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Original Research Article

Validation of a point-of-care serum progesterone cut-off for caesarean section timing in the bitch and its effect on neonatal outcomes

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ABSTRACT

The aim of this study was to evaluate, in a clinical setting, the use of a serum progesterone concentration (PC) cut-off value of 4.6 nmol/L, measured on a point-of-care assay, as an indicator for performing safe preparturient caesarean section (CS) in the bitch. Privately-owned bitches, carrying at least two foetuses, were admitted for prepartum management. A parturient CS was performed once cervical dilatation was observed on vaginoscopy, with vaginoscopies performed at 08h00, 12h00 and 17h00 each day. A preparturient CS was performed when PC was ≤4.6 nmol/L, with PC measured in bitches with a closed cervix at the 17h00 examination. A total of 293 preparturient and 92 parturient CS were performed, with 2102 and 723 puppies delivered, respectively. There was no difference between preparturient and parturient CS in terms of the proportion of stillbirths ($p = 0.91$) or puppies surviving to 2 h post-delivery (p=1.00). Among live puppies delivered via preparturient CS, 99.5% (1986/1996) had normal viability (Apgar score ≥7 out of 10), compared to 98.0% (680/694) in the parturient CS group (p<0.001). The use of vaginoscopy in prepartum bitches is essential to avoid delaying surgical intervention in those bitches that enter Stage I of labour prior to PC dropping to ≤4.6 nmol/L. A PC of ≤6.4 nmol/l (2 ng/ml) is traditionally considered to indicate the expectance of onset of parturition within the next day (24 h), and is widely used as a cut-off for planned CS, however practitioners should be aware of rare instances of PC hovering between 6.4 nmol/L and 5.0 nmol/l up to 100 h before the onset of spontaneous parturition when using a point-of-care assay. Further research to establish safe cut-off values for CS using other progesterone assay systems, and in a wider variety of breeds, is warranted.

1. Introduction

Caesarean section (CS) is a commonly performed surgical procedure in veterinary practice [1]. With the recent expansion of brachycephalic breeds, the incidence of CS is likely to have increased [2]. In the UK, over 80 % of Boston Terrier and English and French Bulldog litters are delivered via CS [3]. Yet, the CS is not solely the domain of brachycephalic breeds. In a Swedish study, data suggest that over 40 % of Scottish Terrier whelpings may require a CS [4]. Other non-brachycephalic breeds prone to dystocia and therefore CS include the Golden Retriever, Labrador Retriever, Miniature Dachshund and Poodle [4,5]. Further risk factors for CS include very large or small litters, and a dam's history of previous CS [6]. For bitches known to be at high risk of dystocia, some owners wish to avoid the risk of foetal loss that may result from trial of labour and therefore prefer elective CS [2,6–9].

Depending on at what point in time in relation to the onset of spontaneous parturition the CS is performed, a CS may be a preparturient CS (the bitch is not yet parturient but is "term" meaning the foetuses have matured to the point where they have equal probability of surviving the extra-uterine environment following delivery as those delivered by parturient CS), parturient CS (bitch is in labour but there is no apparent evidence of foetal distress or demise), or emergency CS (there is already evidence of foetal distress and demise is already present or is imminent). When performing a CS, both a delay in intervention such as occurs in emergency CSs and too early an intervention (before term), may lead to foetal demise and decreased puppy survival [7,10].

Current research suggests that CS is safe if performed within approximately 48 h of the bitch entering Stage I of labour spontaneously [10–12]. Data collected during the bitch's preceding oestrous cycle, including indirect determination of the LH surge and ovulation and/or

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the onset of cytological dioestrus, permit relatively precise prediction of the bitch's subsequent due date [13]. However, many high-risk obstetrical cases present to veterinary clinics with only mating dates available, which are inherently unreliable for predicting whelping dates [14,15]. Currently, the measurement of circulating progesterone concentration (PC) to determine the imminence of parturition is widely practiced. With few exceptions, most notably the singleton pregnancy [16,17], a rapid, prepartum decline in progesterone concentration occurs over the last few days of gestation in the bitch [18–20]. The current convention is to consider CS safe once the bitch's PC has reached 6.36 nmol/L (2 ng/ml) or lower [7,21], despite little evidence that this practice is safe for all bitches under all circumstances.

The decision to perform a CS in practice should ideally be made promptly. The Catalyst® Progesterone (IDEXX Laboratories Inc., Westbrook, Maine, USA) has a turnaround time of under 30 min and has good agreement with chemiluminescent immunoassay (CLIA) when PC is lower than 6.36 nmol/L [22]. A subsequent study established that the Catalyst is comparable to CLIA, with the Catalyst on average 1.7 nmol/L lower than CLIA for PC values (measured by Catalyst assay) under 7 nmol/L [23]. As such, a PC of 6.36 nmol/L measured using CLIA corresponds approximately to a value of 4.6 nmol/L measured using the Catalyst assay.

The aim of this study was to evaluate the safety of a cut-off PC of 4.6 nmol/l, assayed on serum by the Catalyst assay, as a threshold for CS in privately-owned bitches presented to a veterinary clinic for obstetrical management and for which owners had declined a trial of labour. Serial vaginal speculum examinations would also be used in all bitches in the decision-making process.

2. Materials and methods

This retrospective study was approved by the Research Ethics Committee of the Faculty of Veterinary Science, University of Pretoria (REC077-21).

2.1. Animals

Client-owned bitches presented to a private veterinary clinic for management of parturition by CS during the period of April 2019 until November 2022, and for which sufficient data were available, were

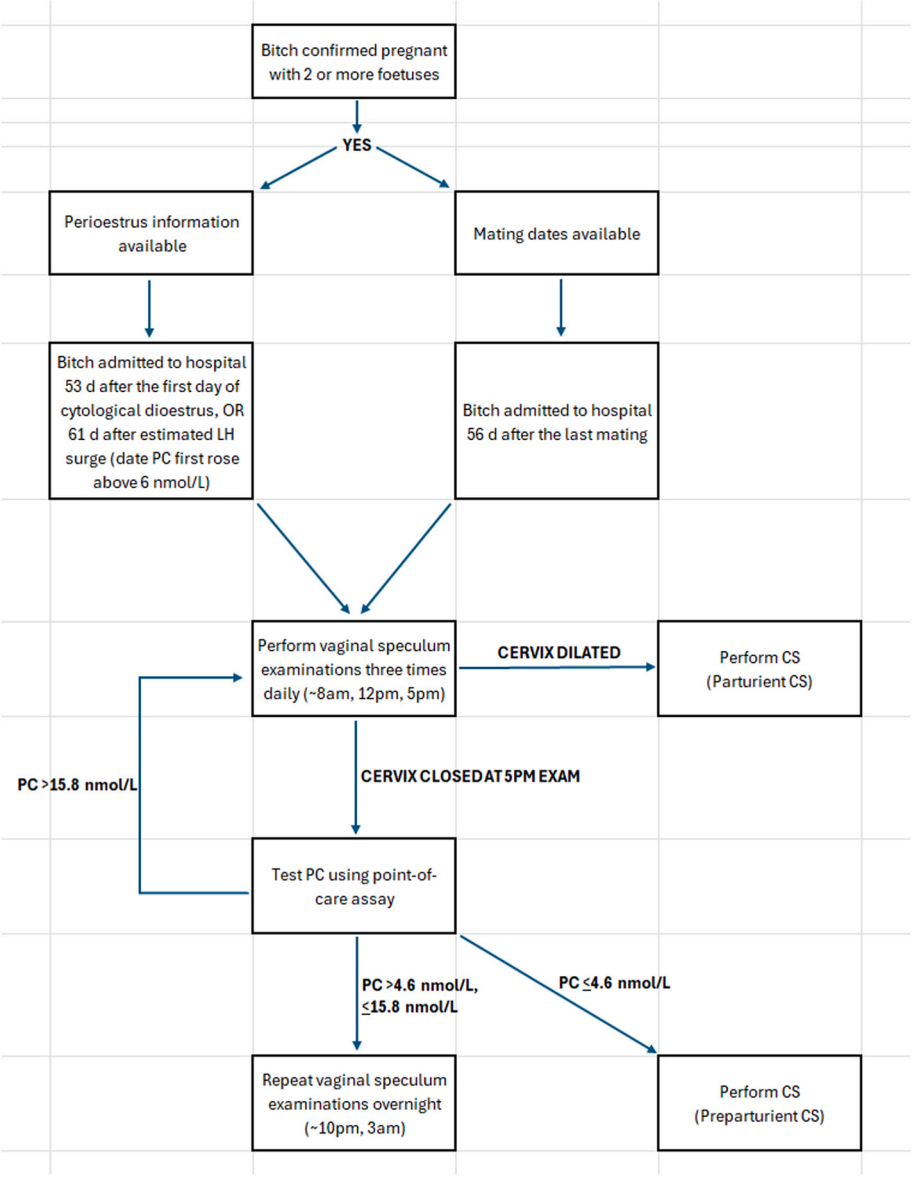

Fig. 1. The study design summarised as a decision tree, from confirmation of (non-singleton) pregnancy to delivery of puppies by preparturient or parturient CS.

included in this study. Caesarean sections on singletons, emergency CSs and those timed on predicted dates (first day of cytological dioestrus or estimated LH surge) were excluded. All owners declined a trial of labour (attempt at spontaneous unassisted parturition), and written consent was obtained to perform a CS based on a drop in PC to ≤4.6 nmol/l, if the bitch's cervix had not dilated during working hours (7:30 a.m. to 7:30 p.m.). Inclusion of CSs in the current study also depended on staff availability on the day and time of the CS to facilitate data collection. Parity, age and prior CS in the bitches were not considered.

2.2. Procedures during management of parturition and caesarean section

The study design is summarised in Fig. 1. Bitches were admitted 53 d after the first day of cytological dioestrus [14] when that day was known, or 61 d after estimated LH surge (PC rose above 6 nmol/l) [24] when that day was known, or 56 days after the last mating when no peri-ovulatory data was known. During hospitalisation, bitches were fed a commercial pelleted diet twice daily with water *ad lib*.

Upon admission, examination by ultrasound was performed to confirm litter sizes of two or more. Abdominal radiography provided final confirmation for any bitches suspected of carrying a singleton.

Vaginal speculum examinations were performed upon admission and thereafter three times daily at around (±1 h) 08h00, 12h00 and 17h00, or more often if the bitch showed signs suggestive of imminent parturition (panting, discomfort, nesting behaviour, inappetence, vomiting, looking at flanks). A parturient CS was performed promptly upon the observation of cervical dilatation (stage one of parturition; see Fig. 2), as previously described [12,25].

Blood was collected from all bitches with a closed cervix at the last vaginal speculum examination of the day (17h00), or at any point before then if the bitch showed signs suggestive of imminent parturition. One ml of blood was drawn by cephalic or jugular venipuncture and, within minutes, assayed for PC using the Catalyst assay (Catalyst® Progesterone, IDEXX Laboratories Inc., Westbrook, Maine, USA) run on a Catalyst Dx Chemistry Analyser, according to the manufacturer's instructions. Quality control for the Catalyst assay was performed monthly using the Catalyst QC clip and VetTrol Control Fluid (IDEXX Laboratories Inc.) throughout the trial period.

A preparturient CS was performed promptly if the PC had reached ≤4.6 nmol/l. If the PC was >4.6 nmol/L but ≤15.8 nmol/l, then vaginal speculum examinations would be repeated at around 22h00–23h00 and 03h00–04h00, with a parturient CS performed if cervical dilatation was observed. If the PC was >15.8 nmol/l, the next speculum examination was scheduled for the following morning. This protocol was deemed safe as results of previous work by the authors showed that when the PC is above 15.8 nmol/l, measured using radioimmunoassay (RIA), there is a 99 % probability that the bitch will not enter spontaneous parturition within the following 12 h [26]. Given that the Catalyst typically measures slightly lower levels than other assay systems [23], use of this cut-off (15.8 nmol/L) represents a conservative approach.

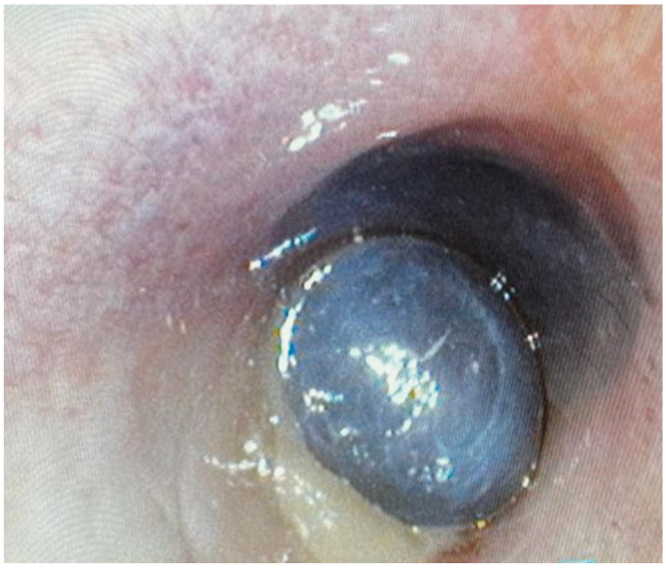

Fig. 2. Image of foetal membranes protruding through a dilating cervix as seen through a vaginal speculum.

The anaesthesia and surgical procedure for CS were as previously described [25,27]. No ovariohysterectomies were performed at the time of CS, and all placentas were removed with each foetus. The processing of puppies following delivery involved immediate administration of atipamezole hydrochloride (Antisedan®, Zoetis Animal Health, Sandton, South Africa) at the dose of 50 μg/puppy sc (irrespective of weight) [25], tying off of the umbilicus and applying 10 % povidone iodine thereto, drying the puppies briskly, removing fluids from their airways and placing them in an air-heated incubator set at 37 °C. No oxygen support was offered to puppies after delivery. Live puppies born with malformations were euthanized at birth. Apgar scores were assessed in all live puppies not euthanized, based on a 10-point scoring system as previously described [28], 15 min after the last puppy was delivered. Bitches and puppies were typically discharged within 2–3 h following surgery.

2.3. Data analysis

The proportion of stillborn and malformed puppies, respectively, within each CS group (preparturient versus parturient CS) were compared using the Chi-square test. Due to the small number of puppies lost between birth and 2 h post-delivery in both groups, this aspect was compared using the Fisher's exact test. Litter sizes recorded for each CS group were compared using the Mann-Whitney *U* test. To compare puppy viability outcomes of each CS group, puppies were classified into one of three categories based on Apgar scores: critical (score ≤3), moderately viable (score between 4 and 6) or normal (score ≥7) [29]. The proportion of puppies delivered with normal viability (Apgar score ≥7) was compared across CS groups using the Chi-square test. A cumulative odds ordinal logistic regression with proportional odds was run to determine the effect of litter size and CS group on the odds of delivery of a puppy with normal viability. In order to further investigate the effect of breed, the analysis was repeated for the two most represented breeds (Boerboel and English Bulldog) individually, as well as the remaining breeds combined into one group. Statistical analyses were performed in IBM SPSS version 29 (Chicago, IL, USA), with significance set at $p<0.05$.

3. Results

After exclusions, 293 preparturient (performed when serum PC reached the cut-off value of 4.6 nmol/L or lower) and 92 parturient (performed upon cervical dilatation) CS were performed, with 2102 and 723 puppies delivered, respectively. The maternal survival rate at clinical discharge was 100%. Complications encountered were wound infection (3), skin wound dehiscence (3), post CS metritis (2), mastitis (2), and haemorrhage (HT below 27%) during or after CS (within 1 h) necessitating blood transfusion (2). Complications were equally distributed between bitches that underwent preparturient or parturient CS (data not shown). The average stay in hospital was 3–4 days but varied from several hours to 7 days. The following breeds were represented (number of CS): Boerboel (222), English Bulldog (74), German Shepherd Dog (25), French Bulldog (17), American Bully (10), Bull Terrier (8), Rottweiler (7), Neapolitan Mastiff (5), Labrador Retriever (4), Chow-Chow (3), Beagle (2), Akita (2), Cocker Spaniel (2), Staffordshire Bull Terrier (2), Dachshund (1) and German Shorthaired Pointer (1).

3.1. Serum progesterone dynamics in late gestation

A scatterplot of all PCs recorded for bitches that underwent a preparturient or parturient CS is shown in Fig. 3. Fig. 4 shows the same data as Fig. 3 but showing only PC values between 4.6 nmol/L and 10.8 nmol/L, to highlight relatively low PC values occurring over time prior to CS. Fig. 5 shows the PC results for six bitches where PC fell to levels below the traditional cut-off of 6.4 nmol/L, more than 48 h before CS.

3.2. Puppy survival and vigour following preparturient and parturient CS

Comparisons between litter size as well as survival and viability outcomes for puppies born by parturient and preparturient CSs are shown in Table 1. Apgar scores were not recorded for two puppies from the preparturient CS group and one from the parturient CS group. For all breeds combined, the odds of delivery of a puppy of normal viability was 4.7 times higher when delivered by preparturient CS compared to parturient CS (95% CI 2.046–10.679), which was statistically significant ($\chi^2(1) = 13.377$, $p<0.001$). In addition, increasing litter size was associated with increasing odds of delivery of a normal neonate, with an odds ratio of 1.241 (95% CI 1.089–1.415; $\chi^2(1) = 10.490$, $p=0.001$). However, when the analysis included data from Boerboels only, neither CS group nor litter size had a significant effect on the odds of delivery of a puppy with normal viability ($p=0.436$ and $p=0.220$ respectively). Similarly, neither CS group nor litter size had an effect on the odds of normal viability when the analysis included all breeds except the Boerboel and English Bulldog ($p=0.998$ and $p=0.498$ respectively). In contrast, for the English Bulldog alone, the odds of delivery of a puppy of normal viability was 6.9 times higher when delivered by preparturient CS compared to parturient CS (95% CI 1.770–27.157), which was statistically significant ($\chi^2(1) = 7.729$, $p=0.005$). In addition, increasing litter size was associated with increasing odds of delivery of a normal neonate in the English Bulldog breed, with an odds ratio of 1.348 (95% CI 1.005–1.809; $\chi^2(1) = 3.965$, $p = 0.046$).

4. Discussion

The decision to perform a CS based on observing cervical dilatation (parturient CS) or the finding of a PC ≤4.6 nmol/l (preparturient CS) results in good outcomes with high neonatal survival rates and Apgar scores, and few maternal complications. In the current study, the rate of stillbirth overall was 2.2 %, with no significant difference between pups born by parturient or preparturient CS. This result is similar to previous studies by this group [12,25]. Other studies have reported rates of stillbirth ranging from 2.3 % to 7 % for planned CS [30–33]. In contrast, rates of stillbirth for emergency CS in some of these studies ranged from 12 % to 21 % [31–33], highlighting the value of timeous intervention in high-risk obstetrical cases.

In the current study, neonatal vitality was assessed 15 min after delivery of the last pup, using an Apgar scoring system adapted for canines by Veronesi et al. [34]. A number of studies have compared Apgar scores measured within 5, 15 or 60 min after delivery, and all demonstrate marked improvements in scores by 1 h after birth [28,35–37]. In the current study, an interval of 15 min was most practical in the context of a busy clinical practice, and was deemed sufficiently close to delivery to avoid masking initial foetal distress [35]. It should be noted that this interval increased for pups delivered prior to the last pup. Delivery of the first to last pup during CS at this clinic has been reported previously and ranged from 88 s to 621 s [25], therefore most pups are likely to have been assessed no more than 25 min after delivery.

Except for Groppetti et al. [38], who used a 14-point system, most studies report on the use of a 10-point Apgar system, with pups classified as critical or severely distressed (score between 0 and 3; low), moderately distressed (score between 4 and 6; medium) or normal neonates (score 7 to 10; high) [28,29,34]. In the current study, over 99.2 % of pups demonstrated an Apgar score of 7 or higher. The Apgar scores observed in the current study are comparable to previous studies by this group [12,25]. In contrast, other workers have reported a wider spread in Apgar scores. For example, Groppetti et al. [38] reported low, medium and high Apgar scores in 45.3 %, 33.7 % and 21 % of pups born by natural delivery or CS and assessed within 10 min of birth. Similarly, Plavec et al. [35] reported low, medium and high Apgar scores in 19.4

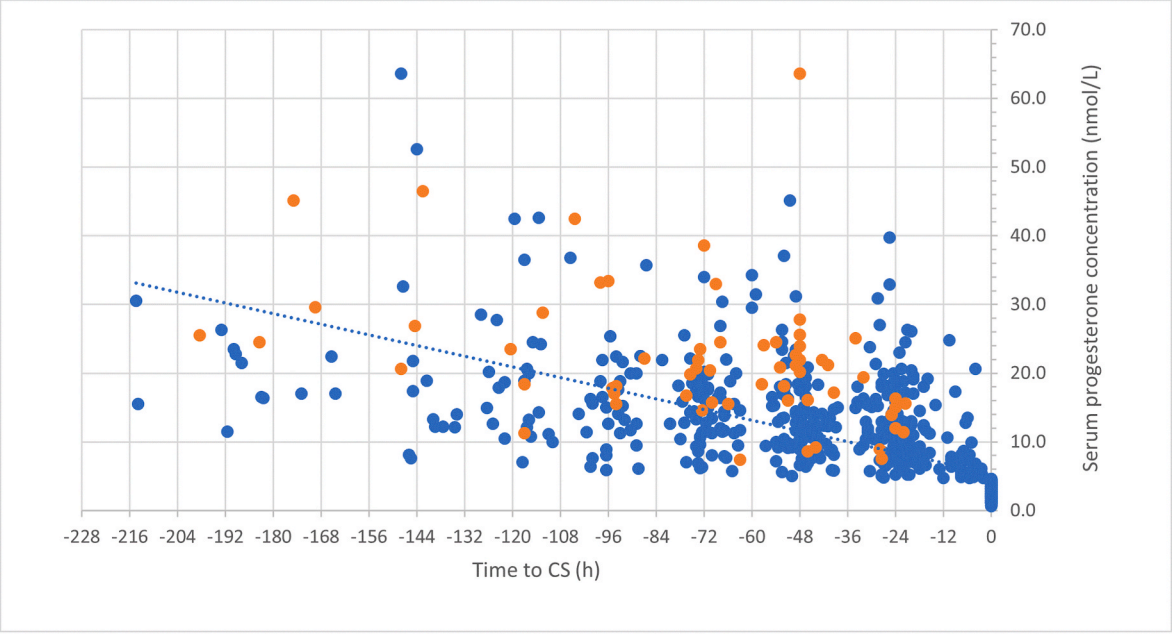

Fig. 3. Serum progesterone concentration (PC) measured in relation to time before caesarean section (CS), for bitches that underwent a CS when PC measured 4.6 nmol/L or less using the IDEXX Catalyst One progesterone assay (preparturient CS) or when the cervix was observed to be dilated on vaginoscopy (parturient CS). The data represents 730 PC results recorded for 293 bitches that underwent a preparturient CS (blue points) and 62 PC results for 92 bitches that underwent a parturient CS (orange points). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

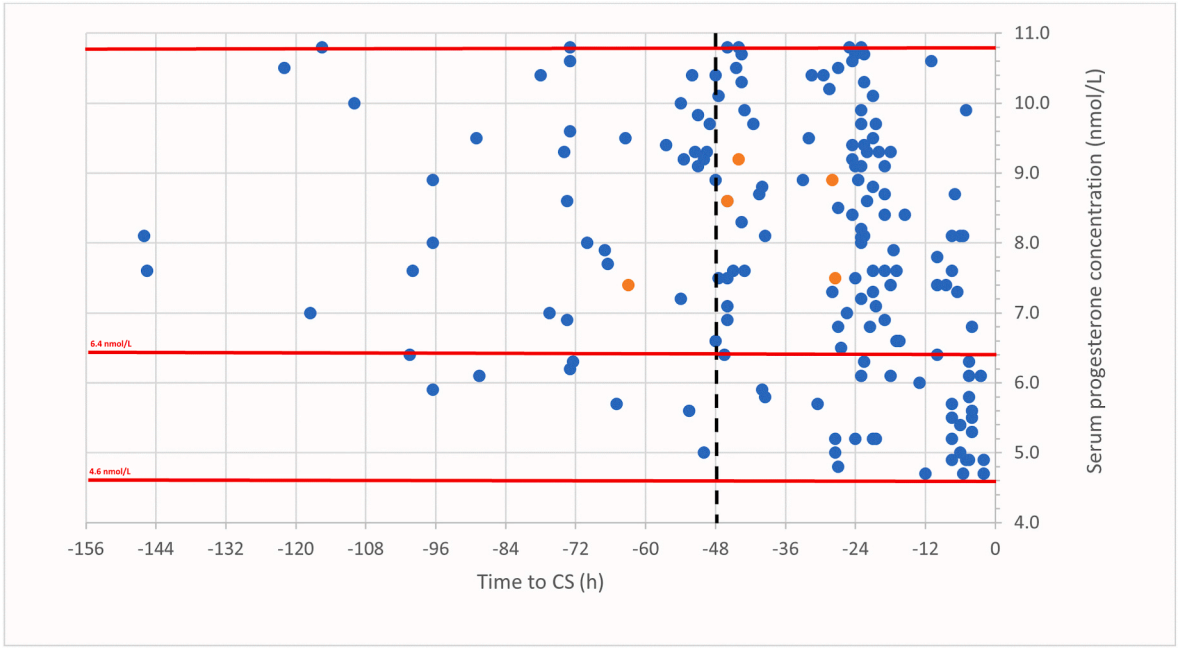

Fig. 4. As for Fig. 3, but showing only PC values between 4.6 nmol/L and 10.8 nmol/L.

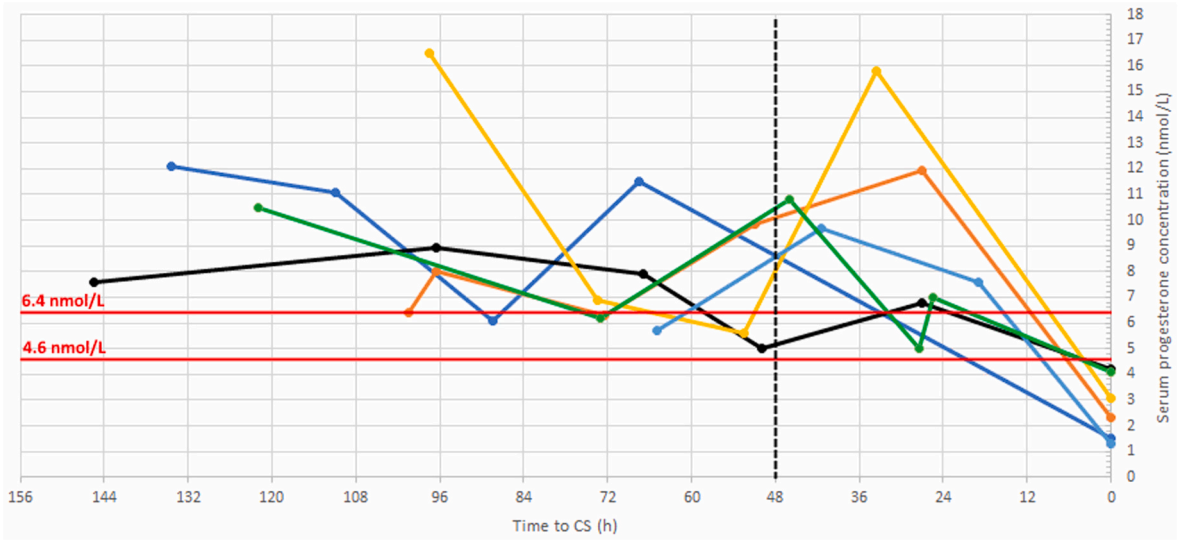

Fig. 5. Serum progesterone concentration (PC; nmol/L) for six bitches that demonstrated one or more PC below the traditional cut-off of 6.4 nmol/L, more than 48 h before CS.

%, 25 % and 55.6 % of pups born by elective CS and assessed 15 min after delivery. Possible reasons for these differences remain speculative, and could be related to variations in anaesthetic protocols or surgical times [39]. In addition, Moon-Massat and Erb [39] found that breed and classification as brachycephalic also played a role in neonatal vitality.

A significant, but only slightly higher, proportion of puppies delivered by preparturient CS had normal Apgar scores in comparison to parturient CS. This result could be taken to suggest that preparturient CS are superior to parturient CS. As a result, one might speculate that vaginal speculum examinations could be replaced with PC assays and use of the 4.6 nmol/l cut-off only. This is, however, not true. Assessing cervical dilatation ensures the timeous identification of bitches that enter Stage I of labour despite PC not having dropped to 4.6 nmol/l or lower. In previous studies, three out of 25 (12 %) bitches had a PC >8.7 nmol/L (measured by RIA) [26], five out of 51 had a PC >6.4 nmol/L (measured by CLIA) [12], and three out of 28 had a PC >5.5 nmol/L (measured by the IDEXX Catalyst) [23], at the time of cervical dilatation. It has been speculated that some of these bitches may have suffered from parturient stress leading to a surge in cortisol with a concomitant rise in PC [19,26]. Alternatively, cervical dilatation may slightly precede a drop in PC and these bitches may have had a drop in PC if assessed a few hours later. By not performing vaginal speculum examinations, bitches that enter Stage I of labour before their PC has dropped to below 4.6 nmol/l would be missed for some period of time, with associated risks to puppy survival and vigour.

Interestingly, preparturient CS and higher litter size was associated with increased odds of delivery of a pup of normal viability (Apgar score ≥7) in the English Bulldog breed, an effect not seen in the Boerboel breed, or all other breeds combined, in this dataset. Brachycephaly has previously been identified as a risk factor for decreased puppy viability

Table 1
Comparison of 293 preparturient (based on a serum progesterone concentration cut-off value) and 92 parturient (based on cervical dilatation) caesarean sections in privately-owned bitches attending a veterinary clinic for prepartum management.

	Total	Preparturient CS	Parturient CS	*p*-value
Number of caesarean sections	385	293	92	n/a
Number of puppies delivered	2825	2102	723	n/a
Median litter size	7	6	8	0.049
Number of puppies stillborn (%)	68 (2.41)	51 (2.43)	17 (2.35)	0.910
Number of puppies euthanized due to congenital malformation (%)	63 (2.23)	51 (2.43)	12 (1.66)	0.229
Number of puppies surviving to 2 h post-delivery (%)[a]	2691 (97.43)	1998 (97.42)	693 (97.47)	1.000
Number of puppies with Apgar score ≥7 (normal viability) 15 min after birth[b]	2666 (99.11)	1986 (99.50)	680 (97.98)	<0.001

[a] Number of puppies surviving to 2 h post-delivery excludes puppies euthanized.
[b] Excludes Apgar scores from two puppies in the preparturient CS groups and one from the parturient CS group (not recorded).

and higher puppy mortality [39]. Larger litter sizes have been associated with decreased puppy viability and increased neonatal mortality [1,29], however Münnich and Küchenmeister [40] linked smaller litter sizes to increased risk of dystocia, regardless of breed. In our study, bitches were monitored closely prepartum in order to deliver pups safely, well before the onset of parturition or possible dystocia. Our data suggest that English Bulldog foetuses may become distressed prior to the onset of cervical dilatation or a drop in PC to low levels, an effect magnified in smaller litters. Practically, increased foetal wellbeing monitoring, such as the frequent tracking of foetal heart rates in late gestation, may be advisable in this breed.

It is crucial that attention be drawn to outliers and exceptions that may occur in the preparturient drop in PC in the bitch. An example of an outlier was demonstrated in one bitch where a fall in PC to undetectable levels was reached as much as 10 d before whelping [41]. In another study, PC was lower than 6 nmol/l for 6 d before parturition [42]. Persistently low PC, starting before 48 h prior to the onset of cervical dilatation, has also been reported more recently [23]. Fig. 5 shows outlier cases in the current study, where the PC hovered around 6–7.4 nmol/l for up to six days and indeed, in all six of these cases, PC had dropped to below 6.4 nmol/l within 100–50 h before the PC finally dropped to below 4.6 nmol/l. This shows that the traditional 6.4 nmol/l cut-off value proposed in the literature may not be universally safe, as this threshold could lead to CS being performed more than 48 h from the onset of spontaneous parturition in some bitches.

In this study, bitches with a PC >15.8 nmol/l at the evening evaluation were left undisturbed overnight. Previous work by the authors showed that when the PC was above 15.8 nmol/l (measured using RIA), there was a 99 % probability that the bitch would not enter spontaneous parturition within the following 12 h [26]. The current study confirmed this finding, with 3 cases out of 293 (1 %) showing a drop to below 4.6 nmol/l despite PC exceeding 15.8 nmol/L within the previous 12 h. Whilst this is reassuring for the vast majority (99 %) of the obstetric population, it also shows that there may be exceptions, albeit rare.

Aglepristone [11,43] and betamethasone [44] have been administered to preparturient bitches as priming agents in an attempt to stimulate foetal maturation prior to induction of parturition or preparturient CS. The results of our study indicate that priming agents appear not to be needed when performing preparturient CSs timed using our 4.6 nmol/l cut-off value assayed using the Catalyst assay. This concurs with findings in another study where safe preparturient CSs were also performed based on the date of onset of dioestrus, without priming agents [12]. However, emergency interventions may be required in late pregnancy due to maternal metabolic or respiratory disorders. In these cases, it was shown that when pregnancy was interrupted five days early, there was a marked degree of prematurity which could be improved by the preterm maternal administration of betamethasone [44,45].

Limitations of the current study are that only one point-of-care assay was assessed, and that two breeds (Boerboel, English Bulldog) were over-represented. Notably, this study did not evaluate bitches <14 kg. In addition, singleton pregnancies were excluded from this study.

5. Conclusion

Using a PC threshold of ≤4.6 nmol/l, measured using the IDEXX Catalyst Progesterone point-of-care assay, to time preparturient CS resulted in good outcomes for puppies and dams. Knowledge of ovulation timing and predicted parturition dates remain helpful for interpreting findings and preventing timing errors in rare outlier cases. Vaginal speculum examinations in late pregnant bitches are essential to avoid missing dams in Stage I of labour despite a PC >4.6 nmol/L. It should not be assumed that the threshold value of 4.6 nmol/L is safe when using other progesterone assay systems.

CRediT authorship contribution statement

Kurt G.M. De Cramer: Writing – original draft, Resources, Project administration, Methodology, Investigation, Conceptualization. **Carolynne J. Joonè:** Writing – review & editing, Formal analysis.

Data availability statement

All data pertaining to this manuscript is available upon reasonable request.

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Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of this manuscript.

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