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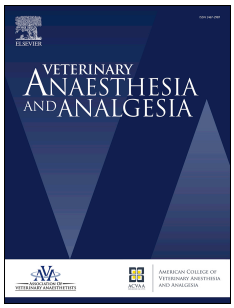

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Evaluation of methadone concentrations in bitches and in umbilical cords after epidural or systemic administration for caesarean section. A randomized trial

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Authors' contributions

All authors participated in data acquisition; moreover CL and AB performed data's analysis and interpretation, and drafted the paper; MC participated in data's interpretation and revised the paper; GB participated in data's analysis and in revising the paper; SA participated in data's interpretation and in drafting the paper; DZ participated in the study design, revised the paper and approved the final version; AZ participated in data interpretation and revised the paper; NR conceived the study, participated in the data's interpretation, revised the paper and approved the final version.

Declaration of interest

Authors declare no conflict of interest

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Abstract

Objective To measure plasma methadone concentrations in bitches and the umbilical cords of their puppies after systemic or epidural administration.

Study design Prospective, randomised, clinical study.

Animals A total of 27 healthy pregnant female dogs undergoing caesarean section, 4.3 ± 2.3 years of age and weighing 19.9 ± 13.2 kg.

Methods The dogs were randomly divided into three groups: 1) intramuscular methadone (0.3 mg kg^{-1}) (group MET; *n* = 9); 2) epidural methadone (0.1 mg kg^{-1}) (group METEPI; *n* = 9) and 3) epidural lidocaine (4.4 mg kg^{-1}) (group CON-control group; *n* = 9). Ten minutes before induction, methadone was administered intramuscularly to the group MET dogs. Anaesthesia was induced with propofol and maintained with isoflurane. Cardiovascular and respiratory parameters were monitored throughout the anaesthesia. After induction, epidural anaesthesia was administered to dogs in groups METEPI and CON. Before any treatment (T0) and, as soon as the last foetus was removed from the uterus (T1), venous blood samples were collected from each dog using heparinised tubes; the umbilical cords were collected and stored at -80°C until pharmacological analysis was carried out. The samples were analysed using ultra performance liquid chromatography.

Results The cardiorespiratory parameters of the bitches and of the puppies at birth, and the Apgar scores did not differ significantly between groups. At T1 both the median maternal methadone plasma concentration and the median methadone umbilical cord

concentration were significantly higher in group MET compared to group METEPI (p=0.0018 and p=0.004). The maternal plasma concentration was significantly higher than the concentration in the umbilical cords (p=0.05) in group METEPI but not in group MET (p=0.25).

Conclusions and clinical relevance Epidural methadone (0.1 mg kg^{-1}) administered to bitches undergoing caesarean section is associated with lower umbilical cord concentrations as compared with intramuscularly administered methadone at higher dosages (0.3 mg kg^{-1}).

Keywords caesarean section, dog, epidural anaesthesia, methadone.

Introduction

In bitches, more than 60% of dystocias result in surgical caesarean section (Münnich & Kuchenmeister 2009; Smith 2012; Martins-Bessa et al. 2016). The anaesthetic protocol adopted for a caesarean section should provide adequate muscle relaxation, analgesia and narcosis to ensure optimal operating conditions (De Cramer et al. 2017). In addition, it must be safe for both the bitch and the foetus since most anaesthetic drugs cross the foetus blood-brain barrier, resulting in neurological and cardiorespiratory depression of the puppies (Luna et al. 2004; Conde Ruiz et al. 2016). Currently, propofol and isoflurane are the anaesthetic drugs most commonly used for the induction and maintenance of general anaesthesia for caesarean sections in dogs (Doebeli et al. 2013). In a previous study, the authors found that the administration of propofol for the induction of general anaesthesia was associated with less depression of the puppies at birth and a lower mortality rate as compared with the administration of thiopentone, or ketamine and midazolam (Luna et al. 2004).

In human medicine, epidural anaesthesia (EA), combining local anaesthetic drugs and opioids, has been used to provide analgesia during labour or caesarean section for many years (Bader et al. 1995; Fernando et al. 1997; Jones et al. 2012). Luna et al. (2004) described that the respiratory rate of puppies born from bitches receiving EA with methadone or lidocaine was higher when compared with those born from bitches in which midazolam/ketamine or propofol for induction and enflurane for maintenance of general anaesthesiawere used (Luna et al. 2004). Epidural anaesthesia, by means of a sparing effect or elimination of inhalant anaesthetics, decreases the risk of excessive neonatal respiratory depression, and improves the comfort of the bitch that is then more likely to nurse the puppies after delivery (Aarnes & Bednarski 2015; Robertson 2016).

However, when local anaesthetic are administered epidurally, hypotension is a common complication, due to a sympathetic blockade, especially in haemodynamic-compromised animals (Jones 2001).

Epidurally administered methadone did not induce significant cardiovascular alterations in healthy dogs (Campagnol et al. 2012). To date, no information is available regarding the placental transfer of methadone in dogs and the respective maternal/foetal plasma concentration ratios. The aim of this study was to compare methadone concentrations in the plasma of the bitches, and those in the veins and arteries of the umbilical cords of their puppies after systemic or epidural administration during caesarean section.

Materials and Methods

Animals

The study was conducted in accordance with the provisions of European Directive 2010/63/UE, adopted by the Italian Government. Privately owned pregnant female dogs which were presented to the Veterinary Teaching Hospital (VTH) of the University of Blogna for dystocia and which underwent emergency caesarean section from December 2014 to December 2016 were included in this study up to a maximum of 30 dogs in accordance with the Local Ethical Committee. The number of animals included in the study was based on the mean of number of dogs admitted for caesarean section at the VTH in the previous five years. Written informed consent was obtained from the owner of each dog before starting the procedure.

The health status of each bitch was assessed by means of clinical examination, and haematological and biochemical parameters. Upon arrival, each bitch underwent a complete obstetrical examination and an ultrasound evaluation.

Bitches under one year of age and those previously treated with methadone (7 days before anaesthesia for caesarean section) were excluded from the study.

Study design

All the bitches included were randomly divided into three groups using a random dice roll. Allocation into a specific group was carried out by the same anaesthetist using online software (http://www.roll-dice-online.com).

Epidural lidocaine 2% (4.4 mg kg^{-1}) was administered to the dogs in the control group (group CON), intramuscular (IM) methadone (0.3 mg kg^{-1}) to the dogs in group MET, and epidural methadone (0.1 mg kg^{-1}) to group METEPI. The same expert anaesthetist who was aware of treatment designation performed the anaesthesia and the epidural puncture.

Anaesthetic protocol

Ten minutes before induction, methadone (Eptadone; Molteni Farmaceutici, Italy) (0.3 mg kg^{-1}) was administered IM in the quadriceps muscles to the bitches in group MET while, in the dogs in the other two groups, an analogous volume of saline solution (NaCl 0.9%-placebo; BBraun, Germany) was administered using the same route. After IM administration, intravenous catheters (22 gauge) were placed into both the left and the right cephalic veins. The left cephalic vein was used for drug injection and for the administration of Lactated Ringer's solution (Ringer lattato; ACME, Italy) at the rate of 10 mL kg^{-1} $hour^{-1}$; the right cephalic vein was used for blood collection.

Anaesthesia was induced with propofol (Propovet; Esteve, Italy), administered intravenously (IV), and titrated to effect to allow endotracheal intubation. Endotracheal

intubation was attempted when masticatory and eyelid muscle tone were decreased, and a ventromedial rotation of the eyeball was observed. General anaesthesia was maintained with isofluorane (Isoflo; Abbott Laboratories Ltd, IL, USA) delivered in oxygen (100%) via a rebreathing system; the vaporizer was adjusted by the anaesthetist in order to obtain a stable surgical anaesthetic depth based on physical signs (reflexes).

During the procedure, the following parameters were continuously monitored with a multiparametric monitor (Datex-Ohmeda- S3; Datex-Ohmeda Inc, WI, USA) and recorded every five minutes: heart rate (HR), respiratory rate (f_R), end-tidal carbon dioxide tension ($P_E'CO_2$), fraction of expired isoflurane in % ($F_E'Iso$), haemoglobin oxygen saturation using a pulse oximeter (SpO_2), non-invasive blood pressure using a Doppler device (Minidrop ES-100 VX; Hadeco, Japan) and body temperature using an oesophageal probe. A forced-air warming blanket (Bair Hugger; 3M, UK) was used to maintain physiologic body temperature. The duration of the anaesthesia was defined as the time from the anaesthetic induction to the extubation of the bitches.

After instrumentation, and five minutes after induction, the bitches in groups METEPI and CON were positioned in sternal recumbency with the hind limbs positioned forward in order to administer the epidural block into the lumbosacral space (L7-S1); the correct placement of the spinal needle (BD Spinal Needle; Becton Dickinson, Spain) was confirmed using the hanging drop technique. Epidural anaesthesia was administered using methadone or lidocaine 2% (Lidocaina cloridrato; S.A.L.F. Spa, Italy) in groups METEPI and CON, respectively. Before injection, the methadone and the lidocaine were both diluted with NaCl 0.9%, if needed, in order to achieve a final volume of 0.25 mL kg^{-1} up to a maximum of 10 mL. The epidural injection was administered slowly over one minute. Hypotension was defined as systolic blood pressure (SAP) lower than

80 mmHg. In the case of hypotension, isoflurane administration was decreased, if possible, and a bolus of crystalloid (10 mL kg^{-1}) was administered intravenously. In the case of persistent hypotension (more than ten minutes), dobutamine was administered (0.005-0.01 mg kg^{-1} $minute^{-1}$).

Within 5 minutes of birth, HR using a stethoscope, f_R by inspection of the thorax and the Apgar score (modified for puppies by Veronesi et al. 2009) were evaluated and recorded. In detail, the Apgar score was applied to evaluate the vitality and distress of the newborns and ranged from 0 to 3 meaning severe distress; 4 to 6 meaning moderate distress and 7 to 10 meaning no distress. After the last puppy was taken from the uterus, methadone was administered IM once at a dose of 0.1 mg kg^{-1} in the bitches in group CON for treating postoperative pain. All the bitches were discharged from the VTH soon after recovery. After discharge, postoperative pain was managed by the referral private veterinarian.

Sample collection

Immediately before the IM administration of methadone (group MET) or a placebo solution (groups METEPI and CON) (T0) and as soon as the foetuses were removed from the uterus (T1), venous blood (2 mL) was collected from each bitch using heparinised tubes and was immediately centrifuged. The plasma was then stored at −80°C until the assay was carried out. Blood samples were collected from the right venous catheter; before each sampling, 2 mL of blood was collected and was then reinjected in order to avoid contamination with the flushing solution. After blood collection, the venous catheter was flushed with 2 mL of NaCl 0.9% saline solution.

As soon as the last puppy had been taken from the uterus, the umbilical cords were removed and stored individually in sterile vials at -80°C until analysis.

Sample analysis

The plasma samples were extracted following a previously published method (Shakleya et al. 2007) with slight modifications. After thawing the samples at 4°C, 200 µL of plasma was transferred to a microtube, and deuterated internal standard (methadone-d3) was added, followed by 600 µL of acetonitrile. The tube was then vortex-mixed for 30 seconds, centrifuged at 7'000 ×*g* at 4°C for 10 minutes; the supernatant was then evaporated to dryness under a gentle nitrogen stream at 35°C. The dry extract was finally reconstituted with 200 µL of mobile phase, consisting of a mixture of 0.1% formic acid in water and acetonitrile (80/20, v/v), and was vortex-mixed for 30 seconds before transferring the contents into a chromatography vial.

A procedure previously validated in humans (De Castro et al. 2013) was adapted to measure the methadone concentrations in the canine umbilical cords. After thawing the collected pools of umbilical cords at 4°C, for each brood 1 g was homogenized in a polypropylene tube containing 5 mL of water using a T25 digital Ultra-Turrax (IKA; Germany) at 24,000 rpm for 2 minutes. The internal standard and 50 µL of formic acid 10% were then added; the tube was then vortex-mixed for 30 seconds and centrifuged for 15 minutes at 5'000 ×*g* at 4°C. The supernatant underwent a clean up step using an SPE Oasis MCX 3cc 60mg cartridge (Waters; Milford, MA, USA) and was eluted with 3 mL of a methanol-ammonium hydroxide (95:5, *v/v*) solution. The sample was then evaporated to dryness under nitrogen and was reconstituted with 200 µL of a 0.1% formic acid aqueous solution-acetonitrile (80/20, *v/v*) mixture. After vortex-mixing and

centrifuging for 10 minutes at 10'000 ×*g* at 4°C, 150 µL of the sample was transferred into a chromatography vial for analysis.

Methadone quantification was carried out using a Waters Aquity ultra performance liquid chromatography (UPLC) binary pump equipped with an Aquity BEH C18 (50 × 2.1 mm, 1.7 µm) column and coupled to a Quattro Premier XE triple quadrupole mass spectrometer (Waters; Milford). The column was kept at 35°C and the mobile phase consisted of a mixture of 0.1% formic acid aqueous solution and acetonitrile at a 0.5 mL minute^{-1} flow rate under programmed conditions. The mass spectrometer operated in positive electrospray ionisation (ESI+) and in MRM (multiple reaction monitoring) mode. The specific transitions observed were: methadone: 310>265 *m/z* and 310>105 *m/z* and methadone-D3: 313.1→268 *m/z*. The capillary voltage was set at 2.00 kV, and the source and desolvation temperatures were 120 and 350°C, respectively; desolvation and cone gas flows were set at 700 and 100 L hour^{-1}, respectively.

The analytical method was validated in accordance with the EMEA/CHMP/EWP/192217/2009 guidelines before the experiment started, providing satisfying performances over a range of 0.5 to 500 ng mL^{-1}.

Statistical analysis

Demographic data and plasma concentrations are reported as mean ± standard deviation (SD). The 95% confidence interval (CI) of the median is reported for plasma and umbilical methadone concentrations. The data were evaluated for normality using a Shapiro-Wilk test. Normal data were compared using one-way ANOVA while not normally distributed data were compared using a Kruskall Wallis test. The plasma concentration of each bitch and that obtained from the respective umbilical cord pool

were compared using a Wilcoxon test for paired samples. The statistical data were calculated using commercial software (MedCalc 6.3; MedCalc Software, Belgium). Data were considered significant at $p<0.05$. At the end of the study, a post hoc power calculation was carried out using computer software (STATA; StataCorp, TX, USA).

Results

Animals

Twenty-seven healthy bitches were included in the study, nine bitches in each group. The mean weight and ages of the bitches was 25.3 ± 13.6 kg, 19.2 ± 12.2 kg and 15.3 ± 13.3 kg, and 3.8 ± 1.6 years, 4.1 ± 1.5 years and 4.8 ± 3.3 years for groups CON, MET and METEPI, respectively. No statistical differences in age and weight were detected among the three groups. All the bitches required an emergency caesarean section due to dystocia. The dogs included belonged to several breeds, with the French bulldog being the most represented. The mean anaesthesia duration did not differ significantly between groups and was 87.7 ± 24.8 minutes in group CON, 95.6 ± 26.0 minutes in group MET and 80.0 ± 32.1 minutes in group METEPI. The number of puppies born in each group, their HRs, their f_Rs, the Apgar scores and the mortality rates did not differ significantly between groups and are reported in Table 1.

The dose or propofol used for induction of general anaesthesia did not differ significantly between groups and was 3.7 ± 2.2 mg kg^{-1} for group MET, 5.1 ± 1.6 mg kg^{-1} for group METEPI and 4.7 ± 1.6 mg kg^{-1} for group CON

The mean F_E´Iso was 1.4 ± 0.2% in group CON and 1.3 ± 0.2% in both group MET and group METEPI without no significant differences between groups. In each dog that was administered an epidural, the aspiration of the “hanging drop” of saline from the needle

hub was observed and increased resistance was felt by the operator while advancing the needle through the *ligamentum flavum*.

In group MET, a preterm caesarean section was performed in a Springer spaniel in which labor began early (approximately 55 days of gestation) because of hypoluteidism and none of her three puppies responded to the resuscitation manoeuvre. In the same group, three puppies from a French bulldog died at birth. In group METEPI, one puppy from a pug and one puppy from an English setter died at birth, but foetal suffering had been diagnosed upon arrival.

In group MET, the last puppy was removed from the uterus and the second blood sample was collected from each bitch (T1) at 35.1 ± 9.9 minutes after IM methadone administration; in group METEPI, the last puppy was removed 25.5 ± 11.9 minutes after epidural administration and a blood sample (T1) was collected. The time interval between methadone administration and T1 did not differ significantly between the two methadone-treated groups.

For all bitches, recovery from anaesthesia was smooth and uneventful. The bitches did not have any complications related to the anaesthesia, to the epidural technique or to the surgical procedure.

Cardiovascular parameters

The mean HR, f_R and SAP of the bitches are reported in Table S1. These parameters did not differ significantly between the groups at any time point.

Some dogs in group METEPI (6/9) experienced transient hypotension (SAP< 80 mmHg) while only 3/9 and 4/9 of the dogs in groups MET and CON, respectively had

hypotension during the procedure. However, the incidence of episode of hypotension did not differ between groups. The transient hypotension was treated by decreasing the isoflurane and by administering a bolus of crystalloid (Lactated Ringer's solution 10 mL kg^{-1}). More dogs in group METEPI (3/6 hypotensive dogs) experienced mild and transient hypotension within five minutes after induction; among the hypotensive animals, the mean SAP was 69 ± 4 mmHg.

Plasma and umbilical cord concentrations

In the samples collected from the animals in group CON, no signal corresponding to methadone was detected.

At T1, the median maternal methadone plasma concentration was 19.0 (range 9.0-56.2; 95% CI: 13.5-31.3) ng mL^{-1} and 6.4 (range 5.1-9.6; 95% CI: 5.2-8.8) ng mL^{-1} in groups MET and METEPI, respectively (Figure 1). The median methadone concentration in the umbilical cords was 15.6 (range 12.1-25.3; 95% CI: 12.3-23.3) ng mL^{-1} in group MET and 3.9 (range 1.2-8.4; 95% CI: 1.9-5.4) ng mL^{-1} in group METEPI (Figure 1). Both the median methadone concentrations in the maternal plasma and in the umbilical cords were statistically higher in group MET compared to group METEPI (p=0.0018 and p=0.004, respectively). In group MET the maternal methadone plasma concentration and the umbilical cord concentration did not differ significantly (p=0.25).

In group METEPI, the methadone concentration was higher in the maternal plasma compared to the concentration in the umbilical cord (p=0.046) and they differed by 39%.

Discussion

In the present study, the methadone concentrations in the plasma of the bitches and in the umbilical cords of their puppies were evaluated after epidural or systemic administration for analgesia during emergency caesarean section.

There is a paucity of information concerning the pharmacokinetics of methadone in dogs after epidural administration (Garrett et al. 1985; Schmidt et al. 1994; Ingvast-Larsson et al. 2010). In human medicine, epidurally administered methadone reached peak plasma concentrations within 10-20 minutes, similar to those observed after IM injection, in the same patients (Max et al. 1985). In the present study, the blood samples for the determination of maternal methadone plasma concentration were collected approximately 9.6 minutes later in group METEPI but IM administered methadone resulted in higher maternal plasma and foetal umbilical cord concentrations compared with those obtained after epidural administration. This difference, despite the 10 minutes of delay for blood collection, might not be only due to due to the different routes of administration but also to the different dosages used. In addition, the plasma methadone concentration after IM injection obtained at the moment in which the last puppies were removed from the uterus was wide: 9.0-56.2 ng mL^{-1}. This is an expression of individual variability, as previously described after extravascular injection of methadone in dogs (Ingvast-Larsson et al. 2010). In fact, absorption after extravascular injection depends on several factors; in particular, on regional perfusion but also on the age, size and breed of the dogs considered (Kukanich & Wiesse 2015).

In the present study, overall mortality among the puppies was 12%, slightly higher than that reported by Luna and colleagues (2004). Since only emergency caesarean sections were included in the present study, the mortality rate might have been influenced by several factors other than the analgesic drug administered, such as the conditions of

labour and the puppies' clinical condition before the anaesthetic procedure. Moreover, in all the dogs, propofol, which crosses the placenta quickly, was administered for anaesthesia induction, and general anaesthesia was maintained with isoflurane in all dogs. Conversely, in the study of Luna and colleagues (2004), dogs in which epidural anaesthesia was performed did not receive general anaesthesia, and the puppies born from those bitches experienced the least respiratory depression compared with those born from bitches receiving propofol, thiopentone or ketamine and midazolam for induction and with enflurane for maintenance of general anaesthesia. In humans, neonatal depression after propofol administration for anaesthesia induction is correlated with the dose administered (Sanchez-Alcaraz et al. 1998). However, the authors did not evaluate the correlation between the dosage of propofol used and the outcome of the puppies as this was beyond the aim of the study.

The present study has several limitations. First, only twenty-seven dogs were included; they all underwent emergency caesarean section, and foetal sufferance had already been diagnosed at presentation. Therefore, morbidity and mortality among the puppies cannot be correlated only with the anaesthetic protocol used. A scheduled caesarean section could have led to different results in the outcomes of the puppies.

In addition, dogs are multiparous and have short umbilical cords; the technique applied allowed evaluation of the umbilical cord concentration using a pool of samples without differentiating among the puppies, or between venous and arterial umbilical samples (Desprats et al. 1991). In humans, the evaluation of the concentration of the drugs in the umbilical cord is of interest for evaluating the correlation between the anaesthetic protocol and the outcome of the foetus.

The cardiorespiratory parameters of the bitches were similar in the three groups. The limited number of animals included might account for the lack of statistically significant differences in cardiorespiratory parameters between groups; however the primary aim of the study was evaluation of the methadone concentration rather than the physiological effect of the anaesthetic protocols used. Hypotension after epidural administration of local anaesthetics is mainly seen in sick animals in which the compensatory mechanisms are unable to counteract the reduced sympathetic tone (Jones 2001). In healthy animals, epidural lidocaine or epidural methadone have been reported to produce only minimal cardiorespiratory changes (Cruz et al. 1997). When methadone was administered epidurally in isoflurane anaesthetised dogs at dosages of 0.1 mg kg^{-1}, a gradual increase in HR and SAP was observed; however, these changes were not significant when compared with placebo-treated dogs (Bosmans et al. 2011). When the effects of methadone (0.5 mg kg^{-1}) administered by an epidural or an intravenous route were compared, no significant differences in HR and blood pressure were reported (Campagnol et al. 2012). In pregnant animals, blood pressure monitoring is pivotal, and hypotension must be promptly corrected. When pregnant animals are positioned in dorsal recumbency, the enlarged uterus can compress the caudal vena cava thus reducing the venous return to the heart chambers and consequently, the cardiac output; therefore, decreased uterine perfusion may result. In the present study the blood pressure was measured non-invasively; however, the doppler device was demonstrated to have a specificity of 97% and a sensitivity of 56% in detecting hypotension in anaesthetised dogs (Kennedy & Barletta 2015). Even if some animals experienced hypotension, it was immediately and successfully corrected by decreasing the isoflurane

and administering a bolus of fluids. Moreover, most of the dogs in which methadone was administered by the epidural route experienced hypotension soon after induction.

In group METEPI, no premedicant drugs were administered prior to induction and a higher dose of propofol was necessary to achieve an adequate anaesthetic plane to perform intubation. Interestingly, both methadone treated groups had a similar mean FE´Iso. This was in accordance with a previous experimental study regarding isoflurane anaesthetised dogs which showed a similar sparing effect of epidural and intravenous methadone up to 2.5 hours after administration, with the epidural methadone providing a longer lasting sparing effect (Campagnol et al. 2012).

Another limitation is the fact that no intraoperative and postoperative pain evaluation was carried out and the correct execution of the epidural anaesthesia was not confirmed by means of a radiographic evaluation. All the epidural punctures were performed by the same expert anaesthetist and their success was confirmed in all dogs in groups METEPI and CON by the hanging drop technique, namely by the operator who felt the change in resistance while passing the *ligamentum flavum* and inspected the needle hub for signs of cerebrospinal fluid or blood before drug injection. In addition, the anaesthetic plane was stable in all patients and no changes in HR, f_R and SAP were observed in response to the surgical stimulation. The hanging drop technique has been described to be an effective method of confirming needle tip location in the extradural space in 88% of dogs in which EA was performed in sternal recumbency (Naganobu & Hagio 2007). Failures of the technique were described to be only false negative results; on the contrary, false positive responses were not observed.

Conclusion

In conclusion, epidurally administered methadone (0.1 mg kg^{-1}) in bitches undergoing caesarean section was associated with lower umbilical cord methadone concentrations as compared to concentrations after IM methadone administration at higher dosages (0.3 mg kg^{-1}). These protocols applied for emergency caesarean section were associated with a puppy mortality rate of 17.7%. More studies are needed to determine the effects of these protocols on the clinical parameters of puppies born from scheduled caesarean sections.

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443 **Figure 1** Box-and-whisker plots of the methadone concentrations in the maternal
444 plasma and the umbilical cords of puppies born from 18 bitches undergoing caesarean
445 section. Blood samples from the bitches and the umbilical cords were collected as soon
446 as the last puppy was removed from the uterus. The bitches received systemic
447 methadone (group MET) or epidural methadone (group METEPI). ( ) Maternal
448 plasmatic concentration; (----) umbilical cord concentration. (*) statistically
449 significant difference ($p< 0.05$).

450 **Table 1** Number of puppies, heart rate (HR), respiratory rate (f_R), Apgar score and
451 mortality in puppies born from bitches undergoing emergency caesarean section and
452 receiving epidural lidocaine 2%, 4.4 mg kg^{-1} (group CON), intramuscular methadone,
453 0.3 mg kg^{-1} (group MET) or epidural methadone, 0.1 mg kg^{-1} (group METEPI). Heart
454 rate, f_R and Apgar score were recorded within 5 minutes after birth. Heart rate and f_R are
455 reported as means ± standard deviation; Apgar scores are reported as median (range).

456

Parameters	**group CON**	**group MET**	**group METEPI**
Number of puppies	35	35	30
HR (beats $minute^{-1}$)	193.7 ± 37.7	165.3 ± 79.1	171 ± 70
f_R (breaths $minute^{-1}$)	10.1 ± 4.1	9.1 ± 5.2	9.2 ± 5.2

Apgar score	7 (0-10)	6 (0-10)	6 (0-10)
Mortality of puppies (n=)	2	6	4
Mortality rate (%)	5.7	17.1	13.3

Table S1 - Systolic blood pressure (SAP), heart rate (HR), respiratory rate (f_R) of 27 bitches undergoing emergency caesarean section and receiving epidural lidocaine (group CON), intramuscular methadone (group MET) or epidural methadone (group METEPI) are reported. Data were collected every five minutes from induction until the end of the anaesthesia and are reported as mean ± SD.

Minutes	SAP (mmHg)			HR (beats minutes^{-1})			f_R (breaths minutes^{-1})		
	CON	MET	METEPI	CON	MET	METEPI	CON	MET	METEPI
5	135.4 ± 20.2	122.4 ± 26.6	104.0 ± 29	126.1 ± 20.7	132.6 ±8.8	128.0 ± 19.8	17.1 ± 6.1	18.7 ± 12.6	25.2 ± 14.5
10	132.6 ± 22.5	129.6 ± 16.4	110.2 ± 22.3	126.4 ± 20.5	128.3 ± 12.4	128.3 ± 15.6	17.6 ± 7.8	20.1 ± 11.7	23.9 ± 13.0
15	126.1 ± 18.9	129.0 ± 16.3	113.6 ± 17.0	122.9 ± 29.2	121.8 ± 13.7	121.7 ± 9.5	15.9 ± 9.0	19.9 ± 11.7	20.6 ± 14.7
20	118.5 ± 25.5	118.2 ± 20	107.1 ± 14.0	122.1 ± 26	120.3 ± 14.5	120.6 ± 16.4	15.1 ± 6.0	18.8 ± 10.4	15.9 ± 5.8
25	116.1 ± 30.6	116.1 ± 21.6	107.2 ± 14.1	117.7 ± 22.9	119.0 ± 15.5	118.1 ± 16.0	13.1 ± 4.1	18.8 ± 10.4	16.2 ± 11.0
30	125.1± 25.8	120.9 ± 20.1	105.7 ± 16.7	116.4 ± 21.7	115.7 ± 17.8	119.9 ± 17.1	13.4 ± 6.7	19.1 ± 10.7	14.1 ± 7.2
35	103 ± 36.5	116.0 ± 17.5	105.6 ± 17.0	121.5 ± 16.4	114.7 ± 13.8	117.8 ± 18.8	15.6 ± 7.4	19.2 ± 10.5	15.5 ± 7.2
40	115.1 ± 40.4	118.0 ± 20.1	110,7 ± 20.1	122.8 ± 13.7	117.1 ± 14.3	116.4 ± 15.7	16.3 ± 8.2	18.4 ± 10.7	15.1 ± 9.0
45	121.5 ± 22.8	117.0 ± 15.9	104.7 ± 18.0	124.4 ± 15.5	115.6 ± 18.7	117.0 ± 17.3	18.9 ± 8.9	17.4 ± 10.8	15.6 ± 7.7
50	114.7 ± 24.5	114.4 ± 18.6	105,4 ± 14.2	120.5 ± 14.0	113.0 ± 17	117.4 ± 19.5	14.5 ± 6.8	19.2 ± 11.6	17.1 ± 7.2
55	123.8 ± 28.4	115.8 ± 22.3	104,5 ± 15.7	124.4 ± 12.0	115.2 ± 15.7	118.4 ± 20.2	16.3 ± 7.5	17.8 ± 11.2	17.0 ± 8.0
60	118.0 ± 20.4	118.8 ± 21,2	107.8 ± 18.8	121.3 ± 10.7	114.1 ± 16.4	115.4 ± 20.6	15.3 ± 8.7	17.0 ± 10.5	17.8 ± 8.0
65	113.5 ± 36.7	121.3 ± 20.1	113.8 ± 19.0	118.4 ± 7.2	110.6 ± 15.6	113.8 ± 26.9	15.0 ± 7.5	18.7 ± 10.1	12.5 ± 7.0
70	108.6 ± 25.8	121.5 ± 15.4	104.5 ± 12.7	118.6 ± 7.8	110.1 ± 17.8	115.5 ± 28.8	13.6 ± 7.9	18.7 ± 10.1	13.5 ± 7.0
75	113.7 ± 22.8	121.6 ± 10.8	113.0 ± 11.6	118.7 ± 8.0	112.6 ± 18	112.5 ± 25.0	16.4 ± 7.8	19.0 ± 10.1	14.0 ± 7.0
80	108.2 ± 24.8	125.6 ± 15.6	110.0 ± 12.0	115.8 ± 8.0	112.4 ± 17.0	125.0 ± 8.7	16.3 ± 7.0	19.0 ± 10.1	14.0 ± 7.0
85	110.0 ± 25.4	122.5 ± 13.0	107.3 ± 7.0	113.3 ± 10.3	110.0 ± 19.0	126.7 ± 11.5	17.8 ± 6.9	20.2 ± 12.0	10.7 ± 5.1
90	123.0 ± 30.40	122.1 ± 9.8	111.3 ± 6.1	112.5 ± 8.7	110.0 ± 19	126.7 ± 11.5	14.0 ± 5.6	19.8 ± 12.0	13.7 ± 5.1
95		121.5 ± 9.4	116.3 ± 11.5	114.3 ± 10.0	108.4	129.3 ± 16.2	11.3 ± 2.3	19.8 ± 12.0	14.3 ± 6.0
100		121.2 ± 11.4	110.7 ± 4.6	108.5 ± 9.1	110.4	130.0 ± 17.3	11.0 ± 1.4	19.8 ± 12.0	12.7 ± 4.0

105	115.0 ± 7.0	108.3 ± 12.3	108.5 ± 9.2	118.0 ± 10.6	130.0 ± 14.1	8.5 ± 4.9	22.8 ± 11.6	11.5 ± 9.1
110	115.5 ± 6.4	118.0 ± 8.5		117.5 ± 10.4	126.5 ± 12.0		22.8 ± 11.6	10.5 ± 7.8
115	115.0 ± 7.4	127.0 ± 4.2		117.0 ± 10.3	127.5 ± 17.7		22.8 ± 11.6	10.5 ± 7.8
120	115.0 ± 6.1	114.5 ± 7.8		117.5 ±10.4	130.0 ± 21.1		22.3 ± 11.9	9.5 ± 6.4
125		112.0 ± 5.6			127.5 ± 17.7			10.0 ± 7.1
130		119.5 ± 6.4			131.5 ± 23.3			11.5 ± 9.2

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