Maternal and neonatal wellbeing during elective C-section induced with a combination of propofol and dexmedetomidine: how effective is the placental barrier in dogs?

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Abstract

Anesthetics administered during C-section (CS) can cross the placenta and the fetal bloodbrain barrier leading to distress up to neonatal mortality. Therefore, to prevent neonatal suffering, sedatives and analgesics are not currently administered to the bitch until all pups are delivered. This study aims to evaluate the effect of a new anesthetic and analgesic protocol for elective CS in dogs, focused on both maternal and neonatal wellbeing. General anesthesia was induced by a combination of propofol (PPF) and dexmedetomidine (DEX), as analgesic, co-inductor, and sparing effect's agent, and maintained with isoflurane. Propofol and DEX concentrations in maternal blood, amniotic fluid, and placenta were correlated to maternal and neonatal parameters. Nine healthy purebred dogs scheduled for elective CS delivered 54 pups. About 78% of pups were vigorous at birth and assigned to the highest Apgar score (AS). The lowest AS was recorded in pups from mothers receiving additional doses of PPF ($p < 0.001$). Apgar scores improved with the increasing of pups' extraction time, starting from 30 minutes after induction ($p < 0.01$). No bitch showed post-operative pain or required additional analgesic doses. Maternal blood PPF and DEX, as well as placental PPF concentrations, decreased over time ($p < 0.01$). Conversely, placental DEX was fair uniformly detected in littermate pups. Both PPF and DEX were not detectable in amniotic fluid. Placenta resulted an effective barrier against fetal DEX exposure, making this protocol safe, analgesic and advisable for elective CS in dogs. Moreover, this study could contribute to clarify the controversy about the optimal extraction's time of pups after PPF induction.

Keywords: Analgesia; Anesthesia; C-section; Dexmedetomidine; Dog; Propofol

1. Introduction

Elective Cesarean section is a common procedure recommended in predisposed breeds, in dogs with previous history of dystocia, in aged primiparous bitches, and in case of singleton pregnancy or very small/large litter size [1]. Different anesthetic protocols and techniques have been reported for canine CS, including epidural anesthesia [2-7]. Epidural anesthesia is mainly appreciated for its analgesic effect leading to minimal maternal and neonatal depression [3,8]. However, addition of morphine which may require 2 to 6 days to be eliminated from the fetus may be needed in order to increase and lengthen post-operative analgesia and to avoid surgical manipulation perception of the patient [8-9]. Anesthetic induction with PPF and maintenance with isoflurane is routinely used during CS in dogs [2,4,10] because of the rapid and smooth maternal recovery due to its fast redistribution and metabolism [4,11]. Propofol and isoflurane have also been associated with a positive effect on neonatal survival and vitality [1,4,12]. However, cardiovascular and respiratory depression are also reported in newborns exposed to PPF [13,14]. Potential adverse effects on the newborn appear to be dependent on the PPF dosage as well as on the time lapse between induction and delivery [15]. In humans, PPF is reported to cross the placenta [13], but specific references in dogs are missing.

While DEX is approved, safe and effective as sedative and preanesthetic drug in dogs undergoing general surgery, there is some reluctance in its use in obstetrics as its effects have not been previously evaluated in this species [16]. Furthermore, DEX shows high placental retention in women [17]. No data are available in dogs.

Besides obvious ethical concerns, maternal and neonatal discomfort results in reduced colostrum/milk production and intake, in reduced maternal care for litter and increased mortality rate of pups [4]. In order to provide adequate anesthesia and analgesia during CS in dogs, we evaluated the impact of a combination of PPF and DEX, as analgesic, co-induction, and sparing effect's agent [18,19], on maternal and neonatal wellbeing. A dedicated Apgar score together with perioperative maternal parameters monitoring and pain assessment with a pain score system, allowed for objective evaluation of pups' viability and maternal comfort. In addition, transplacental transfer of both drugs was investigated. For this purpose, PPF and DEX were quantified in maternal blood, amniotic fluid, and placenta, and compared with the clinical outcomes. Furthermore, uterine position and presentation of the pups, placenta weight, birthweight and neonatal mortality within 48 h from birth were recorded and evaluated.

2. Material and methods

The present study complies with ethical standards and was conducted under the approval of the Ethical Committee of the Università degli Studi di Milano (OPBA_77_2017). Due consent was obtained from the owners of dogs before performing elective CS.

2.1 Animals

Nine healthy purebred bitches scheduled to undergo elective CS at the Reproduction Unit of the Department of Veterinary Medicine, Universita` degli Studi di Milano, Italy, were enrolled in this study. Dogs were deemed healthy according to physical examination and bloodwork. Age of the bitches ranged from 2.2 to 8 years (5.8 ± 1.8), and body weight varied from 31.5 to 62.6 kg (37.7 ± 8.1), as shown in Table 1.

Reproductive cycle was monitored from proestrus to parturition as routine, i.e. vaginal cytology, fetal biometric ultrasound evaluation (Esaote, MyLab™ Five VET, Trezzano,

Italy), and plasma progesterone measurement (ELFA method, miniVIDAS, Biomerieux, Marcy l'Etoile, France) in order to deduce LH surge and accurately estimate the date of delivery [10,20,21]. Aglepristone (Alizin, Virbac, Milano, Italy) SC at 15 mg/kg dose was administered about 20 hours before the expected date of delivery [22].

2.2 Anesthetic and analgesic protocol

Before performing anesthesia, bitches were premedicated with metoclopramide (Vomend, Eurovet Animal Health B.V., Torino, Italy) IM at 0.2 mg/kg dose. Five minutes preoxygenation was provided before anesthesia was inducted IV 1% PPF (PropoVet; Esteve, Bologna, Italy) at 2.5 mg/kg dose together with IV DEX (Dexdomitor; Orion Pharma, Espoo, Finland) at 2 µg/kg dose. If needed, additional doses of PPF were administered to effect in order to achieve tracheal intubation. After tracheal intubation, an adequate depth of anesthesia was maintained with isoflurane in 100% oxygen (IsoFlo; Esteve, Bologna, Italy) in mechanical ventilation as a means to maintain normocapnia during general anesthesia. Surgical procedures were routinely performed [10]. Fifteen minutes were waited between induction and pups' extraction. Whenever reanimation time of a pup overcame five minutes, atipamezole (Antisedan, Pfizer Italia Srl, Milano, Italy) was administered under tongue. After delivery of the last pup, bitches received IV methadone (Semfortan, Dechra Veterinary Products Srl, Torino, Italy) at 0.2 mg/kg dose. Post-surgical inflammatory pain was managed with meloxicam (Metacam, Boehringer Ingelheim, Milano, Italy) SC at 0.2 mg/kg dose at recovery. Atipamezole IM (10 µg/kg) was administered to bitches if needed to speed up recovery. Total duration of anesthesia was defined as the time from anesthetic induction to isoflurane discontinuation. Delivery time was defined as the time from anesthetic induction to extraction of the last pup.

2.3 Maternal and neonatal assessment

Maternal respiratory and heart rate, electrocardiogram (lead II), oxyhemoglobin saturation, end tidal carbon dioxide (Et-CO_2), end tidal isoflurane (Et-Iso) concentration, and noninvasive blood pressure were peri-operatively monitored every five minutes using a GE Datex-Ohmeda S/5 anesthesia monitor (Soma Technology, Inc., Bloomfield, CT, USA). Maternal postoperative pain was assessed by a trained observer using the Glasgow composite pain scale-short form [23]. Pain was assessed every ten minutes from extubation time until the complete recovery of the bitches. According to the scale, pain threshold was set at 6 out of 24 or at 5 out of 20 if mobility was impossible to assess. Maternal blood samples for PPF and DEX measurement were taken concurrently with each pups' extraction and with amniotic fluid's and placenta's collection. Immediately after delivery, fluid from the upper airways and oral cavity of each pup was aspired and pups rubbed, dried and kept warm. All pups were assigned a dedicated Apgar score (considering mucous membrane color, heart and respiratory rate, irritability reflex, motility, suckling, and vocalization) [10] within five minutes from birth. Each parameter was assigned a value of 0, 1, or 2. The resulting Apgar score, summing up each value, ranges from 0 to 14. Pups with a total 0 to 4 AS were considered as severely stressed (grade 0), those with 5 to 9 AS as moderately stressed (grade 1), and the ones with 10 to 14 AS as healthy (grade 2). Furthermore, uterine position, pups' presentation (cephalic or breech), birthweight, placental weight, newborn rectal temperature, and mortality within 48 h were recorded. Uterine position of pups was classified as cranial (uterine apex, that is

close to the ovaries), uterine horn (middle location) and caudal (close to the cervix). Pups in critical conditions were provided with emergency care.

2.4 Sampling procedure

To minimize stress associated with blood sample collection, two 18 G IV catheters were aseptically placed bilaterally into the cephalic vein of the bitches under general anesthesia just before undergoing CS. A venous blood volume of 1 mL was collected each time. Propofol was quantified from whole blood contained in K_2EDTA tubes while DEX from serum tubes centrifuged at 1500 g for 10 min soon after collection. Both whole K_2EDTA blood and serum were stored at -80°C for a maximum of 1 week before analysis. Amniotic fluid was taken from each pup at extraction using a 20 mL sterile syringe. A portion of each placenta 1 to 2 cm in side per side (max 5 g) was sliced at the zoned area. Both amniotic fluid and placenta samples were frozen and stored at -80°C until analysis. Propofol and DEX quantification from maternal blood, amniotic fluid and placental tissue was carried out by high performance liquid chromatography with fluorescence detection (HPLC-FL) and HPLC with mass spectrometry (HPLC-MS/MS), respectively [24-26].

2.5 Statistical analysis

Descriptive statistics for qualitative variables were expressed as percentage, those for quantitative variables as mean, standard deviation, median and quartiles of the distribution. The relationship between Apgar score and clinical variables was estimated by a regression model where AS was the response variable and each of the clinical variables was included as explicative one. The relationship between birth weight and placental weight was estimated by a regression model where birth weight was the response variable and placental weight was included as explicative one. The relationship between drugs' concentrations and extraction time was estimated considering as response variable the difference between current and initial drug concentration, and as explicative variable the extraction time. For continuous explicative variables the possible not linear relationship with the response variable was evaluated by three knots restricted cubic splines regression. The estimated relationship among clinical data and drugs' concentrations were obtained by a regression model based on generalized estimating equations (GEE), allowing to account for the correlation among littermates. The advantage of this approach is the lack of distributional assumption for model results' inference. The significance of regression coefficients was based on Wald test.
Given the low number of examined female dogs, only univariate analysis was performed. In case of significant not linear relationship, the cubic spline estimated coefficients are not of direct and useful interpretation thus results are presented by graphing the estimated relationship with 95% confidence intervals.
Values of $p < 0.05$ and $p < 0.001$ were considered statistically significant and highly significant, respectively.

3. Results

Cesarean section was performed between 64 and 65 days after the estimated LH surge. A total of 54 pups was delivered, 21 females and 33 males. Litter size ranged from 1 to 12 pups (6 ± 3.7) (Table 1). One pup was stillborn (ID. 3.1) and further four pups died within 48 h from birth, so neonatal mortality was 9.26%. Two out of the four dead pups died as a result of trauma caused by the mother (crush). The remaining two pups died for unknown causes.

No pup required atipamezole administration, neither after delivery nor at discharge. The average AS was 11.2 ± 2.7 SD (median 12, Q_1=10, Q_3=13). The 77.8% of pups were vigorous at birth, receiving highest grade of AS (grade 2). The lowest AS (9.4 ± 2.8 SD; $p < 0.001$) was assigned to pups from mothers receiving additional doses of PPF, i.e. from 2.7 up to 3.5 mg/kg. Apgar score of 12.8 ± 0.97 SD was assigned to pups from mother receiving fixed doses of PPF, i.e. 2.5 mg/kg. The relationship between AS and extraction time was not linear. Apgar score improved with increasing of pups' extraction time starting from 30 minutes after induction ($p = 0.0021$; Graph 1). Birthweight ranged from 236 to 732 g (521 ± 132.4 SD g, median 564 g, Q_1=420 g, Q_3=604 g), and placental weight varied from 16 to 150 g (66.2 ± 37.5 SD g, median 68.5, Q_1=30, Q_3=96). Apgar score increased with the pups' weight up to 540 g ($p < 0.01$). Similarly, birthweight increased with placental weight up to pups weighting 530 g ($p < 0.001$), beyond which it was no longer significant. The highest AS were assigned to pups with placental weight ranging from 40 to 100 g. Pups' presentation was recorded for 40 out of 54 pups (Table 2) with breech presentation slightly more frequently observed (52.5%) than cephalic one (47.5%). Four out of five dead pups had breech presentation. Apgar score was higher (11.3 ± 2.2 SD) in cephalic pups than in breech pups (9.7 ± 3 SD) (average decrease -0.961; $p < 0.001$). The position of the pups into the uterus did not influence their Apgar score. The average rectal temperature of pups at birth was 33.5 ± 0.8 °C.

Based on the litter size, time from induction of general anesthesia to extubation varied from 48 to 120 minutes (73.6 ± 20 minutes). Total pups' extraction time ranged from 16 minutes (ID. 4, singleton pregnancy) to 60 minutes (ID. 1, twelve pups). Each pup was extracted from 1 to 7 minutes (2.3 ± 1.1 minutes) after the previous littermate.

Maternal perioperative parameters were all within physiological ranges as shown in Table 3. Additional amounts of PPF were needed in four bitches (ID. 3, 6, 7, and 9) to achieve intubation, for a total dose of 2.7 up to 3.5 mg/kg. Maternal recovery was uneventful and rapid. Post-operative analgesia appeared adequate in all bitches as resulted from Glasgow composite pain scale-short form score less than 4 in all mothers. No bitch required additional analgesic doses. Only one bitch (ID. 4) received atipamezole in order to fast recovery. All bitches showed suitable maternal care.

Maternal blood PPF concentrations (range 0.24-2.8 µg/mL) showed a not linear decrease over time ($p < 0.001$; Graph 2). Considering as reference the initial drug's blood concentration, the decrease becoming significant starting from about 20 minutes after induction. Maternal blood DEX concentrations (range 0.41-2.04 ng/mL) were constantly decreasing over time with respect to the first drug's determination and extraction time. The average increase of the difference between actual and first drug's detection of 0.01 was estimated for each minute ($p < 0.05$; Graph 3). A similar decrease was observed in placental PPF concentrations (range 0.24-2.57 µg/mL) starting from about 30 min after induction ($p < 0.01$; Graph 4). Conversely, placental DEX (range 1.32-6.15 ng/mL) was uniformly detected in littermates regardless of the time. No maternal perioperative parameter was significantly related with PPF or DEX blood concentrations.

The comparison between maternal blood and placental concentrations of both drugs showed higher PPF and DEX values in placenta than in maternal blood over time ($p < 0.001$). This difference was more evident for DEX.

Both PPF and DEX were not detectable in the amniotic fluid.

Placental PPF and DEX concentrations were both not correlated to placental weight.

4. Discussion

Intraoperative selective sedation and analgesia of the mother without fetal involvement represents an idealistic ambition for anesthetic protocols during CS. In order to prevent neonatal drugs exposure, sedatives and analgesics are not administered to bitches before the last pup is delivered. Until now, placental transfer has only been indirectly inferred by ratio of maternal and umbilical venous drug concentrations or by *in vitro* placenta perfusion [14,27], which only provide a measure of the transplacental distribution equilibrium regardless to placental accumulation of the drug [17]. Before suggesting the use of new molecules in pregnant bitches, it is important to obtain information concerning the placental transfer and potential fetal exposure of the drug [17]. To the authors' knowledge, placental PPF and DEX concentrations were never directly obtained from placental tissue titration. Aiming to assess placental crossing and neonatal exposure to PPF and DEX after maternal administration during elective CS, we simultaneously measured concentrations of both drugs from maternal blood, amniotic fluid and placenta at birth. Measure fetal drug concentrations is extremely difficult in dogs as in humans [28]. Blood collection from newborn pups was excluded for ethical reasons. Thus, we opted for some risk-free biological matrices, i.e. amniotic fluid and placenta. Drugs can be detected in amniotic fluid due to placental diffusion and fetal urinary excretion [29,30]. These samplings do not involve hazards for the animals (mother and puppies) when collected at birth. In addition, amniotic fluid and placenta are usually discharged as waste in canine species.

Based on studies in women and ewe, it is known that PPF can quickly cross the placental barrier and reach the fetus [14,27] and has a slower elimination compared with the mother's [31-33]. However, the impact of PPF on newborn is largely undetermined and specific references on dogs are missing. A similar PPF concentration in maternal and umbilical vein blood 18 to 26 minutes after an induction dose of 2.5 mg/kg was reported in human, that is from 0.2 to 0.8 µg/mL [11,28]. Maternal blood PPF levels decreased with delivery time, as well as placental ones although with higher degree than blood. We obtained consistent results and values in the present study (Graph 2). A partial impeding of PPF passage to the fetus can therefore be hypothesized at the placental level. As reported in women [11], also in our samples PPF was not detectable in amniotic fluid. Both slow fetal elimination and single *bolus* PPF administration leading to a short exposure to the drug, can justify this result. In fact, the time interval between the induction of anesthesia and the collection of amniotic fluid, which occurred during the extraction of pups, was between 16 and 60 minutes. The half-life of distribution of PPF IV administered in the dog is 2 to 8 minutes, that of elimination is 30 to 70 minutes [34]. Based on PPF pharmacokinetics, in order to promote maternal clearance of the drug, we started extracting pups 15 minutes after induction. On the other hand, there is only one report in dogs recommending a time lapse of 18 to 20 minutes between PPF induction and pups' extraction to reduce respiratory depression of newborns [35]. According to that paper, we observed a significant decrease in maternal blood PPF concentration over time from 20 minutes after induction. Nonetheless, seen the small sample size, we can only speculate on the best time to perform pups' extraction after PPF induction. However, our results showed that AS improves 30 minutes after PPF induction.

Propofol does not provide analgesia, so additional drugs should be administered for surgical pain management. Currently, in human but not in veterinary medicine, DEX is used as

analgesic during CS due to its fast disappearing from maternal circulation and negligible transferring to fetal circulation [17]. Dexmedetomidine provides sedation, analgesia, and amnesia in women without depressing newborn respiratory function [36]. In human, the concentration of DEX is reported to be lower in maternal blood than in placenta in which it is constantly detected for at least 60 minutes [17]. A similar trend was observed in the present study, with maternal blood DEX concentrations decreasing over the time while placental ones remained constant regardless to extraction time of littermates. This result suggests a high placental retention also in dogs, probably due to the drug's high lipophilicity and affinity to imidazoline-receptors of which the placenta is rich, as reported in humans [17]. The absence of DEX in amniotic fluid can be attributed both to the efficacy of the placenta as a barrier against DEX passage and again to the short exposure time to this drug.

Propofol and DEX combination allowed a reduction in the dose of induction agent and therefore the potential amount reaching the fetuses together with the anesthetic costs. It is important to note that PPF dose for induction of general anesthesia in unpremedicated dogs ranges from 6 to 8 mg/kg [37-39]. The PPF doses required in the present study varied from 2.5 up to a maximum of 3.5 mg/kg. A competitive inhibitory mechanism on the enzyme cytochrome CYP450 involved in hepatic biotransformation and elimination of PPF and DEX has been assumed to explain the reduced doses required when PPF and DEX are given together [36,18,19]. Furthermore, in literature the combination of PPF and DEX is reported to protect the newborn from PPF-induced neurotoxicity and to deep the level of maternal sedation at recovery [36,18,19].

Maternal and neonatal wellbeing are priorities in management of CS. Time interval between birth and the first breath, and the first contact with and acceptance by the mother represent the most critical moments for pups [4]. The ability of the newborns to breathe and of the mothers to take care of their litters significantly depends on the anesthetic protocol [2,4,9,40]. Epidural anesthesia is considered the ideal protocol for CS due to both least effect on the fetuses and to analgesic efficacy on the mother [3,8]. However, no difference in AS and neonatal outcome has been reported comparing neuraxial and general anesthesia [8]. To date, no study highlights intra- and post-operative analgesic effectiveness of epidural analgesia during CS *per se*. The main advantage of epidural anesthesia is in not-requiring systemic drugs [3,41]. On the other hand, avoid general anesthesia involves the impossibility to intubate and ventilate the bitch, leading to decreased oxygenation of the fetuses during surgery and difficult management of anxious mothers [3]. Furthermore, the use of epidural anesthesia technique requires specialized expertise, in particular considering the difficulties in finding bone landmarks in patients with elevated body conditions scores such as pregnant ones. Dedicated material and increase in costs are implied as well. Opioids epidurals, compared to local anesthetics alone, are recommended to prevent maternal movements and to increase and prolong the analgesic effects [8,42]. However, systemic absorption due to rapid vascular uptake of morphine from the epidural space [43] can result in fetal depression. In order to reverse opioid-induced neonatal depression, opioid antagonists can be administered directly to neonates after delivery [9]. However, morphine administration showed serious drawbacks of re-narcotization due to the shorter action of naloxone compared with most opioid agonists and to long time required to fetal elimination (2 to 6 days). Therefore, recurring signs of narcosis after naloxone administration should be carefully monitored both in mother and pups and if needed additional doses should be provided [9].

The present study indicates that PPF and DEX combination is suitable for anesthesia induction and analgesia in bitches undergoing elective CS and safe for canine neonates.

Neonatal mortality in dogs undergoing elective CS is stated from 4 to 15% [1,10,41,44]. We recorded a 9.26% of mortality of pups within 48 h from birth, and 7.4% of mortality among the born alive pups. It should be noted that brachycephalic breeds, litter size higher than five, and aged bitches, as those enrolled in the present study, are associated with increased risk of stillbirth and neonatal mortality [1,41,45,46]. Moreover, two vital pups died for trauma and not due to surgical or anesthesia causes. Compared to our previous study using PPF and isoflurane for anesthesia in dogs during CS, the present protocol showed a higher survival rate of pups (90.7% *versus* 85.7%) [10]. We assigned the highest Apgar grade in about 78% of pups from mothers receiving combination of PPF and DEX compared to no pups from PPF and isoflurane protocol [10].

It is known that low birthweight in pups, as well as in babies, leads to higher risk of neonatal morbidity and mortality compared with normal weight littermates [47]. In agreement with literature, we recorded a positive correlation between AS and birthweight of pups. Furthermore, like in human [48], the weight of pups at birth was related to their placental weight, and the highest AS were assigned to pups with intermediate placental weight (40 to 100 g). In humans, both very low and very high placental weight is associated to fetal distress [48]. We obtained similar results in dogs. We hypothesized that placental tissue growth can affect the amount of nutrients conveyed to the fetus influencing its development and viability. It has been reported an increase in maternal but not fetal PPF clearance related to cardiac maternal frequency in sheep [28]. In our sample, all maternal perioperative parameters showed physiological values, without influencing blood PPF and DEX concentrations. The heart rate-lowering effects of DEX [49] could be implied.

Finally, there are some limitations in the present study. Firstly, it is a pilot study performed in healthy pregnant bitches during elective CS. Therefore, larger canine sample studies are required to confirm these findings in dystocic parturition leading to emergency CS. Furthermore, the different extraction times of pups and subsequently sampling times, complicated the statistical model for the comparison of results. For statistical purposes, sampling at established times, regardless the extraction time of pups, could be more suitable.

5. Conclusions

In conclusion, placenta resulted an effective barrier against fetal DEX exposure, making this protocol safe, analgesic and advisable for elective CS in dogs. It is important to emphasize that maternal comfort has an impact on maternal care for litter and on milk production with obvious implication for pups.

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References

[1] Moon PF, Erb HN, Ludders JW, Gleed RD, Pascoe PJ. Perioperative risk factors for puppies delivered by cesarean section in the United States and Canada. J Am Anim Hosp Assoc 2000;36:359-68.

[2] Luna SP, Cassu RN, Castro GB, Teixeira Neto FJ, Silva Junior JR, Lopes MD. Effects of four anaesthetic protocols on the neurological and cardiorespiratory variables of puppies born by caesarean section. Vet Rec 2004;154:387–89.

[3] Traas AM. Surgical management of canine and feline dystocia. Theriogenology 2008;70:337-42.

[4] Doebeli A, Michel E, Bettschart R, Hartnack S, Reichler IM. Apgar score after induction of anesthesia for canine cesarean section with alfaxalone versus propofol. Theriogenology 2013;80(8):850-4.

[5] Conde Ruiz CC, Del Carro AP, Rosset E, Guyot E, Maroiller L, Buff S, POrtier K. Alfaxalone for total intravenous anaesthesia in bitches undergoing elective caesarean section and its effects on puppies: a randomized clinical trial. Vet Anaesth Analg 2016;43(3), 281290.

[6] De Cramer KGM, Joubert KE, Nöthling JO. Puppy survival and vigor associated with the use of low dose medetomidine premedication, propofol induction and maintenance of anesthesia using sevoflurane gas-inhalation for cesarean section in the bitch. Theriogenology 2017;96:10-15.

[7] Jones RS. Epidural analgesia in the dog and cat. Vet J 2001;161:123-131.

[8] Robertson S. Anaesthetic management for caesarean sections in dogs and cats. In Practice 2016;38:327-339.

[9] Raffe MR and Carpenter RE. In: Tranquilli W, Thurmon J, Grimm KA, editors. Lumb & Jones' Veterinary Anesthesia and Analgesia. Section: Anesthetic management of Caesarean section patients. Blackwell Publishing. Fourth edition. Iowa, USA, 2007; p. 955-68.

[10] Groppetti D, Pecile A, Del Carro AP, Copley K, Minero M, Cremonesi F. Evaluation of newborn canine viability by means of umbilical vein lactate measurement, apgar score and uterine tocodynamometry. Theriogenology 2010;74(7):1187-96.

[11] Ragno G, Cicinelli E, Schonauer S, Vetuschi C. Propofol assay in biological fluids in pregnant women. J Pharm Biomed Anal. 1997;15(11):1633-40.

[12] Moon-Massat PF, Erb HN. Perioperative factors associated with puppy vigor after delivery by cesarean section. JAAHA 2002;38:90–96.

[13] Tumukunde J, Lomangisi DD, Davidson O, Kintu A, Joseph E, Kwizera A. Effects of propofol versus thiopental on Apgar scores in newborns and peri-operative outcomes of women undergoing emergency cesarean section: a randomized clinical trial. BMC Anesthesiol 2015;15:63.

[14] Andaluz A, Tusell J, Trasserres O, Cristòfol C, Capece BP, Arboix M, Garcia F. Transplacental transfer of propofol in pregnant ewes. Vet J 2003;166(2):198-204.

[15] Hu L, Pan J, Zhang S, Yu J, He K, Shu S, Wang R. Propofol in combination with remifentanil for cesarean section: placental transfer and effect on mothers and newborns at different induction to delivery intervals. Taiwan J Obstet Gynecol 2017;56:521-526.

[16] Kuusela E, Raekallio M, Väisänen M, Katja Mykkänen, Hannu Ropponen, Outi Vainio. Comparison of medetomidine and dexmedetomidine as premedicants in dogs undergoing propofol/isofluance anesthesia. Am J Vet Res 2001;62:1073-80.

[17] Ala-Kokko TI, Pienimäki P, Lampela E, Hollmén AI, Pelkonen O, Vähäkangas K. Transfer of clonidine and dexmedetomidine across the isolated perfused human placenta. Acta Anaesthesiol Scand 1997;41(2):313-9.

[18] Yokota H, Yokoyama K, Noguchi H, Nishioka T, Umegaki O, Komatsu H, Sakiki T. Post-operative dexmedetomidine-based sedation after uneventful intracranial surgery for unruptured cerebral aneurysm: comparison with propofol-based sedation. Neurocrit Care 2011;14(2):182-187.

[19] Cremer OL, Moons KG, Bouman EA, Kruijswijk JE, de Smet AM, Kalkman CJ. Longterm propofol infusion and cardiac failure in adult head-injured patients. Lancet 2001;357:117-118.

[20] Groppetti D, Vegetti F, Bronzo V, Pecile A. Breed-specific fetal biometry and factors affecting the prediction of whelping date in the German shepherd dog. Anim Reprod Sci 2015;152:117-22.

[21] Groppetti D, Aralla M, Bronzo V, Bosi G, Pecile A, Arrighi S. Periovulatory time in the bitch: what's new to know?: Comparison between ovarian histology and clinical features. Anim Reprod Sci 2015;152:108-16.

[22] Levy X, Fontaine E, Segalini V, Fontbonne A. Elective caesarean operation in the bitch using aglepristone before the pre-partum decline in peripheral progesterone concentration. Reprod Domest Anim 2009;44 Suppl 2:182-4.

[23] Reid J, Nolan AM, Hughes JML, Lascelles D, Pawson P, Scott EM. Development of the short-form Glasgow Composite Measure Pain Scale (CMPS-SF) and derivation of an analgesic intervention score. Anim Welf 2007;16, 97-104.

[24] Di Cesare F, Villa R, Ravasio G, Lucatello L, Pecile A, Groppetti D, Cagnardi P. Maternal, amniotic and placental concentrations of propofol and dexmedetomidine after administration for elective caesarean section in the bitch. J Vet Pharmacol Ther 2018;41(Suppl.1):161-162.

[25] Cagnardi P, Villa R, Ravasio G., Lucatello L, Di Cesare F, Capolongo F. Boccardo A,

Pravettoni D. Pharmacokinetics and sedative effects of dexmedetomidine in dairy calves. N Z Vet J 2017;65(1):14-17

[26] Zonca A, Ravasio G, Gallo M, Montesissa C, Carli S, Villa R, Cagnardi P. Pharmacokinetics of ketamine and propofol combination administered as ketofol via continuous infusion in cats. J Vet Pharmacol Ther 2012;35 (6):580-587.

[27] Bacon RC, Razis PA. The effect of propofol sedation in pregnancy on neonatal condition. Anaesthesia 1994;49:1058–1060.

[28] Ngamprasertwong P, Dong M, Niu J, Venkatasubramanian R, Vinks AA, Sadhasivam S. Propofol pharmacokinetics and estimation of fetal propofol exposure during mid-gestational fetal surgery: a maternal-fetal sheep model. PLoS One 2016;11(1):e0146563.

[29] Grey T, Huestis M. Bioanalytical procedures for monitoring in utero drug exposure. Anal Bioanal Chem 2007;388(7):1455–1465.

[30] Hostetter A, Ritchie JC, Stowe ZN. Amniotic fluid and umbilical cord blood concentrations of antidepressants in three women. Biol Psychiatry 2000;48:1032-1034.
[31] Juárez-Olguín H, Buendía-Soto E, Lares-Asseff I. Pharmacology for the fetus and the newborn. Gac Med Mex. 2015;151:361-8.

[32] Caruthers B. Kidney development and function in the fetus. The Surgical Technologist 1999:16-19.

[33] Grijalva J, Vakili K. Neonatal liver physiology. Semin Pediatr Surg 2013;22(4):185-9.

[34] Sato R, Aoki T, Kobayashi S, Uchida N, Simamura S, Yamasaki M. The modulating effects of propofol and its lipid carrier on canine neutrophil functions. J Vet Med Sci 2017;78(12):1825-1829.

[35] Short CE and Bufalari A. Propofol anesthesia. Vet Clin North Am Small Anim Pract 1999;29:747-78.

[36] Li C, Li Y, Wang K, Kong X. Comparative Evaluation of Remifentanil and Dexmedetomidine in General Anesthesia for Cesarean Delivery. Med Sci Monit 2015;21:3806-13.

[37] Sams L, Braun C, Allman D, Hofmeister E. A comparison of the effects of propofol and etomidate on the induction of anesthesia and on cardiopulmonary parameters in dogs. Vet Anaesth Analg 2008;35(6):488-94.

[38] Ko JC, Golder FJ, Mandsager RE, Heaton-Jones T, Mattern KL. Anesthetic and cardiorespiratory effects of a 1:1 mixture of propofol and thiopental sodium in dogs. J Am Vet Med Assoc 1999;215(9):1292-6.

[39] Morgan DW, Legge K. Clinical evaluation of propofol as an intravenous anaesthetic agent in cats and dogs. Vet Rec 1989;124(2):31-3.

[40] Pascoe PJ, Moon PF. Periparturient and neonatal anesthesia. Vet Clin North Am Small Anim Pract. 2001;31(2):315-40.

[41] Vilar JM, Batista M, Pérez R, Zagorskaia A, Jouanisson E, Díaz-Bertrana L, Rosales S. Comparison of 3 anesthetic protocols for the elective cesarean-section in the dog: Effects on the bitch and the newborn puppies. Anim Reprod Sci 2018;190:53-62.

[42] Behar M, Olshwang D, Magora F, Davidson JT. Epidural morphine in treatment of pain. The Lancet 1978;313(8115):527-529.

[43] Rawal N, Sjöstrand U, Dahlström B. Postoperative pain relief by epidural morphine. Anesth Analg. 1981;60(10):726-31.

[44] Moon PF, Erb HN, Ludders JW, Gleed RD, Pascoe PJ. Perioperative management and mortality rates of dogs undergoing cesarean section in the United States and Canada. JAVMA 1998;213:365–69.

[45] Batista M, Moreno C, Vilar J, Golding M, Brito C, Santana M, Alamo D. Neonatal viability evaluation by Apgar score in puppiesdelivered by cesarean section in two brachycephalic breeds (English and French bulldog). Anim Reprod Sci 2014;146:218–226.

[46] Tønnessen R, Borge KS, Nødtvedt A, Indrebø A. Canine perinatal mortality: a cohort study of 224 breeds. Theriogenology 2012;77(9):1788-801.

[47] Groppetti D, Pecile A, Palestrini C, Marelli SP, Boracchi P. A National Census of birth weight in purebred dogs in Italy. Animals (Basel) 2017;7(6). pii: E43.

[48] Janthanaphan M, Kor-Anantakul O, Geater A. Placental weight and its ratio to birth weight in normal pregnancy at Songkhlanagarind Hospital. J Med Assoc Thai 2006;89(2):130-7.

[49] Moran-Muñoz R, Valverde A, Ibancovichi JA, Acevedo-Arcique CM, Recillas-Morales S, Sanchez-Aparicio P, Osorio-Avalos J, Chavez-Monteagudo JR. Cardiovascular effects of constant rate infusions of lidocaine, lidocaine and dexmedetomidine, and dexmedetomidine in dogs anesthetized at equipotent doses of sevoflurane. Can Vet J 2017;58(7):729–734.

Fig. 1. Apgar score in relation to pup's extraction time.

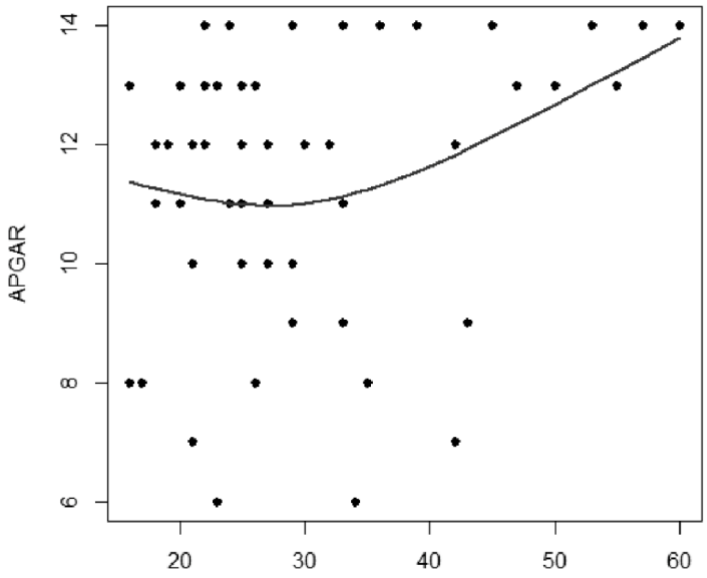

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Table 2 Pups features.

ID.	Gender	Position	Presentation	Apgar score	bBW (g)	Delivery Time (min)
1.1	M	L c	ND	12	575	30
1.2	M	L h	ND	14	579	33
1.3	M	L h	ND	14	563	36
1.4	F	L h	ND	14	629	39
1.5	M	L h	ND	12	590	42
1.6	M	L a	ND	14	565	45
1.7	F	L h	ND	13	553	47
1.8	M	R c	ND	13	604	50
1.9	M	R h	ND	14	573	53
1.10	F	R h	ND	13	600	55
1.11	M	R a	ND	14	616	57
1.12	M	R h	ND	14	630	60
2.1	M	L c	P	11	640	25
2.2	M	R c	P	11	732	27
2.3	M	R a	P	12	698	30
3.1	M	R c	P	0	600	21
3.2	F	R h	C	14	511	22
3.3	M	R a	C	11	380	24
3.4	F	L h	C	12	614	25
4.1	F	R h	C	13	396	16
5.1	F	R c	C	12	490	19
5.2	F	R h	C	13	441	20
5.3	F	L c	ND	12	453	22
5.4	M	L h	ND	13	530	23
5.5	M	L h	P	13	514	25
5.6	M	L a	C	13	450	26
6.1	F	L c	P	11	484	18
6.2	M	L h	C	11	546	20
6.3	F	R h	C	13	434	22
7.1	M	L c	C	8	586	16
7.2	M	L h	P	8	589	17
7.3	F	L h	C	10	600	21
7.4	F	R c	P	8	660	23
7.5	M	R h	P	11	593	25
7.6	M	R h	P	11	502	27
7.7	M	R h	C	10	600	29
7.8	M	R a	P	12	681	33
8.1	M	L c	C	13	673	19
8.2	M	L h	P	14	729	21
8.3	F	L a	P	16	415	24
8.4	F	R c	C	13	604	27
8.5	F	R h	C	16	715	29
8.6	M	R a	P	14	710	32
9.1	M	R b	P	14	330	18
9.2	M	R h	C	8	264	21
9.3	M	R h	C	14	342	23
9.4	M	L b	P	12	310	25
9.5	F	L h	P	9	236	26
9.6	F	L h	P	11	325	29
9.7	M	L h	P	10	317	33
9.8	M	L h	P	7	300	34
9.9	F	L a	C	9	397	35
9.10	F	R h	C	8	367	42
9.11	F	R a	P	10	320	43

‘ID’ identifies the mother followed by the puppies numbered progressively according to delivery time; 'M' means male; 'F' means female; ‘L’ means left uterine horn; ‘R’ means right uterine horn; ‘a’ means cranial apex of the uterine horn (close to the ovary); ‘h’ means middle of the uterine horn; ‘c’ means caudal of the uterine horn (close to the cervix); ‘B’ means caudad; ‘C’ means cephalad; ‘ND’ means not detected; ‘bBW’ means birthweight.

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Table 3
Pre- and intra-operative parameters in bitches undergoing elective CS using a combination of PPF and DEX.

ID.	RT (°C)		HR (bpm)		RR (cpm)		BP (mm Hg)	TDA (min)
	Pre-operative	Intra-operative	Pre-operative	Intra-operative	Pre-operative	Intra-operative	Intra-operative	
1	37.8	36	100	94	40	24	75	120
2	37.9	35.8	130	105	50	25	78	68
3	38	36.1	122	89	25	20	108	65
4	38	37.4	160	112	80	30	94	48
6	37.6	36.3	100	70	100	20	72	60
7	37.8	36.1	120	90	40	29	90	75
8	38.3	35.7	140	100	85	43	79	85
9	37.9	36.5	135	98	70	48	98	74
10	37.7	35.4	156	112	54	18	76	67
Mean	37.9	36.1	129.2	96.7	60.4	28.5	85.5	73.5
SD	0.2	0.6	21.4	13.1	24.7	10.5	12.4	20.2

'RT' means rectal temperature; 'HR' means heart rate; 'RR' means respiratory rate; 'BP' means blood pressure; 'TDA' means total duration of anaesthesia.

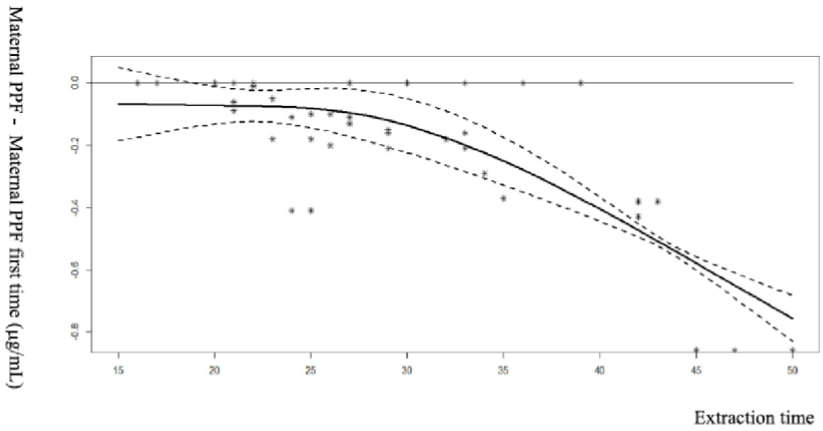

Fig. 2. Maternal blood PPF concentrations over time
This graph represents the change of maternal PPF blood concentration over extraction time with respect to the baseline value. Baseline value of PPF was the concentration in maternal blood collected at the time of the first pup's extraction. The decrease of maternal blood concentration as a function of extraction time was estimated by a regression model (continuous line) with lower and upper (dotted lines) confidence limit (95%). The decrease of PPF concentration was constant till about 25 min after induction and then started to rise with extraction time. The thin horizontal line traced at 0 represents the reference value for "no change in PPF concentration". When 95% confidence limits are below this line a statistically significant decrease in PPF concentration occurs, that is after about 20 min. sample observations (*).

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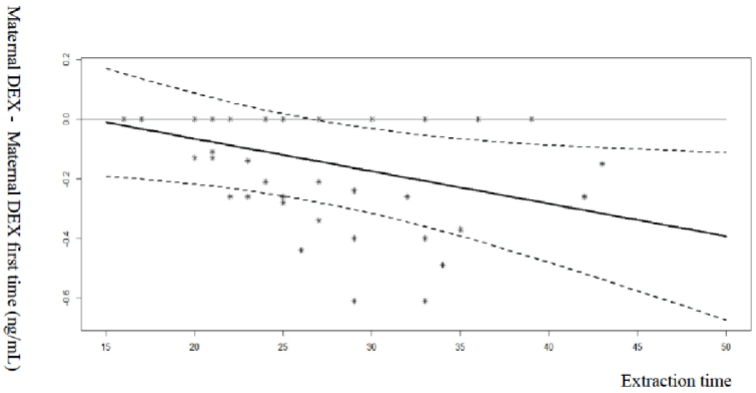

Fig. 3. Maternal blood DEX concentrations over time
This graph represents the change in maternal DEX blood concentration over extraction time with respect to the baseline value. Baseline value of DEX correspond to the concentration in maternal blood collected at the time of the first pup's extraction. The decrease of maternal blood concentration as a function of extraction time was estimated by a regression model (continuous line) with lower and upper (dotted lines) confidence limit (95%). The decrease of DEX concentration rose with extraction time. The thin horizontal line traced at 0 represents the reference value for "no change in DEX concentration". When 95% confidence limits are below the "no change" line a statistically significant decrease in concentration occurs, that is after about 30 min sample observations (*).

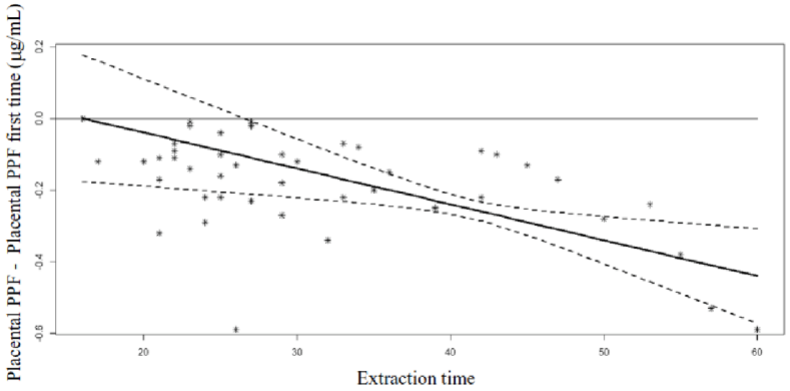

Fig. 4. Placental PPF concentrations over time