Diagnostic Efficacy of a Single Progesterone Determination to Assess Full-Term Pregnancy in the Bitch

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Diagnostic efficacy of a single progesterone determination to assess full term pregnancy in the bitch

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Summary

In clinical settings, when the reproductive history of a near-term bitch is limited to mating dates, the possibility to accurately assess whether pregnancy is at term could be very useful in order to be able to plan a correct management of parturition or to safely perform an elective cesarean section. The aim of this study was to assess the diagnostic efficacy of a single progesterone determination, measured by chemiluminescent immunoassay (CLIA), in predicting the occurrence of parturition on the following day.

At least one blood sample was collected from fifty-one pre-partum bitches during the three days before parturition and day of parturition. The efficacy of progesterone as a marker of the end of pregnancy was tested using a ROC (Receiver Operating Characteristic) analysis. The Youden's index was calculated to select the optimal cut-off value (with 95% Confidence Interval), aiming at maximizing the correct identification of negative events, so not to risk to diagnose as full term a bitch which is not. Progesterone concentration lower than 3.4 ng/ml correctly identified all the bitches whelping the following day; however, because of the obliged prudential approach, sensitivity was low (46.88%), and 17/32 full term bitches were missed. Due to a very large individual variation, a single progesterone determination has low diagnostic efficacy, although it can represent a useful first screening.

Key words

Dog, progesterone, CLIA, parturition

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Single progesterone measure as a marker of parturition

Introduction

The length of canine pregnancy apparently varies between 57 and 72 days because bitches show an extended period of receptivity and viability of spermatozoa in the female reproductive tract is prolonged (Concannon et al. 1983); effective gestation length is 65±1 days when timed from a fixed physiologic event, such as the LH surge (Concannon et al. 1983). The preovulatory LH surge can be detected through the measurement of the concomitant increase in serum progesterone concentrations and this method provided a parturition prediction accuracy of 90% for 65±2 d (Kim et al. 2007). Although LH could be measured directly, it is rather impractical because more than one daily samplings are necessary (Hase et al. 2000).

In clinical settings, veterinarians have frequently to deal with near-term bitches whose history is limited to mating dates. At the end of pregnancy some parameters can be monitored to predict parturition such as body temperature or progesterone concentration. Rectal and vaginal temperature may show a decline around 24 hours before the onset of parturition (Concannon and Hansel 1977; Geiser et al. 2014), although it does not occur in all bitches (Veronesi et al. 2002); a significant decline in progesterone concentration occurs 24 to 40 h before parturition (Nett et al. 1975; Concannon et al., 1978; Veronesi et al. 2002; Brugger et al. 2011), as a possible response to the rapid changes in circulating hormone concentrations (Concannon and Hansel 1977). However the point remains that repeated measures over more days are necessary to detect a decline. In the absence of monitoring, the possibility to predict whether a bitch is going to whelp the following day or not could be extremely useful to a veterinarian that wishes to be prepared and available for the onset of labor or to safely perform an elective cesarean section. Ultrasonographic foetometry has great potential, but the huge morphological and size variability in dogs would require breed-specific foetal growth curves to increase the accuracy of this method (Kutzler et al. 2003b; Socha and Janowski 2014).

The aim of this study was to assess the diagnostic efficacy of a single progesterone determination, measured by chemiluminescent immunoassay (CLIA), in assessing full term pregnancy, i.e the occurrence of parturition the following day.

Materials and Methods.

Animals and sampling

51 pre-partum bitches of different breeds and ages were included in the study. At least one blood sample was collected during the three days before parturition and day of parturition. Blood was collected from the cephalic vein into tubes without separating gel, and allowed to clot at 4°C before centrifugation. Serum progesterone concentration was measured by CLIA (Immulite 2000®; Siemens Medical Solutions Diagnostics, Flanders, New Jersey, USA). Progesterone assay has a sensitivity of 0.1 ng/ml (zero + 2 standard deviations).

Statistical analysis

Progesterone concentrations on the day of parturition and on days -1, -2 and -3 were compared using One-way ANOVA, followed by Bonferroni Multiple Comparisons Test.

The efficacy of progesterone concentration as a marker of the end of pregnancy, that is its ability to detect bitches whelping the following day, was studied using a Receiver Operating Characteristic (ROC) analysis. The ROC curve was developed on progesterone values obtained at days -2 (bitches not whelping the following day) and -1 (bitches whelping the following day); sensitivity, specificity and cut-off value of this potential marker were calculated (with a 95% confidence interval, CI). Sensitivity was defined as the proportion of correctly identified at-term bitches among positive bitches (true positive). Specificity was defined as the proportion of negative events correctly identified by the test, i.e. not-at-term bitches that were correctly identified as not whelping the following day among negative bitches (true negative). To select the optimal cut-off value, with 95% CI, the Youden's index was calculated.

All statistical analyses were done using GraphPad InStat (vers. 3.00) and GraphPad Prism (vers. 4.00) software (GraphPad Inc., San Diego, CA, USA). $P<0.05$ was considered to indicate statistically significant differences.

Results.

The mean values of progesterone concentration on the three days before parturition and on parturition day are reported in Table 1. Statistical analysis showed the occurrence of a highly significant difference between day -2 and -1 and between day -1 and parturition day ($P<0.001$) (Table 1). Conversely, progesterone concentration was similar on days -3 and -2. Large individual variations were observed.

Progesterone concentrations on days -2 and -1 which define the ROC curve appear in Table 2. The area under the curve (AUC) was 0.7677, with a 95% confidence interval (0.6451 - 0.8903; $P < 0.001$) (Fig. 1). The distribution of progesterone values in bitches not whelping the following day (day -2) and bitches whelping the following day (day -1) is shown in Fig. 2. The cut off value that maximizes the Youden's index is 3.4 ng/ml. A clinician can be sure that a bitch with a progesterone concentration below the cut off value will whelp the following day and, especially, to correctly identify negative events, so not to risk to diagnose as full term a bitch which is not.

However, sensitivity is low (46.88%), because many at-term bitches which have a progesterone concentration higher than the cut off value are missed (17/32 bitches). To have 100% sensitivity, i.e. to correctly identify all bitches whelping the following day, progesterone concentration should be lower than 11.95 ng/ml, thus including also not-whelping-the-following-day bitches.

Discussion

Managing parturition in the bitch can be time-consuming in case the beginning of pregnancy is not known. This frequently happens in clinical conditions, when the history of a near-term bitch is limited to the time of multiple matings, implying a possible variation in apparent gestation length higher than ten days (Concannon et al. 1983). The most accurate prediction of parturition date would be based on serial preovulatory serum progesterone measurements to estimate the day of the LH peak (Kim et al. 2007).

Progesterone concentration can be monitored to predict parturition and our results confirm that there is a significant decline in progesterone concentration between 48 and 24 hrs before parturition

and between 24 hrs and parturition day (Nett et al. 1975; Concannon et al. 1978; Veronesi et al. 2002; Brugger et al. 2011); however, the question that was discussed in the present work was the possibility to predict whether a pregnant bitch is at term, having a single progesterone value. In practical settings, the opportunity to assess whether the bitch is going to whelp the following day or not would allow the veterinarian to plan assistance or schedule the time of cesarean section.

Serum progesterone values varied widely between bitches and this means that a single value cannot have high diagnostic efficacy; only about half of our bitches showed progesterone concentration lower than the cut off value on the day before parturition because we chose a precautionary approach, wanting to maximize the correct detection of negative bitches (bitches not whelping the following day). With such an obliged prudential approach, there are no false positives, meaning that there is no risk to perform an elective cesarean section too early, but many bitches whelping the following day are missed.

From literature data, it is common opinion that cesarean sections can be safely performed when progesterone concentration declines below 2 ng/ml, i.e. 24-40 hours before natural parturition, (Concannon et al. 1977; Smith 2007). The gold standard to measure serum progesterone in bitches is radioimmunoassay (RIA) and previous works reporting peri-partum progesterone concentration in bitches has used this methodology (Nett et al. 1975; Concannon et al. 1978; Veronesi et al. 2002). The chemiluminiscent (CLIA- Immulite) assay has been validated for measuring progesterone in canine serum and has the advantage of being safe, fast and accurate enough (Kutzler et al. 2003a; Chapwanya et al. 2008). However, comparing RIA and Immulite serum progesterone values, the average Immulite measurement resulted in significantly higher concentrations than the RIA one, with an average difference of 0.69 ng/ml (Chapwanya et al. 2008). Peri-partum progesterone concentration measured by RIA [3.34 ±1.16 ng/ml at day -1 and 1.15±0.22 at parturition day respectively (Nett et al. 1975); 1.9±0.36 ng/ml 24-16 hours before and 0.55±0.07 12-8 hrs before parturition respectively (Concannon et al. 1978); 2.9±1.7 ng/ml at day -1 and 1.0±0.4 at parturition day respectively (Veronesi et al. 2002)] are numerically lower than our

results. When using CLIA, our data show that a cut off value of 3.4 ng/ml instead of 2 ng/ml could be set for safely performing a caesarean section.

We collected blood samples from the bitches at different hours during the day, according to clinical needs; sampling time might represent a source of variability because progesterone concentration was reported to show diurnal variation in pregnant bitches from weeks 3 to 6, with a.m. values higher than p.m. values (Steinetz et al. 1990); however, in the eighth and ninth week of pregnancy, morning and afternoon values resulted similar (Steinetz et al. 1990). Of course, in clinical settings, sampling time cannot be kept at fixed hours.

At presentation, in the absence of previous monitoring, ultrasound examination can help the clinician to date pregnancy through measuring some foetal parts like the biparietal diameter, in the second half of pregnancy (Lopate 2008): this method is relatively simple but breed-specific foetal growth curves are necessary to increase its accuracy (Kutzler et al. 2003b; Socha and Janowski 2014) because the formulas derive from measures done in small or medium size bitches (Kutzler et al. 2003b; Socha and Janowski 2014). Even when using a specific equation for a single breed, some variables, such as smaller than average litter size, can greatly reduce the accuracy of the prediction (Groppetti et al. 2015). In small and medium size breeds around 70 per cent of parturition dates were predicted within one day and 85% within two days using BP (Beccaglia and Luvoni 2006).

Neither of the two methods alone, i.e progesterone concentration and ultrasound fetometry, appears to be accurate enough to effectively help the clinician; it could be worth to test the association of the two determinations in order to assess whether the diagnostic efficacy becomes higher than with a single one.

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	Days before parturition			
	-3	-2	-1	0
	N=17	N=24	N=32	N=25
P (ng/ml)	7.12±2.79[(a)]	6.81±2.38[(a)]	4.32±2.52[(b)]	1.47±0.67[(c)]
range	3.30-15	3.50-12.40	0.79-10.60	0.43-2.80

222

223 Table 1. Progesterone concentration (mean ± SD) at 3, 2 and 1 day before parturition and on
224 parturition day (0). N= number of bitches. [a,b,c] $P<0.001$

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P (ng/ml)	Sensitivity %	95% CI	Specificity %	95% CI	Youden's index
< 1.04	3.125	0.07909% to 16.22%	100	85.18% to 100.0%	3.125
< 1.35	6.25	0.7661% to 20.81%	100	85.18% to 100.0%	6.25
< 1.50	9.375	1.977% to 25.02%	100	85.18% to 100.0%	9.375
< 1.85	15.63	5.275% to 32.79%	100	85.18% to 100.0%	15.63
< 2.15	18.75	7.208% to 36.44%	100	85.18% to 100.0%	18.75
< 2.30	21.88	9.277% to 39.97%	100	85.18% to 100.0%	21.88
< 2.45	28.13	13.75% to 46.75%	100	85.18% to 100.0%	28.13
< 2.55	31.25	16.12% to 50.01%	100	85.18% to 100.0%	31.25
< 2.80	37.5	21.10% to 56.31%	100	85.18% to 100.0%	37.5
< 3.05	40.63	23.70% to 59.36%	100	85.18% to 100.0%	40.63
< 3.20	43.75	26.36% to 62.34%	100	85.18% to 100.0%	43.75
< 3.40	**46.88**	**29.09% to 65.26%**	**100**	**85.18% to 100.0%**	**46.88**
< 3.55	46.88	29.09% to 65.26%	95.65	78.05% to 99.89%	42.53
< 3.75	50	31.89% to 68.11%	95.65	78.05% to 99.89%	45.65
< 4.15	53.13	34.74% to 70.91%	86.96	66.41% to 97.22%	40.09
< 4.45	53.13	34.74% to 70.91%	82.61	61.22% to 95.05%	35.74
< 4.65	59.38	40.64% to 76.30%	78.26	56.30% to 92.54%	37.64
< 4.90	59.38	40.64% to 76.30%	73.91	51.60% to 89.77%	33.29
< 5.15	68.75	49.99% to 83.88%	73.91	51.60% to 89.77%	42.66
< 5.35	68.75	49.99% to 83.88%	69.57	47.08% to 86.79%	38.32
< 5.45	71.88	53.25% to 86.25%	65.22	42.73% to 83.62%	37.1
< 5.55	71.88	53.25% to 86.25%	60.87	38.54% to 80.29%	32.75
< 5.75	75	56.59% to 88.54%	60.87	38.54% to 80.29%	35.87
< 5.95	75	56.59% to 88.54%	56.52	34.49% to 76.81%	31.52
< 6.20	75	56.59% to 88.54%	52.17	30.59% to 73.18%	27.17
< 6.45	75	56.59% to 88.54%	47.83	26.82% to 69.41%	22.83
< 6.65	81.25	63.56% to 92.79%	43.48	23.19% to 65.51%	24.73
< 6.90	84.38	67.21% to 94.72%	43.48	23.19% to 65.51%	27.86
< 7.10	87.5	71.00% to 96.49%	39.13	19.71% to 61.46%	26.63
< 7.25	90.63	74.98% to 98.02%	39.13	19.71% to 61.46%	29.76
< 7.45	90.63	74.98% to 98.02%	34.78	16.38% to 57.27%	25.41
< 8.15	90.63	74.98% to 98.02%	26.09	10.23% to 48.40%	16.72
< 8.75	90.63	74.98% to 98.02%	21.74	7.460% to 43.70%	12.37
< 8.95	90.63	74.98% to 98.02%	17.39	4.951% to 38.78%	8.02
< 9.15	93.75	79.19% to 99.23%	17.39	4.951% to 38.78%	11.14
< 9.25	96.88	83.78% to 99.92%	17.39	4.951% to 38.78%	14.27
< 9.70	96.88	83.78% to 99.92%	13.04	2.775% to 33.59%	9.92
< 10.35	96.88	83.78% to 99.92%	8.696	1.071% to 28.04%	5.576
< 11.05	100	89.11% to 100.0%	8.696	1.071% to 28.04%	8.696
< 11.95	100	89.11% to 100.0%	4.348	0.1100% to 21.95%	4.348

Table 2. Test performance in % (95% CI) for progesterone (P) concentrations ranging between < 1.04 and < 11.95 ng/ml. Sensitivity: proportion of bitches that whelp within 24 h and are correctly

predicted by the model. Specificity: proportion of bitches that do not whelp within 24 h and are correctly predicted by the model. The optimal cut-off value is printed in bold.

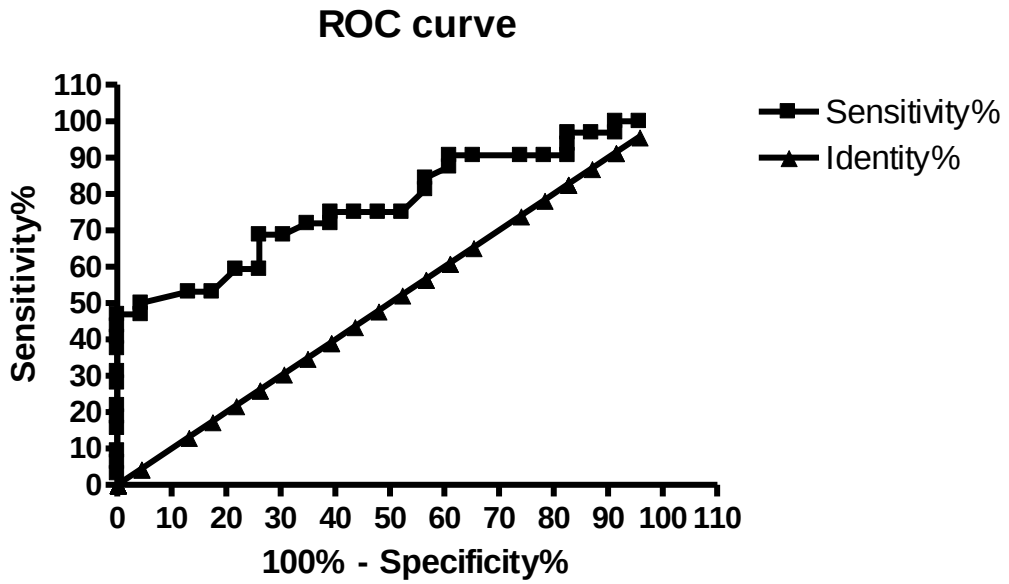

Fig 1. ROC curve: the area under the ROC curve (AUC) was 0.7677 with a 95% CI (0.6451 - 0.8903; P < 0.001). The curve is a schematic representation of the values reported in Table 2.

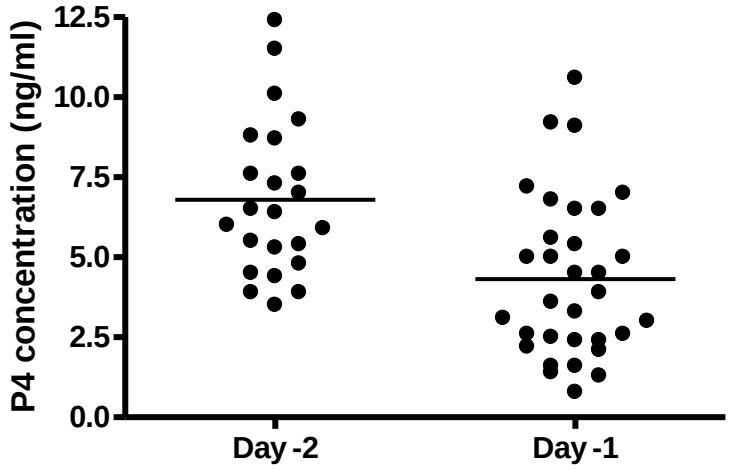

Fig. 2. Distribution of P values in not-at-term bitches (day -2) and at-tem ones (day -1).

Table 1. Progesterone concentration (mean ± SD) at 3, 2 and 1 day before parturition and on parturition day (0). N= number of bitches. [a,b,c] P<0.001

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