

Clinical Studies

Effects of Electro-acupuncture on Shelter Cat Anesthesia Recovery from Ovariohysterectomy: A Randomized and Controlled Clinical Study

Darla Jo Ingerson

ABSTRACT

Spay-neuter programs are crucial to effective community management of unowned animals but face challenges of limited funding and personnel. Identification of cost-effective sterilization protocols while providing comparable quality veterinary care is needed. The objective of the current study was to investigate the effects of atipamezole dose reduction with or without electro-acupuncture (EAP) for comparable anesthetic recovery to a higher dose of atipamezole (greater program cost) following ovariohysterectomy (OHE). A total of 56 shelter cats were enrolled and randomized into 4 anesthetic recovery treatment groups: Group A (control) was treated with 10mcg/kg intramuscular (IM) atipamezole; Group B was treated with 5mcg/kg IM atipamezole + EAP; Group C was treated with EAP only; and Group D was treated with 5mcg/kg IM atipamezole. Recovery time was recorded from post-surgery treatment to first observation of 1) voluntary movement, 2) swallow reflex, 3) head lifting and 4) ambulation. Study results found that anesthesia recovery for patients using EAP only (Group C) had a significantly longer duration than all other groups ($p < 0.001$). Groups B and D (low dose atipamezole with or without EAP) were not significantly different from each other or the high dose animals (Group A). Group B recovery (with EAP), however, was nonsignificantly shorter for all 4 outcome measures when compared to Group D (without EAP). Clinical relevance of study findings suggests 5mcg/kg atipamezole provides an equally effective recovery from general anesthesia when compared to 10mcg/kg for shelter animals undergoing OHE.

Keywords: electroacupuncture, ovariohysterectomy, recovery, reduced atipamezole, shelter medicine, spay recovery, traditional Chinese veterinary medicine

Shelter medicine has gained traction in recent years in response to kill shelters and overpopulation of pets. To assist with overpopulation and to help lower the number of animals being euthanized, clinics that perform high-quality, high-volume spay-neuter programs have been established. These facilities specialize in efficient systems and surgical techniques to provide affordable sterilization for pets, Trap-Neuter-Release programs or feral colony care. The availability of services provided by these low-cost, high-volume clinics is primarily limited by cost of materials and pharmaceuticals required to perform the surgical procedures. Finding alternative methods to reduce cost and maintain quality of care would permit low-cost clinics to offer more services in a more economical and efficient way.

Current treatment for performing an ovariohysterectomy (OHE) requires sedation, analgesia, anesthesia and recovery support. These procedures create inherent risks and potential complications for the patient.¹ The 5 most common complications are hypothermia, arrhythmia, hypotension, hypoventilation and delayed recovery.²⁻⁴ Of these, delayed recovery is one of the most important and can exacerbate hypothermia, hypoventilation and

hypotension.^{2,3,5} To avoid complications, recovery treatments are started as soon as possible after completion of the surgical procedure with recovery time goals between 15 to 30 minutes.^{3,6} Reversal agents such as the alpha2-antagonist atipamezole help provide smoother recovery with a shorter duration from the OHE procedure. The cost of this drug, however, is often a limiting factor for the number of spays that can be performed at low-cost, high-volume clinics.

Traditional dry needle acupuncture (DNAP) provides stimulation to nerve endings and bundles. Stimulation of the nerves can be caused by several factors, the most common being direct stimulation of the needle and stimulation through the release of histamine.⁷ This effect can be increased by passing an electrical current through the needle. This is referred to as electro-acupuncture (EAP),

Author Professional Degrees and Certifications: DVM, MS-TCVM, CTCVMP; **From:** Louisiana State University School of Veterinary Medicine, Baton Rouge, Louisiana, USA; and Chi University, Reddick, Florida, USA; ***Address correspondence to** Dr. Darla Jo Ingerson (drdingers79@gmail.com).

which is designed to stimulate nerves using a small electrical current with alternating frequencies, facilitating the release of endogenous opioids such as endorphins and serotonin, shown to reduce pain and inflammation.⁸⁻¹⁰

In traditional Chinese medicine and traditional Chinese veterinary medicine, energy flows through Channels. There are 12 regular Channels and 8 extraordinary Channels.¹¹ The 8 extraordinary Channels connect and coordinate the 12 regular Channels.¹¹ These Channels also store and regulate Blood and *Qi*. The *Du* (Governing Vessel) and *Ren* (Conception Vessel) Channels are the only extraordinary Channels to have their own acupuncture points.¹¹

There are 28 acupuncture points on the *Du* Channel and 2 were selected for use in this study. One of them was GV-1b, also known as *Wei-jian* (tail tip). *Wei-jian* is located at the tip of the tail and is indicated for shock, heatstroke and paralysis (Figure 1).¹¹ The depth of the point is 0.1-0.3 *cun* with the needle inserted perpendicularly.¹² The second acupuncture point used was GV-26 (*Shui-gou/Ren-zhong*). GV-26 is located at the philtrum in animals

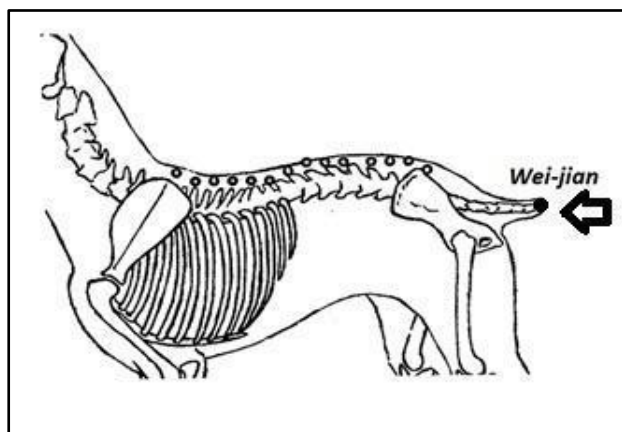


Figure 1: Illustration of the location of acupuncture point, *Wei-jian*, which is located at the tip of the tail and is indicated for shock, heatstroke and paralysis.¹¹

MATERIALS AND METHODS

Animals

The study subjects were cats admitted to local shelters Cat Haven and Companion Animal Alliance (CAA) located in Baton Rouge, Louisiana, USA. These shelters work closely with the Louisiana State University School of Veterinary Medicine (LSU-SVM) conducting spay and neuter clinics. The bulk of the research was performed at LSU-SVM and authorized by Cat Haven and the veterinarians in the Shelter Medicine Department (IACUC Protocol# 17-026). Cat Haven and CAA were willing to assist in the research. All female cats that were scheduled to undergo OHE were enrolled with no age, size or breed restrictions. The only exclusion criterion was pregnancy since the condition might have

(Figure 2).¹¹ This point is used for shock, collapse, coma and mania. The needle is inserted perpendicular and to a depth of 0.3 *cun*.¹² GV-26 and *Wei-jian* were chosen because they are on the same Channel, are indicated for shock and assist with anesthesia recovery.¹⁰ These acupoints specifically are indicated for helping re-establish *Qi* flow and help stabilize patients in shock. The rationale is that activating the beginning and the end of a Channel increases the flow of *Qi* (life energy) and thus could shorten the recovery period from anesthesia.

The aim of this study was to evaluate atipamezole dose reduction as a less expensive effective option for general anesthesia recovery in shelter cats. The study objective was to investigate atipamezole reduction and compare the result to atipamezole reduction combined with EAP stimulation at GV-26 and *Wei-jian* on recovery from general anesthesia in shelter cats undergoing OHE. The study hypothesis was that the integrative treatment (EAP + 5mcg/kg atipamezole) would be as effective as a 10mcg/kg dose of atipamezole.

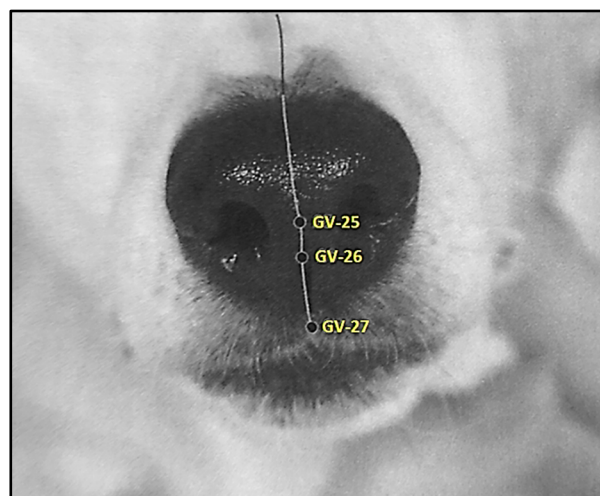


Figure 2: Picture of a dog demonstrating the acupuncture point, GV-26, which is located at the philtrum in animals and is used for shock, collapse, coma and mania.¹¹

lengthened the surgery time and therefore impacted outcome measurements.

Transportation and housing prior to study start for the cats was provided by the shelters and ranged from cats already housed at the shelter to feral animals transported in covered traps or other transport carriers arriving on the day of surgery. Handling and physical examinations were not performed until animals were premedicated for surgery. Withholding water was not recommended by shelter veterinarian protocols and although withholding food is recommended (minimum of 4 hours), exceptions were made for the feral cats because of safety risks removing uneaten bait.⁶

Study Design

All patients were premedicated with a single intramuscular (IM) injection of dexmedetomidine^a (20mcg/kg), ketamine^b (5mg/kg) and butorphanol^c (0.5mg/kg) before the surgical procedure. After sedation, meloxicam^d (0.1mg/kg, SC) was given for post-surgery analgesia. A physical examination was performed that included verification of sex along with measurement of body weight, heart rate, respiratory rate and body temperature, which was recorded for each cat.

Once the physical exam was completed, study cats were intubated and during surgery were maintained on 1% to 2% isoflurane inhalant anesthesia with 1 to 2 L of oxygen (subject to surgeon discretion). They did not have intravenous catheters placed and did not receive fluid therapy during the procedure. After surgery completion, the study cats were removed from the surgery suite and taken to the recovery room where they were extubated and placed on tables for observation. The patients were wrapped in warm blankets and kept warm with water heater pads, heat rice socks and hot water bottles to help maintain body temperature during recovery. Postoperative treatment was begun immediately (denoted as T₀) according to the randomly assigned treatment groups.

Group A received the current neuter program dose of atipamezole^e [10mcg/kg, IM] and served as the Control Group. The other 3 study groups included Group B, which was treated with EAP at GV-26 and *Wei-jian* acupoints plus half dose (5mcg/kg IM) atipamezole; Group C was treated with EAP only at GV-26 and *Wei-jian* acupoints; or Group D treated only with half dose atipamezole (5mcg/kg IM) (Table 1). The patients were monitored for adverse reactions to drugs and procedures used during surgery and the recovery period as this might affect recovery times. All assessments, including recording study outcome data, during the recovery period was performed by the author with assistance from student volunteers. Randomization for group assignment was conducted by drawing tokens in a container filled with an equal number of tokens to represent each group.

Table 1: Summary table of the experimental treatment groups; a total of 56 cats were randomized and assigned to 4 experimental groups (14 per group) that investigated duration of post-surgical recovery associated with atipamezole dose and/or electro-acupuncture.

Group	Atipamezole Dose	EAP Treatment	Recovery Parameters
A Control	10 mcg/kg (IM)	None	TPVM, TPSR, TPHL, TPA
B	5 mcg/kg (IM)	GV-26 + <i>Wei-jian</i> (30 minutes) F1=2Hz, F2=20Hz, Intensity= 2-3	TPVM, TPSR, TPHL, TPA
C	None	GV-26 + <i>Wei-jian</i> (30 minutes) F1=2Hz, F2=20Hz, Intensity= 2-3	TPVM, TPSR, TPHL, TPA
D	5 mcg/kg (IM)	None	TPVM, TPSR, TPHL, TPA

TPVM = voluntary movement, TPSR = swallow reflex, TPHL = head lifting, TPA = ambulation, IM = intramuscular

Patients treated with acupuncture (groups B and C), had sterile disposable acupuncture needles (0.25 mm × 15 mm^f) placed in the *Wei-jian* and GV-26 acupoints. Electrode wires were clamped to the needles that were stimulated with an acupunctoscope^g for 30 minutes immediately after surgery. The stimulation settings used were F1 set at 2Hz, F2 set at 20Hz and the intensity between 2-3 mA, dependent on patient localized response. For cases where the patients fulfilled all criteria for recovery before the full 30 minutes of post-operative treatment, the treatment was terminated.

Data Analysis

The outcome measurements for group comparisons included: 1) duration from T₀ to a post-treatment voluntary movement (TPVM); 2) duration from T₀ to a post-treatment swallow reflex (TPSR); 3) duration from T₀ to a post-treatment head lifting (TPHL); and 4) duration from T₀ to ambulation (TPA). For each of these recovery measurements, the study tested the statistical hypotheses: H₀: There exist no differences in the population mean of TPVM/TPSR/TPHL/TPA among treatment groups vs. H_A: There exists a difference in the population mean of TPVM/TPSR/TPHL/TPA among treatment groups.

To test the hypothesis that compares means of numerical outcome among 4 subject groups, nonparametric Kruskal-Wallis Test was used. Normality test was not necessary as nonparametric tests were used. When the null hypothesis was rejected (i.e. at least one group difference exists), Tukey and Kramer-Nemenyi test was employed to identify which pairs of groups were significantly different. Assuming that a group difference exists with at least 50% greater than the overall standard deviation (i.e., $\Delta/s = 1.5$), it was determined that a sample size of 14 per group would reject the null hypothesis with a 90% power under a significance level = 0.05. Statistical software R was used for all statistical computing and graphics^h.

Patients were monitored for adverse reactions to experimental procedures which included administration of reversal medication and electro-acupuncture. Pain was not assessed during recovery and was not included in this study.

RESULTS

A total of 56 shelter cats (predominantly feral) scheduled for OHE surgery were enrolled in this randomized controlled study with a mean age of 12.0±10.2 months and a mean body weight of 1.93±0.86 kg. By design, the group randomization procedure allocated 14 patients in each treatment group. All patients completed the study. Subject characteristic data included age, body weight, pre-surgery heart rate, pre-surgery respiratory rate and pre-surgery body temperature, which were recorded and compared among groups (Table 2). The statistical comparisons did not find significant differences among groups except body weight, which was significantly higher in the Group B patients ($p=0.006$). None of the study patients had adverse reactions to the experimental procedures, which included the reversal medication and electro-acupuncture.

The TPVM values (Mean±SD) were 6.1±3.5, 7.8±4.6, 37.7±23.1, and 8.6±2.8 minutes in groups A, B, C and D, respectively. Based on the Kruskal-Wallis test there existed a significant difference among the 4 groups ($p = 0.000015$). *Post hoc* multiple comparisons test revealed that significant differences existed between groups A and C ($p = 0.00003$), B and C ($p = 0.0004$), D and C ($p = 0.0095$). Groups A, B and D were not significantly different. Comparison of percent improvement of TPVM recovery time in Group B (with EAP) compared to Group D (without EAP) was 9.3% (Table 3).

The TPSR values (Mean±SD) were 4.5±3.2, 5.1±2.5, 26.3±25.4, and 6.6±2.7 minutes in groups A, B, C and D, respectively. Based on the Kruskal-Wallis test there existed a significant difference among the 4 groups ($p = 0.00003$). *Post hoc* multiple comparisons test revealed that significant differences existed between groups A and C ($p = 0.00007$), B and C ($p = 0.0004$), and groups C and D ($p = 0.019$). Groups A, B and D were not significantly different. Comparison of percent improvement of TPSR recovery time in Group B compared to Group D was 22.7%.

The TPHL values (Mean±SD) were 7.1±5.3, 8.1±4.8, 37.2±23.0, and 8.4±2.6 minutes in groups A, B, C and D, respectively. Based on the Kruskal-Wallis test, there

existed a significant difference among the 4 groups ($p = 0.00003$). *Post hoc* multiple comparisons test revealed that significant differences existed between groups A and C ($p = 0.00007$), B and C ($p = 0.0005$) and groups C and D ($p = 0.0077$). Groups A, B and D were not significantly different. Comparison of percent improvement of TPHL recovery time in Group B compared to Group D was 3.6%.

The TPA values (Mean±SD) were 8.6±6.2, 9.5±6.2, 39.3±23.0, and 10.5±4.6 minutes in groups A, B, C and D, respectively. Based on the Kruskal-Wallis test there existed a significant difference among the 4 groups ($p = 0.00003$). *Post hoc* multiple comparisons test revealed that significant differences existed between groups A and C ($p = 0.00008$), B and C ($p = 0.0004$) and groups C and D ($p = 0.0076$). Groups A, B and D were not significantly different. Comparison of percent improvement of TPA recovery time in Group B compared to Group D was 9.5%.

Further analyses based on the 95% confidence interval of group difference between groups A (10mcg/kg, atipamezole) and B (5mcg/kg, atipamezole + EAP) suggested that Group B's recovery durations with respect to TPSR, TPHL and TPA, respectively, were not statistically greater than Group A's (within Group A's standard deviation).

Table 2: Summary statistics of subject characteristic data (all measured before surgery)

Group	Age (months) (Mean±SD)	Weight (kg) (Mean±SD)	HR (Mean±SD)	RR (Mean±SD)	BT (Mean±SD)
A	7.8±6.2	2.0±0.9	116.7±27.9	34.9±9.6	97.6±2.6
B	10.4±9.3	2.3±1.0*	105.3±23.5	30.7±4.5	97.5±2.4
C	11.6±9.7	1.5±0.3	104.2±14.9	32.3±7.6	96.1±2.5
D	13.8±11.4	1.4±0.5	92.9±17.8	30.0±8.4	96.0±2.3
<i>p</i> -value [^]	0.232	0.006	0.114	0.485	0.130

*significant difference; [^] based on nonparametric Kruskal-Wallis test on between-group differences; HR=heart rate; RR=respiratory rate; BT=body temperature

Table 3: The Mean±SD recovery time (minutes) for each study group from post-surgery treatment to first observation of 1) voluntary movement (TPVM), 2) swallow reflex (TPSR), 3) head lifting (TPHL) and 4) ambulation (TPA) is presented in this table.

Group	TPVM (Mean±SD)	TPSR	TPHL	TPA
A	6.1±3.5	4.5±3.2	7.1±5.3	8.6±6.2
B	7.8±4.6#	5.1±2.5#	8.1±4.8#	9.5±6.2#
C	37.7±23.1*	26.3±25.4*	37.2±23.0*	39.3±23.0*
D	8.6±2.8	6.6±2.7	8.4±2.6	10.5±4.6
<i>p</i> -value [^]	0.000015	0.00003	0.00003	0.00003

* significantly different from the other groups; [^] based on nonparametric Kruskal-Wallis test on between-group differences. # Group B recovery had nonsignificantly shorter duration for all 4 outcome measures when compared to Group D. * Group C recovery had significantly longer duration for all 4 outcome measures when compared to the other groups.

DISCUSSION

As public efforts to reduce the overpopulation of community cats have increased, many veterinarians have increasingly focused their clinical efforts on the provision of cost effective spay-neuter services that can optimally manage these populations. This clinical study in shelter cats (predominantly feral) investigated effective anesthesia recovery from OHE surgery, but at a lower cost, by using reduced doses of atipamezole both combined with electro-acupuncture or administration of low dose atipamezole only.

Study results documented no statistically significant difference for the 4 outcome measurements (time to swallow, voluntary movement, head lift, ambulation) among patient groups treated with 10mcg/kg IM of atipamezole, 5mcg/kg IM atipamezole with EAP or 5mcg/kg IM atipamezole only following general anesthesia. The only group with inferior recovery outcome parameters ($p < 0.001$) was Group C (EAP only).

Even though the study results indicated that the use of a low dose, 5mcg/kg atipamezole only, had comparable results to a 10mcg/kg dose, the addition of EAP to low dose atipamezole treatment (Group B) was associated with a non-significantly shorter recovery duration for all 4 outcome measures (TPVM = 9.3% improved, TPSR = 22.7% improved, TPHL = 3.6% improved, TPA = 9.5% improved) when compared to Group D (low dose atipamezole only). These findings suggest 5mcg/kg atipamezole (less high-cost recovery drug) provides an equally effective recovery from general anesthesia when compared to 10mcg/kg (control) for shelter animals undergoing OHE; however, the addition of EAP to low dose drug treatment appears to marginally improve recovery times under the experimental conditions of the present study.

The minimal superiority of Group B over Group D was an unexpected study outcome. The study hypothesis predicted that Group B would be significantly superior to Group D due to the addition of EAP treatment. This was based on literature support of the beneficial effects of EAP on anesthesia. In non-human species, a study performing EAP on wild caught snapping turtles found EAP treatment shortened recovery time when compared to the administration of epinephrine.¹³ Several studies in humans have found that patients recovered from anesthesia faster with the addition of EAP or DNAP.⁸⁻¹⁰ Additionally, German anesthesiologists have documented the use of transcutaneous electrical stimulation of an acupuncture point near the tragus reduces anesthetic requirements, while other investigators found that AP had the ability to reduce the amount of anesthesia and pharmaceuticals required for procedures.^{12,14} These studies support that integration of EAP or DNAP with conventional pharmaceuticals can be more effective than using conventional medications alone and suggest that the marginal recovery improvement times found in the present study, when EAP was added to a lower drug dose, may suggest a biological trend in the study cats.

Contributing factors, to only a minimal difference between Group B and Group D, to be considered and

modified in future studies would include: 1) the small sample size; 2) longer surgery times associated with longer recovery for some animals (related to variable surgeon skills in this study); 3) not monitoring body temperatures during recovery (hypothermic animals associated with longer recovery); and 4) significant increased body weight in Group B (increased weight in Group B cats could impact anesthesia complications or recovery differently compared to other groups). A final contributing factor to consider, was EAP stimulation of acupoints (2 Hz and 20 Hz). This creates release of neurotransmitters which can compete for receptors with pre-anesthetic drugs (i.e. ketamine with opioid receptors) leading to unexpected EAP outcomes.¹⁵ To date, there have been a paucity of studies investigating the combination of EAP and pharmaceuticals.¹⁵

Limitations to this study included the common challenges of conducting a clinical investigation under conditions that could not be tightly controlled. There was marked animal variability with most cats feral (significant fear, anxiety, and stress); no medical history was available; or ability to assess surgical candidate status per American Society of Anesthesiology (ASA) criteria; all of which complicated study outcomes. The limited funds for shelter spay-neuter programs meant no pre-surgery blood work, along with variability of anesthesia machines and monitoring equipment. Due to the nature of the volunteer work that was utilized for the surgery, the skills and experiences of each surgeon were varied. Some of the surgeons were fourth-year students under the tutelage of a veterinarian. Other surgeons were well seasoned veterinarians able to complete the surgery efficiently. The lack of personnel also caused the inability to create effective blinding for the study with the author and student volunteers (who knew treatment groups) evaluating and recording outcome data. The data that was documented was mostly objective, therefore, it is thought that this limitation did not greatly affect study outcome data results.

An additional significant limitation of the study was the variability in thermoregulation and lack of body temperature monitoring, particularly during the anesthetic recovery period. It has been noted that hypothermia and a higher ASA score can have a significant impact on recovery time.^{14,16,17} One study focused on postanesthetic hypothermia in cats. This retrospective study determined that abdominal surgery, increased surgery duration and a higher ASA score will all increase hypothermic risk in cats.¹⁶

Even with the significant challenges that were presented during the conduct of this study, the research offers encouraging information on the potential to use acupuncture during post-anesthetic recovery to reduce costly medications and provide comparable shortened anesthetic recovery. More studies, especially larger randomized, controlled, blinded trials using a more uniform study population, surgical conditions and recovery areas with better monitoring of patient thermoregulation are necessary to evaluate the present study's findings.

In summary, the effect of EAP added to reduced doses of atipamezole on length of general anesthesia recovery in shelter cats undergoing OHE was investigated. There was no statistically significant difference in the recovery parameters except the EAP only group had significantly longer recovery times. Clinical relevance of these study findings and study hypothesis suggest 5mcg/kg atipamezole (less high-cost recovery drug) provides an equally effective recovery from general anesthesia when compared to 10mcg/kg (commonly used dose) for shelter animals undergoing OHE; and the addition of EAP to low dose atipamezole was associated with non-significantly shorter recovery times under the experimental conditions of this study.

ACKNOWLEDGEMENTS

I would like to thank Dr. Ronald Koh, who first introduced me to TCVM, has mentored me during my TCVM journey and provided me with the basis for this research project. I would also like to thank the previous researcher, Dr. Alison Mikes who started the original research project as a summer scholar. Great appreciation goes to the doctors and students at the Louisiana State University School of Veterinary Medicine who dedicate their time, energy, knowledge and expertise to assist with Spay Day. Specifically, I particularly want to thank Dr. Jeannette Cremer, Dr. Brandy Duhon and Dr. Wendy Wolfson for their contributions and support. A heart-felt thank you goes to Companion Animal Alliance and Cat Haven, of Baton Rouge, LA for their assistance with this project.

Declaration of Interest and Funding

The author declares that there is no conflict of interest that could be perceived as prejudicing the impartiality of this paper and the author did not receive any specific grant of funding for authorship of this paper.

FOOTNOTES

- ^a Dexmedetomidine, Zoetis, Parsipanny, New Jersey, USA
- ^b Ketamine HCl, generic, several sources available in USA
- ^c Butorphanol tartrate, generic, several sources available in USA
- ^d Meloxicam, generic, several sources available in USA
- ^e Atipamezole, Zoetis, Parsipanny, New Jersey, USA
- ^f DBC, DongBang™ Corporation, Koreas
- ^g Electro-acupuncture Stimulator, JM-2A model, Wuxi Jiajian Medical Instrument Co, LTD, China
- ^h R version 3.5.2. The R Foundation for Statistical Computing, Vienna, Austria (<http://www.R-project.org>)

REFERENCES

1. Broadbelt D, Blisset K, Hammond R et al. The risk of death: The confidential enquiry into perioperative small animal fatalities. *Veterinary Analgesia and Anesthesia* 2008; 35(5):365-373.
2. Sisak D. The top 5 anesthetic complications. In *Proceedings of the University of Pennsylvania Vet Annual Conference*, Philadelphia, Pennsylvania; University of Pennsylvania 2017:1-5.
3. Robertson S, Gogolski S, Pascoe P et al. AAFP Feline anesthesia guidelines. *J Feline Med Surg* 2018; 20(7):602-634. doi: 10.1177/1098612X18781391
4. Dyson D, Maxie M, Schnurr D. Morbidity and mortality associated with anesthetic management in small animal veterinary practice in Ontario. *J Am Anim Hosp Assoc* 1998; 34(4):325-335. doi: 10.5326/15473317-34-4-32
5. Brodbelt D. The Confidential Enquiry into Perioperative Small Animal Fatalities. Thesis for Royal Veterinary College, University of London. London, England 2008; 105-106.
6. Griffin B, Bushby P, McCobb E et al. The Association of Shelter Veterinarians' 2016 veterinary medical care guidelines for spay-neuter programs. *J Am Vet Med Assoc* 2016; 249(2):165-188. doi: 10.2460/javma.249.2.165
7. Huang M, Wang X, Xing B et al. Critical Roles of TRPV2 Channels, Histamine H1 and Adenosine A1 receptors in the initiation of acupoint signals for acupuncture analgesia. *Sci Rep* 2018; 8(1):6523. doi: 10.1038/s41598-018-24654-y
8. Lu Z, Dong H, Wang Q et al. Perioperative acupuncture modulation: More than anesthesia. *Br J Anaesth* 2015; 115(2): 183-193. doi: 10.1093/bja/aeu227
9. Zhang R, Lao L, Ren K et al. Mechanisms of acupuncture-electroacupuncture on persistent pain. *Anesthesiology* 2014; 120(2):482-503. doi: 10.1097/ALN.000000000000101
10. Chernyak G, Sessler D, Warltier D. Perioperative acupuncture and related techniques. *Anesthesiology* 2005; 102:1031-1049. doi.org/10.1097/00000542-200505000-00024
11. Xie H, Preast V. Xie's Veterinary Acupuncture. Ames, Iowa: Blackwell Publishing 2007:3-5, 6-7, 199, 226.
12. Kotani N, Hashimoto H, Sato Y et al. Preoperative intradermal acupuncture reduces postoperative pain, nausea vomiting, analgesic requirement, and sympathoadrenal responses. *Anesthesiology* 2001; 95(2):349-356. doi: 10.1097/00000542-200108000-00015
13. Goe A, Shmalberg J, Gaston B et al. Epinephrine for GV-26 electrical stimulation reduces inhalant anesthetic recovery time in common snapping turtles (*Chelydra serpentina*). *J Zoo and Wildl Med* 2016; 47(2):501-507. doi: 10.1638/2015-0264.1
14. Greif R, Laciny S, Mokhtarani M et al. Transcutaneous electrical stimulation of an auricular acupuncture point decreases anesthetic requirement. *Anesthesiology* 2002; 96(2):306-312. doi: 10.1097/00000542-200202000-00014
15. Nascimento F, Marques V, Crocioli G et al. Analgesic efficacy of laser acupuncture and electroacupuncture in cats undergoing ovariohysterectomy. *J Vet Med Sci* 2019; 81(5):764-770. doi: 10.1292/jvms.18-0744
16. Redondo J, Suesta P, Gil L et al. Retrospective study of the prevalence of postanesthetic hypothermia in cats. *Vet Rec* 2012; 170(8):206. doi: 10.1136/vr.100184
17. Bednarski R, Grimm K, Harvey R et al. AAHA anesthesia guidelines for dogs and cats. *J Am Anim Hosp Assoc* 2011; 47(6): 377-385. doi: 10.5326/JAAHA-MS-5846