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Combination of ketamine and xylazine to reduce pain during electroejaculation in dogs

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Abstract. The present study evaluated the efficiency of a ketamine-xylazine combination to attenuate the pain associated to electroejaculation (EEJ) in dogs. To that end, 10 dogs of undetermined breed were anesthetized (i.m.) with a mixture of 8mg.kg^{-1} ketamine hydrochloride (Cetamin®, Syntec, Brazil) and 1mg.kg^{-1} IM xylazine hydrochloride (Xilazin®, Syntec, Brazil) and subjected to EEJ procedures. Painful stimuli were detected by cardiorespiratory rate, measured every 5 min, and body temperature, taken before and after EEJ. Mean values for the parameters evaluated decreased, suggesting that the anesthesia protocol used is safe for semen collection by EEJ and can relieve the pain associated to this procedure.

Keywords: Collection, semen, mammals

Combinação da cetamina e da xilazina para reduzir a dor durante a eletroejaculação em cães

Resumo. Este trabalho avaliou a eficiência da combinação entre a cetamina e a xilazina para reduzir a dor associada à eletroejaculação (EEJ) em cães. Para tanto, 10 cães sem raças definidas foram anestesiados (I.M.) com a associação de 8,0 mg. kg^{-1} de cloridrato de cetamina (Cetamin®, Syntec, Brasil), e 1,0 mg.kg^{-1} IM de cloridrato de xilazina (Xilazin®, Syntec, Brazil), sendo então submetidos ao procedimento de eletroejaculação. Os estímulos dolorosos foram avaliados pela aferição das frequências cardíacas e respiratórias, mensuradas a cada 5 minutos; e temperatura retal, antes e após a EEJ. A média das variáveis avaliadas diminuiram, sugerindo que este protocolo anestésico usado é seguro para a coleta de sêmen por eletrojaculação e pode evitar a dor associada a este procedimento.

Palavras chave: Coleta, sêmen, mamíferos

Combinación de ketamina y xilacina para reducir el dolor durante la electroeyaculación en perros

Resumen. Este trabajo evaluó la eficacia de la combinación entre ketamina y xilazina para reducir el dolor asociado con la electroeyaculación (EEJ) en perros. Para eso, se anestesiaron 10 perros sin razas definidas (I.M.) con la asociación de 8,0 mg. kg^{-1} de hidrocloruro de ketamina (Cetamin®, Syntec, Brasil) y 1,0 mg.kg^{-1} IM de hidrocloruro de xilazina (Xilazin®, Syntec, Brasil), siendo luego sometidos al procedimiento de

electroeyaculación. Los estímulos dolorosos se evaluaron midiendo las frecuencias cardíaca y respiratoria, medidas cada 5 minutos y temperatura rectal, antes y después de EEJ. El promedio de las variables evaluadas disminuyó, sugiriendo que este protocolo anestésico utilizado es seguro para la recolección de semen por electroyaculación y puede evitar el dolor asociado a este procedimiento.

Palabras clave: Colectar, semen, mamíferos

Introduction

Pain is an unpleasant sensorial or emotional experience associated to total or potential tissue destruction (Lozano et al., 2020). Electrical stimuli applied in electroejaculation (EEJ) procedures to collect semen causes extreme discomfort or pain in humans (Halpern et al., 2020); consequently, this procedure is assumed to be painful to animals as well. Cortisol is used as an indicator of animal welfare, but this is not suitable to measure pain associated to EEJ. Because EEJ is usually carried out in the same treatment areas that animals associate to routine stressful experiences such as vaccination (Whitlock et al., 2012), cortisol levels may increase irrespective of the procedure applied. Heart frequency of awake animals is another parameter that can be altered by a particular environment.

In bulls subjected to EEJ, adrenal progesterone production has been used as an endocrine pain indicator (Whitlock et al., 2012), while the main variables used to indicate pain in dogs are heart rate, respiratory rate and body temperature (Grubb et al., 2020). These physiological variables are determined before and after the potentially painful stimuli (Grubb et al., 2020).

Ketamine is a dissociative anesthetic frequently applied along with α2-adrenergic agonists such as xylazine and romifidine. Some studies monitoring cardiorespiratory rates in dogs showed that pain perception, which lasts from 30 to 45 min in response to mechanical stimuli (using a forceps clamped to the skin of the abdominal region close to the umbilicus, anal sphincter and interdigital space) (Hahn & Warner, 2010) reaching up to 60 min in elective castration (Kropf & Hughes, 2019) can be blocked by ketamine/xylazine anesthesia.

A range of anesthetics can be used alone or in combination for chemical pain relief during dog EEJ. In rabbit, for example, epidural xylazine administration eliminates EEJ-caused pain and reduces heart rate, however there was no anesthetic effect when administered intravenously (Orihuela & Ungerfeld, 2019). Another common problem observed during EEJ procedures is urine-contaminated semen. In order to avoid this, α-2 adrenergic action of xylazine combined with ketamine is used to induce bladder neck closure (Martin et al., 2003).

The present study assessed the use of a ketamine/xylazine combination to attenuate pain in dogs during sine-wave EEJ by monitoring physiological pain indicators.

Material and methods

Ten dogs of undetermined breed, apparently healthy, weighing 7.6 to 22.2kg (mean=15.7 ± 1.5kg) and considered suitable for semen collection by electroejaculation, were used. Dogs underwent clinical and ultrasonographic examination at a veterinary hospital before the experiment, which was approved by the Ethics Committee for Animal Use of the local university (Universidade Estadual do Norte Fluminense - UENF), Protocol 67/2009.

Animals were held in individual cages for 3 days to acclimatize them. They were fed a commercial diet (BillDog Premium®, Extrutecnica, Brazil) twice a day and provided with water ad libitum for the two first days. On the third day, dogs were deprived of solid food for 12h before being anesthetized and undergoing the EEJ protocol.

Semen samples were collected in the Reproductive Clinic for Small Animals of the Veterinary Hospital - UENF, under controlled temperature (±23° C). The electroejaculator (Santa Lydia Laboratórios – Brazil) operated with a voltage of 0-10.9V, current of 100 and 200 mA and sine wave frequency of 50 and 60 Hz. It was equipped with a 25.4 cm probe (1.6cm diameter) and two longitudinal 8-cm electrodes (Newell-Fugate, 2009; Ohl et al., 1994).

Before initiating the EEJ protocol, dogs were chemically restrained using i.m. application of 8 $mg.kg^{-1}$ ketamine hydrochloride (Cetamin®, Syntec, Brazil) and 1$mg.kg^{-1}$ xylamine hydrochloride (Xilazin®, Syntec, Brazil). After anesthesia induction, radial artery catheterization was performed to administer a saline solution at flow rate of 5 mL/kg/h. Animals were considered to be under deep anesthesia when they lost postural control and stayed in dorsal decubitus position. At this stage, they were subjected to EEJ.

The probe was lubricated with KY gel® (Johnson & Johnson, Brazil), inserted through the rectum and positioned over the prostate. The entire protocol consisted of 10 series of 10 electrical stimuli using 100mA current at 50Hz for 3s and 3s of rest. Electrical voltages applied in the 1 to 10 series were: 1.5V, 2.4V, 3.0V, 4.0V, 4.2V, 4.9V, 5.9V, 7.3V, 8.8V, and 10.9V. Stimulus intensity was gradually increased until the dog started to ejaculated. At this stage, electrical stimuli continued to be applied at the same voltage until the end of ejaculation. After ejaculation, stimulation stopped even though the sequence of 10 series had not been completed. If ejaculation was not achieved by the 8th stimulus application, the probe was removed and repositioned over the prostate to apply the last two stimuli.

A stethoscope was used to auscultate heart and respiration rates every 5 min. Rectal temperature was measured before and after this procedure.

Chemical restraint lasted 20 min. If the animal showed voluntary head movements or any vocalization, electroejaculation was stopped and half of the anesthetic dosage initially used was applied i.m. Only after animals recovered from deep anesthesia were electrical stimuli continued until ejaculation.

After semen collection and volume measurement, sperm was subjectively examined through microscopy to assess total motility (%) and vigor, an indicator of motility quality classified on a 0- to-5 scale ranging from stationary cells (irrespective of tail movements) to progressive swimming. To that end, 10µL aliquots of semen were placed on 22 x 22 mm slides, covered with a coverslip and observed at 100x and 400x magnification. Sperm concentration was also determined by diluting semen samples in formol-saline solution (1:10) and counting sperm in a Neubauer chamber. To assess sperm morphology in each sample, 200 sperm in wet preparation were evaluated and classified (Oettlé, 1993) under phase contrast microscopy at 1000x magnification.

Data were analyzed using version 2.11.1 of the R software package (2010). Data normality was analyzed using the Shapiro-Wilk test at the 0.05 level of significance. Because data on respiratory rate did not show a normal distribution (even after transformation), the non-parametric Wilcoxon signed rank test was used to compare data between initial and subsequent experimental phases, at the 0.05 level of significance. Temperature data exhibited normal distribution and was evaluated using ANOVA and Student's t test for paired samples. Data are shown as mean and standard error.

Results and discussion

Data on heart and respiratory rates during EJC are shown in Table 1. Rectal temperature was 39.0±0.1°C at the beginning and 38.7±0.2°C at the end of the experiment (P=0.09).

Table 1. Vital signals (mean ± S.E.) of dogs during electroejaculation (N =10).

Variables	Time (min.)				
	0	5	10	15	20
Heart rate (HR)	96±7	89±6	78±6†	86±11	76±6
Respiratory rate (RR)	24±2	18±2†	13±1†	13±1†	15±3†

†In a same row, indicates statistical difference from time 0 (Wilcoxon signed rank test, P < 0.05).

Ejaculation by EEJ was obtained in 100% of the dogs. Antegrade ejaculation was observed in 80% of the dogs, while only 20% exhibited retrograde ejaculation (when the ejaculate is redirected to the urinary bladder).

Mean motility of the recovered sperm was 10.0 ± 4.5% (0-40%), semen volume was 1.9 ± 0.8mL (0.07-5 mL) and sperm count 68.1 ± 23.8 (7.5-150) million spermatozoon.

Heart and respiratory rates did not increase during EEJ, indicating that the animals were anesthetized and did not experience pain in response to the electrical stimuli. These results are consistent with the study carried out by Perry et al. (2019) who used the EEJ technique under anesthesia (*alphaxalone* 15 mg/kg intravenously) once a week, for four weeks in *Chamaeleo calyptratus*, and did not observed differences in morphology between the ventilation scores after the first week. The authors concluded that serial electroejaculation procedures under anesthesia with *alfaxalone* can be safely performed on *Chamaeleo calyptratus*. In general, the administration of anesthetics, sedatives or hormones reduces the pain and stress caused by EEJ, and can improve sperm quality, but results may vary depending on the species (Abril-Sánchez et al., 2019).

Anesthesia protocols are known to depress the cardiorespiratory system (Haskins et al., 1986), which explains the decrease in the respiratory rate 5min after anesthesia ($P < 0.05$). This finding corroborates other studies reporting that animals under deep anesthesia experience a reduced heart rate 15 min after ketamin-xylazine injection, irrespective of the use of levomepromazine as pre-anesthetic medication (Krause et al., 2016). In addition, the ketamine-xylazine combination is considered safe for maintaining the cardiorespiratory functions of dogs (Abdelhakiem et al., 2019). As observed in the present study, the xylazine-ketamine combination had a neutral effect on both HR and RR, causing non-significant changes (Abdelhakiem et al., 2019). Sinus dysrhythmia, an early indicator of cardiac arrest, is an important aspect to be considered (McEvoy et al., 2018), but this was not evaluated here.

The University of Glasgow, UK, developed a pain scale based on behavioral responses to painful stimuli such as surgery (Morton et al., 2005). The Glasgow pain scale was modified by Murrell et al. (2008) to include parameters such as attitude (aggressive, uninterested, nervous/anxious, quiet/indifferent, happy/content), posture (rigid, hunched, normal), comfort (uncomfortable, comfortable), vocalization (cry, groan, scream, quiet), attention to the surgical wound (chewing, licking/looking/rubbing, ignoring), mobility (refusing to move, stiff, slow/reluctant, lame, normal), response to touch (cry, flinch, snap, growl/guard, nothing). However, the physiological pain indicators adopted in the present study were important to indicate pain perception. Variables such as vocalization and cortisol increase can demonstrate the animal's reaction to determinate discomfort or recall of painful practices associated to the area where EEJ procedures were applied rather than pain (Amaral et al., 2017).

One noteworthy aspect of the present study is that EEJ was performed with sine waves, which increase semen collection efficiency, since they require lower voltage than quadratic waves to trigger ejaculation (Castelo et al., 2015). Therefore, the dog may ejaculate in a short period of time, before recovering from anesthesia. This avoids the application of another anesthetic dose to continue the EEJ protocol.

Conclusion

In conclusion, the ketamine/xylazine combination is efficient for pain control in dogs subjected to semen collection by electroejaculation.

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