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Maternal and Neonatal Outcome Following Elective Cesarean Section based on Progesterone Levels in High Risk Pregnant Dogs

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ABSTRACT

Background: Elective cesarean section (CS) is an appropriate therapeutic modality in high risk pregnant dogs and it is scheduled following a drop in serum progesterone to less than 2ng/ml. However, a series of progesterone assays are required to confirm this drop in serum progesterone levels and this voluntary waiting period often results in foetal loss. The present study aimed to assess the innocuity of elective CS in dogs before the prepartum decline of serum progesterone, without administration of any priming agents.

Methods: Elective CS was performed on an estimated day 63 of ovulation in seven high risk pregnant dogs with a serum progesterone level between 2-5 ng/ml (Group I) and maternal and neonatal outcomes were assessed in comparison with nine dogs that underwent elective CS at serum progesterone less than 2 ng/ml (Group II). Histopathological examination of lung tissue from dead neonates was performed to ascertain the presence of type II pneumocytes for assessing lung maturity.

Result: Even though progesterone levels were above 2 ng/ml in dogs of Group I at the time of elective CS (3.11±0.29 ng/mL), the live birth rate was 96.97 per cent with acceptable neonatal survival. The histopathology of lung tissue from dead neonates from Group I revealed the presence of type II pneumocytes. The study suggested that elective CS in dogs could be done on day 63 after ovulation without compromising foetal lung maturity and a drop in serum progesterone level to less than 2 ng/ml was not mandatory for puppy survivability.

Key words: Dog, Elective cesarean section, Serum progesterone levels.

INTRODUCTION

Dogs likely to have a higher prevalence of maternal and foetal perinatal morbidity or mortality than that of the general obstetrical population are considered to be having high risk pregnancies. History of pregnancy loss, dystocia, caesarean section, advanced maternal age, brachycephalism, pelvic fracture, metabolic disorders, vaginal strictures and unusually small or large litter size are factors contributing to high risk pregnancy (Johnson, 2008).

In the case of high risk pregnant dogs, a properly planned elective CS is considered to be a safe, effective and justified intervention. Planning CS in the dog is crucial because early intervention can lead to reduced puppy survival due to lung immaturity. The criteria mooted for determining the timing of elective cesarean and ensuring delivery of mature puppies was a dip of serum progesterone to values below 2 ng/ml (Smith, 2007). However, the procedure requires repetitive measurements of serum progesterone over several days to identify the decline to 2 ng/ml. In a clinical setting, such diagnostic support could be challenging and could lead to foetal distress during the waiting period resulting in reduced live birth rate or survival of the distressed neonates following CS.

Though studies have reported safe CS in bitches on an average of two days before the prepartum dip in progesterone to below 2 ng/ml, these studies had incorporated progesterone receptor antagonists (Levy *et al.*, 2009; Roos *et al.,* 2018). These studies questioned the necessity of prepartum decline in serum progesterone as a requirement of foetal maturation and advocated further studies without the incorporation of progesterone receptor antagonists. Taking these factors into account, this study was carried out to assess the prospect of performing elective CS in high risk pregnant dogs on an estimated day 63 after ovulation, before the drop of serum progesterone level to less than 2 ng/ml and without any priming agents.

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MATERIALS AND METHODS

This study was conducted at University Veterinary Hospitals,

Kokkalai and Mannuthy, Kerala Veterinary and Animal Sciences University, from March 2019 to March 2020. High risk pregnant dogs (brachycephalic breeds, history of stillbirth, dystocia and CS) were followed up during oestrus to determine the date of ovulation by estimating serum progesterone with chemiluminescence immunoassay (CLIA). Scheduled mating with stud dogs was advised based on cytology findings, behaviour and ovulatory progesterone levels of 4 to 8 ng/ml. Pregnancy was confirmed 25 days post ovulation by trans-abdominal sonography.

The pregnant dogs were reviewed on day 63 after the estimated day of ovulation. Dogs were selected for elective CS based on serum progesterone levels measured using CLIA. Those bitches with a progesterone (P_4) level exceeding 5ng/ml were excluded from the study. Bitches with a serum P_4 of less than 5ng/ml (n=16) were selected for the study and categorized into two groups, Group I (n=7) with P_4 levels between 2 and 5 ng/ml and Group II (n=9) with P_4 level $\leq$ 2 ng/ml.

Foetal viability in pregnant dogs, in terms of foetal heart rate, was assessed before the CS by trans-abdominal sonography (Lamm and Makloski, 2012) and litter size was estimated by radiography. Bitches were preoperatively examined for drop in rectal temperature, initiation of lactation and presence of vaginal discharges. The vaginal examination was performed using a rigid sigmoidoscope to confirm dilatation of the cervix, indicated with the presence of a foetal bag at the anterior vagina (Cramer and Nothling, 2019).

Dogs in both groups were pre-medicated with glycopyrrolate (0.01 mg/kg, IM) followed 10 min later by butorphanol (0.2 mg/kg, IV) and midazolam (0.2 mg/kg, IV). General anaesthesia was induced with propofol (3.5 mg/kg, IV) followed by maintenance with 2% isoflurane. A midventral laparotomy approach followed by an incision on the dorsal aspect of the uterine body was performed in all the surgeries (Fossum, 2013). Placental characteristics were recorded as either detachable or non-detachable and bleeding from placental sites was recorded as copious or negligible. The placenta was left *in situ* for spontaneous expulsion in dogs having non-detachable placenta and bleeding from placental sites.

Neonatal evaluation in the immediate postoperative period was done with blood gas analysis and Apgar scoring. To estimate the blood gases, samples were collected as soon as possible after separating the neonates from the uterus. A total of 0.25- 0.3 ml of blood was collected from the jugular vein of neonates and subjected to immediate analysis using an EPOC blood gas analyzer. The modified Apgar scoring adopted for canine neonates by Groppetti *et al*. (2010) was used for the assessment of neonatal viability. Litter size, number of stillborn and live pups were recorded in both groups and puppy mortality was recorded up to day 14. Carcasses of dead puppies were collected and post mortem examination was conducted to determine the cause of death. Foetal lung samples were collected from all the dead puppies and preserved in 10 per cent neutral buffered formalin and subjected to haematoxylin-eosin (H&E) staining. Histopathological examination was done to ascertain the presence of type II pneumocytes, which are indicative of foetal lung maturity (Sipriani *et al*., 2009).

The dams were examined regularly for any post-partum complications including suture dehiscence, agalactia, mammary gland affections or any other metabolic disorders post-operatively till day 14.

The data obtained were tabulated and analysed statistically (Snedecor and Cochran, 1994) using the SPSS version 24 statistics software package.

RESULTS AND DISCUSSION

A total of 16 dogs belonging to four different breeds were included in the study. The mean serum P_4 levels on the day of CS was 3.11±0.29 ng/ml (Range of 2.21 to 4.18 ng/mL) in Group I animals whereas, in Group II animals the mean serum P_4 level on the day of CS was 1.25±0.12 ng/ml (Range of 0.82 to 1.84 ng/ml), the difference being significant ($p<0.01$). This signifies that the elective CS was performed in Group I dogs when the gestation was not as advanced as in dogs of Group II.

A prepartum drop in rectal temperature (≤99°F) and the onset of lactation are considered to be clinical indicators of impending whelping (Wykes and Olson, 2003). However, in 42.86 per cent of dogs in Group I (progesterone levels >2 ng/ml), rectal temperature was below 99°F. In Group II (progesterone ≤ 2 ng/ml), though it was expected that most bitches would have a rectal temperature below 99°F, only 55.36 per cent had a rectal temperature below 99°F. 71.4 per cent of dogs in Group I and 77.8 per cent dogs in Group II had active mammary glands before surgical intervention. The absence of a clear correlation between progesterone levels, rectal temperature and prepartum lactation in the study corroborates with the observations of Cramer and Nothling (2019) that drop in rectal temperature and presence of lactation could not be used as sole predictors of impending parturition or readiness for CS.

Smith (2007) stated cervical dilation to be an objective parameter, which could be monitored to indicate if the bitch was parturient and considered it to be safe for CS if the cervix had dilated. In 55.6 per cent of dogs in Group II, the cervix remained closed even after a drop in serum progesterone levels to less than 2 ng/ml. While in 42.9 per cent bitches of Group I, the cervix was open though serum P_4 levels were higher than 2 ng/ml. This finding questions the relation between P_4 level and cervical dilatation and were following the interpretation of Cramer and Nothling (2019) that prepartum drop in serum P_4 levels do not consistently precede cervical dilatation.

The Apgar score of puppies (Mean±SE) in Group I at birth, 30 min and 60 min after birth were 2.7±0.24, 6.75±0.48 and 10.73±0.42, respectively. The corresponding values in Group II puppies were 3.6±0.26, 8.88±0.34 and 11.95±0.24. In comparison of the mean Apgar score, Group II puppies

had a significantly higher score compared to Group I puppies. Notwithstanding the type of CS, the significant increase in Apgar score as time advanced indicate that the neonates, irrespective of the groups, were capable of overcoming this initial depression. The relatively high proportions of pups acquiring high scores at 30 and 60 min could be attributable to the rapid clearance of gaseous anaesthetic from the circulation (Moon *et al*., 2000).

In the current study, a total of 76 pups (33 in Group I and 43 in Group II) were born from 16 dams by elective CS. The live birth rate was 96.97 per cent in Group I and hundred per cent in Group II. Stillbirth constitutes the major proportion of pup losses and should ordinarily constitute less than 30 per cent of full-term puppies that do not survive to wean (Lawler, 2008). In terms of live birth rate, elective CS has advantages over emergency CS, spontaneous whelping or induction protocols as the factors which can induce stress to the pups and those which increase the odds of stillbirth are under control (Fontbonne *et al*., 2009).

Findings of blood gas analysis (Table 1) indicated that during delivery through elective CS, irrespective of the pre-parturient progesterone levels, puppies experienced a stage of respiratory acidosis as indicated by the low pH, high PCO_2, low PO_2 and normal bicarbonate. The transient respiratory acidosis in the neonates could be attributed to hypoxic-ischemia effects of anaesthesia (Kredatusova *et al.,* 2011). The puppies born of elective CS in this study, irrespective of the groups, benefitted from the lack of uterine contractions of first and second stage labour and compression stimulus during labour, which otherwise would have exacerbated the foetal acidosis (Groppetti *et al.,* 2010).

For instituting a safe protocol for elective CS, it becomes imperative to schedule the CS without compromising foetal maturity. Neonatal mortality could be used as an indicator of faults in scheduling CS. In the present study, no neonatal mortality was recorded up to 14 days in Group II. However in Group I, four puppies died within the first two weeks of birth, of which one death was due to a congenital anomaly of the imperforated anus and occurred within 24 h of birth. Two puppies died within 24 h and another puppy death was recorded by the third day. Excluding the puppy death from the congenital anomaly, the overall neonatal survival recorded in Group I was 90.63% (29/32). Neonatal death noticed belonged to two different litters; two puppies from a litter with P_4 level 4.18 ng/ml and a single pup from a litter with progesterone level 3.25 ng/ml. On critical analysis of the neonatal survival in Group I, it could be deduced that between prepartum progesterone concentrations of 2.0 to 3.71 ng/ml, the neonatal survival was 96.55 per cent (28/29), which declined to 89.66 per cent (26/29), when the dog with P_4 concentration of 4.18 ng/ml was also considered.

Neonatal maturity can be assessed subjectively based on the extent and amount of hair cover on the face, ears, trunk and feet of the pups (Smith, 2007). None of the dead puppies had any external signs of prematurity. Post mortem could not establish any specific cause of death for the two neonatal mortalities which occurred within 24 h of birth. However, post mortem of the pup which died within three days after birth revealed pulmonary haemorrhage, pneumonia, hepatic congestion and petechial haemorrhages on the kidney. Blood smear examination confirmed the presence of numerous bipolar and bacilli organisms suggestive of systemic bacterial infection.

A feature that accounts for more than 60 per cent of neonatal loss in canines is respiratory distress syndrome (RDS) or hypoxia (Munnich and Kuchenmeister, 2014). The condition is attributed to impaired surfactant production by type II pneumocytes. The development of lung tissue in the canine foetus is characterized by pseudo glandular phase between the 35th and 46th day of gestation and the onset of canalicular and saccular periods from the 48th day and 60th day of gestation (Sipriani *et al.,* 2009). The canalicular phase is characterized by the development of type I and type II pneumocytes, which are responsible for surfactant production. Identiûcation of type II pneumocytes indicated the degree of foetal lung maturation. It was concluded in studies by Kutzler and Volkmann (2008) that foetal maturity in canines was achieved at 59 days post ovulation in the female foetus and by 60 days in the male foetus. In our study, the presence of type II pneumocytes was evident in the histopathology of lung samples (Plate 1) collected from all four dead neonates. Hence, pulmonary immaturity as a cause of neonatal mortality in group I could be ruled out.

Though the above-stated observations indicated maturity of the foetus despite serum progesterone levels remaining above 2 ng/ml in dogs of Group I, a lack of placental maturation, as evidenced by lack of detachability at the time of CS, was apparent in a major proportion of

Table 1: Blood gas values of neonates delivered of elective CS at progesterone level 2-5 ng/ml (Group I) and below 2 ng/mL(Group II).

Parameters	Group I	Group II	t-value	p-value
pH	7.16±0.02	7.14±0.02	0.434^{ns}	0.667
pO_2 (mmHg)	16.73±0.71	18.64±0.91	1.485^{ns}	0.148
pCO_2 (mmHg)	62.26±2.32	61.02±1.88	0.412^{ns}	0.683
TCO_2 (mmol/L)	22.68±0.3	23.36±0.78	0.822^{ns}	0.420
HCO_3^- (mmol/L)	21.48±0.5	21.3±0.78	0.165^{ns}	0.870
BE (mmol/L)	-10.72±0.36	-10.12±0.45	0.926^{ns}	0.363
sO_2 (%)	18.43±0.7	17.1±0.61	1.409^{ns}	0.169

ns: Non-significant (p>0.05).

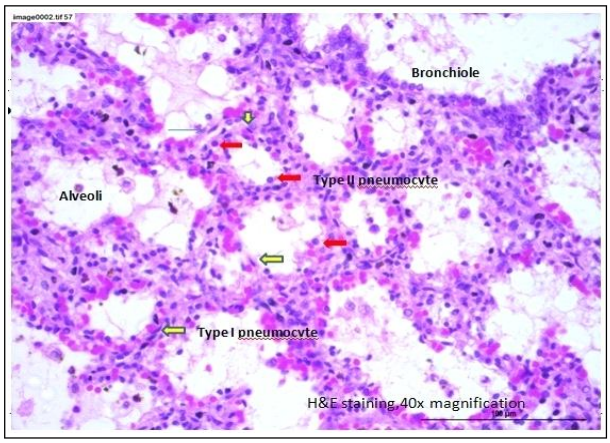


Plate 1: Histopathology of lung tissue collected from dead neonate (H&E staining, 400 ×), revealing type II pneumocytes, indicative of lung maturation. yellow arrow - type I pneumocyte, red arrow - type II pneumocytes

bitches. In 71.4 per cent of the dogs belonging to Group I, foetal membranes were not readily detachable during CS and copious bleeding from placental sites were noted when the detachment was attempted; whereas in bitches of Group II, bleeding from placental sites were negligible. Dogs in which placenta was left *in situ*, cervix dilation and expulsion of uterine contents were noticed 15 to 35 min (mean 25 min) after uterine closure.

No postoperative complications, including suture site infection, mammary gland affections or metabolic disorders were observed in any of the dogs that underwent elective CS and skin sutures were removed on day 10 postoperatively in all the dogs.

CONCLUSION

The current study showed that a decrease in serum progesterone below 2 ng/ml by day 63 post-ovulation was not a prerequisite for safe maternal and neonatal outcomes following elective CS in dogs. A good neonatal outcome obtained with prepartum P_4 levels between 2.0 to 3.71 ng/ml indicated the safety in the conduct of elective CS at P_4 levels ≤ 3.71 ng/ml. Identification of type II pneumocytes on lung samples of neonates indicate that foetal lung maturity is complete by day 63 after ovulation in canine fetuses and elective CS could be successfully attempted without the aid of any priming agents. However, reduced neonatal survival rates obtained in elective CS performed at P_4 levels of 4.18 ng/ml even in the presence of confirmed pulmonary maturity, needs further investigations.

Conflict of interest

All authors declare that they have no conflict of interest.

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