Revised manuscript

Curtailing parturition observation and performing preparturient cesarean section in bitches

K.G.M. De Cramer[1]*and J.O. Nöthling[1]

[1]**Corresponding author. Department of Production Animals, Faculty of Veterinary Science, University of Pretoria, South Africa, kdcramer@mweb.co.za*

[1]*Department of Production Animals, Faculty of Veterinary Science, University of Pretoria, South Africa,* Johan.Nothling@up.ac.za

**Current address of corresponding author. Tel +27 0116603110. P.O. Box 704, Rant en dal, 1751, Mogale city, South Africa. Email address:* kdcramer@mweb.co.za*. Rant en Dal Animal Hospital, 51 Cecil Knight Street, Mogale City, Gauteng, South Africa*

Highlights

• Preparturient cesarean section were performed in 67 bitches and compared to 205 parturient cesarean sections.

• High Apgar scores were achieved and high puppy survival rates with 99% of puppies born alive and 98.8% surviving to 2 h.

•Progesterone level was significantly higher at the time of preparturient CSs than at the time of parturient CSs.

Abstract

Properly planned elective cesarean section (CS) in bitches is considered safe and justified for some breeds. Therefore, planning a scheduled (fixed date and time) preparturient CS in bitches belonging to a subpopulation where a CS is unavoidable, seems justified. The first day of cytological diestrus (D0) was used to predict the date of parturition. The aims of this study were to (1) compare the mortality of puppies delivered by preparturient CS to those delivered by parturient CS, (2) determine the extent to which a preparturient CS (performed at 08:00 on D57, while the cervix is closed) would shorten gestation, (3) compare the level of progesterone at the time of preparturient CS to that at the time of parturient CS, (4) compare the change in hematocrit before and after CS for parturient- and preparturient CSs. Out of 99 gestations for which D0 was known and a preparturient CS planned, the CS was performed at the scheduled time in 61%, before the scheduled time because cervical dilatation had started in 32% and before the scheduled time in 7% because the bitches had started showing signs of impending parturition, although their cervices were still closed. This study showed high neonatal survival ratios and good Apgar scores after preparturient CS with 99% of puppies delivered by preparturient CS born alive and 98.8% surviving to 2 hours. Performing preparturient CSs shortened gestation by four to 52 hours. Progesterone level was significantly higher at the time of preparturient CSs than at the time of parturient CSs. Further studies are required to determine whether preparturient CSs on D57 are routinely safe in all breeds, does not affect long term survival of the puppies and to timeously identify bitches in which parturition starts prior to the date and time for the scheduled elective preparturient CS.

Keywords: Progesterone; preparturient; cesarean section; puppy survival; Apgar scores; bitch

1. Introduction

The veterinary community and dog breeding fraternities have thus far failed to reduce the high proportion of deliveries requiring cesarean section (CS) in many breeds [1-8]. In the Boston terrier, English bulldog and French bulldog more than 80% of litters are delivered by CS [1, 2, 9]. These breeds have high -risk parturitions because they have a high risk of dystocia necessitating high prevalence of CS and failing to intervene timeously may result in fetal demise. For such high-risk parturitions, properly planned elective CS is considered a safe, effective and justified intervention [3, 4]. Apart from these breeds, there exist subpopulations of pregnant bitches in which the prevalence of obstetric complications necessitating CS, is likely to be higher than that of the general obstetric population. Such pregnancies include pregnancies in bitches with a history of prior CS and those with known large litters [10]. Although unreported in the literature, Boerboel bitches left to whelp naturally often develop uterine inertia, resulting in the death of valuable foetuses. Some owners avoid the risk of fetal loss that may result from trial of labour and prefer elective CS [11].

Even when litters are delivered by elective CS, stillbirths may occur. Wydooghe (2013) reported that 13% of English bulldog fetuses delivered by CS at full term were stillborn. Moon et al. (1988) reported puppy mortality data on a wide variety of breeds: 8% of fetuses delivered by CS at full term were stillborn, 13% died before 2 h and 20% died before 7 d.

Using the signs of parturition to time elective CSs in the bitch is problematic: fetal distress or -demise may occur in some bitches before the first signs of parturition, resulting in reduced survival of the offspring in such elective CSs [12]. Objective fetal monitoring during parturition observation may not always be available to breeders. Preparturient CS may reduce the prevalence of parturient fetal demise and stillbirths in pregnancies destined for CS delivery. The time of day during which CSs are performed influences the proportion of still born fetuses because a full staff complement during normal working hours help maximize puppy survival [11]. These arguments form the basis for planning elective CSs as preparturient CSs occurring at a fixed date and time before the onset of parturient cervical dilatation, in bitches where a CS is unavoidable. The timing of CS exposes clinicians to criticism if their intervention or lack thereof is thought to have reduced puppy survival. The concept of a reliable protocol allowing planned preparturient CS therefore resonates with clinicians' needs. Planned preparturient CS obviates the need for after-hour interventions and allows for timeous delivery of the puppies potentially reducing the prevalence of fetal compromise and still births.

The current study assumed that a dog fetus can be delivered 48 h before the expected time of spontaneous parturition and remain viable without assistance. This assumption was based on current knowledge that fetuses born naturally or by CS as much as 48 h prior to the expected time of spontaneous parturition from bitches treated with aglepristone [13, 14] or betamethasone [15] survive without special assistance once born.

In a previous study it was shown that the first day of cytological diestrus (D0) allowed the prediction of the date of cervical dilatation with a precision of ± 1 d, ± 2 d and ± 3 d in 88%, 99% and 100% in 242 estrous cycles with a mean interval of 56.7 d (95% CI 54.9–

58.6 d, with intervals ranging from 54 to 60 d) [16]. No study thus far reports on using D0 to time CS nor on performing preparturient CS without the antenatal maternal administration of either aglepristone or betamethasone.

Serious uterine hemorrhage may result from cesarean sections [11]. It is not known whether or not the placentas will be at a suitable state to detach without serious hemorrhage in bitches undergoing CS before cervical dilatation had occurred. The current study undertook to evaluate hematocrit before and after parturient and preparturient CSs.

The first aim of this study was to compare the mortality of puppies delivered by preparturient CS (CS performed on D57 or D56, while the cervix was still closed), to that of puppies delivered by parturient CS (CS performed once cervical dilatation has started). The second aim was to determine by how much gestation would be shortened in those bitches undergoing CS while their cervices are still closed at 08:00 on D57 or before. The third aim was to compare the level of progesterone in the serum at the time of CS for bitches undergoing preparturient CS to that of parturient CS. The fourth aim was to compare the change in hematocrit before and after CS of parturient CSs to preparturient CSs.

2. Materials and methods

2.1 Bitches

The protocols were approved by the Animal Ethics Committee of the Faculty of Veterinary Science of the University of Pretoria (Project numbers v071-13, v010-14, v010-14 amend1 and v048-14 pertaining to the non-restricted group as defined below and v079-15 pertaining to the restricted group as defined below). The experimental animals were all housed and fed commercial dry pellets twice daily and had access to ad-lib water. They were privately owned bitches presented to a private veterinary clinic for routine estrus monitoring, artificial insemination and elective CS. Only English bulldog and Boerboel bitches were included in the current study. Parity, age and prior cesarean section was not considered. All bitches used in the current study were well habituated, used to travelling, showing and residing at the obstetric clinic for purposes of artificial insemination and management of parturition. Bitches that were combative or resisted the minimal restraint required to perform vaginal speculum examinations six-hourly were not included in the study. The owners declined a trial of labor (attempt at spontaneous unassisted parturition) for all the bitches in the current study. Bitches with a singleton pregnancy were excluded.

2.2 Procedures during estrus and the preparturient period

During pro-estrus and estrus, vaginal speculum examination [17] was used to time AI and vaginal cytology was performed to establish D0 [18]. The vaginal speculum examination and vaginal cytology was performed at 08:00 a.m. This standardizing of time of collecting data for D0 was necessary to obtain accurate relevant time intervals between D0 and CS.

In the pre-parturient period, all bitches were monitored for behavioral changes associated with impending parturition (sustained panting for > 2 h, restlessness, nesting behavior, depression, vomition, anorexia and looking towards their flanks). The expected day of cervical dilatation was taken as D57 (D0 + 57 d) [16]. In all the bitches, vaginal speculum examinations were performed every 6 h, starting at 08:00 (thus a minimum of 4 times per day around the clock) on D54 or more frequent if it was suspected that the onset of cervical dilatation was imminent. The vaginal speculum examination was performed using a Perspex tube (size 12–18 mm inner diameter, dependent on the size of the bitch and

220 – 330 mm long). The speculum was advanced in the vagina as far cranial as possible and light from a cold light source shone through the most caudal part of the speculum to illuminate the vaginal mucous membrane cranial to the speculum to visualize the cervix and observe for the first sign of any degree of dilatation of the cervix. The cervix was considered to have started to dilate when fetal membranes could be visualized within the *os cervices*.

2.3 Allocation of bitches and cesarean sections to groups

2.3.1 Restricted group (bitches in which the duration of gestation was restricted)

Based on CSs the primary investigator had performed prior to applying for ethical approval, we estimated that we would require about 67 preparturient CSs to demonstrate a two-hours survival ratio higher than the ratio of 0.87 reported by Moon et al., (2000) for a large number of CSs performed on a large variety of bitches for a variety of reasons by a large number of veterinarians under a diversity of conditions. Having obtained ethical approval to perform 67 prepaturient CSs within one year, we decided prospectively that each following CS on an English bulldog or Boerboel bitch destined for elective CS would be performed by 08:00 on D57 by the latest, irrespective of whether her cervix had started to dilate by then or not. We therefore prospectively decided to restrict the duration of gestation in these bitches to end no later than 08:00 on the morning of D57. In some of these bitches their cervices had started to dilate before 08:00 on D57 and a parturient CS was performed right away. In others their cervices were still closed by 08:00 on D57 and a preparturient CS was performed right away. Yet others showed signs of imminent parturition before 08:00 on D57, while their cervices were still closed, and a preparturient CS was performed right away. The signs of impending parturition prompting immediate CSs were nesting behaviour or excessive panting.

By the time that the 67th preparturient CS had been performed, a total of 99 CSs had been performed on bitches in which the intention was to restrict the duration of gestation. These 99 CSs of the restricted group eventually consisted of the following three subgroups:

1. Sixty CSs performed on 60 bitches (17 English bulldogs and 43 Boerboels) at 08:00 on D57, while their cervices were still closed. These 60 CSs were allocated to the restricted D57 group).

2. Seven CSs on seven bitches (two English bulldogs and five Boerboels) that were performed on D56 while their cervices were still closed because they showed signs of imminent parturition. These seven CSs were allocated to the restricted D56 group.

3. Thirty-two parturient CSs on 32 bitches (four English bulldogs and 28 Boerboels) that were performed before or at 08:00 on D57, once cervical dilatation had started. Nine of these 32 CSs were performed at 08:00 on D57, whereas the other 23 were performed before then. These 32 CSs were allocated to the group named restricted parturient group

2.3.2 Group in which the duration of gestation was not restricted

Prior to having obtained ethical approval to perform the required 67 preparturient CSs we had performed 173 elective parturient CSs once cervical dilation had first been noticed following six hourly vaginal speculum examinations round the clock. All the procedures as described for parturient CSs described in the current paper were followed on these 173 CSs. Because the duration of gestation was in no way restricted in these 173 CSs, we named them the non-restricted group, which consisted of 74 CSs on 53 English bulldog bitches and 99 on 85 Boerboel bitches. All these CSs were thus parturient.

2.3.3 Parturient- and preparturient groups

The 173 CSs of the non-restricted group and the 32 CSs of the restricted parturient subgroup were combined into the parturient group, consisting of 205 CSs in 163 bitches. The 60 CSs of the restricted D57 group and the seven of restricted D56 group were combined to form the preparturient group, consisting of 67 CSs in 67 bitches.

2.4 Cesarean sections and critical data collected

Only healthy bitches destined for elective CS were included in the current study. The hematocrit was assessed before and after CS as previously described [19]. The bitches where anesthetized using the standard anesthetic protocol in the practice, consisting of medetomidine hydrochloride (7 µg/kg iv) as premedicant, propofol, (1–2 mg/kg iv) as induction agent and sevoflurane, at 2% in oxygen for maintenance of anesthesia [20]. The CS was performed in standard fashion as previously described [21]. Once the decision to perform a CS was made, an ultrasound examination of the abdomen was performed to establish whether there were any dead fetuses (absence of detectable heartbeat). The bitches were weighed before surgery. In all bitches, fluid administration (Ringer lactate, Fresenius Kabi, Midrand, South Africa) commenced starting at induction and continued for 1½–2 h following induction for surgery until the set amount of fluids (35 ml/kg bw) had been infused. The peri-operative use of antibiotics included cefazolin (Zefkol®, Brimpharm, Claremont, South Africa) administered iv at 10 mg/kg at the time of induction followed by oral amoxycillin (Betamox, Be-tabs, Roodepoort, South Africa) at 20 mg/kg b.i.d for five days. Meloxicam (Metacam®, Boehringer Ingelheim, Randburg, South Africa) was administered iv (0.1 mg/kg) intra-operatively as proposed [22], immediately after delivery of the last puppy. The fetuses were freed from their membranes, allowed to breathe, the placenta was dislodged from its uterine attachment by gentle traction after which the umbilicus was cut and tied off. 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 [20], tying off of the umbilicus and applying 10% povidone iodine thereto, drying the puppies, shaking fluids from their airways and placing them in an air-heated incubator set at 35°C. No oxygen support was offered to the puppies after delivery.

Live puppies born with malformations were euthanized within 15 min of delivery at the time of taking the Apgar score but hydrops fetalis fetuses were euthanized at birth. After delivery of the puppies the following records were made; total number of puppies delivered, live puppies, dead puppies, deformed puppies and puppies euthanized. The Apgar scores were assessed starting with the first puppy 15 min after the last puppy was delivered according to the method adapted by Veronesi et al, (2009) and later used by Doebeli et al, (2013) [23, 24]. The bitch and puppies were discharged usually 2 to 3 h following surgery.

No ovariohysterectomies were performed.

Immediately before performing each of 25 non-restricted CSs, a blood sample was collected in a 10-ml green-stoppered heparinized glass vial (BD Vacutainer ® (170 IU lithium heparin), BD Plymouth, UK), the plasma separated by centrifugation and frozen (-20 °C) in 1.8 ml cryo vials (Catalogue number 750273, PlastPro Scientific, Edenvale

South Africa). The progesterone levels in these 25 plasma samples were later determined using the last available Coat-A-Count® radioimmunoassay (Siemens Health Care Diagnostics Inc. Los Angeles, CA 90045 USA) before the assay was removed from the market [25]. Immediately before 26 non-restricted parturient- and 52 preparturient CSs, a blood sample was collected blood (BD VAC PLAIN glass tube, BD Plymouth, UK), left at room temperature for 2 h and then centrifuged and the serum transferred to a cryovial and frozen at -20°C. The progesterone levels were later measured (two replicates each) using a chemiluminescent immunoassay (Immulite LKPW1 ®; Siemens Medical Solutions Diagnostics, 5210 Pacific Concourse Drive Los Angeles, CA 90045-6900 USA). The two replicates of each serum or plasma sample were used to determine the intra-assay CV. Fifteen serum samples were assayed in two different batches of Immulite and the interassay CV determined, whereas the interassay CV for the Coat-A-Count radioimmunoassay has previously been determined in this laboratory and found to vary from 5.7% for progesterone levels of 2–4 nmol/L to 12.45% for levels of 22–23 nmol/L.

Critical data collected were the date and time at which the CS was performed; progesterone level in the plasma or serum at the time of CS; hematocrit before and after CS; state of the cervix immediately before CS (dilating or closed); litter size, numbers of stillborn fetuses, number of live puppies, puppies that were deformed and euthanized; maternal survival and puppy survival at birth and 2 h after CS.

2.5 Data analysis

The frequencies of puppies born alive and surviving to 2 h were summarized and reported as percentages for English bulldogs and Boerboels and both breeds combined. This was done for parturient CSs (those performed at the time of spontaneous cervical dilatation) and for preparturient CSs (those performed prior to the onset of cervical dilatation).

A mixed-effects logistic regression was done to determine the effect of day of CS, breed and litter size on the log odds of fetuses being stillborn. Day of CS had three levels: Zero for CSs performed at the first observation of spontaneous cervical dilatation; one for CSs performed at 08:00 on D57, while the cervix was still closed and two for CSs performed on D56, while the cervix was still closed. Breed had two levels: Zero for English bulldogs and one for Boerboels. Litter size stated the number of fetuses born, irrespective of whether they had died before or during CS. Data were clustered in litters.

The same mixed-effects logistic model was used to assess the effect on the log odds of puppies spontaneously dying before 2 h. Stillborn fetuses were included as losses prior to 2 h, but puppies that were born alive but euthanized due to serious congenital defects were excluded.

For each mixed-effect logistic regression model the first-order interactions between day of CS and breed, between day of CS and litter size, and between breed and litter size were first included in the model, following which each model was finally run again without the interaction terms.

We used a mixed-effect multiple regression model to determine whether puppies delivered by CSs in the restricted group had different Apgar scores than those delivered by CSs in the non-restricted group. Apgar score was the outcome variable, parturient CSs in the non-restricted group were the baseline group to which the other groups were compared. The other groups were those of the restricted group, which included the subgroups of preparturient CSs performed on D57 (restricted D57 group), preparturient CSs performed on D56 (restricted D56 group) and parturient CSs performed at 08:00 on D57 or earlier (restricted parturient group). Breed and litter size were included as covariates. All first-order interactions were initially included following which all non-significant interactions ($P > 0.05$) were removed from the model.

A Kruskal-Wallis test was used to determine whether the levels of progesterone differed among the four subgroups. They were the non-restricted group, restricted D57 group, restricted D56 group and the restricted parturient group. Wilcoxon's rank sum test, with Bonferroni's correction was used for pairwise comparisons ($P < 0.05$).

Multiple regression was used to compare the decline in hematocrit during preparturient CSs to the decline occurring during parturient CSs. Breed (Boerboel compared to English bulldog) and litter size as well as the interaction between them were included as covariates. The interaction was first removed from the model, sequentially followed by the covariates that did not significantly affect the decline in hematocrit ($P > 0.05$). The five largest declines in hematocrit (one each of 13, 14 and 16 percentage points associated with CSs in parturient bitches and one each of 13 and 14 percentage points associated with CSs performed on preparturient bitches) were removed from the model to avoid heteroskedasticity and residuals that were not normally distributed.

3. Results

Table 1 shows the numbers and percentages of puppies alive at birth and 2 h following parturient and preparturient CS. Table 1 also shows the numbers and percentages of puppies that were euthanized shortly after birth due to severe congenital malformation.

Overall, 7.4% of English Bulldog puppies coming from 31% of the litters and 1.3% of Boerboel puppies coming from 9.7% of the litters were euthanized due to such malformations.

3.1 Effects of day of CS, breed and litter size on the odds of stillbirth

No interaction was significant ($P \geq 0.12$).

After controlling for breed and litter size, the odds of stillbirth for preparturient CSs performed on D57 is similar to that for parturient CSs (95% CI 0.086 times as high to 1.092 times higher, $P = 0.07$). No puppies were stillborn from preparturient CSs performed on D56 and the odds were not determined for that group of CSs.

Controlling for day of CS and litter size, the odds of stillbirths for English bull dog puppies was similar to that for Boerboel puppies (95% CI from 0.5 times as high to 3.1 times higher, $P = 0.60$).

After controlling for day of CS and breed, the odds of stillbirth remained similar with each increase by one in litter size (95% CI decreasing by 23 percentage points to increasing by 0.5 percentage points, $P = 0.06$).

3.2 Effects of day of CS, breed and litter size on the log odds of neonatal death before 2 hours

No interaction was significant ($P \geq 0.17$).

The odds of neonatal death prior to 2 h for preparturient CSs performed on D57 is 0.22 times as high as that for parturient CSs (95% CI 0.068 times as high to 0.730 as high, P =

0.01). No puppies delivered by preparturient CSs performed on D56 died before 2 h and the odds were not determined for that group of CSs.

Controlling for day of CS and litter size, the odds of neonatal deaths before 2 h was similar for English bulldogs and Boerboels (95% CI 0.9 times as high to 4.1 times higher, P = 0.10).

After controlling for day of CS and breed, the odds of neonatal death before 2 h remained similar for each increase by one in litter size (95% CI changing by 0 percentage points to decreasing by 20 percentage points, P = 0.05).

3.3 Effects of day of CS, breed and litter size on Apgar scores

In total, 2023 Apgar scores were recorded on puppies from 271 CSs, with an average of 7.5 per CS. The average of the 2023 Apgar scores was 9.7 (SD 0.88, ranging from 2 in 3 puppies to 10 in 1703).

The Apgar scores of puppies delivered by preparturient CSs on D57, by preparturient CSs performed on D56 and by parturient CSs performed at 08:00 on D57 or earlier were similar to those of puppies delivered by parturient CS in the non-restricted group (P > 0.7). There was a breed effect on Apgar score; Apgar scores of Boerboel puppies were 1.03 higher than those of English Bulldog puppies (95% CI 0.53 to 1.53, P < 0.001). Apgar scores increased by 0.09 for each increase by one in litter size (95% CI 0.03 to 0.16, P = 0.003). Breed and litter size interacted in their effect on Apgar score; the breed effect decreased by 0.08 (95% CI 0.02 to 0.15, P = 0.02) for each increase by one in litter size.

Table 1. Frequency and percentage of puppies alive at birth and 2 h following parturient cesarean sections (performed once the cervix had started to dilate) and preparturient cesarean sections (performed while the cervix was still closed 56 or 57 d after the onset of cytological diestrus) in bitches

	Parturient CS group (dilating cervix)			Preparturient CS (closed cervix)		
	English bulldog (78 litters from 54 bitches)	Boerboel (127 litters from 109 bitches)	Both breeds (205 litters from 163 bitches)	English bulldog (19 litters from 19 bitches)	Boerboel (48 litters from 48 bitches)	Both breeds (67 litters from 67 bitches)
Born alive	476 of 491 (97.0)[a]	1189 of 1215 (97.9)	1665 of 1706 (97.6)	105 of 108 (97.2)	310 of 311 (99.7)	415 of 419 (99.0)
Malformed[b]	32 in 21 litters	15 in 13 litters	47 in 34 litters	12 in 9 litters	4 in 4 litters	16 in 13 litters
Percentage malformed	6.5	1.2	2.8	11.1	1.3	3.8
Alive at 2 hours[c]	431 of 459 (93.9)	1164 of 1200 (97.0)	1595 of 1659 (96.1)	92 of 96 (95.8)	306 of 307 (99.7)	398 of 403 (98.8)

[a] The first number in each cell shows the number of live puppies, the second the total number of puppies and percentages are between parentheses

[b] Puppies that were euthanized due to severe congenital malformation

[c] Numbers in this row exclude puppies that were euthanized due to severe congenital defects

Table 2. Predictive margins (population average probabilities) for still births or neonatal deaths for parturient cesarean sections (performed once the cervix had started to dilate) or preparturient cesarean sections (performed while the cervix was still closed 57 or 56 d after the onset of cytological diestrus)

	Stillborn	Died before 2 hours
English bulldog		
Parturient	0.029 (0.015–0.044)[a]	0.057 (0.035–0.080)
Preparturient D57	0.010 (0.000–0.021)	0.015 (0.001–0.030)
Preparturient D56		
Boerboel		
Parturient	0.024 (0.014–0.034)	0.033 (0.022–0.045)
Preparturient D57	0.008 (0.000–0.015)	0.008 (0.001–0.016)
Preparturient D56		

[a] The first number shows the expected probability of death and the figures between parentheses its 90% confidence interval

3.4 The extent of shortening gestation by preparturient cesarean sections

Figure 1 shows the interval between D0 and cesarean section in the 173 parturient cesarean sections of the non-restricted group. The shaded bars represent the 88 cesarean sections that were performed later than they would have been if they were performed at 08:00 on D57. Half of these 88 CSs took place 4–10 h later than they would have if they were performed at 08:00 on D57 and the other half 10–52 h later. The central 50% of these 88 CSs (those lying between the 25th and the 75th percentiles), were performed 4–24 h later than would have if they were performed at 08:00 on D57.

Of the 99 restricted group CSs, 69 (70%) took place at the scheduled date and time of 08:00 on D57; 60 of which while the cervix was still closed and nine of which had started to dilate by then. Ninety-two (93%) of the 99 restricted group CSs took place within 24 h before or at 08:00 on D57 and all occurred within 48 h before or at that time.

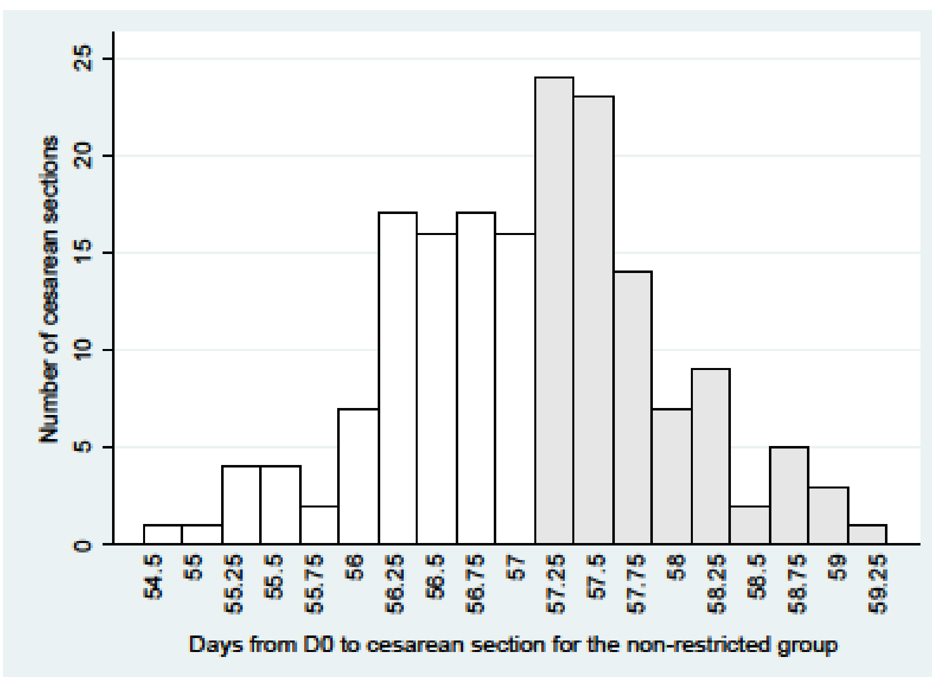

Figure 1. Interval between D0 (the day of onset of cytological diestrus) and each of 173 cesarean sections from the non-restricted group, performed once cervical dilatation was first observed

3.5 Progesterone at the time of CS

The intra-assay coefficient of variation was 7.77% for the 24 plasma samples analyzed with RIA and 7.35% for the 79 serum samples analyzed with Immulite. The interassay CV for Immulite was 6.06% for serum samples with progesterone levels from 10 to 46 nmol/L (n = 8) and 10.77% for 7 serum samples with progesterone levels from 1.1 to 9.96 nmol/L.

Figure 2 shows the difference in progesterone level at the time of CS in 51 parturient- and 52 preparturient CSs. The progesterone level was above 6.4 nmol/L in 31 (60%) of the preparturient CS, compared to five (10%) of the parturient CS (P < 0.001). Twenty-three (44% of) preparturient CSs had progesterone levels above 10 nmol/L compared to 3 (6% of) parturient CSs (P < 0.001).

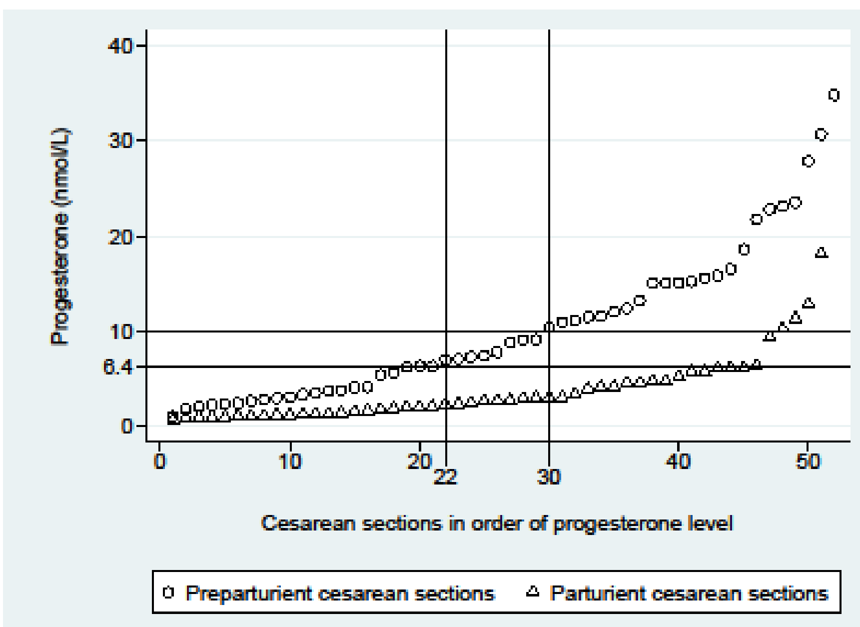

Figure 2. The progesterone level in the serum or plasma of bitches at the time of cesarean section, plotted in order of increasing progesterone level for 51 parturient- and 52 preparturient cesarean sections

Table 3 shows that the levels of progesterone in the serum of bitches that underwent preparturient CS was higher than the levels in bitches that underwent parturient CS ($P < 0.001$). Table 3 also shows that all the data were positively skewed.

Considering preparturient CSs, the odds of stillbirth and of puppies dying at any time from birth to 7 d is independent of the level of progesterone in the serum at the time of CS ($P > 0.30$).

Table 3. Level of progesterone (nmol/L) in the serum or plasma at the time of CS

	Min	Q1	Med	Q3	Max	n	
Parturient CS group							
Non-restricted group	0.72	1.59	3.43	6.11	12.84	25	a
Restricted parturient group	0.90	1.27	2.19	4.14	18.10	26	a
Both subgroups combined	0.72	1.28	2.73	4.76	18.10	51	c
Preparturient CS group							
Restricted D57 subgroup	1.08	3.58	7.52	15.10	34.80	47	b
Restricted D56 subgroup	3.79	11.50	13.20	15.55	27.85	5	b
Both subgroups combined	1.08	3.80	8.33	15.10	34.80	52	d

a, b Subgroups with different letters differ ($P < 0.05$)

c, d Groups with different letters differ ($P < 0.001$)

3.6 The effect of preparturient CS on hematocrit

Twelve hematocrits before and 13 after CS were missing. After removal of the non-significant terms (interaction between breed and litter size ($P = 0.08$), breed ($P = 0.97$) and litter size ($P = 0.98$) from the model, CS on preparturient bitches resulted in a 1.3 (95% CI

0.55–2.02) percentage points smaller decline in hematocrit than CSs performed on parturient bitches (P = 0.001). Hematocrit declined from 43.9 (SD 4.27) to 37.6 (SD 4.31) during 193 CSs on parturient bitches and from 44.2 (SD 4.31) to 39.2 (SD 4.02) during 66 CSs on preparturient bitches.

4. Discussion

The advantages of parturition induction protocols [26, 27] and preparturient CS [14] are similar. Both allow for a shorter period of supervision prior to the delivery of puppies and allows the clinician to expect a bitch to whelp or to perform a CS on a predictable time. For bitches in which an elective CS is planned, it is ideal to perform a preparturient CS to avoid fetal compromise. This notion concurs with the suggestion that a substantial proportion of pregnant brachycephalic bitches undergo elective cesarean section before natural parturition begins [2]. Once the decision has been made that an elective CS will be planned for a bitch, the problem of timing the elective CS manifests. Historically this decision was based on detecting a drop in rectal temperature, the display of imminent signs of parturition (panting, nesting behavior), having observed the presence of a dilating cervix or having observed ruptured fetal membranes [11]. Behavioral signs of impending parturition are inconsistently displayed in bitches and parturition supervisors may fail to observe them [28]. Also, a decline in rectal temperature was of some value but was found to be too variable between individuals to use as the sole criterion in predicting onset of parturition [29-31].

The preparturient drop in progesterone levels to below 6.4 nmol/L in the last 24–36 h before parturition has been adequately demonstrated [32-34]. The only exception may be the singleton pregnancy [6]. It is suspected that incomplete luteolysis in singleton pregnancies may be the cause of fetal demise before onset of spontaneous parturition [6, 35] and therefore singleton pregnancies were eliminated from the current study. Although it has been suggested that preparturient progesterone levels can be used to confirm that a bitch is at term [36], no controlled studies have been published showing that it is safe to perform a CS when the progesterone level has decreased to below 6.4 nmol/L or that is unsafe to perform a CS when it is above 6.4 nmol/L. The current study showed that good Apgar scores and puppy survival rates can be achieved following CS performed when progesterone level is above 6.4 nmol/L as 31 bitches (60%) of the preparturient group and five (10%) of the parturient group had progesterone levels of above 6.4 nmol/L in their blood at the time of CS.

Using D0 as predictor of the day of cervical dilatation [16] or other methods of predicting the day of parturition [37-39], allows the clinician to predict the day of parturition to within 3 d on either side of the day on which parturition would actually occur. In the current study, 60 preparturient CSs (Experimental preparturient subgroup D57) were performed at a fixed time and day (08:00 on D57), which curtailed the period of parturition observation to 72 h (from 08:00 on D54 until 08:00 on D57) or less.

Out of 99 gestations for which D0 was known and a preparturient CS planned for 08:00 on D57, the CS was performed at the scheduled time in 69 (70%), and before then in 23 (23%) because cervical dilatation had started. Sixty preparturient CSs (Experimental preparturient subgroup D57) were performed at a fixed time and day (08:00 on D57), which curtailed the period of parturition observation to 72 h (from 08:00 on D54 until 08:00 on D57) or less.

In addition, our protocol allowed for safe preparturient CS on D56 in seven bitches showing clinical signs of impending parturition. These are the seven restricted D56 group CSs that were scheduled for 08:00 on D57 but were actually performed before then because they had started showing signs of impending parturition, even though their cervices were still closed. Nesting behavior associated with uterine contractions may occur as much as 7 d before spontaneous parturition [40]. Therefore, behavioral signs cannot be used to time CSs on their own. However, signs of imminent parturition close to D57 (within 24 h of D57 as these seven bitches suggest) may be more significant than when no prediction date is available. It is not known whether it would have been safe to also perform a CS on D56 in the 60 bitches of the restricted D57 subgroup. Preparturient CS on D56 was only considered in bitches that displayed signs of impending parturition and with the knowledge that they were within 24 h of D57. It is not known how many of the Preparturient D56 subgroup would have had dilating cervices before or at 08:00 on D57. It is fair to assume to that some would have.

Unless the interval between D0 and cervical dilatation differ among breeds, this study provides a protocol that enables clinicians to perform CSs on a scheduled day and at a time of the day where there is adequate support staff available on approximately two thirds of bitches in which an elective CS has been planned.

In the current study, our scheduling of fixed time CS is not perfect. This is because roughly a third (32/99) of CSs were performed before or at 08:00 on D57, suggesting that it was necessary to perform vaginal speculum examinations on all bitches every six hours to identify those that required CSs before then because their cervices had started to dilate. We deemed it necessary to perform vaginal speculum examinations once every six hours and not less frequently because we were concerned that fetal compromise, puppy death and the need for emergency CSs may increase if the interval between vaginal speculum examinations is increased from every 6 h to every 12 h. It would therefore be useful to have a means of predicting that a bitch would not undergo cervical dilatation during the next 12 hours, thereby negating the necessity to observe bitches overnight. Preparturient progesterone levels holds promise as such a predictor as it was shown that, among 25 bitches that were within 12 hours prior to cervical dilatation, only one had a serum progesterone level above 15.8 nmol/L and only three had progesterone levels of 8.7 nmol/L or higher [25].

This study shows that the odds of stillbirth following preparturient CSs performed at 08:00, 57 days after the onset of cytological diestrus were similar to those after parturient CSs but that the odds of dying before two hours were lower. This study showed high neonatal survival ratios after preparturient CS with 99% of puppies delivered by preparturient CS born alive and 98.8% surviving to 2 h. Apgar scores depend on the anesthetic protocol and lung maturation at birth [41, 42]. The good Apgar scores achieved showed that both the anesthetic protocol and timing of CSs in both the parturient and preparturient CSs did not adversely affect our results. The probability of puppy mortality with preparturient CSs compared favorably to those reported in the literature for CSs performed at the time of parturition [9, 12].

The high neonatal survival rates confirm that the timing of the preparturient CSs in this study was good and resulted in delivery of the fetuses before fetal demise and compromise could occur during onset of parturition. More data on preparturient CSs performed on D57 and on D56 in bitches showing impending signs of parturition are required to conclude whether this is routinely safe and routinely safe in all breeds.

The odds of stillbirth or puppies dying before they were two hours' old was similar in English bulldogs and Boerboels. Litter size did not affect the odds of stillbirths or puppies dying before they were two hours old.

The timing of preparturient CSs is important. Performing preparturient CSs too early may yield premature, non-viable puppies, cause failure of placental release and increase the risk of serious uterine hemorrhage [11] and death of the dam [43]. In the current study both, preparturient CSs and parturient CSs yielded high puppy survival ratios at birth and up to two hours and good Apgar scores suggesting that the preparturient CSs on D57 does not affect these parameters. Further long term puppy survival studies are required to establish safety of this protocol for routine in practice use. The good Apgar scores achieved following preparturient CSs suggest that significant lung maturation, allowing normal extra-uterine survival, occurs in the dog fetuses some time before spontaneous parturition.

The current study shows that preparturient CSs, performed at 08:00 on the morning of D57, while the cervix is still closed, shorten gestation by 4–52 h relative to what would have been the case had they been performed upon first noticing cervical dilatation. Yet, it does not show by how long gestation was curtailed by a particular preparturient CS. More research is required to better define the safe period of intervention in the bitch.

The higher serum progesterone levels and the higher proportions of bitches with levels above 6.4 or 10 nmol/L at the time of preparturient CSs than at the time of parturient CSs confirm that gestation of the preparturient group has not progressed as far by the time of cesarean section as it did in the parturient group. The current study shows that it is not a prerequisite for the level of progesterone in the serum to decrease below 6.4 nmol/L in order to obtain high survival ratios at birth and by two hours following preparturient CS. Indeed, 60% of bitches that underwent CSs before cervical dilatation had started and 10% of those that underwent CSs once cervical dilatation had started, still had progesterone levels above 6.4 nmol/L.

Aglepristone [13, 14, 26] and betamethasone [15] have been administered to preparturient bitches as priming agents in attempt to stimulate fetal maturation. It is not known whether they are beneficial close to the time of onset of spontaneous parturition and, if they are, at what time before the onset spontaneous parturition. In these studies, the time of priming or CS was derived from the peri-ovulatory level of progesterone in blood plasma or serum on its own or together with LH. Unfortunately, none of these studies are large or convincing enough to conclude that performing CS based on their timing method is safe for routine use in clinical practice. Also, these studies do not show whether good Apgar scores and survival of the puppies could have been achieved when not using antenatal maternal priming. However, emergency interventions may be required in late pregnancy due to maternal metabolic and 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].

The current study achieved good Apgar scores at 15 minutes' post CS without priming. It is not known whether priming may influence the outcome of preparturient CSs performed earlier than was the case in the current study.

Fixed date CSs have advantages over current parturition induction protocols as it requires no time to monitor the delivery of puppies and no ecbolic support to complete parturition as was required in other studies [13, 26, 27].

In the current study, preparturient CS did not lead to complications in the dams. The maternal survival rate was 100%. The decline in hematocrit during preparturient CSs was slightly smaller (1.3 percentage points) than during parturient CSs.

It is not known whether performing a CS while the cervix is closed would pose a risk of retained placentas and metritis if the cervix does not dilate adequately to allow for placentas and lochia to escape. Retained placentas were avoided in the current study as each placenta was removed together with its fetus and no post-operative uterine infections were noted during the 7 d following CS. The removal of placentas was not associated with complications and renders it more likely to identify placental abnormalities and fetuses sharing placental sites [45, 46]. This does however not mean that routine removal of placentas can be advocated in all circumstances. This is because it cannot be excluded that the use of the alpha 2-agonist, medetomidine, may have influenced uterine tone and the risk of placental site hemorrhage.

5. Conclusions

The current study showed that good Apgar scores could be achieved and high puppy survival rates at birth and 2h post-delivery when performing preparturient CSs at 08:00, 57 days after the onset of cytological diestrus, if cervical dilatation had not started before then. The current study also showed that the time of parturition observation could be curtailed in approximately two thirds of the obstetric population. Further studies are required to determine whether preparturient CSs on D57 are routinely safe in all breeds, does not affect long term survival of the puppies and to timeously identify bitches in which parturition starts prior to the date and time for the scheduled elective preparturient CS.

Author contributions

K.G.M. De Cramer was the main person involved in experimental work and wrote the protocol and manuscript. J.O. Nöthling assisted in designing the experiment and preparing the research protocol, performed the statistical analyses and assisted in preparing the manuscript.

Conflicts of interest

Neither 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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