–28.1
35	46.8	46.1–47.4	22.2	21.8–22.5	31.9	31.5–32.2	18.0	17.5–18.4	33.7	33.3–33.9	3.9	3.8–4.0	26.3	24.9–27.6
36	48.5	47.7–49.1	22.7	22.3–23.0	32.8	32.3–33.1	19.3	18.8–19.7	34.7	34.5–34.9	4.0	3.9–4.1	25.7	24.4–27.1
37	50.2	49.4–50.8	23.2	22.8–23.5	33.6	33.1–33.9	20.6	20.1–21.0	35.7	35.2–35.9	4.1	4.0–4.1	25.2	23.8–26.5
38	51.8	51.0–52.3	23.7	23.2–24.0	34.3	33.9–34.7	21.9	21.4–22.3	36.6	36.2–36.9	4.1	4.0–4.2	24.6	23.2–26.0
39	53.3	52.5–54.0	24.1	23.7–24.5	35.0	34.6–35.4	23.2	22.6–23.6	37.5	37.1–37.8	4.2	4.1–4.3	23.9	22.5–25.4
40	54.7	53.9–55.3	24.6	24.1–24.9	35.7	35.3–36.1	24.5	23.9–24.9	38.4	38.0–38.6	4.3	4.1–4.4	23.3	21.9–24.7
41	56.1	55.3–56.7	25.0	24.6–25.3	36.4	35.9–36.7	25.8	25.2–26.2	39.2	38.8–39.4	4.3	4.1–4.4	22.6	21.2–24.1
42	57.4	56.6–58.0	25.4	25.0–25.7	37.0	36.5–37.3	27.1	26.5–27.5	39.9	39.6–40.2	4.4	4.2–4.4	21.9	20.5–23.4
43	58.7	57.9–59.3	25.8	25.4–26.1	37.6	37.1–37.9	28.4	27.7–28.8	40.7	40.3–40.9	4.4	4.3–4.5	21.2	19.8–22.6
44	59.9	59.1–60.5	26.2	25.8–26.5	38.1	37.6–38.4	29.7	29.0–30.1	41.4	41.0–41.6	4.4	4.3–4.5	20.5	19.1–21.9
45	61.0	60.2–61.6	26.6	26.1–26.9	38.6	38.1–38.9	30.9	30.2–31.4	42.1	41.6–42.3	4.9	4.3–4.5	19.7	18.3–21.1
46	62.1	61.3–62.6	26.9	26.5–27.9	39.0	38.5–39.3	32.2	31.5–32.7	42.7	42.3–42.9	4.5	4.4–4.5	18.9	17.5–20.3
47	63.1	62.2–63.4	27.3	26.8–27.3	39.4	38.9–39.7	33.5	32.8–34.0	43.3	42.8–43.5	4.5	4.4–4.6	18.1	16.7–19.5
48	64.0	63.1–64.7	27.6	27.2–27.9	39.8	39.3–40.1	34.8	34.0–35.3	43.8	43.4–44.0	4.5	4.4–4.6	17.3	15.9–18.6
49	64.9	64.0–65.4	27.9	27.5–28.2	40.1	39.6–40.4	36.1	35.3–36.6	44.4	43.9–44.5	4.5	4.4–4.6	15.1	15.0–17.8
50	65.6	64.8–66.2	28.2	27.7–28.6	40.4	39.8–40.7	37.4	36.5–38.0	44.8	44.3–45.0	4.6	4.4–4.6	14.4	14.1–16.9
51	66.4	65.5–67.0	28.5	28.0–28.9	40.6	40.0–40.9	38.7	37.8–39.3	45.2	44.7–45.4	4.6	4.4–4.6	13.6	13.1–16.0
52	67.0	66.0–67.7	28.8	28.3–29.2	40.8	40.2–41.2	40.0	39.0–40.6	45.6	45.0–45.8	4.6	4.4–4.6	12.9	12.1–15.2
53	67.6	66.6–68.3	29.0	28.5–29.5	40.9	40.3–41.3	41.3	40.3–41.9	46.0	45.3–46.2	4.6	4.4–4.6	12.1	11.1–14.3
54	68.2	67.0–69.0	29.3	28.7–29.7	41.0	40.3–41.5	42.6	41.5–43.2	46.3	45.6–46.5	4.6	4.4–4.6	11.3	10.0–13.4
55	68.7	67.4–69.5	29.5	28.9–30.0	41.1	40.4–41.6	43.9	42.8–44.6	46.6	45.8–46.8	4.6	4.3–4.6	10.6	8.8–12.6
56	69.1	67.7–70.0	29.7	29.0–30.3	41.1	40.3–41.7	45.2	44.0–45.9	46.8	46.0–47.0	4.5	4.3–4.6	9.8	7.6–11.6
57	69.4	67.9–70.5	29.9	29.2–30.5	41.1	40.2–41.7	46.5	45.3–47.2	47.0	46.1–47.3	4.5	4.3–4.6	9.0	6.4–10.8
58	69.7	68.1–70.9	30.1	29.3–30.8	41.0	40.1–41.7	47.8	46.5–48.5	47.1	46.2–47.5	4.4	4.2–4.6	8.2	5.1–9.9
59	69.9	68.2–71.2	30.3	29.4–31.1	41.0	39.9–41.7	49.1	47.7–49.8	47.2	46.2–47.6	4.4	4.2–4.6	7.4	3.7–9.0
60	70.0	68.1–71.5	30.4	29.5–31.3	40.9	30.7–41.7	50.4	49.0–51.2	47.3	46.2–47.7	4.4	4.1–4.6	6.6	2.3–8.1
61	70.1	68.0–71.7	30.6	29.5–31.5	40.6	39.4–41.6	51.6	50.2–52.5	47.3	46.1–47.8	4.3	4.0–4.5	5.8	0.9–7.3
62	70.1	67.9–71.9	30.7	29.6–31.7	40.4	39.1–41.4	52.9	51.5–53.8	47.3	46.0–47.9	4.3	4.0–4.5	4.9	0.6–6.4
63	70.0	67.6–72.0	30.8	29.6–32.0	40.1	38.7–41.3	54.2	52.7–55.1	47.3	45.8–47.9	4.2	3.9–4.5	4.1	0.5–5.5

All measurements in mm and P_4 in ng/mL.
Abbreviations: ATP, anterior–posterior; DG, day of gestation; GSD, gestational sac diameter; GSV, gestational sac volume ($\times 10^3$); LEN, length; P_4, progesterone; UWT, uterine wall thickness; WID, width.

Table 4
Predicted values and 95% confidence interval (CI) of ultrasound fetal measurements from 35 to 63 days after the first mating in queens (n = 16) drawn from regression equations using polynomial equations shown in Table 1.

DG	Ultrasound fetal measurements					
	CRL		HD		BD	
	Predicted	95% CI	Predicted	95% CI	Predicted	95% CI
35	31.7	29.8–33.4	10.1	9.9–10.2	11.3	10.1–12.2
36	33.6	31.9–35.0	10.5	10.3–10.6	12.5	11.4–13.2
37	35.4	33.9–36.5	11.0	10.8–11.1	13.6	12.7–14.2
38	37.1	35.9–38.1	11.4	11.2–11.5	14.7	13.9–15.2
39	38.8	37.7–39.6	11.9	11.7–12.0	15.8	15.0–16.1
40	40.4	39.5–41.1	12.3	12.2–12.4	16.8	16.1–17.1
41	42.0	41.1–42.6	12.8	12.6–12.9	17.8	17.2–18.1
42	43.5	42.7–44.1	13.2	13.1–13.3	18.8	18.2–19.0
43	45.0	44.1–45.5	13.7	13.6–13.8	19.8	19.1–20.0
44	46.4	45.5–46.9	14.2	14.0–14.3	20.7	20.0–20.9
45	47.7	46.8–48.3	14.6	14.5–14.7	21.6	20.9–21.8
46	49.0	48.0–49.6	15.1	14.9–15.2	22.5	21.8–22.7
47	50.2	49.2–50.8	15.5	15.4–15.6	23.3	22.6–23.5
48	51.4	50.3–52.0	16.0	15.8–16.0	24.1	23.4–24.3
49	52.5	51.4–53.1	16.4	16.3–16.5	24.9	24.1–25.1
50	53.5	52.4–54.1	16.9	16.7–17.0	25.6	24.9–25.9
51	54.5	53.4–55.1	17.3	17.2–17.4	26.4	25.6–26.6
52	55.4	54.3–56.0	17.8	17.6–17.9	27.1	26.3–27.3
53	56.3	55.2–56.9	18.2	18.1–18.3	27.7	26.9–27.9
54	57.1	55.9–57.7	18.7	18.5–18.8	28.4	27.6–28.6
55	57.8	56.6–58.5	19.1	19.0–19.2	29.0	28.1–29.2
56	58.5	57.2–59.2	19.6	19.4–19.7	29.6	28.7–29.8
57	59.1	57.8–60.0	20.0	19.9–20.1	30.1	29.2–30.4
58	59.7	58.2–60.7	20.5	20.3–20.6	30.7	29.6–31.0
59	60.2	58.5–61.3	21.0	20.8–21.4	31.8	30.0–31.6
60	60.7	58.7–62.0	21.4	21.2–21.6	31.6	30.3–32.2
61	61.1	58.9–62.6	21.9	21.6–22.0	32.1	30.6–32.8
62	61.4	58.9–63.2	22.3	22.1–22.5	32.5	30.8–33.3
63	61.7	58.9–63.7	22.8	22.5–23.0	32.9	31.0–33.9

All measurements in mm.
Abbreviations: BD, body diameter; CRL, crown–rump length; DG, day of gestation; HD, head diameter.

between 21 and 63. Likewise, using the same table, it is possible to assess the normal or abnormal embryo/fetal development. Interestingly enough, equations derived using days after the first mating instead of day before parturition had a higher coefficient of determination (r^2) in all GS and fetal measurements.

Table 5
Accuracy prediction of parturition day at different gestational ages in queens (n = 16).

Weeks of pregnancy	Gestational sac measurements							
	0 days		±1 day		±2 days		±3 days	
	GSV, n (%)	GSD, n (%)	GSV, n (%)	GSD, n (%)	GSV, n (%)	GSD, n (%)	GSV, n (%)	GSD, n (%)
3	4/40 (10.0)	4/40 (10.0)	16/40 (40.0)	22/40 (55.0)	36/40 (90.0)	37/40 (92.5)	40/40 (100)	40/40 (100)
4	31/157 (19.7)	31/157 (19.7)	79/157 (50.3)	85/157 (54.1)	130/157 (82.8)	124/157 (79.0)	152/157 (96.8)	142/157 (90.4)
5	14/124 (11.3)	16/124 (13.0)	46/124 (37.1)	51/124 (41.1)	68/124 (54.8)	80/124 (64.5)	85/124 (68.5)	96/124 (77.4)
6	7/103 (6.8)	7/103 (6.8)	22/103 (21.3)	26/103 (25.2)	39/103 (37.8)	45/103 (43.7)	52/103 (50.5)	60/103 (58.2)
7	4/92 (4.3)	6/93 (6.4)	17/92 (18.5)	20/93 (21.5)	24/92 (26.1)	26/93 (28.0)	37/92 (40.2)	39/93 (41.9)
8	4/53 (7.5)	1/52 (1.9)	9/53 (17.0)	7/52 (13.4)	16/53 (30.2)	10/52 (19.2)	18/53 (34.0)	16/52 (30.7)
9	1/26 (3.8)	0/25 (0.0)	1/26 (3.8)	1/25 (4.0)	3/26 (11.5)	1/25 (4.0)	6/26 (23.0)	2/25 (8.0)
P	0.0023	0.0005	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Weeks of pregnancy	**Fetal measurements**							
	0 days		±1 day		±2 days		±3 days	
	HD, n (%)	BD, n (%)	HD, n (%)	BD, n (%)	HD, n (%)	BD, n (%)	HD, n (%)	BD, n (%)
5	6/20 (30.0)	2/20 (10.0)	16/20 (80.0)	10/20 (50.0)	19/20 (95.0)	18/20 (90.0)	20/20 (100)	19/20 (95)
6	15/106 (14.1)	19/103 (18.4)	57/106 (53.7)	46/103 (44.6)	82/106 (77.3)	63/103 (61.2)	96/106 (90.5)	73/103 (70.9)
7	21/101 (20.8)	7/94 (7.4)	45/101 (44.5)	28/94 (29.8)	75/101 (74.3)	43/94 (45.7)	91/101 (90.1)	56/94 (59.6)
8	8/62 (12.9)	5/58 (8.6)	26/62 (41.9)	13/58 (22.4)	44/62 (71.0)	23/58 (39.7)	50/62 (80.6)	25/58 (43.1)
9	6/43 (13.9)	4/34 (11.8)	19/43 (44.2)	8/34 (23.5)	28/43 (65.1)	12/34 (35.3)	34/43 (79.1)	14/34 (41.2)
P	0.2790	0.1576	0.0256	0.0096	0.1202	<0.0001	0.0414	<0.0001

All measurements in mm.
Abbreviations: BD, body diameter; GSD, gestational sac diameter; GSV, gestational sac volume; HD, head diameter; P, probability.

Furthermore, the high coefficients of determination ($r^2 > 0.85$) obtained for LEN, WID, HD, GSV, GVD, and P_4 clearly indicate goodness of fit of models to predict gestational age with these models. To our knowledge, this is the first study to make available these data and equations with such high coefficients of determinations for practitioners to use in clinical practice. It is noteworthy to point out that the small variability observed in embryo/fetal measurements could be explained because in our study, there was a very homogeneous population of pregnant queens with similar size, weight, and kitten number per pregnancy. These factors should be included between the limits of our study when used in clinical work.

These findings are in agreement with the results obtained by Beck et al. [13] who found a lineal regression between HD and BD and prepartum day. However, the resolution of ultrasound equipment used in that study did not allow differentiation of the head earlier than 38 days prepartum. In the same way, Zambelli et al. [14] correlated head, stomach, and abdominal diameters of the fetus with gestational age during the second half of pregnancy and found a parabolic and exponential regression analysis showed higher coefficients of regression than the linear one. Although that study showed a relationship between ultrasonographic measurement and gestational age, the measurements were performed only in some days of pregnancy and using small numbers of observations. In this study, we performed daily ultrasound examinations on the last two-thirds of pregnancy, which provides a complete study of embryo–fetal evolution and the relationship between different ultrasonographic measurements with gestational age.

The accuracy of gestational age prediction by ultrasonography changes on gestational week basis, and this observation in our work is in agreement with the findings of Beccaglia et al. [16] and Beccaglia and Luvoni [17]. The predictions obtained by Beccaglia et al. using HD were similar to our result. In the same way, according to Beccaglia et al., the application of the formulae described in this work resulted in high accuracy at $\pm$ 2 days (GSD week 3, 92.5%; HD week 5, 95%) suggesting GSD measurement provides reliable information on the gestational age since week 3 and HD since week 5 [17]. In agreement with Topie et al. [27], considering the first mating like the start of gestation in our work, the accuracy of GSD was high at week 3. Fetal development progresses rapidly, allowing recognition of organogenesis ultrasonographically. From Day 30 after the first mating, it becomes possible to recognize different fetal organs [8,9]. These data could be related with the use of formulas for the prediction of gestational age and days before parturition. Therefore, we developed easy-to-use formulas for the prediction of gestational age and days before parturition in the cat which can be supplemented with fetal morphology changes.

Brito et al. [18] observed an effect of day after mating on fetal measurements as well as the same observed in our results. In Brito et al. study like in our work, GS and fetal measurements increased as gestational aged advanced. The linear increase in biparietal diameter during pregnancy founded by Gatel et al. [15] is in agreement with our results in which the same parameter increases with the days after mating.

Thereby the positive correlation found between ultrasonographic measurements and gestational age could be used for monitoring pregnancy in the queen. Gestational sac and fetal ultrasonographic measurements have been used to estimate gestational age. This work provides important information for clinical monitoring of fetal development in the last two-thirds of pregnancy and for dating gestational age.

The ultrasonographic data derived in this study can be used to estimate gestational age and to evaluate the progression of embryo/fetal development (Tables 3 and 4). Furthermore, the results from this study show that from 21 days after the first mating to parturition, GSD was the most useful measurement for estimation of gestational age because it was the most accurate predictor. In addition, in agreement with the findings of Brito et al. [18], in our study, HD was the most accurate measurement for estimation of gestational age from 35 days after the first mating to parturition. Using recorded measurements, GSD and HD, and equations derived in Table 1, accurate estimation of the gestational age and date for parturition could be performed in practice. Our results show that GSD and HD are accurate if there are used in the week 3 and 5 respectively.

In conclusion, the equations derived from this study will be useful for monitoring pregnancy in the queen from 21 days after the first mating to the end of pregnancy.

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