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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