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Evaluation of serum C-reactive protein concentration as a marker of impending parturition and correlation with progesterone profile in peri-partum bitches

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ABSTRACT

C-reactive protein (CRP) is one of the major acute phase proteins in dogs. It is produced by the liver and rapidly increases in response to an inflammatory stimulus. The aim of this study was to measure CRP concentrations around parturition and to verify whether this protein could be useful, together with progesterone (P), to detect the onset of parturition in bitches. The CRP and P concentrations were measured in 66 serum samples from 28 healthy pregnant bitches, collected between -5 and +2 days from parturition (day of parturition = day 0). The effect of 'days from parturition', parity, and litter size on P and CRP concentration was analyzed and the correlation between CRP and P values was calculated. The P and CRP values were affected by 'days from parturition'. While P decreased during the last days of pregnancy, CRP concentration was greater than the normal range (0–1.07 mg/dl) starting the parturition day with the increase starting on day -1. The CRP concentration profiles during the days around parturition have not been previously reported in dogs. The increase in CRP very near the time of parturition and the low magnitude of the increase do not allow for it to be useful in clinical practice to assess the onset of parturition in the bitch.

1. Introduction

The C-reactive protein (CRP) is an acute-phase protein (APP) that is mainly produced in the liver upon stimulation by pro-inflammatory cytokines. The acute phase response is a nonspecific reaction that is induced by any tissue injury and develops following either infectious, immunologic, neoplastic, traumatic events or from other causes (Ceron et al., 2005). The APPs are mediators and inhibitors of inflammation, and have a protective effect through the opsonization of apoptotic or necrotic cells, by binding to bacterial proteins and affecting the immune response which accompanies inflammation (Gabay and Kushner, 1999). There is an early and marked response with increasing concentrations of CRP in dogs with a 20- to 100-fold increase, depending on the cause of inflammation that can be detected after 4 h from the time of the insult with a peak concentration at 24–48 h (Ceron et al., 2005). The CRP can be evaluated in clinical exams as a marker of both acute and chronic inflammatory disorders (Ceron et al., 2008).

An increase in CRP concentration has been detected in the first (Eckersall et al., 1993) or second (Kuribayashi et al., 2003)

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trimester of pregnancy in the bitch, followed by a decrease before parturition (Eckersall et al., 1993; Kuribayashi et al., 2003). A second increase after parturition has been occasionally observed (Eckersall et al., 1993). Implantation of the developing embryo in the endometrium and placental development were suggested as the likely cause of an acute phase response (Eckersall et al., 1993) and it has also been ascribed as 'the influence of endocrine hormones during pregnancy' (Kuribayashi et al., 2003). In the past, the analysis of CRP and other APP concentrations was proposed as an approach for early pregnancy diagnosis in the bitch (Evans and Anderton, 1992; Vannucchi et al., 2002). Although the exact mechanisms responsible for parturition are still to be elucidated, the presence of an inflammatory response in the myometrium occurs in women (Thomson et al., 1999; Mendelson and Condon, 2005; Leong et al., 2008) and could also occur in other mammalian species. When measured before, during and after women give birth to a child, assessments of CRP and another acute-phase protein, serum amyloid A, confirmed there was a physiological occurrence of a major acute-phase response (De Villiers et al., 1990; Cicarelli et al., 2004).

A reliable estimate of parturition date is rather difficult in the bitch when only mating dates are available for the clinician to assess. A precise prediction of impending parturition could be very useful to avoid long observation periods and is very important when planning a cesarean section. Serum progesterone concentration decreases before parturition (Onclin and Verstegen, 1997) and some cut-off values have been calculated so that when progesterone concentrations are less than this value, parturition is likely to occur within a given time interval (Rota et al., 2015; De Cramer and Nöthling, 2018). A large individual variability exists in progesterone concentration, particularly in the last days of pregnancy (Rota et al., 2015; De Cramer and Nöthling, 2018), and other easily measurable variables that could allow for determination of the onset of parturition would be very useful in clinical practice.

Because CRP measurement is part of the routine biochemical profiles performed by many veterinary laboratories, the aim of this study was to measure peri-partum CRP concentration in the bitch, to establish a relationship between CRP and progesterone profiles and to assess its use in clinical practice for determination of the impending time of parturition in the bitch.

2. Materials and methods

2.1. Animals and samples

Twenty-eight healthy pregnant bitches that whelped live puppies were included in the study. Exclusion criteria were dystocia, oxytocin administration and postpartum disorders. The bitches, of various breed [Staffordshire Bull Terrier (n = 6), Flat Coated Retriever (n = 4), Boxer (n = 4), Jack Russell Terrier (n = 3), Bouvier des Flandres (n = 2), Australian Shepherd (n = 2), and one each of the following: American Staffordshire Terrier, Bloodhound, Bassett Hound, Labrador Retriever, Golden Retriever, Samoyed, Rough Collie] and parity, ranging in age from 2 to 8 years (3.9 ± 1.6 mean ± SD) were brought to the veterinary hospitals of the University of Padova or Torino for pregnancy monitoring and parturition assistance, in the period from June 2016 to October 2017. Blood had been collected by cephalic venipuncture for routine progesterone assay and biochemistry evaluation and the remaining sera had been stored frozen at −20 °C. The frequency of blood collection varied among dogs, depending on the reason of the sampling, from twice a week to daily.

On the case sheet of each bitch, there was recording of the day of parturition and the number of puppies to which the bitch gave birth. Serum samples collected between 5 days before and 2 days after parturition were selected for analysis, resulting in a total number of 66 samples, with a minimum of one and a maximum of five samples for each dog. The number of samples for each of the 8 days of observation was the following: 8, 9, 9, 10, 8, 8, 8, 6. Written informed consent to use the stored samples was obtained from dog owners.

The study was performed in accordance with the guidelines for the care and use of animals of the Department of Veterinary Science of the University of Turin and of the Department of Animal Medicine Production and Health of Padova.

2.2. Measurement of progesterone and CRP

The CRP was quantified using the turbidimetric method (BT1500®, Biotecnica instruments SpA, Roma, Italy); normal reference values are considered when in the range of 0–1.07 mg/dl (Wong et al., 2011). The values obtained using this assay had been previously correlated with those when there was use of a canine CRP turbidimetric assay (Randox canine CRP reagents, RANDOX, Milan, Italy) and validated for the canine species (Kjelgaard-Hansen et al., 2003). Repeatability was CV < 7.5%; Linearity (O/A) y = 0.9795x – 0.0074 R^2 = 0.9935. The relationships between the two kits was obtained by the analysis of 91 samples and described by the linear regression as follows: y = 1.5135x – 0.0123 with and R^2 = 0.9431. Progesterone was quantified using a Chemiluminescence immunoassay (CLIA) (Immulite 1000®; Siemens Diagnostics, Flanders, NJ, USA) validated for the canine species (Kutzler et al., 2003).

2.3. Statistical analysis

The statistical analysis was performed using a repeated mixed linear model where days from parturition (days -5 to +2), parity (primiparous compared with multiparous), and number of puppies born from each bitch (< 4 compared 4–8 compared > 8) were considered as fixed effects and dog was considered as random and repeated effect. Hypotheses of linear model on residuals were graphically assessed. *Post hoc* pairwise contrasts among levels were calculated using Bonferroni correction. Data were reported as least-squares means ± standard error (ls-means ± SE). Day 0 was the day of parturition. Significance was set at P < 0.05.

The Spearman rank correlation was calculated between CRP and progesterone values. Using the threshold of 2 ng/ml to identify

Table 1
Mean C-reactive protein (CRP) concentration (mg/dl) and standard error of the mean (SEM) from 5 days before to 2 days after parturition (day 0).

day	CRP (mg/dl)	SEM
−5	0.63	0.19
−4	0.86	0.19
−3	0.73	0.18
−2	0.67	0.17
−1	0.79	0.19
0	1.2	0.19
1	1.39	0.2
2	1.39	0.22

bitches that were approaching the time of parturition (Concannon et al., 1977), the animals were divided into two groups (at term/not at term). The capacity for use of CRP concentration to distinguish between bitches at term and not at term was evaluated using receiver operating characteristic (ROC) curve analysis. To select the optimal cut-off value, with 95% CI, Youden's Index was calculated. The value of the area under the curve (AUC) as a criterion of the accuracy of the marker was defined as low (0.5 − 0.7), moderate (0.7 − 0.9) and high (> 0.9) (Gardner and Greiner, 2006). All analyses were performed using the statistical software packages of SAS V.9.3 (SAS Institute Inc., Cary, NC) and MedCalc v.12.4.0 (Ostend, Belgium).

3. Results

The CRP concentrations ranged from 0/0.04–2.74 mg/dl and were affected by 'days from parturition' ($P = 0.019$), parity ($P = 0.044$) and number of puppies whelped by the bitch ($P = 0.0036$). Serum CRP values increased over time (Table 1). Mean CRP concentrations were greater than the normal range subsequent to parturition with the initiation of the increase being on day -1.

Primiparous bitches had greater CRP concentrations than pluriparous bitches (Fig. 1). Bitches that whelped less than four puppies had lesser CRP concentrations than bitches with larger litters (Fig. 2).

Progesterone concentration was affected by 'days from parturition' ($P < 0.0001$) and there was a decrease during the last days of pregnancy. In particular, there was a significant decrease between 4 and -1 days pre-partum (5.01 ± 0.48 ng/ml to 2.74 ± 0.48ng/ml) and there was a further decrease on day 1 post parturition (0.35 ± 0.51 ng/ml; Fig. 3). Postpartum values were less than 1 ng/ml and did not differ between days. Neither parity nor litter size affected progesterone concentration. There was an inverse correlation between CRP and progesterone concentrations ($r = -0.52$; $P < 0.001$. The ROC curve analysis indicated that area under the curve (AUC) was 0.73, with 0.591–0.835 as 95%CI ($P = 0.001$)1, meaning that CRP has a moderate accuracy as a marker of impending parturition. The cutoff value that maximizes the Youden's Index is 1.41 mg/dl, with a sensitivity of 87.5% (71–96.5; 95% CI) and a specificity of 56% (34.9 − 75-6; 95% CI).

4. Discussion

Serum progesterone concentrations can be used by clinicians to assess the time of the impending parturition of bitches (Rota et al., 2015; De Cramer and Nöthling, 2018), however, data from the present study indicate that the progesterone concentrations are not different during the last 3 days of pregnancy. Pre-partum luteolysis occurs around 60 days after ovulation, following PGF2α release from the utero/placental compartment (Kowalewski, 2014). Activation of the placental prostaglandin system follows the decrease in progesterone concentration to less than a critical threshold (2–3 ng/ml) which leads to alterations in the placental feto-maternal

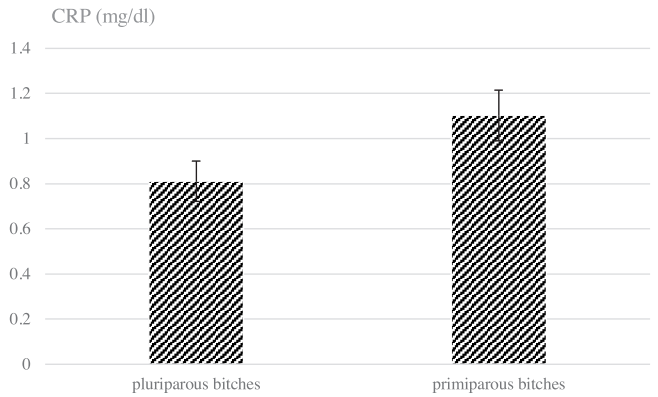

Fig. 1. Mean C-reactive protein (CRP) concentration (mg/dl) in pluriparous ($n = 14$) and primiparous ($n = 14$) bitches ($P = 0.044$). Bars represent the standard error of the mean (SEM).

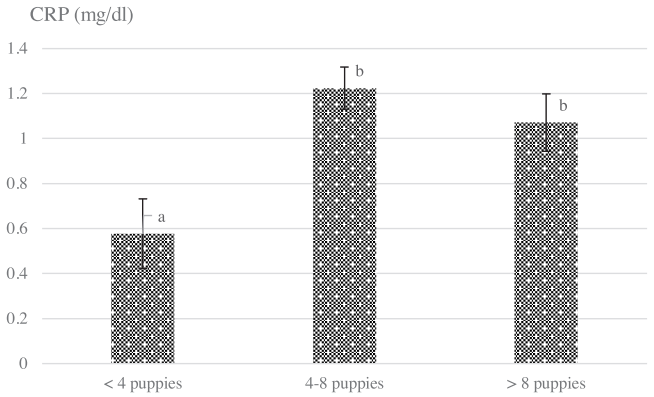

Fig. 2. Mean C-reactive protein (CRP) concentration (mg/dl) in bitches with litters of different size ($P = 0.0036$); different letters indicate values that differ ($P < 0.01$); bars represent the standard error of the mean (SEM).
Number of bitches for each category of litter size: < 4 puppies $n = 6$; 4–8 puppies $n = 15$; > 8 puppies $n = 7$.

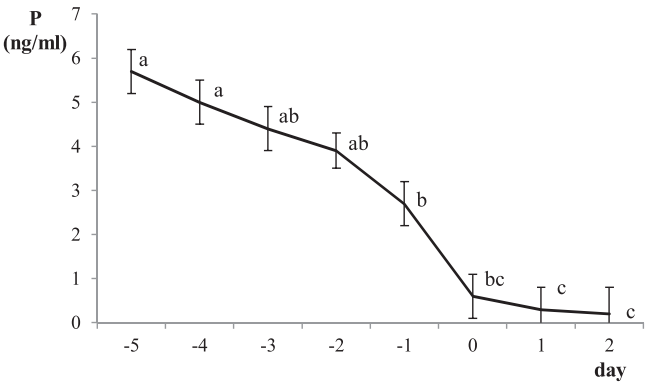

Fig. 3. Mean progesterone concentration (ng/ml) from 5 days before to 2 days after parturition (day 0); different letters indicate values that differ ($P < 0.01$); bars represent the standard error of the mean (SEM).

communication (Kowalewski, 2014) and possibly to an inflammatory response.

Other easy-to measure serum variables could be useful to increase the accuracy of the diagnosis with use CRP concentrations because the values for these variables can be ascertained in any facility having instruments for biochemistry serum analysis. Our hypothesis was that uterine inflammation is a characteristic feature of parturition in bitches, as occurs in women (De Villiers et al., 1990; Thomson et al., 1999; Cicarelli et al., 2004; Mendelson and Condon, 2005), and that CRP, which is a reliable and early marker of inflammation in dogs (Ceron et al., 2005, 2008) could also be a marker of the onset of parturition. Serum concentrations of CRP during pregnancy in dogs have been reported in various studies, with inconsistent results, but daily values around parturition have not been previously measured in dogs. Different methods for CRP determination were used, resulting in different values. Data from the present study are similar to those of Eckersall et al. (1993) who also used the technique of immuno-turbidimetry, although with a different commercial kit. With a 'solid sandwich' immunoassay, Ulutas et al. (2009) obtained different absolute values of CRP concentrations. Although technical differences may explain these differences, this does not allow for an explanation of the different serum profiles that have been reported from previous investigations. In some studies there was a marked seven- to ten-fold increase of CRP serum concentrations beginning around the third to fourth week after ovulation, followed by a decrease in the last third of pregnancy (Eckersall et al., 1993; Kuribayashi et al., 2003). In six of nine beagle bitches in a previous study, there was an increase in CRP after parturition (Eckersall et al., 1993). Results from other studies (Concannon et al., 1996; Ulutas et al., 2009) did not support that there was such a distinct serum CRP profile. Ulutas et al. (2009) reported that there was a greater CRP serum concentration in pregnant bitches than in bitches in proestrus, but the increase in CRP concentrations was very low, both in the first and in second half of pregnancy.

The rate of the increase in serum CRP concentration that was detected in the present study after the day of parturition was less than the value reported by Eckersall et al. (1993) where there was a three- to ten-fold increase. Data from the present study did not indicate that there was a marked increase of CRP serum values, making it difficult to use this variable as a marker of impending parturition. This result is, however, consistent with the trend of progesterone concentration change around the time of parturition in bitches and there was a weak negative relationship between CRP and progesterone concentrations meaning that CRP concentration increases during the time when there are decreasing concentrations of progesterone around the time of parturition in bitches.

The peri-partum pattern of CRP concentration that occurred in the bitch is similar to what has been reported for other species,

irrespective of different placental types and if the species are monotocous or polytocous. An increase of serum CRP concentrations has been detected in sows, where increased concentrations are evident from the day of parturition until day 7 after farrowing (Wierzchosławski et al., 2018). The concentrations of CRP increased just before foaling (≤2days) in pregnant mares and there was a decrease starting 7 days after foaling (Yamashita et al., 1991). In women, the CRP concentrations are generally greater during pregnancy, and there is a significant increase during the postpartum period, approximately10-fold greater than the concentrations during the second and third trimester (Skarżyńska et al., 2018). The CRP concentration was also greater during the first month after calving than in the last trimester of pregnancy in cows (Dębski et al., 2016).

These previous data and data from the present study indicate parturition induces an increase in CRP concentrations, likely due to uterine physiological inflammatory conditions (Thomson et al., 1999; Leong et al., 2008). It is speculated that the inflammatory response in primiparous bitches can be greater because there are disruptions of tissues that for the first time undergo modifications that are associated with the impending parturition. Also the influence of litter size on CRP concentration is consistent with a greater inflammatory response as a result of a larger number of feto-placental units.

The results from the ROC analysis in the present study indicated that CRP is a marker of impending parturition that has a 'moderate' accuracy (Kjelgaard-Hansen et al., 2003). Even though the sensitivity (87.5%) is quite good there is low specificity (56%), meaning that when CRP concentration decreases to less than the cutoff value (identified as 1.41 mg/dl) a clinician can distinguish when there is an impending parturition in 87.5% of cases, while bitches in which there is not an impending parturition can be correctly identified in 56% of cases.

The increase shortly preceding the time of parturition and the low magnitude of serum concentrations of CRP that was detected in the present study indicate this variable cannot be used alone in clinical practice to assess the onset of parturition in the bitch. Further studies are, however, needed to correlate CRP concentrations with values for other variables of impending parturition, and especially with the physiological or pathological outcome of parturition.

Conflict of interest

The authors have no conflict of interest to declare.

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