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

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(Article begins on next page)

1 **Grugliasco (Torino)**

2 **Italy**

3

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

6

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13

14 **Summary**

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

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

31

32

33 **Key words**

34 Dog, progesterone, CLIA, parturition

35

36 **Running head**

37 Single progesterone measure as a marker of parturition

38

39 **Introduction**

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

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

63

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

67

68 **Materials and Methods.**

69 Animals and sampling

70 51 pre-partum bitches of different breeds and ages were included in the study. At least one blood
71 sample was collected during the three days before parturition and day of parturition. Blood was
72 collected from the cephalic vein into tubes without separating gel, and allowed to clot at 4°C before
73 centrifugation. Serum progesterone concentration was measured by CLIA (Immulite 2000®;
74 Siemens Medical Solutions Diagnostics, Flanders, New Jersey, USA). Progesterone assay has a
75 sensitivity of 0.1 ng/ml (zero + 2 standard deviations).

76

77 Statistical analysis

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

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

90 All statistical analyses were done using GraphPad InStat (vers. 3.00) and GraphPad Prism (vers.
91 4.00) software (GraphPad Inc., San Diego, CA, USA). $P < 0.05$ was considered to indicate
92 statistically significant differences.

93

94 **Results.**

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

100 Progesterone concentrations on days -2 and -1 which define the ROC curve appear in Table 2. The
101 area under the curve (AUC) was 0.7677, with a 95% confidence interval (0.6451 - 0.8903; $P <$
102 0.001) (Fig. 1). The distribution of progesterone values in bitches not whelping the following day
103 (day -2) and bitches whelping the following day (day -1) is shown in Fig. 2. The cut off value that
104 maximizes the Youden's index is 3.4 ng/ml. A clinician can be sure that a bitch with a progesterone
105 concentration below the cut off value will whelp the following day and, especially, to correctly
106 identify negative events, so not to risk to diagnose as full term a bitch which is not.

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

111

112 **Discussion**

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

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

121 and between 24 hrs and parturition day (Nett et al. 1975; Concannon et al. 1978; Veronesi et al.
122 2002; Brugger et al. 2011); however, the question that was discussed in the present work was the
123 possibility to predict whether a pregnant bitch is at term, having a single progesterone value. In
124 practical settings, the opportunity to assess whether the bitch is going to whelp the following day or
125 not would allow the veterinarian to plan assistance or schedule the time of cesarean section.

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

133 From literature data, it is common opinion that cesarean sections can be safely performed when
134 progesterone concentration declines below 2 ng/ml, i.e. 24-40 hours before natural parturition,
135 (Concannon et al. 1977; Smith 2007). The gold standard to measure serum progesterone in bitches
136 is radioimmunoassay (RIA) and previous works reporting peri-partum progesterone concentration
137 in bitches has used this methodology (Nett et al. 1975; Concannon et al. 1978; Veronesi et al.
138 2002). The chemiluminiscent (CLIA- Immulite) assay has been validated for measuring
139 progesterone in canine serum and has the advantage of being safe, fast and accurate enough
140 (Kutzler et al. 2003a; Chapwanya et al. 2008). However, comparing RIA and Immulite serum
141 progesterone values, the average Immulite measurement resulted in significantly higher
142 concentrations than the RIA one, with an average difference of 0.69 ng/ml (Chapwanya et al. 2008).

143 Peri-partum progesterone concentration measured by RIA [3.34 ±1.16 ng/ml at day -1 and
144 1.15±0.22 at parturition day respectively (Nett et al. 1975); 1.9±0.36 ng/ml 24-16 hours before and
145 0.55±0.07 12-8 hrs before parturition respectively (Concannon et al. 1978); 2.9±1.7 ng/ml at day -1
146 and 1.0±0.4 at parturition day respectively (Veronesi et al. 2002)] are numerically lower than our

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

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

155 At presentation, in the absence of previous monitoring, ultrasound examination can help the
156 clinician to date pregnancy through measuring some foetal parts like the biparietal diameter, in the
157 second half of pregnancy (Lopate 2008): this method is relatively simple but breed-specific foetal
158 growth curves are necessary to increase its accuracy (Kutzler et al. 2003b; Socha and Janowski
159 2014) because the formulas derive from measures done in small or medium size bitches (Kutzler et
160 al. 2003b; Socha and Janowski 2014). Even when using a specific equation for a single breed, some
161 variables, such as smaller than average litter size, can greatly reduce the accuracy of the prediction
162 (Groppetti et al. 2015). In small and medium size breeds around 70 per cent of parturition dates
163 were predicted within one day and 85% within two days using BP (Beccaglia and Luvoni 2006).

164 Neither of the two methods alone, i.e progesterone concentration and ultrasound fetometry, appears
165 to be accurate enough to effectively help the clinician; it could be worth to test the association of the
166 two determinations in order to assess whether the diagnostic efficacy becomes higher than with a
167 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

225

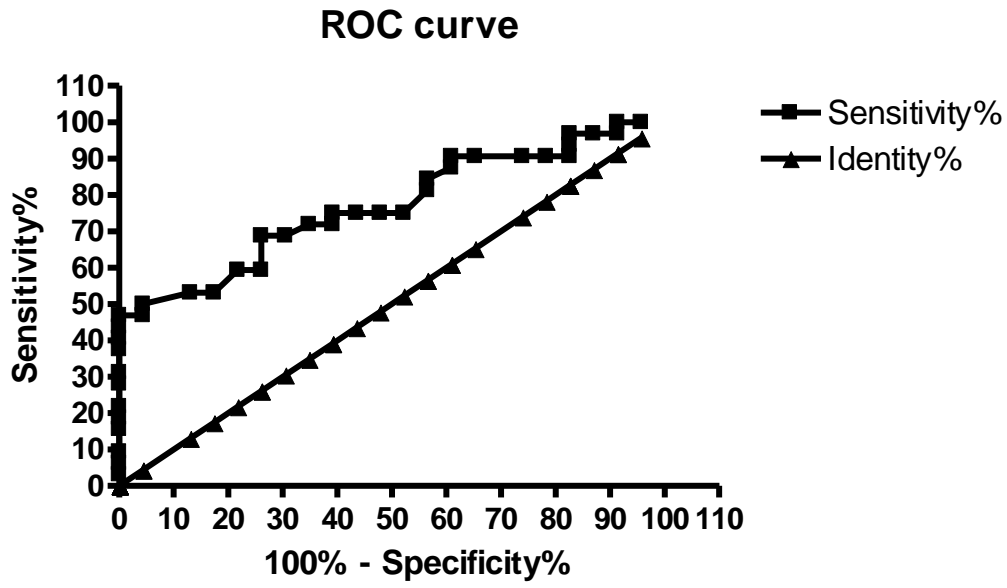
P (ng/ml)	Sensitivity		Specificity		Youden's index
	%	95% CI	%	95% CI	
< 1.04	3.125	0.07909% to 16.22%	100	85.18% to 100.0%	3.125
< 1.35	6.25	0.7661% to 20.81%	100	85.18% to 100.0%	6.25
< 1.50	9.375	1.977% to 25.02%	100	85.18% to 100.0%	9.375
< 1.85	15.63	5.275% to 32.79%	100	85.18% to 100.0%	15.63
< 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

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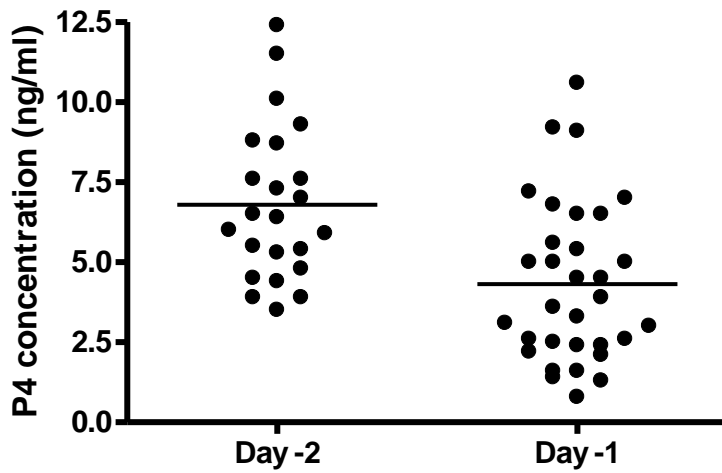


238

239 Fig 1. ROC curve: the area under the ROC curve (AUC) was 0.7677 with a 95% CI (0.6451 -
240 0.8903; $P < 0.001$). The curve is a schematic representation of the values reported in Table 2.

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244

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

246

247 Table 1. Progesterone concentration (mean \pm SD) at 3, 2 and 1 day before parturition and on
248 parturition day (0). N= number of bitches. ^{a,b,c} P<0.001

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